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Helmithfauna of the American mink (*Neovison vison*), the Iberian wolf (*Canis lupus signatus*) and the red fox (*Vulpes vulpes*) in the Northwest of Iberian Peninsula

Helmintofauna del visón americano (*Neovison vison*), el lobo ibérico (*Canis lupus signatus*) y el zorro (*Vulpes vulpes*) en el noroeste de la península ibérica.

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A mi abuelo Salvador, quien me preguntaba por qué estudié Veterinaria si lo que quería era ser "Doctor".

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Según una definición estricta, una Tesis Doctoral es un trabajo original de investigación en un área de conocimiento. Para mí, ha sido mucho más, ha sido una etapa de mi vida, una etapa de aprendizaje y mejora como persona. Al echar la vista atrás de estos años, me queda más claro todo el esfuerzo y todas las personas que, de forma directa o indirecta, han influido en la realización de este "trabajo original de investigación en un área de conocimiento", a las cuales quiero hacer mención en estas líneas.

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RESUMEN/SUMMARY

RESUMEN

En esta Tesis Doctoral se ha estudiado la helmitofauna de tres especies de carnívoros silvestres presentes en el noroeste de España: el visón americano (*Neovison vison*), el zorro (*Vulpes vulpes*) y el lobo ibérico (*Canis lupus signatus*). El visón americano es un mustélido procedente de Norteamérica que fue introducido en la península ibérica y otras partes del mundo para ser criados en granjas peleteras. Con el paso del tiempo, las fugas y las liberaciones intencionadas de estos animales han dado lugar a la formación de poblaciones silvestres establecidas en áreas del centro y norte de España. Por otra parte, el zorro y el lobo son las dos únicas especies de cánidos silvestres autóctonas de la península ibérica. Las poblaciones principales de lobo se localizan en el norte y noroeste, además de una pequeña población residual en el sur de España, mientras que el zorro se distribuye de forma homogénea por todo el territorio peninsular.

En la actualidad, existe una tendencia cada vez mayor a la transformación de hábitats naturales en ambientes fragmentados y humanizados que favorecen la presencia de especies generalistas, como el zorro, en detrimento de las que son especialistas. Sin embargo, durante los últimos años se ha producido una mayor concienciación de la población de nuestro país, y también de las Administraciones, sobre la necesidad de conservar especies emblemáticas, como el lobo. Esta creciente sensibilidad con la conservación de la biodiversidad ha coincidido con el abandono de muchas áreas rurales, lo que ha permitido la expansión de estos carnívoros silvestres hacia zonas antiguamente ocupadas por la actividad humana. Por estos motivos, las poblaciones de estos tres carnívoros silvestres se encuentran en expansión, a pesar de la presión cinegética o de control de sus poblaciones a las que se encuentran sometidas.

El acercamiento de la fauna silvestre a las áreas antropizadas conlleva un mayor contacto, ya sea directo o indirecto, entre los animales domésticos y silvestres, además del propio ser humano, de manera que el riesgo epidemiológico de transmisión de agentes patógenos se incrementa en la interfaz doméstico-silvestre-humano. La gran mayoría de los agentes patógenos de los carnívoros silvestres son compartidos con el perro y el gato, por lo que las especies silvestres pueden participar perfectamente en la transmisión de estos patógenos desde un ciclo selvático hacia uno rural o periurbano. Además, algunos de estos agentes son zoonósicos, con las implicaciones que esto conlleva desde un punto de vista de la salud pública. Debemos indicar, no obstante, que el contacto entre carnívoros silvestre y domésticos no siempre implica que los primeros sean el origen del patógeno transmitido, sino que pueden ser víctimas de agentes infectocontagiosos procedentes de las poblaciones de perros y gatos. Es decir, nos encontramos ante nuevas situaciones epidemiológicas cuyas consecuencias pueden ser importantes, debido a cambios en el papel de las especies como reservorio, amplificador o transmisor de dichos patógenos que, en situaciones concretas, pueden desencadenar efectos spill-over o spill-back. Además, en el caso concreto del visón americano, se produce una circunstancia especial ya que, al tratarse de una especie invasora en expansión, potencialmente es reservorio y/o transmisor de patógenos exóticos específicos de este mustélido americano. Por otra parte, la ocupación del hábitat por parte del visón americano supone el contacto de esta especie hospedadora con los agentes patógenos propios de estas áreas peninsulares, lo que puede suponer un factor epidemiológico nuevo, hasta ahora no estudiado, que influya en la dinámica del anidamiento natural y dispersión de todos estos patógenos nativos. Por tanto, el papel epidemiológico de esta especie invasora en la implantación del ciclo selvático de patógenos foráneos o, por otra parte, la integración en el ciclo de los patógenos propios de las especies carnívoras peninsulares, podría acarrear cambios epidemiológicos impredecibles cuyas consecuencias sobre las poblaciones de las especies autóctonas son una incógnita.

Por todo lo anteriormente mencionado, el objetivo principal de esta Tesis Doctoral es la descripción de las especies de helmintos cardiopulmonares y gastrointestinales que afectan al visón americano, al zorro y al lobo del noroeste de la península ibérica, discutiendo el papel epidemiológico que cada uno de ellos desempeña en el anidamiento natural y la transmisión de estos parásitos, e intentando explicar la helmintofauna hallada desde una perspectiva ecológica, sobre todo en base al comportamiento trófico que tiene cada una de estas tres especies hospedadoras.

En el Capítulo 1 se estudian los helmintos gastrointestinales y cardiopulmonares del visón americano en Galicia (noroeste de España), con el fin de dilucidar si este mustélido actúa como reservorio y diseminador de parásitos propios de esta especie invasora hasta ahora no descritos en la península ibérica, o bien si se trata de una especie que, al integrarse en el hábitat que ha invadido, se ha incorporado al ciclo biológico selvático de los helmintos propios de los mustélidos autóctonos y otros carnívoros silvestres del área colonizada. Para tal fin, durante el periodo 2008-2014 fueron capturados y sacrificados cincuenta visones americanos (35 machos y 15 hembras) de diferentes edades (22 inmaduros y 28 adultos) procedentes de las provincias de Lugo, Ourense y Pontevedra. Se encontraron ocho especies de parásitos (seis nematodos y dos trematodos) con las siguientes prevalencias: *Molineus patens* (68%), *Aonchotheca putorii* (54%), *Crenosoma melesi* (10%), *Aonchotheca annulosa* (8%), *Angiostrongylus daskalovi* (6%), *Aelurostrongylus* spp. (2%), *Troglotrema acutum* (2%) y un trematodo digestivo que no pudo ser identificado (2%). El 82% de los visones albergaban helmintos, incluyendo 15 animales (30%) infectados por una sola especie de parásito, 19 (38%) por dos especies, cinco (10%) por tres especies, y dos visones (4%)

por cuatro especies de helmintos. Todas las especies de parásitos identificados son nativos de los mustélidos europeos. Se utilizaron modelos estadísticos para evaluar si las características de los animales (edad, sexo y peso), la fecha y el área de captura influían en la prevalencia, intensidad o riqueza de parásitos. Sólo se detectaron diferencias significativas en los modelos de intensidad de *M. patens*, *A. putorii* y *C. melesi*. Este trabajo es la primera descripción en el visón americano de *A. daskalovi*, un nematodo cardiopulmonar, y *A. annulosa*, un nematodo gastrointestinal propio de roedores. Además, aunque el trematodo *T. acutum* ya había sido descrito previamente en el visón americano, nuestro estudio es la primera cita de este trematodo en el pulmón, una ubicación ectópica nunca antes mencionada.

En el Capítulo 2 se describen las especies de nematodos cardiopulmonares presentes en el lobo ibérico y el zorro de Galicia y Asturias (noroeste de España), discutiendo el papel epidemiológico que cada uno de estos cánidos silvestres desempeña en el anidamiento natural y la dispersión de estos parásitos, los cuales también afectan al perro y desarrollan una destacada acción patógena sobre el hospedador. Durante el periodo 2008-2014 se realizó la necropsia de un total de 257 zorros y 74 lobos, y se examinó la tráquea, pulmones y corazón de cada animal. Se identificaron cuatro especies de nematodos cardiopulmonares: *Angiostrongylus vasorum*, *Eucoleus aerophilus*, *Crenosoma vulpis* y *Filaroides hirthi*. No se detectó *Dirofilaria immitis* en ninguno de los animales. Las dos especies hospedadoras compartieron las mismas especies de nematodos, excepto *F. hirthi*, que sólo fue encontrada en lobos. La prevalencia global de parásitos cardiopulmonares fue significativamente mayor en zorros (70,4%) que en lobos (28,4%). En concreto, las prevalencias en zorros y lobos fueron, respectivamente, 43,2% y 21,6% para *A. vasorum*, 33,4% y 5,4% para *E. aerophilus*, y 30,0% y 9,4% para *C. vulpis*. La prevalencia de *F. hirthi* fue del 16,2%, siendo la primera vez que se cita este nematodo, a nivel mundial, en la especie *C. lupus*. La intensidad de *A. vasorum* fue significativamente mayor en zorros que en lobos, y también en el caso de *C. vulpis*, pero sólo marginalmente. Los modelos de regresión logística mixta confirmaron el riesgo significativamente mayor de que los zorros se infecten con estos parásitos cardiopulmonares en comparación con los lobos. La prevalencia de estos parásitos en ambas especies de cánidos silvestres está probablemente relacionada con las diferencias en el comportamiento trófico. En base a los resultados obtenidos, se plantea la hipótesis de que los nematodos cardiopulmonares han coevolucionado con sus respectivos hospedadores definitivos, intermediarios y paraténicos, de manera que los zorros se han convertido en los hospedadores que presentan las mayores intensidades de parásitos y el mayor número de animales infectados, en comparación con el lobo. Es decir, los nematodos cardiopulmonares, aunque adaptados evolutivamente a varias especies de cánidos silvestres como lobos, zorros, coyotes o chacales, han logrado vincular sus ciclos

biológicos a un tipo de presa que es más probable que sea consumida por el depredador más pequeño (el zorro). Esto impide que la capacidad cardiorrespiratoria de un gran depredador (el lobo), situado en la parte superior de la cadena trófica, se vea comprometida, lo que se podría interpretar como una adaptación por parte del lobo basada en una estrategia trófica que reduce el riesgo de infección.

En el Capítulo 3 se describe la helmintofauna gastrointestinal del zorro y del lobo ibérico del noroeste de la península ibérica (Galicia y Asturias), analizando el papel epidemiológico que estos cánidos silvestres desempeñan en el mantenimiento del ciclo biológico selvático y transmisión de estos parásitos. Los helmintos gastrointestinales son parásitos frecuentemente descritos en estos cánidos silvestres. Tienen una especial importancia desde el punto de vista sanitario, ya que muchos de estos patógenos también pueden afectar a los animales domésticos e incluso, en algunos casos, tienen potencial zoonósico. Durante el periodo 2008-2014 se realizó la necropsia de un total de 257 zorros y 83 lobos ibéricos, y se examinó el tracto gastrointestinal de cada animal (esófago, estómago, intestino delgado e intestino grueso). Además, mediante PCR clásica se analizaron 94 cestodos individuales obtenidos de 27 lobos para su identificación molecular. Se obtuvieron productos amplificados en 63 de estas muestras, y las secuencias confirmaron la presencia de dos especies de cestodos: *Taenia hydatigena* (16/63 secuencias) y *Taenia krabbei* (47/63 secuencias). Se detectaron un total de 13 especies de helmintos (cinco nematodos y ocho cestodos). Los nematodos *Uncinaria stenocephala*, *Toxocara canis*, *Trichuris vulpis* y los cestodos *Taenia hydatigena*, *Taenia pisiformis*, *Taenia crassiceps*, *Mesocestoides* spp. y *Dipylidium caninum* fueron identificados en ambos cánidos silvestres, mientras que *Ancylostoma caninum* y *Taenia krabbei* sólo se aislaron en lobos. Por último, *Toxascaris leonina*, *Taenia polyacantha* y *Taenia taeniaeformis* se encontraron exclusivamente en zorros. Esta es la primera vez que se describe *T. krabbei* en lobos de la península ibérica. No se detectó la presencia de cestodos pertenecientes al género *Echinococcus* en ninguno de los animales estudiados. La prevalencia general de nematodos gastrointestinales fue mayor en lobos (80,7%) que en zorros (61,9%), así como la prevalencia global de cestodos (68,7% y 44,0%, respectivamente). La intensidad de parasitación por cestodos fue significativamente mayor en los lobos (14 cestodos por hospedador infectado) que en los zorros (7 cestodos por hospedador infectado), pero no hubo diferencias respecto a la intensidad de parasitación por nematodos. La mayoría de los helmintos identificados son transmitidos a través de las redes tróficas, por predación sobre hospedadores intermediarios o paraténicos. En este sentido, los zorros presentaron una helmintofauna típica de carnívoros que consumen pequeñas presas, como roedores, mientras que los lobos tuvieron principalmente parásitos transmitidos por la ingestión de presas de gran tamaño, como son los ungulados. De esta forma, se evidencia que, pese a que el zorro y el lobo son susceptibles a los mismos helmintos gastrointestinales, las particularidades

ecológicas, principalmente tróficas, de la especie hospedadora son determinantes del tipo de helmintofauna gastrointestinal de cada uno de estos cánidos silvestres y, por tanto, también condicionan el papel epidemiológico que cada uno de ellos desempeña en el mantenimiento del ciclo biológico de estos parásitos, así como en su dispersión.

Los hallazgos obtenidos a lo largo de los tres capítulos que conforman esta Tesis Doctoral aportan una valiosa información que permite conocer mejor cuál es el papel epidemiológico que pueden tener estas tres especies de carnívoros silvestres en el noroeste de la península ibérica, destacando la importancia de cada uno de estos hospedadores en el mantenimiento del ciclo biológico de los helmintos encontrados. Además, considerando que son especies en expansión, se evidencia la necesidad de tener en cuenta la presencia de estos carnívoros como un factor de riesgo en la interfaz silvestre-doméstico-humano. Los datos obtenidos podrán servir como base para la elaboración de programas de vigilancia epidemiológica de enfermedades zoonósicas o de especial relevancia en la conservación de estos carnívoros o de otras especies, ya sean domésticas o silvestres, con las que pudieran entrar en contacto.

SUMMARY

In this PhD dissertation we have studied the helminthfauna of three wild carnivore species present in northwestern Spain: the American mink (*Neovison vison*), the red fox (*Vulpes vulpes*) and the Iberian wolf (*Canis lupus signatus*). The American mink is a mustelid from North America which was introduced in the Iberian Peninsula and other parts of the world to be raised on fur farms. Over time, escapes and intentional releases of these animals have resulted in the formation of established wild populations in areas of central and northern Spain. On the other hand, red foxes and wolves are the only two native species of wild canids to the Iberian Peninsula. The main wolf populations are located in the north and northwest, in addition to a small residual population in southern Spain, while the fox is distributed homogeneously throughout the peninsular territory.

Currently, there is an increasing trend to transform natural habitats into fragmented and humanized environments that favour the presence of generalist species, such as the fox, in detriment of those who are specialists. However, in recent years, society and administrations have become more aware of the need to conserve emblematic species, such as the Iberian wolf. This increasing sensitivity for biodiversity conservation has coincided with the abandonment of many rural areas, which has allowed the expansion of wildlife into areas formerly occupied by human activity. For these reasons, the populations of these three wild carnivores are expanding, despite the pressure of hunting or control of their populations.

The approach of wildlife to anthropized areas entails greater contact, either direct or indirect, between domestic and wild animals, as well as the human being, so the epidemiological risk of pathogen transmission is increased at the domestic-wild-human interface. Most pathogens of wild carnivores are shared with dogs and cats, so wild species can be perfectly involved in the transmission of these pathogens from a sylvatic to a rural or peri-urban cycle. In addition, some of these agents are zoonotic, with implications for public health. It should be noted, however, that contact between wild and domestic carnivores does not always imply that the former are the origin of transmitted pathogens, but that they may be targets of infectious agents from dog and cat populations. In other words, we are facing new epidemiological situations whose consequences may be significant, due to changes in the role of species as reservoir, amplifier or transmitter of these pathogens which, in specific situations, may cause spill-over or spill-back effects. Furthermore, in the specific case of American mink, there is a special circumstance since, being an expanding invasive species, it is a potential reservoir and/or transmitter of exotic pathogens specific to this American mustelid. On the other hand, the occupation of the habitat by the American mink supposes the contact of this host species with the native pathogens of these peninsular areas, which can imply a new epidemiological factor, until now unstudied, that influences the dynamics of the natural nesting and dispersion of all these native pathogens. Therefore, the epidemiological role of this invasive species in the introduction of the sylvatic cycle of foreign pathogens or, on the other hand, the integration in the cycle of pathogens typical of peninsular carnivorous species, could lead to unpredictable epidemiological changes whose consequences on the populations of native species are unknown.

For all the above-mentioned reasons, the main objective of this PhD dissertation is the description of cardiopulmonary and gastrointestinal helminth species which affect the American mink, the fox and the wolf in the northwest of the Iberian Peninsula, discussing the epidemiological role played by each of them in the natural nesting and transmission of these parasites, and explaining the helminthfauna found from an ecological perspective, especially based on the trophic behaviour of each host species.

In Chapter 1 we study the gastrointestinal and cardiopulmonary helminths of the American mink in Galicia (northwestern Spain), in order to determine if this mustelid acts as a reservoir and spreader of parasites specific to this invasive species not yet described in the Iberian Peninsula, or if it is a species which, when integrated into the habitat it has invaded, has been incorporated into the sylvatic life cycle of the helminths of the autochthonous mustelids and other wild carnivores of the colonised area. For this purpose, during the period 2008-2014, fifty American minks (35 males and 15 females) of different ages (22 juveniles and 28 adults) from the provinces of Lugo, Ourense and Pontevedra were captured and sacrificed. Eight species of parasites (six nematodes and two trematodes) were found with the following prevalences: *Molineus patens* (68%), *Aonchotheca putorii* (54%), *Crenosoma melesi* (10%), *Aonchotheca annulosa* (8%), *Angiostrongylus daskalovi* (6%), *Aelurostrongylus* spp. (2%), *Troglotrema acutum* (2%) and a digestive trematode that could not be identified (2%). Eighty-two percent of the minks housed helminths, including 15 animals (30%) infected by a single species of parasite, 19 (38%) by two species, five (10%) by three species, and two minks (4%) by four species of helminths. All identified parasite species are native to European mustelids. Statistical models were used to evaluate whether animal characteristics (age, sex and weight), date and area of capture influenced the prevalence, intensity or richness of parasites. Only significant differences were detected in the intensity models of *M. patens*, *A. putorii* and *C. melesi*. This study is the first description in the American mink of *A. daskalovi*, a cardiopulmonary nematode, and *A. annulosa*, a rodent-specific gastrointestinal nematode. In addition, although the trematode *T. acutum* had previously been described in the American mink, our study is the first to detect this trematode in the lung, an ectopic location never before mentioned.

In Chapter 2 we describe the cardiopulmonary nematode species present in the Iberian wolf and the fox from Galicia and Asturias (northwestern Spain), discussing the

epidemiological role played by each of these wild canids in the natural nesting and spread of these parasites, which also affect dogs and cause an important pathogenic action on the host. During the period 2008-2014 a total of 257 foxes and 74 Iberian wolves were necropsied, and the trachea, lungs and heart of each animal were examined. Four species of cardiopulmonary nematodes were identified: *Angiostrongylus vasorum*, *Eucoleus aerophilus*, *Crenosoma vulpis* and *Filaroides hirthi*. *Dirofilaria immitis* was not detected in any animal. Both host species shared the same nematode species, except *F. hirthi*, which was only found in wolves. The overall prevalence of cardiopulmonary parasites was significantly higher in foxes (70.4%) than in wolves (28.4%). Specifically, the prevalences in foxes and wolves were 43.2% and 21.6% for *A. vasorum*, 33.4% and 5.4% for *E. aerophilus*, and 30.0% and 9.4% for *C. vulpis*, respectively. The prevalence of *F. hirthi* was 16.2%, the first report of this nematode in *C. lupus* worldwide. The intensity of *A. vasorum* was significantly higher in foxes than in wolves, and also for *C. vulpis*, but only marginally. Mixed logistic regression models confirmed the significantly higher risk of foxes becoming infected with these cardiopulmonary parasites compared to wolves. The prevalence of these parasites in both wild canid species is probably related to differences in trophic behaviour. Based on the results obtained, we hypothesized that cardiopulmonary nematodes have co-evolved with their respective definitive, intermediate and paratenic hosts, so that foxes have become the host with the highest parasite intensities and the highest number of infected animals compared to wolves. In other words, cardiopulmonary nematodes, although evolutionarily adapted to various species of wild canids, have succeeded in linking their biological cycles to a type of prey which is more likely to be consumed by the smallest predator (the fox). This prevents the cardiorespiratory capacity of a large predator (the wolf), located at the top of the food chain, being compromised, which could be interpreted as an adaptation by the wolf based on a trophic strategy that reduces infection risk.

Chapter 3 describes the gastrointestinal helminthfauna of foxes and Iberian wolves from the northwest of the Iberian Peninsula (Galicia and Asturias), analysing the epidemiological role that these wild dogs play in the maintenance of the sylvatic life cycle and transmission of these parasites. Gastrointestinal helminths are parasites frequently described in these wild canids. They have a special importance from an animal and public health point of view, as many of these pathogens can also affect domestic animals and even, in some cases, have zoonotic potential. During the period 2008-2014, a total of 257 foxes and 83 Iberian wolves were necropsied, and the gastrointestinal tract of each animal (esophagus, stomach, small intestine and large intestine) was examined. In addition, 94 individual cestodes obtained from 27 wolves were analysed by classical PCR for molecular identification. Amplified products were obtained in 63 of these samples, and the sequences confirmed the presence of two

cestode species: *Taenia hydatigena* (16/63 sequences) and *Taenia krabbei* (47/63 sequences). A total of 13 helminth species (5 nematodes and 8 cestodes) were detected. The nematodes *Uncinaria stenocephala*, *Toxocara canis*, *Trichuris vulpis* and the cestodes *Taenia hydatigena*, *Taenia pisiformis*, *Taenia crassiceps*, *Mesocestoides* spp. and *Dipylidium caninum* were identified in both wild canids, whereas *Ancylostoma caninum* and *Taenia krabbei* were only isolated in wolves. Finally, *Toxascaris leonina*, *Taenia polyacantha* and *Taenia taeniaeformis* were found exclusively in foxes. This is the first time that *T. krabbei* has been detected in wolves from the Iberian Peninsula. Cestodes belonging to genus *Echinococcus* were not detected in any of the studied animals. The overall prevalence of gastrointestinal nematodes was higher in wolves (80.7%) than in foxes (61.9%), as well as the overall prevalence of cestodes (68.7% and 44.0%, respectively). The parasite intensity of cestodes was significantly higher in wolves (14 cestodes per infected host) than in foxes (7 cestodes per infected host), but there was no difference in the intensity of nematodes. Most of the identified helminths are transmitted through trophic networks, by predation on intermediate or paratenic hosts. In this sense, foxes presented a typical helminthfauna of carnivores which consume small prey, such as rodents, while wolves had mainly parasites transmitted by the ingestion of large prey, such as ungulates. In this way, it is evident that, although foxes and wolves are susceptible to the same gastrointestinal helminths, the ecological characteristics, mainly the trophic behaviour, of the host species are determinants of the type of gastrointestinal helminthfauna of each of these wild canids and, therefore, also condition the epidemiological role that each of them plays in the maintenance of these parasite life cycle, as well as in their spread.

The findings obtained throughout the three chapters constituting this doctoral thesis provide valuable information that helps to better understand the epidemiological role played by these three wild carnivore species in the northwest of the Iberian Peninsula, highlighting the importance of each of these hosts in the maintenance of the life cycle of the helminths found. In addition, considering that they are species in expansion, it is evident the need to consider the presence of these carnivores as a risk factor in the wild-domestic-human interface. The data obtained may serve as a source for the development of epidemiological surveillance programs for zoonoses or diseases of special relevance to conservation of these carnivores or other species, either domestic or wild, with whom they might come into contact.

INTRODUCCIÓN

Este trabajo aborda el estudio de los helmintos gastrointestinales y cardiorrespiratorios que parasitan tres especies de mamíferos carnívoros presentes en el noroeste de España: el zorro (*Vulpes vulpes*, Linnaeus, 1758) y el lobo ibérico (*Canis lupus signatus*, Linnaeus, 1758), que son las dos únicas especies autóctonas de cánidos silvestres de la península ibérica, y el visón americano (*Neovison vison*, Schreber, 1777), un mustélido alóctono considerado como especie invasora. Las partes de que consta esta introducción son una breve revisión del papel ecológico que desempeñan los mamíferos carnívoros en los ecosistemas, así como las consecuencias que tiene la expansión de determinadas especies de fauna silvestre en los entornos antropizados. Además, abordaremos de una forma sintética el papel que pueden desempeñar las especies de fauna silvestre, desde un punto de vista epidemiológico, en la interfaz doméstico-silvestre-humano, y finalizaremos con la descripción de las características biológicas más destacadas de las tres especies de carnívoros silvestres sobre las que se centra el presente estudio.

PAPEL ECOLÓGICO DE LOS MAMÍFEROS CARNÍVOROS

Los carnívoros mamíferos son, desde un punto de vista ecológico, piezas clave en el mantenimiento del equilibrio en las interacciones biológicas. Según Van Valkenburgh (2007), los mamíferos carnívoros se pueden dividir en tres categorías en función del porcentaje de su dieta que proporciona la predación sobre vertebrados y, por tanto, la capacidad para adaptar su dieta a fuentes alimentarias distintas. En este caso, los carnívoros que presentan una dieta compuesta por vertebrados en más del 70% se conocen como hipercarnívoros, entre los cuales se encuentran los grandes predadores como lobos, leones o hienas. Por otra parte, los mesocarnívoros son los que presentan una dieta compuesta por vertebrados en un 50-70%; en este grupo se enmarcan carnívoros de pequeño y mediano tamaño, como el zorro o la mayoría de mustélidos. Por último, los hipocarnívoros son el grupo cuya dieta está formada por vertebrados en menos del 30% de su composición, estando clasificados en esta categoría los úrsidos. En términos generales, y refiriéndonos sobre todo a los grandes carnívoros, unos pocos individuos pueden causar destacados efectos directos debido a la depredación o, de forma indirecta, influir en otras especies animales del ecosistema que, por un efecto de evitación, reducen su presencia en el área ocupada por dichos grandes predadores (Ripple y Beschta, 2004; Ray *et al.*, 2005; Roemer *et al.*, 2009). Mediante la depredación, estos carnívoros regulan directamente la población de presas y, a su vez, pueden alterar los patrones de producción primaria al influenciar sobre la interacción vegetación-herbívoro, lo que desencadena unos efectos indirectos conocidos como cascadas tróficas (Fortin *et al.*, 2005) (Figura 1). Por otro lado, de acuerdo con Roemer *et al.* (2009), los mesocarnívoros también desempeñan un papel regulador de las poblaciones de presas pequeñas, desarrollando además funciones únicas que no son realizadas por grandes carnívoros y que, en definitiva, también influyen en las interacciones ecológicas, como por ejemplo el papel en la dispersión directa de semillas o la predación sobre especies que son importantes dispersores de semillas. Por otra parte, en los ecosistemas donde coexisten ambos tipos de carnívoros, la población de mesocarnívoros está regulada por la presencia de grandes carnívoros; no obstante, debido principalmente a la presión realizada por los seres humanos sobre los grandes carnívoros, estos han desaparecido de muchos ecosistemas y su papel ha sido ocupado por los mesocarnívoros (Prugh *et al.*, 2009).

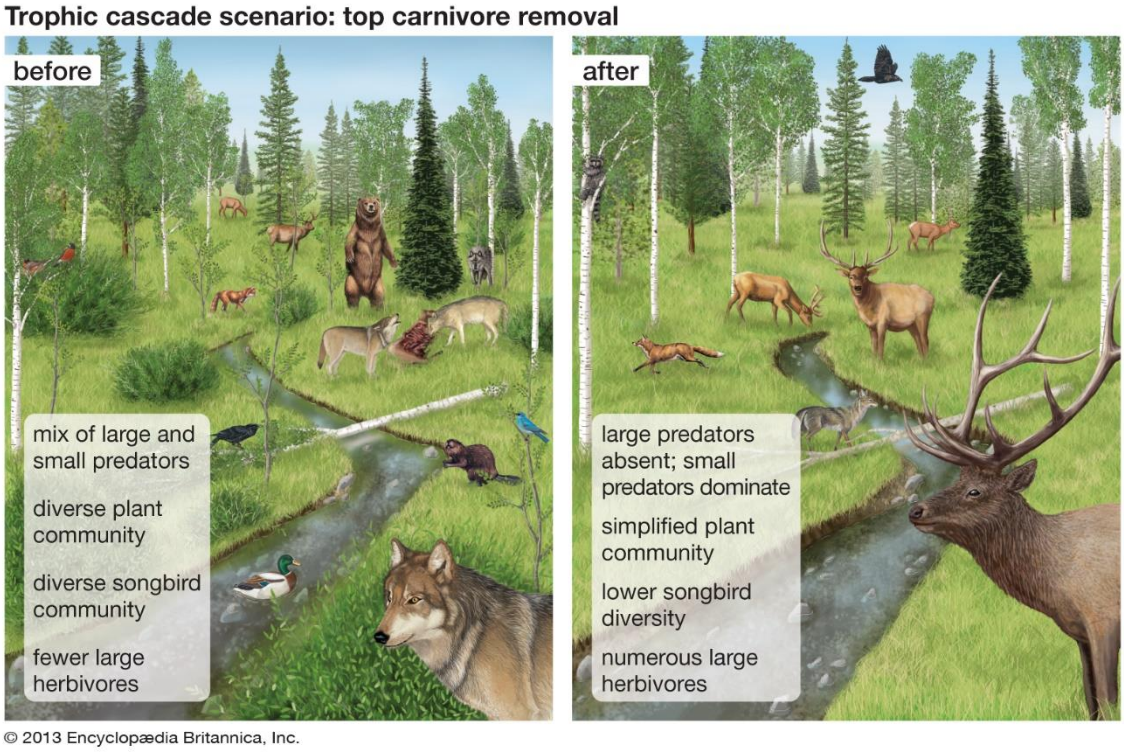

Figura 1 Efecto de una cascada trófica: Antes de la eliminación de depredadores situados en la cúspide de la pirámide alimenticia, el ecosistema muestra una mayor diversidad de carnívoros, herbívoros y plantas. Cuando se eliminan estos depredadores son sustituidos por mesocarnívoros, aumenta la población de herbívoros y se pierde diversidad vegetal. Fuente: Encyclopaedia Britannica.

LA EXPANSIÓN DE LA FAUNA SILVESTRE A LOS AMBIENTES HUMANIZADOS

La conversión antropogénica de hábitats naturales y seminaturales en áreas urbanizadas es una de las principales causas del cambio de uso de la tierra en todo el mundo (Pickett *et al.,* 2001; Piorr *et al.,* 2011; Šálek *et al.*, 2015) y afecta gravemente a la estructura y función de los ecosistemas. Este proceso puede causar una pérdida sustancial, modificación y fragmentación de los hábitats naturales que finalmente repercute negativamente en la conservación de la biodiversidad (McKinney, 2008; Plumer *et al.,* 2014). No obstante, la presencia de este tipo de ambientes resulta atractiva para muchas especies de fauna silvestre (Mackenstedt *et al.,* 2015) ya que el ecosistema urbano ofrece una diversidad y heterogeneidad aún mayor de hábitats y nichos ecológicos potenciales en comparación con un paisaje agrícola homogéneo y de uso intensivo (Červinka *et al.,* 2014). Los tres aspectos que más influyen en la capacidad de una especie para ocupar hábitats urbanos son, de forma general, la disponibilidad de recursos, la falta de competidores naturales y, además, factores abióticos como, por ejemplo, las condiciones microclimáticas urbanas específicas (Shea y Chesson, 2002; Šálek *et al.*, 2015). En este sentido, los ambientes urbanos proporcionan recursos alimentarios relacionados con la actividad humana, como la disponibilidad de basura o fuentes de agua no estacionales (Plumer *et al.,* 2014), condiciones microclimáticas favorables todo el año (Berry, 2008), así como estructuras artificiales que proporcionan sitios de madriguera y refugios suplementarios (Šálek *et al.*, 2015). Además, en el caso concreto de los carnívoros urbanos, pueden beneficiarse de la reducción de la competencia intraespecífica y la depredación por parte de otros carnívoros ("scarecrow effect"; Leighton *et al.,* 2010), así como de la reducción o incluso ausencia de persecución por parte de los humanos (Gompper, 2002; Gehrt, 2007).

Las especies de carnívoros que se sitúan en la zona alta de la pirámide trófica se consideran comúnmente sensibles a la pérdida y fragmentación del hábitat debido a sus grandes requerimientos espaciales, baja tasa de reproducción, especialización en la dieta y/o la persecución humana a la que se ven sometidas (Crooks, 2002). Estas características ecológicas los hacen particularmente vulnerables a las actividades humanas asociadas a la urbanización. Sin embargo, la respuesta de las especies carnívoras frente a la humanización del entorno varía significativamente de acuerdo con su capacidad para vivir en hábitats modificados por el hombre, lo que depende de la plasticidad ecológica o conductual específica de cada especie (Bateman y Fleming, 2012). Entre las especies que han conseguido adaptarse a los ambientes antropizados destacan los mesocarnívoros, ya que exhiben un alto grado de flexibilidad para adaptarse a nuevos hábitats, poseen una dieta generalista y, además, se muestran tolerantes respecto a la actividad humana (Bateman y Fleming, 2012). Son muchas las especies de carnívoros que han sido descritas en ambientes urbanos y periurbanos, como el coyote (*Canis latrans*), zorro, mofeta (*Mephitis mephitis*), marta (*Martes martes*), garduña (*Martes foina*), tejón euroasiático (*Meles meles*) y mapache (*Procyon lotor*), hasta el punto de que pueden alcanzar densidades más altas en los hábitats urbanos que en los rurales (Bateman y Fleming, 2012; Šálek *et al.,* 2015). El zorro, el tejón, la garduña y la marta son las especies de carnívoros presentes en la península ibérica que cumplen con estas características. En este sentido, según Recio *et al.* (2015), el zorro es la especie que mejor se ha adaptado a los ambientes suburbanos de España.

Por otra parte, existe otro fenómeno que ha permitido la expansión de las poblaciones de fauna silvestre en los últimos años y, consecuentemente su acercamiento a áreas antropizadas. Es lo que se ha denominado “Pax Romana” por parte de Martínez-Abraín *et al.* (2019). Según estos autores, la presión ejercida históricamente por los seres humanos sobre las poblaciones de fauna silvestre habría provocado una selección hacia individuos con un comportamiento huidizo frente a la presencia humana, alejándose de las zonas antropizadas. Pero en los últimos años, con el cambio de sensibilidad que se ha producido en la relación entre seres humanos y fauna silvestre, hay una mayor concienciación y aplicación de políticas en favor de la conservación. Esto, junto al abandono del medio rural, ha conllevado una reducción de la presión sobre la fauna, lo que ha permitido que las especies silvestres desarrollen comportamientos que permiten que sus poblaciones se expandan hacia zonas rurales deshabitadas y, progresivamente, hacia zonas en las que la presencia humana es mayor y constante en el tiempo. Concretamente, este proceso ha sido observado en el lobo ibérico en áreas del norte y noroeste de la península ibérica, siendo posible localizar este cánido en zonas donde la presencia humana es escasa y no supone una amenaza para la especie, pero no muy lejos de zonas propiamente antropizadas.

El impacto de las especies invasoras es una de las principales causas de pérdida de biodiversidad en todo el mundo, lo que ha generado un gran interés en comprender los patrones y los ritmos de expansión de las especies fuera de su área de distribución nativa (Genovesi, 2009; Fraser *et al.*, 2015). Concretamente, en España existe un caso muy importante de especie exótica invasora, el visón americano, un mustélido procedente de Norteamérica que cuenta con una gran capacidad de adaptación, siendo su expansión un importante factor de amenaza para las comunidades de vertebrados autóctonos, especialmente para los mamíferos semiacuáticos sobre los que actúa, bien por depredación, bien por exclusión competitiva (Balmori *et al.*, 2015).

LA FAUNA SILVESTRE Y LA TRANSMISIÓN DE ENFERMEDADES

La fauna silvestre ha sido considerada históricamente como una potencial fuente de transmisión de agentes infectocontagiosos a los seres humanos y a los animales domésticos. Por tanto, las enfermedades propias de animales silvestres han sido tenidas muy en cuenta cuando repercutían sobre los intereses económicos, sociales o sanitarios humanos (Thomson *et al.,* 2009). Desde este punto de vista antropocentrista, se ha prestado menos atención al estudio del patógeno desde una perspectiva ecológica-epidemiológica, considerándolo como componente de la biodiversidad y, en consecuencia, como un actor más que interviene en la evolución de las poblaciones de animales silvestres. Esta escasez de información, en el caso de los parásitos, redunda en el desconocimiento de aspectos relevantes de su ciclo biológico y, en consecuencia, en las medidas de prevención y control que pudieran acometerse en circunstancias epidemiológicas que lo requiriesen (Thompson *et al.,* 2010). Afortunadamente, durante la presente década ha adquirido una mayor importancia el concepto “One Health” (“Una Sola Salud”), que consiste en un enfoque multidisciplinar que reconoce que la salud de los seres humanos, animales y ecosistemas está íntimamente relacionada (Zinsstag, 2012; Jenkins *et al.,* 2015) y, por tanto, el control de la transmisión de enfermedades debe realizarse en todos estos niveles (Figura 2). En este contexto adquiere importancia el concepto “spillover”, que se emplea para señalar la transmisión de patógenos entre un hospedador que actúa de principal reservorio de una enfermedad hacia otro hospedador que no es el habitual. En este sentido, existen enfermedades en las que la fauna silvestre es el principal reservorio y la presencia del patógeno es perpetuada principalmente en ambientes silvestres, existiendo la posibilidad de que puedan transmitirse a los seres humanos o animales domésticos. Del mismo modo, también se describen casos de patógenos que son transmitidos a la fauna silvestre como consecuencia de la actividad humana (Thompson, 2013; Otranto y Deplazes, 2019). Puesto que existe esta reciprocidad en la transmisión de las enfermedades entre ambientes silvestres y humanizados, se debe reconocer la posibilidad de que, por ejemplo, un patógeno que circula mayoritariamente entre animales domésticos pueda ser transmitido a los animales silvestres y, de la misma forma, el animal silvestre participaría en el mantenimiento del patógeno para, finalmente, actuar como fuente de infección para los domésticos; en estos casos en se produce el retorno del agente infeccioso al hospedador original, el proceso se denomina “spillback” (Rhyan y Spraker, 2010).

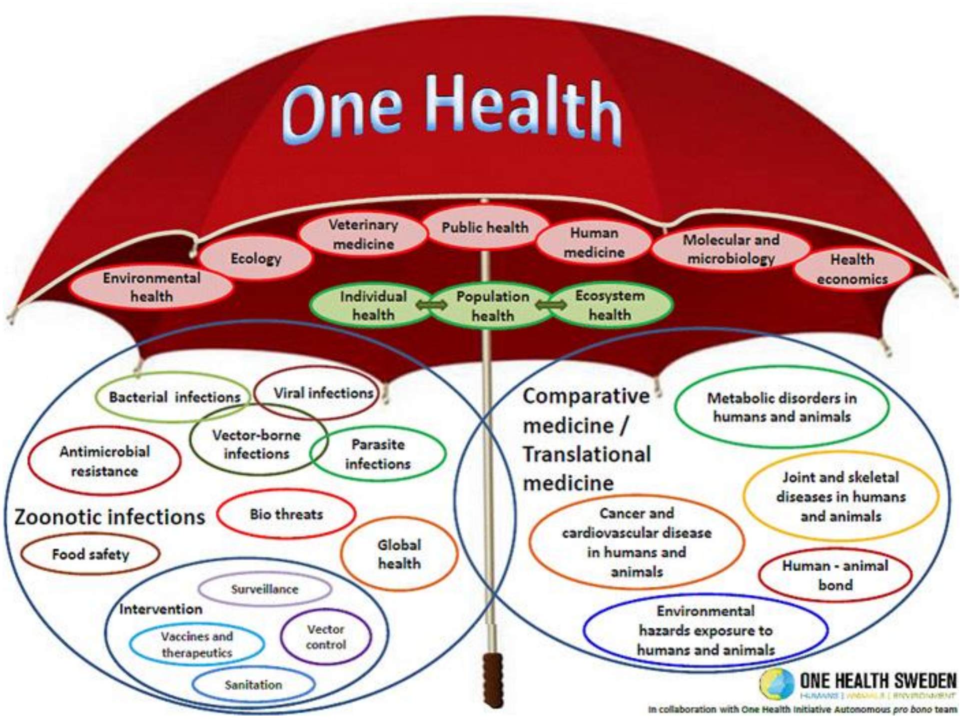

Figura 2 El concepto One Health como enfoque multidisciplinar que engloba la salud humana, animal y medioambiental. Fuente: The One Health Initiative.

De acuerdo con Duscher *et al.* (2015), los zorros juegan un papel clave en la interfaz entre fauna silvestre, animales domésticos y los seres humanos. Las razones para ello incluyen el aumento de la densidad de población de los zorros que da lugar a una mayor distribución y proximidad a los asentamientos humanos como consecuencia de su comportamiento sinantrópico. En el aspecto sanitario, destaca la susceptibilidad del zorro a patógenos relevantes relacionada con su preferencia por alimentarse de pequeños mamíferos, que suelen actuar como hospedadores intermediarios de multitud de agentes patógenos, sobre todo en el caso de parásitos de ciclo biológico indirecto o que presentan hospedadores paraténicos en su ciclo (Duscher *et al.,* 2006). No obstante, zorros, lobos y perros domésticos comparten multitud de especies parasitarias debido a que son especies filogenéticamente próximas y presentan pautas de comportamiento similares (Huang *et al.*, 2014). En este nuevo escenario epidemiológico, caracterizado por la expansión de las poblaciones de fauna silvestre y una mayor interacción en zonas antropizadas, así como el aumento de actividades al aire libre en compañía de animales domésticos (Rizzoli *et al.*, 2014), los cánidos silvestres y domésticos podrían actuar como nexo de unión entre los ciclos selvático y doméstico de estos agentes parasitarios y, en consecuencia, suponer un riesgo de flujo de patógenos con importante capacidad patogénica para los propios cánidos e incluso algunos con especial repercusión en la sanidad humana, debido a su potencial zoonósico (Figura 3).

En el caso de los agentes parasitarios de especial relevancia para el estado sanitario de los cánidos silvestres y domésticos se encuentran los nematodos cardiopulmonares, debido a su poder patogénico sobre los cánidos. En este sentido, nematodos como *Angiostrongylus vasorum* y *Dirofilaria immitis*, que se localizan en el lado derecho del corazón y las arterias pulmonares, pueden causar sintomatología cardíaca severa, como insuficiencia cardíaca congestiva, e incluso la muerte (Traversa *et al.*, 2010). La circulación en el medio de estos agentes patógenos podría suponer un riesgo para la salud de las poblaciones de cánidos silvestres y domésticos.

Por otra parte, estos cánidos intervienen en los ciclos biológicos de agentes parasitarios de reconocido potencial zoonósico. Entre estos patógenos se encuentran nematodos como *Ancylostoma caninum,* causante de *larva migrans* cutánea (Bowman *et al.*, 2010), o *Toxocara canis*, asociado a *larva migrans* visceral y ocular, así como causante de cuadros clínicos neurológicos (Overgaauw y Knapen, 2013). Además de estos ejemplos, cabe destacar la equinococosis como una de las principales parasitosis que se transmite en la interfaz fauna silvestre, animales domésticos y seres humanos. Se trata de una enfermedad clínicamente importante y una de las más frecuentes de las helmintosis zoonósicas; se estima que de 2 a 3 millones de personas en todo el mundo podrían estar infectados con *Echinococcus granulosus*, y entre 0,3 y 0,5 millones con *Echinococcus multilocularis*, que son las dos principales especies pertenecientes a este género (Craig *et al.,* 2007; Gordon *et al.,* 2016). Los hospedadores definitivos son principalmente cánidos, entre los cuales destacan el lobo y el zorro siendo ambas especies objeto de estudio de este trabajo, pero también otros como el perro doméstico (*Canis lupus familiaris*), el coyote y el chacal, por ejemplo (Eckert *et al.,* 2001). Por otra parte, los hospedadores intermediarios en el caso de *E. granulosus* son una amplia variedad de ungulados silvestres y domésticos, mientras que los de *E. multilocularis* son roedores (Eckert *et al.*, 2001). Las especies hospedadoras que intervienen en los ciclos biológicos de estos cestodos pueden estar presentes en ambientes antropizados y permitir el solapamiento entre el ciclo selvático y el doméstico (Mackenstedt *et al.,* 2015). Estas dos especies de cestodo han sido descritas en varios países europeos (Otranto *et al.,* 2015), aunque en España actualmente solo ha sido mencionada la presencia de *E. granulosus* en lobos (Sobrino *et al.,* 2006) y zorros (Segovia *et al.,* 2004).

La importancia desde el punto de vista sanitario del visón americano en España es distinta al papel desempeñado por zorros y lobos, puesto que este mustélido no está implicado directamente en la dispersión de importantes agentes zoonósicos. Su importancia radica principalmente en la posibilidad de introducir agentes patógenos exóticos en los ecosistemas ibéricos, así como en el desconocimiento sobre el papel que puede desempeñar en los ciclos biológicos de parásitos autóctonos, pudiendo actuar a modo de reservorio y fuente de transmisión para otros mamíferos autóctonos, algunos de los cuales son especies vulnerables o amenazadas. Las especies invasoras pueden afectar a las especies nativas directamente, a través de la competencia o la depredación, o indirectamente, alterando el hábitat o cambiando la dinámica de las enfermedades (Lymbery *et al.,* 2014). Los hospedadores exóticos introducidos suelen tener menos especies de parásitos y una menor prevalencia de parásitos que los hospedadores nativos, ya que la población del hospedador introducido habitualmente procede de una pequeña parte de la población original, que incluso podría estar libre de parásitos previamente. Por otra parte, los parásitos de ciclos complejos introducidos con el hospedador exótico encuentran la dificultad de que en el hábitat colonizado puede que no existan los hospedadores intermediarios adecuados (Torchin *et al.,* 2003), lo que dificultaría su implantación en el nuevo territorio invadido por su hospedador. Según Lymbery *et al.* (2014), una vez que se ha producido la introducción de un hospedador exótico en un ecosistema, los patógenos puede transmitirse de hospedadores nativos a exóticos, lo que conduce a un aumento de la infección de los nativos si los exóticos amplifican la transmisión ("spillback"; Kelly *et al.,* 2009; Mastisky y Veres, 2010) o a una disminución de la infección de los nativos si los exóticos reducen la transmisión, en un efecto de dilución (Paterson *et al.,* 2011; Poulin *et al.,* 2011). Si los hospedadores exóticos introducen nuevos parásitos, estos pueden transmitirse a los nativos, lo que da lugar a la aparición de nuevas enfermedades en los nativos, produciéndose un fenómeno de "spillover" o "pathogen pollution" (Daszak *et al.,* 2000; Taraschewski, 2006).

Existen varios ejemplos de parásitos introducidos por una especie exótica invasora, pero uno de los casos más importantes desde el punto de vista sanitario es el nematodo *Bayliscaris procyonis*. El hospedador definitivo de este nematodo es el mapache (Kristen-Page, 2013). La larva de este parásito puede realizar migraciones intraorgánicas en hospedadores paraténicos y accidentales, causando casos de *larva migrans* visceral, ocular, cutánea o neural (Gavin *et al.,* 2005), que en seres humanos puede provocar enfermedades neurológicas graves e incluso la muerte (Kazacos *et al.,* 2013). Con la introducción y establecimiento de poblaciones silvestres de mapache en áreas diferentes a la de su distribución natural, este nematodo ha podido hallarse en países europeos como Alemania o Dinamarca (Beltrán-Beck *et al.,* 2012; Al-Sabi *et al.,* 2015).

Debido a la posibilidad de que los tres carnívoros silvestres que son objeto de este estudio puedan ser transmisores de agentes parasitarios que son relevantes desde el punto de vista sanitario de las poblaciones de carnívoros silvestres, animales domésticos e incluso seres humanos, es necesario el estudio de estos agentes patógenos para conocer sus ciclos biológicos, los cuales se encuentran ligados a la ecología y etología trófica de las especies hospedadoras, ya que muchos de estos agentes parasitarios se transmiten a través de las interacciones predador-presa. Además, es muy importante el estudio de la presencia de posibles focos endémicos que faciliten la persistencia de estos agentes de especial relevancia sanitaria.

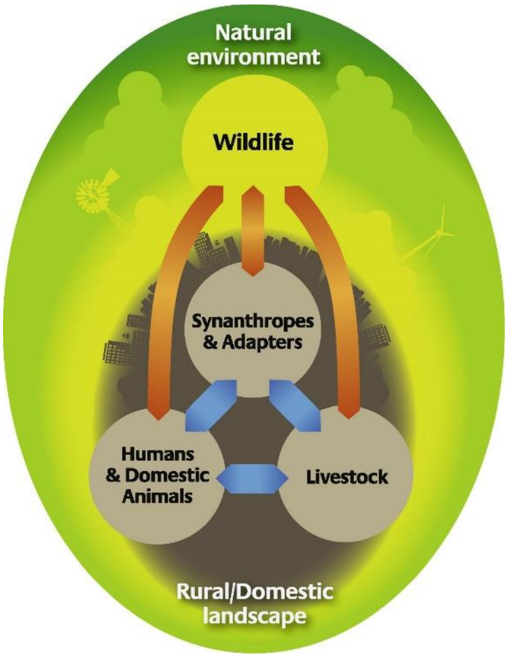

Figura 3 Diagrama que indica el posible flujo de agentes patógenos entre humanos, animales domésticos y silvestres en ambientes rurales y urbanos. Fuente: Thompson *et al.*, 2013

ESPECIES HOSPEDADORAS

EL ZORRO ROJO

El zorro rojo es la especie miembro del orden Carnivora que presenta la mayor distribución geográfica mundial (Figura 2); se puede encontrar prácticamente en todo el hemisferio norte, desde el Círculo Polar Ártico hasta el norte de África, América Central y las estepas asiáticas (Macdonald y Reynolds, 2004). En Europa se distribuye por todo el continente, excepto en las islas de Creta, Malta, Elba, Lipari, Baleares, Shetland, Feroe, Hébridas, Orkney e Islandia (Mitchell-Jones *et al.,* 1999). En España, este cánido es frecuente a lo largo de todo el territorio peninsular, los territorios de Ceuta y Melilla (Gortázar *et al.,* 2007), y se consideraba ausente de las Islas Baleares, aunque se ha registrado un caso en la isla de Mallorca, posiblemente introducido de forma no natural (Pinya y Bestard, 2010).

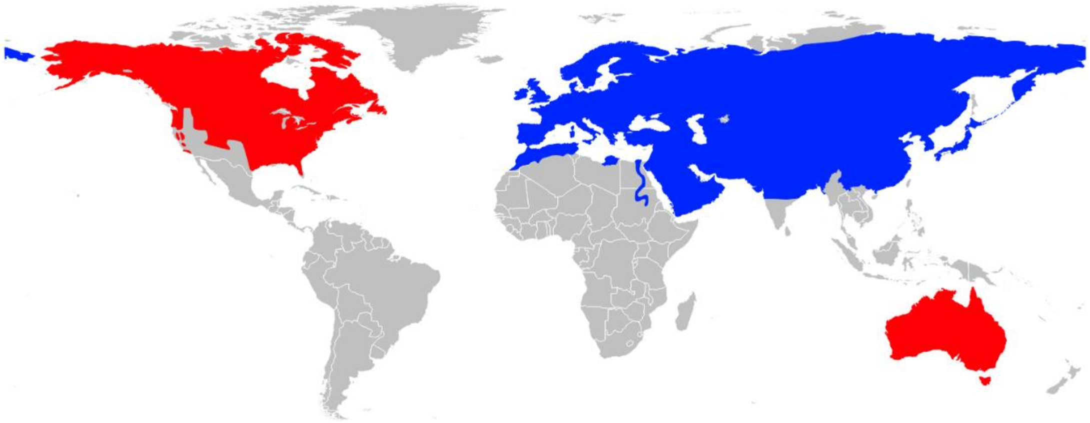

Figura 4 Mapa de la distribución del zorro rojo a nivel mundial. El color azul muestra las zonas de distribución originaria de este cánido y el color rojo los lugares donde ha sido introducido. Fuente: Elaborado a partir de la información disponible en la web IUCN Red List (Hoffmann y Sillero-Zubiri, 2016)

Se trata de una de las especies de carnívoros con mayor plasticidad ecológica, habituada a vivir en todo tipo de hábitats naturales, desde desiertos semiáridos hasta la tundra, zonas de cultivo y en bosques boreales. Incluso se ha adaptado a ecosistemas urbanos o periurbanos (Bateman y Fleming, 2012; Plumer *et al.*, 2014). De forma general, los hábitats fragmentados y heterogéneos, con ecotonos y márgenes de bosque y matorral constituyen mejores hábitats para el zorro que los ambientes homogéneos (Harris y Smith, 1987; Gloor *et al.,* 2001; López-Martín, 2017).

Los estudios realizados sobre las fuentes tróficas del zorro en la península ibérica demuestran que la dieta es típica de un carnívoro generalista en la que los micromamíferos, los lagomorfos y los residuos orgánicos de áreas humanizadas representan la mayor proporción de la alimentación de esta especie (López-Martín, 2017). Otras fuentes de alimento como frutos, reptiles, insectos, lombrices, cangrejos de río y peces completan la dieta del zorro en la península ibérica, con variaciones en las proporciones debidas, principalmente, al tipo de hábitat y la época del año (López-Martín, 2017). Así, por ejemplo, la dieta de este cánido silvestre presenta un patrón latitudinal caracterizado por un mayor consumo de lagomorfos e invertebrados en las zonas meridionales que en las septentrionales, donde los micromamíferos, frutos y semillas son más frecuentes como recursos tróficos (Díaz-Ruíz *et al.,* 2013).

La densidad y abundancia del zorro son muy variables en función de los recursos disponibles en el hábitat, lo que influye en la cantidad y tipo de alimentación (Gortázar *et al.,* 2003), del período del año (reproducción, dispersión juvenil…), de la presencia de enfermedades como la sarna sarcóptica o el moquillo canino (Nouvellet *et al.*, 2013; Devenish-Nelson *et al.*, 2014), así como de las medidas de gestión de sus poblaciones (caza y control) (Heydon *et al.,* 2000). En la península ibérica, las densidades que se han estimado coinciden con los valores medios obtenidos en otras zonas de Europa con condiciones ambientales similares (Blanco, 1998). Así, en el valle del Ebro se han estimado densidades entre 0,8 zorros/km^2 en zonas de secano y 2,5 zorros/km^2 en zonas de regadío (Gortázar, 1997); en la Reserva de Doñana se estimaron densidades de 1,4-1,7 zorros/km^2 (Rau, 1987), y en la sierra de Baza (SE península ibérica) la abundancia de zorros jóvenes fue 0,9 individuos/km^2 y la de zorros adultos fue 1,6 individuos/km^2 (Palomares y Ruiz-Martínez, 1994). En estudios más recientes, Peris y Del Amo (2003), estimaron que la densidad era de 0,53 zorros/km^2 en el litoral catalán, o Sarmento *et al.* (2009), que obtuvieron densidades que variaron entre 0,74 y 0,91 zorros/ km^2 en la Serra da Malcata (Portugal).

Con respecto al estado de conservación del zorro rojo, a nivel mundial y según la clasificación de la International Union for Conservation of Nature (IUCN), es una especie de categoría Preocupación Menor (Macdonald y Reynolds, 2010). En España se encuentra catalogado en este mismo nivel (Blanco, 2007). En la actualidad no hay ninguna amenaza para la conservación de las poblaciones de zorro en la mayoría de zonas donde habita (Macdonald y Reynolds, 2004). No obstante, sus poblaciones han sido históricamente perseguidas, ya que es una especie trasmisora de la rabia (Europa), por su carácter invasor (Australia) o por ser considerada una amenaza para la conservación de otras especies, como por ejemplo de aves coloniales que anidan en el suelo o especies cinegéticas (Europa). Sin embargo, a pesar de la presión a la que el ser humano ha sometido a esta especie, las poblaciones de zorro logran recuperarse, volviendo a alcanzar el tamaño poblacional original (Macdonald y Reynolds, 2004).

EL LOBO

Originariamente el lobo ocupaba la mayor parte de Norteamérica y Eurasia; no obstante, ha sido prácticamente erradicado de México, la mayor parte de los EEUU y gran parte de Europa Occidental (Mech y Boitani, 2004) (Figura 3). En los últimos 30 años, las poblaciones se han empezado a recuperar parcialmente en los países europeos. En España existe la subespecie ibérica, que sufrió una persecución que hizo que desapareciera de la mayor parte del territorio, salvo en el área noroccidental peninsular. A partir de los años 80 del siglo pasado, su población ha experimentado un aumento en el número de individuos y en su área de distribución (Blanco y Cortés, 2002; Llaneza *et al.*, 2012). De acuerdo con Blanco y Cortés (2012), la población estimada de lobos en España era de unos 2000 ejemplares en 2010, organizados en unas 262 manadas, de las cuales el mayor número se encontraría en Castilla y León (149 manadas), Galicia (68 manadas) y Asturias (36 manadas), pero también en otras Comunidades Autónomas, como Cantabria, Andalucía, País Vasco, Madrid y Castilla-La Mancha, aunque con números significativamente inferiores.

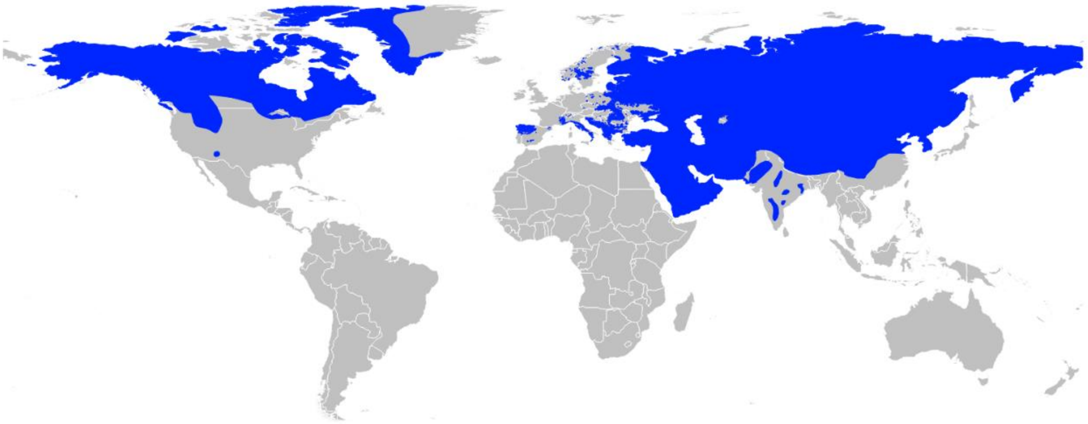

Figura 5 Mapa de la distribución mundial del lobo. Fuente: Elaborado a partir de la información disponible en la web IUCN Red List (Boitani *et al.,* 2018).

El lobo es una especie con una alta plasticidad ecológica, capaz de ocupar una gran variedad de hábitats, siempre y cuando le aporten alimento y protección frente al hombre (Grilo *et al.*, 2019). Se pueden encontrar ejemplares de esta especie desde zonas polares hasta desérticas, pasando por casi todos los medios excepto el bosque tropical (Mech, 1970). En la península ibérica también ocupa hábitats muy diversos, incluyendo desde áreas agrícolas con escasa cobertura y casi desprovistas de ungulados silvestres, hasta montañas cubiertas de matorrales, bosques y pastizales (Blanco *et al.*, 2007).

El lobo es el predador más importante de grandes mamíferos del hemisferio norte (Mech, 1970). En los hábitats menos alterados por la mano del hombre, las presas principales del lobo son casi siempre medianos y grandes ungulados, pero su gran capacidad de adaptación se refleja en una dieta que varía en función de las particularidades ambientales del área que ocupa (Blanco, 2017). Por ejemplo, la proporción de ungulados silvestres y domésticos en la dieta de los lobos depende de la disponibilidad de ambos tipos de presas. En un área con abundante ganado, principalmente caballos semisalvajes, los ungulados domésticos son las presas más abundantes o podrían ser consumidos como carroña, mientras que los ungulados silvestres son menos frecuentes; en contraste, donde el ganado es escaso, los corzos y los jabalíes son las presas más frecuentes de los lobos (Llaneza *et al.,* 1996). Sin embargo, Barja (2009) describe una predilección de lobos por alimentarse de ungulados silvestres, principalmente corzos, ciervos rojos y jabalíes, en lugar de presas domésticas en una zona del noroeste de España donde existe ganado extensivo.

En 1996, esta especie fue eliminada de la Lista Roja, pasando a considerarse desde entonces en la categoría "Riesgo menor: mínima preocupación". La población ibérica de lobos, sin embargo, fue incluida en la categoría "Riesgo menor: dependiente de conservación". Entre las principales amenazas para la conservación del lobo hay que destacar la caza ilegal, generalmente como medio de represalia por la tendencia del lobo a depredar sobre el ganado, pero también son frecuentes las muertes por atropello o envenenamiento (Colino-Rabanal *et al.*, 2011; Torres y Fonseca, 2016).

EL VISÓN AMERICANO

El visón americano es un mustélido originario de Norteamérica, donde tiene una amplia distribución que ocupa casi todo el subcontinente, excepto el Círculo Polar Ártico y la zona meridional de Estados Unidos y México (Banfield, 1974; Linscombe *et al.,* 1982; Melero y Palazón, 2017) (Figura 4). Este mustélido fue introducido en Europa y en la antigua URSS durante la década de los años 20 del siglo XX con el fin de criarlo en granjas para la industria peletera (Vidal-Figueroa y Delibes, 1987; Dunstone, 1993; Melero y Palazón, 2011). En España, la instalación de dichas granjas se realizó a finales de la década de los años cincuenta (Bravo y Bueno, 1992). La primera cita de esta especie en estado libre en España tuvo lugar en Segovia, a final de la década de los 70 del siglo pasado (Delibes y Amores, 1978). Con el paso de los años, el visón americano fue colonizando nuevas áreas a partir de los núcleos iniciales de invasión; de hecho, a mediados de la década de los 80 ya existían en España tres núcleos poblacionales estables en libertad: suroeste de Galicia (Vidal-Figueroa y Delibes, 1987), noreste de Cataluña (Ruiz-Olmo, 1987) y España central (Bueno y Bravo, 1990). En los años siguientes se establecieron tres nuevas poblaciones: en Cantabria, noroeste de Galicia y Teruel-Castellón (Ruiz-Olmo *et al.*, 1997).

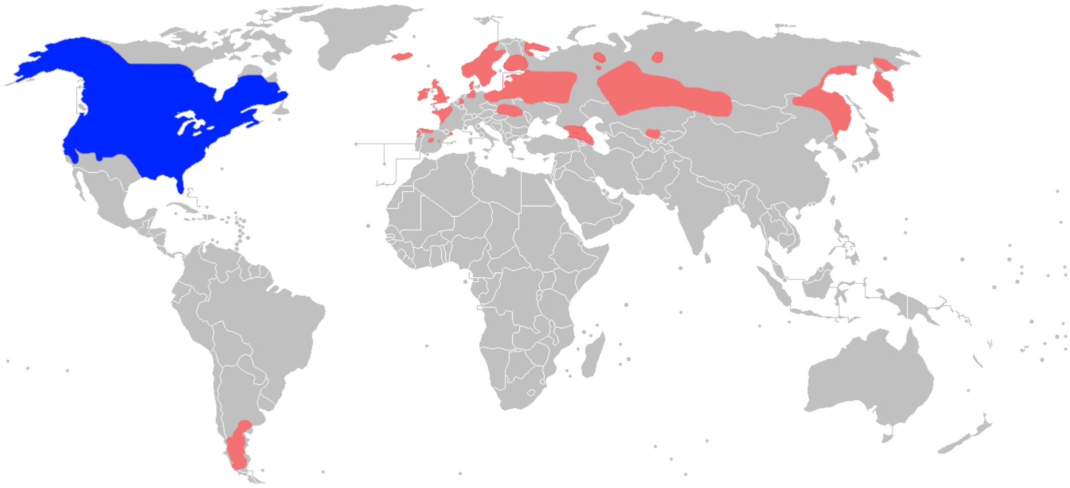

Figura 6 Mapa de la distribución mundial del visón americano. El color azul muestra las áreas originarias de este mustélido y el color rojo las zonas donde ha sido introducido. Fuente: Elaborado a partir de la información disponible en la web IUCN Red List (Reid *et al.,* 2016) y Melero y Palazón (2017).

El visón es un animal de hábitos semiacuáticos y, por ello, su distribución está ligada al agua. Puede ocupar todo tipo de hábitats acuáticos: arroyos, ríos, embalses, lagos, lagunas y también el mar, siempre que exista suficiente alimento disponible (Bravo y Bueno, 1992). En la costa ocupa zonas de rocas y peñas (Dunstone, 1993); en concreto, en la costa gallega se sitúa en zonas de rocas graníticas, donde las densidades de visón son elevadas (Romero, 2009; Melero y Palazón, 2017). Además, esta especie puede tolerar ambientes contaminados o degradados por la actividad industrial y urbana, incluso en los propios cascos urbanos (Vidal-Figueroa y Delibes, 1987).

La dieta del visón americano es muy variada, pudiendo predar sobre un amplio espectro de presas, tanto acuáticas como terrestres. En Galicia y en las áreas de distribución de clima mediterráneo consume principalmente cangrejos, peces y micromamíferos (Vidal-Figueroa y Delibes, 1987; Melero *et al.,* 2008). Es una especie generalista y oportunista, capaz de modificar su dieta en función del tipo de hábitat, la disponibilidad de presas y la presencia de competidores (Bonesi *et al.*, 2004).

Ha sido demostrado en numerosos estudios que la presencia del visón americano provoca efectos negativos sobre las poblaciones de otras especies autóctonas de la península ibérica, que son sus presas potenciales; es el caso del cangrejo de río (*Austropotamobius pallipes*), el desmán ibérico (*Galemys pyrenaicus*) o la rata de agua (*Arvicola sapidus*) (Palazón y Ruiz-Olmo, 1997; Palomo y Gisbert, 2002). Por otra parte, se considera que el visón americano tiene un impacto negativo sobre las poblaciones de otros carnívoros, como la nutria (*Lutra lutra*), visón europeo (*Mustela lutreola*) y turón europeo (*Mustela putorius*), debido a la competencia por el espacio o los recursos tróficos (Balmori *et al.*, 2015). Por este motivo, en el año 2013 el Ministerio de

Agricultura, Alimentación y Medio Ambiente desarrolló, un programa de control de sus poblaciones denominado "Estrategia de gestión, control y erradicación del visón americano (*Neovison vison*) en España", cuyo objetivo principal es la reducción de los núcleos poblacionales estables y su potencial colonizador de otras áreas poniendo especial énfasis en las zonas donde su presencia afecta directamente a otros vertebrados autóctonos.

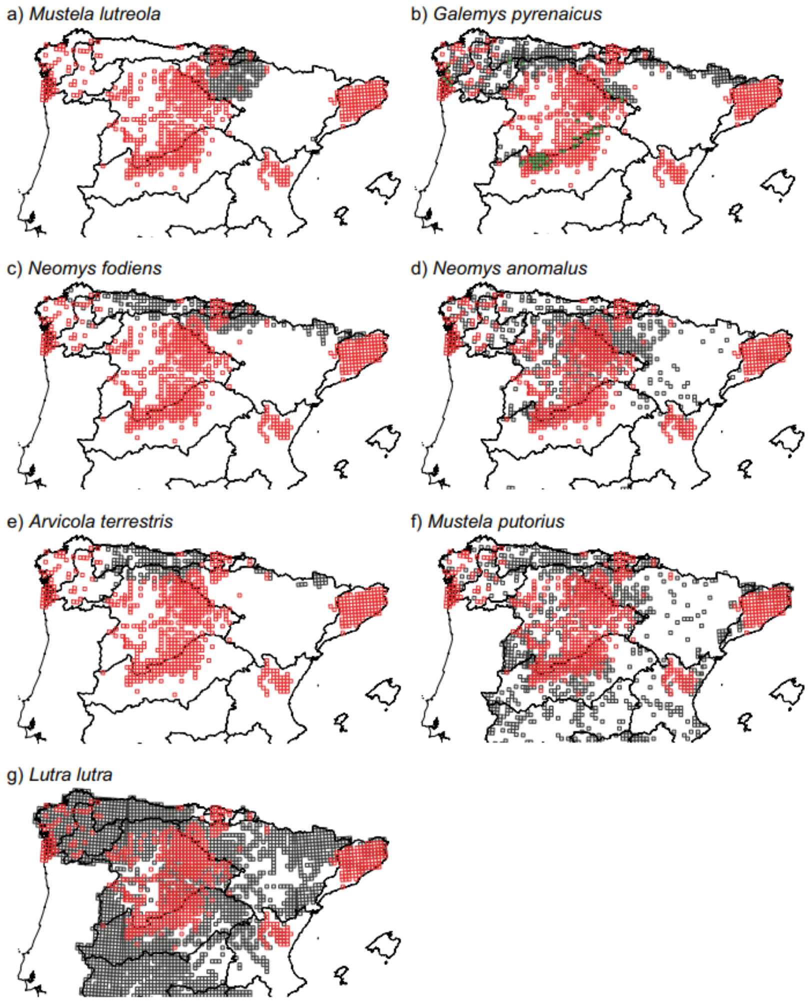

Figura 7 Mapas de distribución de diferentes mamíferos semiacuáticos de la península ibérica (cuadrados de color negro) y del visón americano (cuadrados de color rojo) que muestran el solapamiento espacial de estas especies autóctonas y el visón americano. Las especies mostradas son: a) visón europeo, b) desmán ibérico, c) musgaño patiblanco, d) musgaño de Cabrera, e) rata topera, f) turón europeo, g) nutria euroasíatica. El área de color verde en el mapa b indica las zonas donde el desmán ibérico ha desaparecido. Fuente: Balmori *et al.,* 2015.

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OBJETIVOS

El impacto que tienen los parásitos sobre las poblaciones de carnívoros silvestres es difícil de cuantificar. Esto es debido a que no se puede desvincular la presencia de los parásitos de los factores dependientes del propio hospedador y del ambiente.

Abordar el estudio de la interacción parásito-hospedador-ambiente, en un entorno singular como es el noroeste de España, en el que conviven estas tres especies de carnívoros silvestres, una alóctona (el visón americano) y dos autóctonas (el zorro y el lobo ibérico), es, sin duda, una gran oportunidad para valorar las consecuencias que tiene el anidamiento de los parásitos de este hospedador exótico sobre las especies autóctonas que ocupan el mismo hábitat al que se ha adaptado perfectamente este mustélido.

El visón americano, especie incluida en el Catálogo español de especies exóticas invasoras (Real Decreto 630/2013), no ha sido suficientemente estudiado en Europa y se desconoce el papel epidemiológico que puede desempeñar en el mantenimiento de los ciclos biológicos de algunas especies de parásitos, tanto los presentes de forma natural en los hábitats del noroeste de España como los parásitos que hayan podido ser introducidos junto con el propio visón americano. Por lo tanto, se desconoce el impacto que la distribución de los parásitos albergados por la especie de estudio puede tener en la conservación de otras especies de carnívoros autóctonos, entre los cuales se incluyen las otras dos especies objeto de esta Tesis, ya que su distribución coincide en áreas del noroeste de España, además de la especial atención necesaria al caso de los mustélidos autóctonos, algunos de los cuales se encuentran en peligro de extinción, como el visón europeo. Así pues, el primer objetivo de esta Tesis Doctoral es:

- Describir las comunidades de los helmintos gastrointestinales y cardiorrespiratorios del visón americano presentes en Galicia para conocer si puede desempeñar un papel importante en el mantenimiento y dispersión de los estos agentes parasitarios hacia otras especies de carnívoros autóctonas. Este objetivo se desarrollará en el Capítulo 1.

En Europa se han realizado varios estudios que describen los parásitos que afectan a zorros y lobos, pero muy pocos han centrado su atención en los parásitos cardiopulmonares. Además, la tendencia hasta ahora ha sido estudiar ambos hospedadores por separado, sin tener en cuenta que estas dos especies silvestres son susceptibles a los mismos nematodos cardiopulmonares y, además, comparten hábitat. Diversos estudios han confirmado que los parásitos cardiopulmonares pueden afectar negativamente la capacidad física de los cánidos hospedadores, siendo algunos de estos agentes muy patógenos y que pueden suponer un riesgo para la conservación de los cánidos silvestres. Por tanto, el segundo objetivo de esta Tesis Doctoral es:

- Describir los parásitos cardiopulmonares de lobos y zorros del noroeste de la península ibérica, discutiendo el papel epidemiológico que cada uno de estos cánidos silvestres tiene en el anidamiento natural de estos parásitos. Este objetivo se desarrollará en el Capítulo 2.

En el área de estudio, que se corresponde con la parte noroccidental de la península ibérica, el zorro y el lobo ibérico comparten hábitat y pueden tener parte de su dieta en común. Por ello, resulta interesante poder establecer una comparación entre la helmintofauna digestiva de ambas especies mediante un estudio de la estructura de las comunidades, con índices de riqueza, dominancia, etc. Igualmente, debemos conocer la posible presencia de especies de parásitos con repercusión negativa sobre la sanidad de los animales domésticos y del propio ser humano (debido al carácter zoonósico de algunos de estos agentes parasitarios), ya que el lobo, y particularmente el zorro, son especies que conviven cercanas al ganado, a las mascotas y a las poblaciones humanas. Por todo ello, el tercer objetivo de esta Tesis Doctoral es:

- Describir la helmintofauna digestiva de zorros y lobos en el noroeste de la península ibérica, área que está compartida por ambos cánidos, además de los animales domésticos y la población humana, analizando el papel epidemiológico que cada uno de estos cánidos silvestres tiene en el mantenimiento del ciclo biológico selvático de estos parásitos, explicando los hallazgos parasitológicos en base a las características ecológicas y comportamiento trófico de ambos hospedadores. Este objetivo se desarrollará en el Capítulo 3.

CAPÍTULO 1: The American mink (*Neovison vison*) is a competent host for native European parasites

Martínez-Rondán, F. J., Ruiz de Ybáñez, M. R., Tizzani, P., López-Beceiro, A. M., Fidalgo, L. E., Martínez-Carrasco, C. (2017). The American mink (*Neovison vison*) is a competent host for native European parasites. *Veterinary Parasitology*, *247*: 93–99.

The American mink (Neovison vison) is a competent host for native European parasites

ABSTRACT

The American mink (*Neovison vison*) is a mustelid native to North America that was introduced in Europe and the former USSR for fur farming. Throughout the last century, accidental or deliberate escapes of mink from farms caused the establishment of stable feral populations. In fact, the American mink is considered an invasive alien species in 28 European countries. The present study evaluates the gastrointestinal and cardiopulmonary helminth fauna of the American mink in Galicia (NW Spain) to understand its role as a potential reservoir for parasites affecting other autochthonous mustelids. In the period 2008–2014, fifty American mink (35 males and 15 females) of different ages (22 immatures and 28 adults) from the provinces of Lugo, Ourense and Pontevedra were captured and sacrificed. Eight parasite species were found (6 nematodes and 2 trematodes) with the following prevalences: *Molineus patens* (68%), *Aonchotheca putorii* (54%), *Crenosoma melesi* (10%), *Aonchotheca annulosa* (8%), *Angiostrongylus daskalovi* (6%), *Aelurostrongylus* spp. (2%), *Troglotrema acutum* (2%) and an unidentified trematode (2%). Eighty-two per cent of the mink harboured helminths, including 15 animals (30%) infected by only one parasite species, 19 (38%) by two species, 5 (10%) by three species and 2 mink (4%) by four species. All helminth species identified are native to European mustelids. Statistical models were used to evaluate if animal characteristics (age, sex and weight), date and capture area influenced the prevalence, intensity or parasite richness. Statistical differences were detected only in models for intensity of *M. patens*, *A. putorii* and *C. melesi*. This is the first report of *Angiostrongylus daskalovi*, a cardiopulmonary nematode, and *A. annulosa*, a gastrointestinal nematode specific of rodents, in American mink. Moreover, although the fluke *T. acutum* has already been cited in American mink, to our knowledge, the present study represents the first report of this trematode in the lung.

INTRODUCTION

The American mink (*Neovison vison* Schreber, 1777) is a semi-aquatic mustelid native to North America, widespread in this area with the exception of the Arctic Circle, the southern of United States and Mexico (Banfield, 1974; Linscombe *et al.*, 1982; Melero and Palazón, 2011). It was introduced in Europe and the former USSR in the early 20s of last century for fur industry purposes (Vidal-Figueroa and Delibes, 1987;

Dunstone, 1993; Melero and Palazón, 2011). In Spain, first fur farms were established during the late 1950s (Bravo and Bueno, 1992).

Accidental or deliberate releases consecutive to accidents (fires, windstorms, etc.), limited security measures and/or cessation of farm's activity led to the establishment of feral populations (Vidal-Figueroa and Delibes, 1987; Palazón and Ruíz-Olmo, 1997). In fact, the American mink is considered an invasive species in 28 European countries (Bonesi and Palazón, 2007). In Spain, the first feral mink was reported in Central Spain (Segovia) in 1978 (Delibes and Amores, 1978) and, since then, this mustelid has progressively colonized Southwest of Galicia (Vidal-Figueroa and Delibes, 1987), Northeastern Catalonia (Ruíz- Olmo, 1987) and Central Spain (Bueno and Bravo, 1990). Currently, three other core populations are established in Cantabria, North of Galicia and Teruel-Castellón (Ruíz-Olmo *et al.*, 1997).

American mink is a generalist and opportunistic species which consumes a wide spectrum of both aquatic and terrestrial preys. Its diet varies depending on the habitat, the prey availability and the presence of other competitor species (Bonesi *et al.*, 2004). Fragmented landscapes, with a wide variety of habitats and food resources, favor its presence (Rodríguez and Zuberogoitia, 2011). This mustelid preferably inhabits territories with well-structured riparian vegetation (Zuberogoitia *et al.*, 2006; Zabala *et al.*, 2007; Melero *et al.*, 2008b; Melero and Palazón, 2011).

American mink can cause adverse effects on autochthonous prey populations (Bonesi and Palazón, 2007), such as European crayfish (*Austropotamobius pallipes*), Pyrenean desman (*Galemys pyrenaicus*), water vole (*Arvicola sapidus*) and Mediterranean water shrew (*Neomys anomalus*) (Palazón and Ruíz-Olmo, 1997; Bergmans and Blom, 2001; Palomo and Gisbert, 2002; García-Díaz *et al.*, 2013). Moreover, it could have a negative impact on the populations of other carnivores, such as the European mink (*Mustela lutreola*), the otter (*Lutra lutra*) and the European polecat (Mustela putorius), due to the competition for space or food resources (Sidorovich *et al.*, 1999; Melero *et al.*, 2012). Furthermore, the introduction of an invasive species can have unpredictable epidemiological consequences, because it can carry alien agents potentially pathogenic for native host species or, alternatively, the same alien species can act as a new reservoir for autochthonous infectious agents (Sepúlveda *et al.*, 2014; Sherrard-Smith *et al.*, 2015). For all these reasons, a national specific regulation was published in Spain, and the Ministry of Agriculture, Food and Environment developed the program "Management strategy, control and eradication of the American mink in Spain".

The American mink's control programs in mainland areas are quite complex and, moreover, the area from which the species is removed can be easily recolonized (Bryce *et al.*, 2011). For these reasons, this carnivore is likely to persist in Spain over a long period of time. Therefore, it is important to acquire further information about the epidemiological role of this mustelid in the maintenance and diffusion of infectious pathogens. The aim of this study was to describe the gastrointestinal and cardiorespiratory macroparasites of the American mink in Galicia (NW Spain), an area where the species is present since the 1980s (Figure 8).

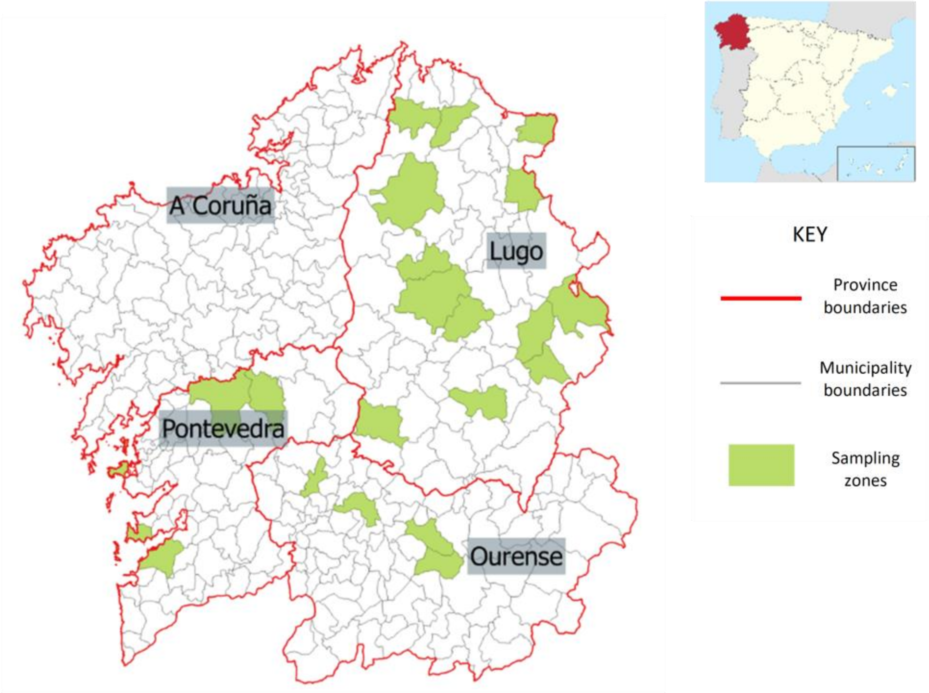

Figura 8 Map showing the sampling zones in Galicia (northwestern Spain).

MATERIAL AND METHODS

Sampled animals

American mink included in this study were caught in river basins from Lugo, Ourense and Pontevedra provinces (Galicia, northwest Spain). The climate in Galicia is predominantly oceanic.

The average annual rainfall is 1180 mm, with a homogeneous distribution throughout the year, and the average annual temperature is 13.3 °C, with limited yearly variations (Castillo-Rodríguez *et al.*, 2007).

Between 2008 and 2014, fifty American mink (the sample distribution by sex, age and month of capture is shown in Table 1) were captured during the official control plan implemented by the Galician administrative authority (Consellería de Medio Ambiente, e Ordenación do Territorio).

Captured mink were sent to the Wildlife Recovery Centers of O Veral (Lugo), O Rodicio (Ourense) and Cotorredondo (Pontevedra), where animals were euthanized by the veterinary staff, under current Spanish and European Animal Welfare Legislation.

Tabla 1 Distribution of the American mink (n = 50) by month of capture, sex and age.

Month	Number of minks				
	Sex		Age		Total
	Male	Female	Immature	Adult	
January	1	0	0	1	1
February	10	2	5	7	12
March	6	2	3	5	8
April	1	0	1	0	1
May	4	2	2	4	6
June	0	2	2	0	2
July	3	4	5	2	7
August	2	0	0	2	2
September	2	0	0	2	2
October	1	0	0	1	1
November	3	2	2	3	5
December	2	1	1	2	3
Total	35	15	21	29	50

Laboratory procedures

Digestive and cardiorespiratory tracts of the necropsied mink were removed and individually stored in labelled plastic bags. All samples were frozen (−20 °C) and sent to the Veterinary Faculty of Murcia for parasitological evaluation.

The digestive viscera (stomach, small and large intestine, liver and esophagus) were analyzed. Sections of the alimentary tract were longitudinally opened and scraped, the content was washed over a sieve (mesh size 0.3 mm diameter), and both content and mucosa were observed under a stereomicroscope to detect helminth specimens. Liver parenchyma was sliced and bile ducts and gallbladder were opened and also examined using a stereomicroscope. Heart, pulmonary veins and pulmonary arteries were carefully separated from trachea and lungs. Trachea, bronchial tree and pulmonary blood vessels were longitudinally opened, washed over a sieve 0.3 mm diameter and, subsequently, the retained material was observed by stereomicroscope. To investigate the presence of lung larvae, the filtrated liquid was collected and examined using the Baermann larval migration technique. Once completed the examination of the lung parenchyma by stereomicroscope, this tissue was also analyzed with the same technique. In cases where bronchopulmonary larvae were detected, lung parenchyma was cut into small stripes (approximately 0.5 cm diameter) and, subsequently, enzymatically digested in freshly prepared 1.5% (w/v) pepsin (1: 10,000 activity) and 1.5% (w/v) chlorhydric acid in distilled water in order to isolate adult nematodes. Digestion was carried out at 40 °C for 1 h under gentle shaking, after which the undigested remains were filtered through a sieve of 63 µm diameter to collect the nematodes. Finally, the heart chambers and the major blood vessels were carefully opened, washed, and the resultant liquid was filtered through a 63 µm sieve before stereomicroscopic examination of the retained material.

Parasite identification

Parasites collected from each American mink were washed in distilled water, counted and stored in tubes with 70% ethanol for further examination. For taxonomic identification purposes, nematodes were cleared with lactophenol, while flukes were stained with Semichon's acetic carmine (Schmidt, 1986). Parasites were morphometrically identified attending Skrjabin (1964), Skrjabin and Petrow (1928), Yamaguti (1971), Butterworth and Beverley-Burton (1980), Janchev and Genov (1988), Mascato *et al.* (1993), Popiolek *et al.* (2009) and Gherman *et al.* (2016).

Statistical analyses

Prevalence, intensity and abundance were calculated for each parasite genus or species according to Bush *et al.* (1997), as well as parasite richness (number of parasite species per host). Data obtained from parasitological analysis (prevalence, intensity, richness—considered as dependent variables) and host characteristics (sex, age, weight, capture area, month and season of trapping—considered as risk factors) were analyzed using a Generalized Linear Models (GLM).

Different distribution families were used for the GLM analysis, according to the distribution of the dependent variable. A binomial distribution was used to analyse prevalence, both Poisson and negative binomial distributions were tested for intensity and, finally, a Poisson distribution was used for richness. The multivariate analysis for prevalence was carried out only for the species whose prevalence was higher than 10%, while the analysis of intensity and richness con sidered all parasite species. A stepwise approach and the "Akaike information criterion (AIC) were used to select the best model" (Akaike, 1974). Significant level was considered for $p < 0.05$

Statistical analysis was performed using the software R 3.1.0 (R Core Team, 2014). The package MASS was used to apply the negative binomial GLM.

RESULTS

Six nematode and two trematode species were recovered. Eighty-two percent of mink (41/50, C.I. 95% = 70.9–93) harboured at least one helminth species, 52% (26/50, C.I. 95% = 37.6–66.3) were infected with two or more species, and a maximum of four species were detected in 4% of mink (2/50, C.I. 95% = 0–9.6%).

Prevalence, median intensity and median abundance of these helminths are shown in Table 2.

Angiostrongylus daskalovi, *Aelurostrongylus* spp., *Troglotrema acutum* and the unidentified trematode were found only in male mink. *Aelurostrongylus* spp. was found only in one adult mink, whereas *T. acutum* and the unidentified trematode were only found in one immature animal, respectively.

Statistically significant influence of some risk factors were detected in models only for intensity of *Molineus patens* and, *Aonchotheca (Capillaria) putorii*. Concretely, the best model explaining the intensity of *M. patens* selected the sex and season (AIC = 253.6; explained variance = 46.6%), with males and spring showing a lower *M. patens* intensity. Regarding *A. putorii*, the best models selected the age and season, detecting the lower intensity in subadult mink and in winter (AIC = 192.34, explained variance = 31.5%); specifically, mink captured in July showed higher intensity of this nematode species ($p < 0.001$). No significant effect of the studied factors was observed for prevalence and parasite richness.

Tabla 2 Gastrointestinal (*) and cardiorespiratory (**) parasites recovered from 50 American mink captured in Galicia, Spain.

	Prevalence % (95% C.I.)					Range	Median intensity ±SD	Median abundance ±SD
	Total	Sex		Age				
		Male	Female	Immature	Adult			
*Molineus patens**	68 (54.6-81.3)	77 (62.5-91.7)	46.6 (18.0-75.2)	59.0 (36.7-81.4)	75.0 (57.9-92.0)	1-193	8.0±33.6	5.0±28.8
*Aonchotheca putorii**	54 (39.6-68.3)	57.1 (39.8-74.3)	46.6 (18.0-75.2)	50.0 (27.3-72.6)	57.1 (37.6-76.6)	1-83	5.0±21.7	1.0±17.1
*Crenosoma melesi***	10 (1.3-18.6)	11.4 (0.3-22.5)	6.6 (0.0-20.9)	9.0 (0.0-22.1)	10.7 (0.0-22.9)	1-42	1.0±18.2	0.0±5.9
*Aonchotheca annulosa**	8 (2-15.7)	8.5 (0.0-18.3)	6.6 (0.0-20.9)	4.5 (0.0-13.9)	10.7 (0.0-22.9)	2-29	10.5±11.7	0.0±4.6
*Angiostrongylus daskalovi***	6 (0-12)	8.5 (0.0-18.3)	0	9.0 (0.0-22.1)	3.5 (0.0-10.8)	1-3	2.0±1.0	0.0±0.52
Aelurostrongylus spp.**	2 (0-6)	2.8 (0.0-8.6)	0	0	3.5 (0.0-10.8)	1	-	-
*Troglotrema acutum***	2 (0-6)	2.8 (0.0-8.6)	0	4.5 (0.0-13.9)	0	7	-	-
Trematode spp.*[a]	2 (0-6)	2.8 (0.0-8.6)	0	4.5 (0.0-13.9)	0	27	-	-

[a] Genus was not determined due to the poor preservation status of these specimens.

DISCUSSION

The overall prevalence of helminths in our study is similar to the one described in American mink from Southwest of France (81%) (Torres *et al.*, 2008) and Belarus (78%) (Shimalov and Shimalov, 2001). However, it should be noted that the number of parasitized animals in our study differs considerably from the results described by Torres *et al.* (2003) in American mink from 12 Spanish provinces; these authors divided their sample in two groups: one including American mink from areas where European mink was present, and the other group with American mink captured in areas where the European mink was absent. In this study, the prevalence was significantly lower than in our study (respectively 35.5% and 48.0%).

Moreover, parasite richness (eight species) and helminths identified in our study differ from the values recorded in other European areas. Concretely, Shimalov and Shimalov (2001) found higher parasite richness (19 species) in Belarus. In the same country, Sidorovich and Anisimova (1997) found a higher richness in American mink captured in less polluted areas than in those from contaminated ones (17 and 12 species, respectively). Our results are closer to those described in Torres *et al.* (2008) in France (richness of nine species) and to the seven species that had been previously described in American mink captured in Spain (Torres *et al.*, 2003). According to these authors, a greater number of species may be associated to an earlier host adaptation to the area. In this sense, American mink has been described as invasive species in Belarus since the 50 s of last century (Sidorovich, 1993) and, therefore, this nonindigenous mustelid has shared habitat for a longer time with the native parasite fauna than the American mink in Galicia. However, this wide parasite richness also includes some helminths that could have been introduced in Belarus carried by the American mink from its natural distribution area, as may happen with *Baylisascaris devosi*, *Capillaria mustelorum*, *Metorchis albidus* and *Apophallus donicus* (Anisimova, 2004). Furthermore, differences in parasite richness between Galicia and Belarus could be due to the wide expansion of American mink in Belarus, since parasite richness tends to increase when the host species is distributed along greater territories (Morand, 2015). In this sense, according to Bonesi and Palazón (2007), American mink is much more spread in Belarus than in Spain. So, it is reasonable to assume that, if American mink continues its expansion across the Iberian Peninsula, it could increase its parasite richness, acquiring a new epidemiological role in the maintenance and diffusion of parasites shared by other autochthonous mustelids.

So far, all the helminth species found in American mink captured in Spain are native from European mustelids (Torres *et al.*, 2003, 2006), as also in our study. These findings suggest that American mink lost their original helminths during its adaptation to Iberian environments (Torchin *et al.*, 2003) or, as the feral populations comes from animals raised in fur farms, it is possible that these American mink were treated against parasites, eliminating their specific parasites. It is well known that, in the case of alien invasive species released without previous antiparasitic treatment, it is possible the parasite adaptation to new habitats and autochthonous hosts; for example, Tizzani *et al.* (2011) found *Obeliscoides cuniculi* in European brown hare, probably introduced by the invasive Eastern cottontail rabbit (*Sylvilagus floridanus*) in areas where both lagomorphs live in sympatry (Tizzani *et al.*, 2011, 2014). Our results suggest that American mink from Galicia is currently playing an epidemiological role in the maintenance of the life cycle of native European parasites, but fortunately not of alien parasites whose establishment in the invaded areas could have unpredictable consequences on European mustelids, with the risk of changes in the composition of host-parasite communities, and the incidence of polyparasitism within individual hosts (Polley and Thompson, 2015).

Molineus patens was the most prevalent parasite in our study (68%). This gastrointestinal nematode is frequently found in Iberian mustelids (Feliú *et al.*, 1991 Torres *et al.*, 1997). In the authors knowledge, this is the greatest record described in European studies to date: 50% in France (Torres *et al.*, 2008), 9.7% in Spanish areas where American mink share habitat with European mink and 36% in the rest of Spain (Torres *et al.*, 2003), and 8% in Belarus (Shimalov and Shimalov, 2001). According to Poulin and Mouillot (2003) and Sherrard-Smith *et al.* (2015), nonindigenous host species that have lost their natural parasites tend to acquire generalist parasites from other local host species. Our results indicate that American mink is a competent host for *M. patens*, a parasite common to Palearctic mustelids, contributing to its transmission and natural nidality in Galicia. In our study, males showed a significantly lower *M. patens* intensity than females, and spring was the season with a lower intensity. However, the heterogeneity of the sample not allow to establish any conclusion, and therefore further studies are necessary in order to improve the knowledge of these and other epidemiological aspects.

We also found the gastric nematode *A. putorii*, one of the most common parasite of mustelids worldwide. Although host's infection is usually associated to the direct ingestion of this parasite's eggs, it has also been described the role of earthworms as paratenic hosts (Anderson, 2000). Prevalence of *A. putorii* recorded in Galicia was considerably higher than the ones observed in other European areas: 25.8% in American mink captured in shared areas with European mink in Spain, and 12% in other Spanish territories (Torres *et al.*, 2003); 20% in Belarus (Shimalov and Shimalov, 2001); and 18.42% in France (Torres *et al.*, 2008). As in the case of *M. patens*, the high prevalence of *A. putorii* indicates that American mink is a well host involved in the natural nidality of this parasite in Galicia. In our study, the intensity of parasitic infection by *A. putorii* was significantly lower in subadult mink and in winter but, as in the abovementioned case of *M. patens*, we have no sound arguments to justify these results that should be contrasted with future studies.

Crenosoma melesi is the third more prevalent (10%) parasite found in American mink from Galicia. Until now, this cardiorespiratory nematode has been cited only in American mink captured in Europe (prevalence of 2%) by Torres *et al.* (2003), but not in America. It has also been described in badger and other Iberian mustelids (Torres *et al.*, 1997, 2001). This parasite has an indirect life cycle with slugs and snails as intermediate hosts (Anderson, 2000), indicating that these gastropods are part of the trophic resources of American mink in Galicia (Melero *et al.*, 2008a). The remarkable prevalence found in our study suggests that American mink is a competent host for *C. melesi*, and provides evidence that this alien mustelid could impact on native mustelid populations by means of a spillback effect (Kelly *et al.*, 2009).

To our knowledge, this is the first report of a nematode belonging to the genus Angiostrongylus in American mink. The enzymatic digestion of lung parenchyma has probably facilitated the isolation of this cardiorespiratory nematode since it is frequently found into the small vessels of lungs. In addition, this procedure is highly useful to avoid the under- estimation of mustelid's respiratory helminths due to the scarce amount of pulmonary tissue usually obtained, allowing the detection of most of these small nematodes. The morphometric characteristics do not adjust to those of A. vasorum, a parasite recovered from domestic and wild canids worldwide (Segovia *et al.*, 2001; Conboy, 2004; Bourque *et al.*, 2008; Morgan *et al.*, 2008; Gerrikagoitia *et al.*, 2010; Eleni *et al.*, 2013), and also described in Eurasian badger (*Meles meles*) from Spain (Miquel *et al.*, 1993; Feliú *et al.*, 1996; Torres *et al.*, 2001). Specimens isolated in our study were identified as *A. daskalovi* according to Janchev and Genov (1988) and Gherman *et al.* (2016). This nematode has been previously cited in pine marten (*Martes martes*), stone marten (*Martes foina*) and badger from Bulgaria (Janchev and Genov, 1988), and in badgers from Spain and Romania (Gerrikagoitia *et al.*, 2010; Gherman *et al.*, 2016). Our findings are consistent with the hypothesis that an alien host species can be a competent host for native parasites, increasing the risk of transmission to other indigenous mustelids by parasite spillback (Kelly *et al.*, 2009). In other words, American mink is a new host that participates in the indirect life cycle of *A. daskalovi*, acting as reservoir host that could change the epidemiological dynamics of this parasite and, consequently, producing a negative impact on native mustelids.

We have also identified *Aelurostrongylus* spp. in one adult American mink. Morphometric characteristics of the unique specimen found do not match with Aelurostrongylus pridhami, the species previously described in Eurasian badger (Torres *et al.*, 2001) and European mink (Torres *et al.*, 2003). Unfortunately, the nematode was cleared in lactophenol for the morphometric exam and it was not possible to analyze by PCR. Aelurostrongylus shows an indirect life cycle with slugs and snails as intermediate hosts (Anderson, 2000). As for *A. daskalovi* and *C. melesi*, the presence of this respiratory nematode in American mink indicates that it is a well-adapted host for native metastrongyloid species present in Galicia, participating in its life cycle owing to the abundance of gastropods in riparian areas, which probably form part of the mink's diet.

In our study, *A. annulosa* was isolated from the small intestine of four mink, even though this capillarid nematode is usually located in the small intestine of various species of rodents (Mascato *et al.*, 1993; Feliu *et al.*, 1997). Taking into account that rodents are part of the diet of American mink (Melero *et al.*, 2008a; Morales *et al.*, 2010), the finding of *A. annulosa* in this mustelid could be considered as a pseudoparasite due to the ingestion of infected rodents. However, nematodes in our study did not show any kind of degradation attributable to the digestion process, and all specimens were found in the small intestine (the usual microhabitat of *A. annulosa*), but not in stomach or large intestine; moreover, fertile female nematodes (with eggs inside) were recovered, as a proof of the species viability and its reproductive capacity into the small intestine of the American mink. In fact, *A. annulosa* has been previously described in other mammals different to rodents; in this sense, Umur *et al.* (2012) found *A. annulosa* in a Hamadryas baboon (*Papio hamadryas*) from a zoo in northern Turkey, attributing the infection to the ingestion of infective eggs expelled in the feces by a rodent. In our opinion, the finding of *A. annulosa* in the American mink indicates that this mustelid could have acquired the infection through the ingestion of embryonated eggs or invertebrates infected with third-stage larvae (Anderson, 2000). It could be interpreted as further evidence that native parasites are able to take advantage of the mink's presence to acquire a new host in the invaded habitat.

This is the first time that *T. acutum* is recorded in American mink from the Northwest of the Iberian Peninsula. This trematode was detected in one immature mink. Considering that we did not have the opportunity to examine the head of mink, our results probably underestimate the prevalence of *T. acutum* in Galicia, because its usual anatomical location are the nasolacrimal sinuses (Koubek *et al.*, 2004; Torres *et al.*, 2006). According to Koubek *et al.* (2004), the European polecat is the main definitive host, although other mustelid species could act as hosts and may contribute to the maintenance of this cranial fluke in the environment (Ribas *et al.*, 2012), whose life cycle includes prosobranch snails and amphibians as intermediate hosts (Vogel and Voelker, 1978; Koubek *et al.*, 2004). This parasite causes suppurative osteomyelitis and severe injuries to the skull bones (Jubb and Kennedy, 1963; Koubek *et al.*, 2004; Torres *et al.*, 2008). In Europe, the highest prevalences of *T. acutum* in American mink have been recorded in France (33%) by Torres *et al.* (2008) and in Álava (North of Spain), where the prevalence was 30.4% (Torres *et al.*, 2006). In our study, we found seven specimens of *T. acutum*, all of them collected from the lung parenchyma. Since three of these trematodes were mature specimens, this finding indicates that *T. acutum* is able to originate ectopic foci and, moreover, to complete its development in the lung parenchyma of the American mink. This anatomical location could drive to even more severe pathological consequences than those assumed so far; unfortunately, the histopathological study to confirm the tissue damage could not be carried out as lung tissue was not preserved after dissection.

We found only one species of digestive trematode in our study. Concretely, twenty-seven immature specimens were isolated from the liver and the small intestine of a mink but, unfortunately, the morphometric identification was not completed due to their poor preservation status. In Europe, *Ascocotyle* spp., *Euparyphium melis*, *Pseudamphistomum truncatum* (whose intermediate hosts are fish) and *Euryhelmis squamula* (with amphibians as intermediate hosts) have been detected in American mink (Shimalov and Shimalov, 2001; Torres *et al.*, 2008; Hawkins *et al.*, 2010). In Spain, *E. squamula* is the only trematode species cited in American mink, showing a low prevalence (2–3.2%) (Torres *et al.*, 2003). Despite fish and amphibians have been described as a main component of the diet of American mink in Galicia (Vidal-Figueroa and Delibes, 1987), recent studies (Romero, 2013, 2015) have demonstrated that its diet is focused mainly on rodents and other terrestrial animals, reducing the ingestion of aquatic and riparian preys in habitats where otter populations are expanding (Bonesi *et al.*, 2004). So, the scarcity of digestive flukes in American mink from Galicia could be due to the diet of this allochthonous mustelid.

Any of the analyzed American mink was parasitized by cestodes. However, *Taenia martis* and *Taenia tenuicollis* have been previously cited in this mustelid from Spain with a prevalence of 2% and 3.2%, respectively (Torres *et al.*, 2003). In France, Torres *et al.* (2008) found *T. tenuicollis* in European polecat (12.1%), but not in American mink. In Belarus, Shimalov and Shimalov (2001) detected *T. mustelae* (synonym *T. martis*) with a low prevalence (4%), and *Spirometra erinacei* larvae (10% prevalence) in American mink. According to Vidal- Figueroa and Delibes (1987) and Romero (2013, 2015), the diet of the American mink in Galicia includes small mammals (the intermediate hosts of all these tapeworms) but, as mentioned above, these helminths were not detected in our study. These results could indicate that cestodes are not frequent in the mustelid species present in Galicia.

As shown in the present study, American mink is an invasive species that can acquire parasites from the indigenous host species with which this mustelid shares habitat, and thus, may achieve a relevant epidemiological role on these parasites' natural nidality. Moreover, our results pointed to an unpredictable environmental impact of these adaptive phenomena, mainly in other mustelid species that inhabit the same area, since parasites from different host species are included, as happens with *A. daskalovi*. The American mink can contribute to the dispersion of these parasites among populations of native mustelids in Spain. This can turn into a severe conservation challenge for endangered species such as the European mink. In this sense, we recommend the parasite monitoring of American mink populations in habitats where this allochthonous mustelid are present, mainly in new areas of invasion.

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CAPÍTULO 2: Cardiopulmonary nematode infections in wild canids: Does the key lie on host-prey-parasite evolution?

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Cardiopulmonary nematode infections in wild canids: Does the key lie on host-prey-parasite evolution?

ABSTRACT

Cardiopulmonary nematodes are among the most pathogenic parasites of domestic and wild canids. The aim of this study was to describe the species diversity, prevalence and infection intensity of these parasites in the Iberian wolf (*Canis lupus signatus*) and the red fox (*Vulpes vulpes*) in the northwest of the Iberian Peninsula. 257 foxes and 74 wolves were necropsied between 2008 and 2014. Four nematode species were identified: *Angiostrongylus vasorum*, *Eucoleus aerophilus*, *Crenosoma vulpis* and *Filaroides hirthi*. This last species was only found in wolves, being the first time that is cited worldwide in this wild canid. The overall parasite prevalence was significantly higher in foxes (70%) than in wolves (28%). Specifically, prevalences in foxes and wolves were, respectively, 43% and 22% for *A. vasorum*, 33% and 5% for *E. aerophilus*, and 30% and 9% for *C. vulpis*. The prevalence of *F. hirthi* was 16%. The *A. vasorum* intensity was significantly higher in foxes than in wolves. Differences between host species in the risk of infection would be associated to diverging feeding behavior, and possibly reflects a parasite-host adaptation related to host's hunting strategies and cardiorespiratory require- ments. This study revealed an association between infection and environmental factors, and highlighted a wide variation in the spatial distribution of *A. vasorum*. Our results indicate that cardiopulmonary parasites are widespread in wild canids in northwest Spain, and further agrees with other studies indicating the expansion of *A. vasorum* in Europe and, therefore, the urgent need to investigate infection in dogs in sympatric areas.

INTRODUCTION

The Iberian wolf (*Canis lupus signatus*) and the red fox (*Vulpes vulpes*) are the only two wild canid species present in the Iberian Peninsula. There are around 2000 wolves in Spain, with the main population located in the northwest (Blanco and Cortés, 2012). In contrast, red fox can be found throughout the Iberian Peninsula (Gortázar, 2007). Both species occupy a wide variety of habitats, including anthropogenic landscapes (Blanco, 2017; Harris and Smith, 1987; Gloor *et al.*, 2001; López-Martín, 2017).

Parasite distributions in wildlife are highly conditioned by the nature of their life-cycle. Specifically, in the case of trophic transmitted parasites of carnivores, the kind of prey affects both predator-prey and host-parasite interactions and, thus, potentially influence the dynamics and trophic web structure (Friesen and Roth, 2016). In this regard, it has been suggested that vertebrate carnivores are able to balance their nutrient intake through selective predation or feeding to obtain specific nutrients (Kohl *et al.*, 2015; Potter *et al.*, 2018). On the other hand, the size of the prey caught by carnivores and, consequently, the foraging strategy are influenced by the ecological niches available to them and the encounter rate with prey. In fact, large terrestrial mammalian carnivores over 21.5 kg usually prey on large-sized animals to maximize their energetic intake while minimizing their energy expenditure (Carbone *et al.*, 1999; Carbone *et al.*, 2007).

There are remarkable differences between the diet of the wolf and the fox; the wolf prefers to prey on domestic and wild ungulates (Llaneza *et al.*, 2012; Torres *et al.*, 2015; López-Martín, 2017), whereas the fox is a generalist predator with a high ecological plasticity (Dell'Arte *et al.*, 2007) that feeds on a broad trophic spectrum, including vegetables, small prey, carrion and garbage (Díaz-Ruiz *et al.*, 2013; López-Martín, 2017).

Cardiopulmonary nematodes are among the most important parasites of canids, specially Angiostrongylus vasorum and Dirofilaria immitis, which are highly pathogenic and emerging parasites that cause congestive heart failure, severe dyspnea and even death (Traversa *et al.*, 2010). The impact of these and other cardiopulmonary parasites as *Crenosoma vulpis* and *Eucoleus aerophilus* on wild canids has not been analyzed but, based on the evolutionary proximity of wolves, foxes and dog, we could assume a comparable pathogenicity in all species. Moreover, considering that wolves and, to a lesser extent, foxes require optimal cardiorespiratory capacity for successful predatory activity, it is reasonable to infer that cardiopulmonary parasites could have a significant impact on the species survival and especially on individuals with high parasite loads and co-infections. The trend so far has been to study cardiopulmonary nematodes in wolves and foxes separately, without considering that both species share habitats and are susceptible to the same parasites. For all these reasons, the objective of this study was to describe the cardiopulmonary parasites of wolves and foxes in the northwestern Iberian Peninsula, discussing the epidemiological role that each of these wild canids have in the natural nidality of these parasites.

MATERIAL AND METHODS

The study was performed on Iberian wolves and red foxes from the regions of Asturias and Galicia (northwest Spain), covering an area of approximately 11,000 km^2 and 30,000 km^2, respectively. Galicia includes the provinces of A Coruña, Lugo, Ourense and Pontevedra (Fig. 1). Climate is predominantly oceanic with average annual rainfall and temperature ranging 960–1688 mm and 11.4–13.3 °C (Castillo-Rodríguez *et al.*, 2006; Valenzuela *et al.*, 2018). The study was performed in 257 red foxes hunted in January and February 2008 in municipalities of A Coruña (34), Lugo (156) and Pontevedra (67), in the context of official hunting tournaments, and 74 Iberian wolves from Asturias (40) A Coruña (18), Lugo (8), Ourense (1) and Pontevedra (7), mostly shot in officially authorized hunts between 2009 and 2014, and some found dead due to road traffic accidents.

Animals were necropsied, and the trachea, lungs and heart were removed and stored frozen at −20 °C until examined for parasites. To this effect, following defrosting, the heart chambers and its great vessels were carefully dissected, washed and filtered through a 62 µm mesh sieve. Trachea, bronchial tree and pulmonary blood vessels were longitudinally opened and observed under a stereomicroscope to collect parasites. Subsequently, in order to remove nematodes that may have remained in the bronchi and of smaller diameter vessels, the lungs were washed through a sieve and the retained material was examined under a stereomicroscope. Finally, to ensure that no nematode was left undetected, pulmonary parenchyma was enzymatically digested in pepsin and chlorhydric acid, according to Martínez-Rondán *et al.* (2017).

Nematodes were cleared with lactophenol and identified according to Georgi (1979), Butterworth and Beverley-Burton (1980), Costa *et al.* (2003) and Popiolek *et al.* (2009). Prevalence (percentage of infected animals), parasite intensity (number of parasites in infected animals) and species richness (number of species in infected animals) were calculated (Bush *et al.*, 1997) and Yate's-corrected chi-square test and the non-parametric Kruskal-Wallis test were used to compare proportions and medians, respectively. Mixed logistic regression models were developed to investigate the relationship between infection with a particular parasite species (outcome variable) and host species (fox or wolf), adjusted for sex, age and environmental variables of the area were animals were collected, and including municipality as a random effect to allow for spatial clustering of infection (Agresti, 2013). Environmental variables, included the human population density, average annual temperature and average annual rainfall, were categorized prior to inclusion in the model. Parameters were estimated using the maximum likelihood method, p-values were calculated with the chi-squared test and significance was considered at the 5% ($p < .05$) level for a two- tailed test. Analyses were carried out using R 3.4.3 software (R core Team, 201).

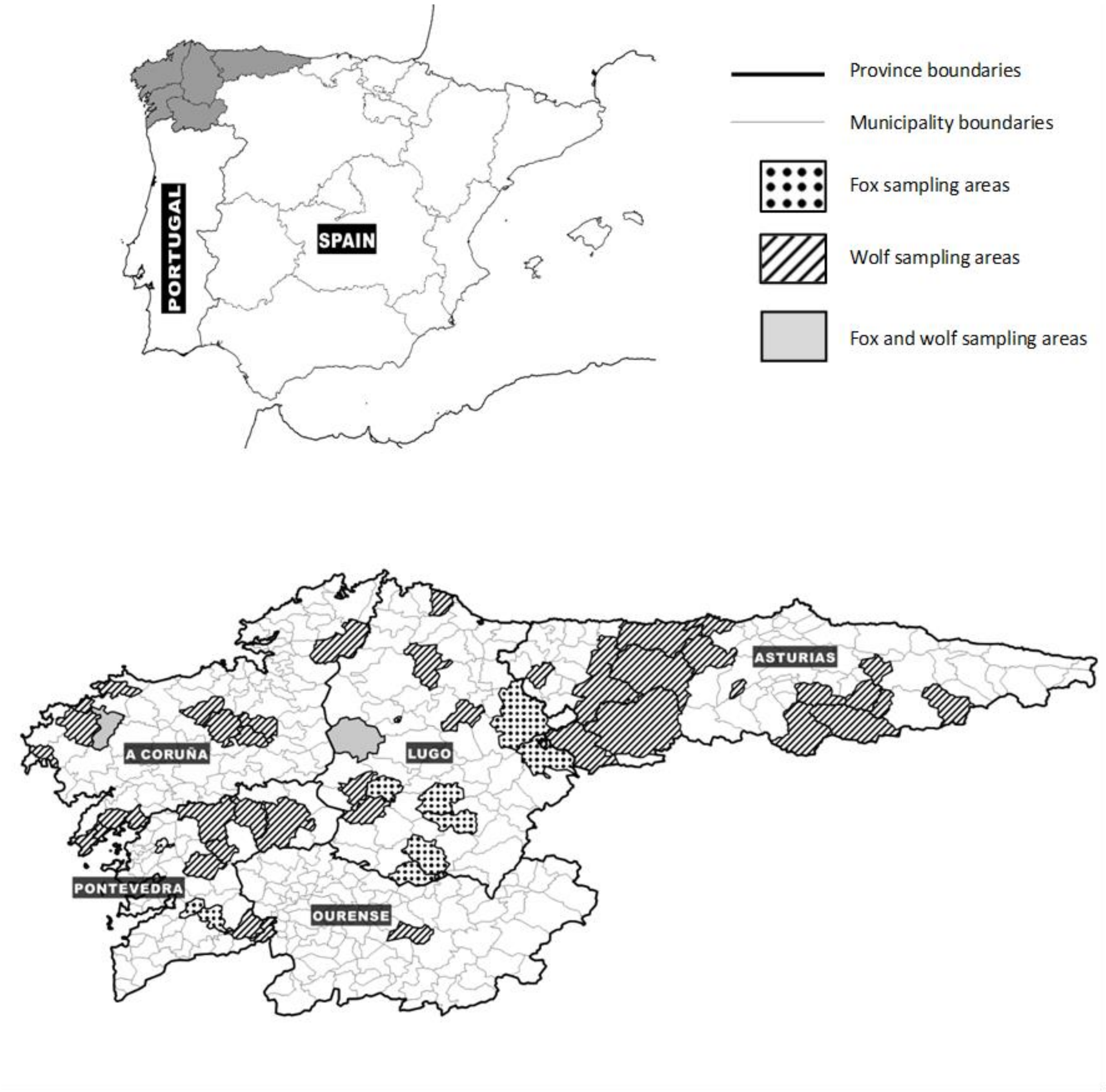

Figura 9 Municipalities in northwestern Spain where wolves and foxes were sampled to study the cardiopulmonary nematode species.

RESULTS

Four nematode species were identified: *Angiostrongylus vasorum*, *Eucoleus aerophilus*, *Crenosoma vulpis* and *Filaroides hirthi* (Table 1). Parasite prevalence (95% CI) was 70% (65–76) in foxes and 28% (18–39) in wolves ($p < .05$). The percentage of foxes infected with one, two or three nematode species were 54% (98/181), 40% (73/181) and 6% (10/181), respectively. These same prevalences for wolves were 62% (13/21), 24% (5/21), and 5% (1/21), and 10% (2/21) of wolves were infected with the four nematode species.

The parasite distribution in infected foxes and wolves was widely variable and positively skewed, with most animals having low parasite burdens. Foxes had the largest number of *A. vasorum*, *C. vulpis* and *E. aerophilus*, and median *A. vasorum* and *C. vulpis* intensity were higher ($p < .05$) and marginally higher (p-value=.06), respectively, in this host species compared to wolves. It was not possible to estimate the median intensity of *F. hirthi*, because most nematodes were fragmented or damaged after the enzymatic digestion of the pulmonary parenchyma required to detect this small nematode species.

The prevalence and median intensity of *A. vasorum*, *C. vulpis* and *E. aerophilus* in wolves and foxes varied according to some of the environmental variables, but they did not follow an increasing or decreasing trend with any of the variables analyzed except for *E. aerophilus* prevalence in foxes, which decreased with increasing annual rainfall (Tables 2 and 3).

The logistic regression models confirmed the significantly higher risk of foxes being infected with cardiopulmonary parasites compared to wolves, and the association between *A. vasorum* infection and areas with medium human population density, *C. vulpis* and juvenile hosts, and *E. aerophilus* and low temperature and precipitation ($p < .05$). Moreover, *A. vasorum* models revealed additional, unexplained variation in the risk of infection between municipalities, highlighting the strong spatial aggregation of this parasite species (Table 4).

Tabla 3 Prevalence (95% CI), median intensity and range of cardiopulmonary nematode species detected in foxes and wolves from northwestern Spain (n=331).

	Foxes (n=257)			Wolves (n=74)			All (n=331)		
Parasite species	Prevalence (%)	Median intensity	Range	Prevalence (%)	Median intensity	Range	Prevalence (%)	Median intensity	Range
Angiostrongylus vasorum	43.2 (37.0-49.2)	7.0	1-422	21.6 (12.0-31.2)	1.5	1-48	38.4 (33.1-43.6)	6.0	1-422
Crenosoma vulpis	30 (24.3-35.6)	3.0	1-140	9.4 (2.6-16.2)	1.0	1-8	25.4 (20.6-30.1)	3.0	1-140
Eucoleus aerophilus	33.4 (27.6-39.2)	4.0	1-36	5.4 (0.1-10.6)	8.5	1-14	27.2 (22.4-32.0)	4.0	1-36
Filaroides hirthi	0	-	-	16.2 (7.6-24.8)	ND	ND	3.6 (1.6-5.6)	ND	ND

Prevalence refers to the percentage of parasitized animals, and intensity to the number of nematode specimens in parasitized individuals; ND: not determined.

Tabla 4 Prevalence and median intensity of cardiopulmonary nematode species found in foxes (n=257) according to host and environmental features

Variable	Levels	n	*Angiostrongylus vasorum*					*Crenosoma vulpis*					*Eucoleus aerophilus*				
			% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value	Median	Maximum	P value
Age	Adult	186	44.1 (82/186)	0.743	7.0	163	0.677	24.2 (45/186)	0.002	3.0	110	0.623	36.6 (68/186)	0.12	5.0	36	0.034
	Juvenile	71	40.8 (29/71)		6.0	433		45.1 (32/71)		3.0	140		25.4 (18/71)		2.0	15	
Sex	Female	108	48.1 (52/108)	0.216	7.5	53	0.631	27.8 (30/108)	0.608	3.5	92	0.627	32.4 (35/108)	0.864	2.0	30	0.021
	Male	149	39.6 (59 /149)		6.0	422		31.5 (47/149)		3.0	140		34.2 (51/149)		5.0	36	
Human population density (people/km^2)	3.1-13.3	105	28.6 (30/105)	0.000	4.0	163	0.013	41.9 (44/105)	0.001	3.0	140	0.173	48.6 (51/105)	0.000	3.0	30	0.439
	13.4-54.6	71	73.2 (52/71)		10.0	422		26.8 (19/71)		5.0	65		14.1 (10/71)		2.0	12	
	54.7-294	81	35.8 (29/81)		6.0	50		17.3 (14/81)		2.0	15		30.9 (25/81)		5.0	36	
Altitude (m)	0-316	130	53.8 (70/130)	0.000	9.0	422	0.024	20.0 (26/130)	0.000	3.5	65	0.731	18.5 (24/130)	0.000	6.0	36	0.020
	317-633	98	28.6 (28/98)		3.5	51		44.9 (44/98)		3.0	140		37.8 (37/98)		2.0	30	
	634-950	29	44.8 (13/29)		5.0	163		24.1 (7/29)		3.0	12		86.2 (25/29)		5.0	21	
Annual temperature (°C)	8.8-10.7	44	40.9 (18/44)	0.440	5.5	163	0.238	27.3 (12/44)	0.000	3.0	12	0.484	72.7 (32/44)	0.000	4.0	21	0.043
	10.8-12.6	111	39.6 (44/111)		12.5	78		44.1 (49/111)		3.0	140		26.1 (29/111)		2.0	30	
	12.7-14.4	102	48.0 (49/102)		6.0	422		15.7 (16/102)		3.0	65		24.5 (25/102)		5.0	36	
Annual rainfall (mm)	894-1270	114	40.4 (46/114)	0.506	5.0	163	0.025	33.3 (38/114)	0.017	3.0	140	0.405	54.4 (62/114)	0.000	5.0	36	0.034
	1271-1660	76	48.7 (37/76)		13.0	78		36.8 (28/76)		2.0	110		26.3 (20/76)		2.5	13	
	1661-2040	67	41.8 (28/67)		6.0	422		16.4 (11/67)		3.0	65		6.0 (4/67)		1.5	4	

[a] **% p (pos/n):** % prevalence (positive animals/studied animals)

Tabla 5 Prevalence and median intensity of cardiopulmonary nematode species found in wolves (n=74) according to host and environmental features

Variable	Levels	n	*Angiostrongylus vasorum*					*Crenosoma vulpis*					*Eucoleus aerophilus*					*Filaroides hirthi*	
			% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value
Age	Adult	31	19.4 (6/31)	0.908	1.0	2	0.035	0.0 (0/31)	0.050	-	-	-	3.2 (1/31)	0.855	2.0	2	0.180	9.7 (3/31)	0.329
	Juvenile	43	23.3 (10/43)		2.0	48		16.3 (7/43)		1.0	8		7.0 (3/43)		9.0	14		21.0 (9/43)	
Sex	Female	38	18.4 (7/38)	0.686	1.0	4	0.424	10.5 (4/38)	1	1.0	5	0.659	5.3 (2/38)	1	11.0	14	0.439	18.4 (7/38)	0.831
	Male	36	25.0 (9/36)		2.0	48		8.3 (3/36)		1.0	8		5.6 (2/36)		5.5	9		13.9 (5/36)	
Human population density (people/km²)	3.1-13.3	18	5.6 (1/18)	0.014	4.0	4	0.349	11.1 (2/18)	0.906	1.0	1	0.417	0.0 (0/18)	0.182	-	-	-	11.1 (2/18)	0.310
	13.4-54.6	41	34.1 (14/41)		1.5	48		9.8 (4/41)		3.0	8		9.8 (4/41)		8.5	14		22.0 (9/41)	
	54.7-294	15	6.7 (1/15)		1.0	1		6.7 (1/15)		1.0	1		0.0 (0/15)		-	-		6.7 (1/15)	
Altitude (m)	0-316	38	21.1 (8/38)	0.515	2.0	48	0.213	13.2 (5/38)	0.494	1.0	8	0.629	7.9 (3/38)	0.602	9.0	14	0.655	18.4 (7/38)	0.632
	317-633	32	25.0 (8/32)		1.0	4		6.3 (2/32)		3.0	5		3.1 (1/32)		8.0	8		15.6 (5/32)	
	634-950	4	0.0 (0/4)		-	-		0.0 (0/4)		-	-		0.0 (0/4)		-	-		0.0 (0/4)	
Annual temperature (°C)	8.8-10.7	20	10.0 (2/20)	0.331	1.5	2	0.224	5.0 (1/20)	0.426	1.0	1	0.779	5.0 (1/20)	0.796	8.0	8	0.259	5.0 (1/20)	0.127
	10.8-12.6	28	25.0 (7/28)		1.0	4		7.1 (2/28)		3.0	5		3.6 (1/28)		2.0	2		14.3 (4/28)	
	12.7-14.4	26	26.9 (7/26)		2.0	48		15.4 (4/26)		1.0	8		7.7 (2/26)		11.5	14		26.9 (7/26)	
Annual rainfall (mm)	894-1270	16	18.8 (3/16)	0.267	1.0	1	0.213	6.3 (1/16)	0.792	5.0	5	0.420	6.3 (1/16)	0.608	2.0	2	0.180	18.8 (3/16)	0.186
	1271-1660	44	27.3 (12/44)		2.0	48		11.4 (5/44)		1.0	8		6.8 (3/44)		9.0	14		20.5 (9/44)	
	1661-2040	14	7.1 (1/14)		2.0	2		7.1 (1/14)		1.0	1		0.0 (0/14)		-	-		0.0 (0/14)	

[a] **% p (pos/n):** % prevalence (positive animals/studied animals)

Tabla 6 Estimates of logistic regression models investigating the effect of host dependent variables (sex, age and canid species) and environmental variables (average annual temperature, average annual rainfall and human population density) over the prevalence of A. vasorum, C. vulpis and E. aerophilus (n=331).

Variable	Levels	*Angiostrongylus vasorum*			*Crenosoma vulpis*			*Eucoleus aerophilus*		
		Estimate	Std. Error	P value	Estimate	Std. Error	P value	Estimate	Std. Error	P value
Fixed components										
Intercept		-2.5633	0.6245	0.0000	-3.0612	0.6089	0.0000	-0.7097	0.6979	0.3092
Sex	Female	0.0000			0.0000			0.0000		
	Male	-0.1210	0.2595	0.6410	0.1666	0.2791	0.5504	0.0532	0.3135	0.8653
Age	Adult	0.0000			0.0000			0.0000		
	Juvenile	-0.0324	0.2843	0.9094	0.9878	0.2945	0.0008	-0.2148	0.3499	0.5392
Host species	Wolf	0.0000			0.0000			0.0000		
	Fox	1.9509	0.4373	0.0000	1.7527	0.4690	0.0002	2.4230	0.6387	0.0001
Annual temperature (ºC)	8.89-10.7	0.0000			0.0000			0.0000		
	10.8-12.6	-0.2429	0.4657	0.6020	0.6141	0.3983	0.1231	-2.1265	0.4558	0.0000
	12.7-14.4	0.2558	0.5788	0.6585	0.2894	0.6411	0.6518	-0.3551	0.6731	0.5978
Annual rainfall (mm)	894-1270	0.0000			0.0000			0.0000		
	1271-1660	-0.1021	0.4008	0.7990	0.3263	0.3785	0.3886	-1.0007	0.4415	0.0234
	1661-2040	-0.9809	0.5018	0.0506	-0.2696	0.5078	0.5954	-3.3379	0.6197	0.0000
Human density (people/km^2)	3.1-13.3	0.0000			0.0000			0.0000		
	13.4-54.6	2.1026	0.5008	0.0000	-0.6111	0.4324	0.1575	-0.8051	0.5472	0.1412
	54.7-294	0.4406	0.5108	0.3884	-0.8072	0.5052	0.1101	-0.7544	0.5527	0.1723
Random effect		Standard deviation			Standard deviation			Standard deviation		
Municipality		0.3404			<0.0001			<0.0001		

DISCUSSION

Angiostrongylus vasorum has been described in canids worldwide (Spratt, 2015). However, until a few decades ago, its geographical distribution in Europe was localized to a few endemic foci (Morgan *et al.*, 2009). The study confirms that *A. vasorum* is endemic in north-west Spain with higher prevalence in foxes (43%) than elsewhere in the Iberian Peninsula (16–36%) (Gortázar *et al.*, 1998; Segovia *et al.*, 2004; Mañas *et al.*, 2005; Eira *et al.*, 2006; Gerrikagoitia *et al.*, 2010). Likewise, prevalence of this species in wolves (22%) was higher than in previous surveys in northwest Spain (2–5%) (Torres *et al.*, 2000; Segovia *et al.*, 2001; Segovia *et al.*, 2007; Garrido-Castañé *et al.*, 2015). The sensitivity of detection of *A. vasorum* in foxes by dissection of the heart and pulmonary arteries is estimated at 84% (Houpin *et al.*, 2016). We additionally used enzymatic digestion of lung parenchyma to reduce the number of false-negative cases. It is likely that the prevalence of *A. vasorum* was underestimated in other studies in which enzymatic digestion was not used.

The prevalence of *A. vasorum* was higher in foxes than in wolves, coinciding in general terms with the literature mentioned above. This result reflects dietary differences between species, with foxes feeding more on intermediate and paratenic hosts of this parasite than wolves, including a wide variety of gastropod species (Ferdushy and Hasan, 2010), and frogs and birds (Bolt *et al.*, 1993; Elsheikha *et al.*, 2014; Mozzer and Lima, 2015), respectively. Moreover, the similar prevalence and *A. vasorum* intensity in juvenile and adult foxes indicates that these prevalence and median intensity was higher in juvenile compared to adult wolves, supporting that gastropods and paratenic hosts of *A. vasorum* constitute part of the diet of juveniles during the developmental period until they become accomplished adult hunters of large prey (MacNulty *et al.*, 2012; Zimmermann *et al.*, 2015). It is important to point out, moreover, that carnivores modify their nutrient intake through selective predation or feeding in order to obtain a balanced diet (Kohl *et al.*, 2015). This could partly explain that 19% of adult wolves were parasitized by *A. vasorum*; that is, although adults usually hunt large animals, they also eat smaller prey.

The geographic distribution of *A. vasorum* is characterized by stable foci of high prevalence (Morgan *et al.*, 2009), mostly in areas with mild and wet climates, as is our study area, since the invertebrate hosts are highly sensitive to temperature and moisture (Jeffery *et al.*, 2004; Ferdushy and Hasan, 2010). In Europe, the distribution of *A. vasorum* has increased in recent decades, probably due to the fox's expansion (Van Doorn *et al.*, 2009; Al-Sabi *et al.*, 2013, Al-Sabi *et al.* 2014). Although the pathogenicity of *A. vasorum* has been described in foxes (Morgan *et al.*, 2008) and wolves (Eleni *et al.*, 2014; De Liberato *et al.*, 2017), the impact on the wider population is unknown. Also, the role of foxes and wolves in the epidemiology of infection in dogs living in close-by areas is also discussed (McCarthy *et al.*, 2016; Schug *et al.*, 2018; Lange *et al.*, 2018). Although few studies compared *A. vasorum* prevalence in synanthropic wild and domestic canids, a wide molecular study of strains from dogs and foxes revealed no evidence of genetic segregation suggesting a common transmission cycle (Jefferies *et al.*, 2010). In our study, prevalence was highest in rural areas with medium human density, as well as the highest intensities in foxes. We hypothesize that, in these rural areas, there are probably more dogs that are allowed to roam freely and, therefore, have the possibility of ingesting intermediate hosts, playing a summative role in the local *A. vasorum* life cycle involving domestic and wild canids.

Crenosoma vulpis has been described in canids from temperate regions worldwide (Shimalov and Shimalov, 2000; Latrofa *et al.*, 2015; Figueiredo *et al.*, 2016; Maksimov *et al.*, 2017), including the Iberian Peninsula, in both foxes (Gortázar *et al.*, 1998; Segovia *et al.*, 2004; Mañas *et al.*, 2005; Eira *et al.*, 2006; Garrido-Castañé *et al.*, 2015) and wolves (Segovia *et al.*, 2007). Like *A. vasorum*, gastropods are the intermediate hosts of this nematode species (Jeffery *et al.*, 2004). The higher *C. vulpis* prevalence in foxes compared to wolves indicates than this host species plays a more prominent role than wolves in the epidemiological dynamics of this parasite. Similar to what has been argued in *A. vasorum*, this result suggests that both canids prey on gastropods, but more notably in foxes (Colella *et al.*, 2016; Lange *et al.*, 2018). *C. vulpis* was more prevalent in juvenile foxes than in adults, and was only found in juvenile wolves. This could be due to differences in trophic behavior and a more effective immune response in adults (Jeffery *et al.*, 2004; Davidson *et al.*, 2006; Hodžić *et al.*, 2016). Notwithstanding this, some adult foxes had a high parasite burden which, although not fatal, would reduce the host's respiratory capacity significantly (Traversa *et al.*, 2010).

Eucoleus aerophilus has been reported in wild and domestic canids (Torres *et al.*, 2000; Morgan *et al.*, 2008; Conboy, 2009; Traversa *et al.*, 2009; Di Cesare *et al.*, 2014). The prevalence in foxes was similar to those reported in other Iberian areas (Gortázar *et al.*, 1998; Segovia *et al.*, 2004; Mañas *et al.*, 2005), contrasting with the low prevalence described by other authors (Eira *et al.*, 2006; Martínez-Carrasco *et al.*, 2007). High prevalences (65–84%) have been described in foxes in other European countries (Davidson *et al.*, 2006; Saeed *et al.*, 2006; Lalošević *et al.*, 2013; Al-Sabi *et al.*, 2014; Hodžić *et al.*, 2016; Schug *et al.*, 2018). In our study, *E. aerophilus* was the least prevalent cardiopulmonary nematode in wolves; in other European countries, the prevalence in wolves (8–36%) is usually lower than that described in foxes (Shimalov and Shimalov, 2000; Popiołek *et al.*, 2007; Bagrade *et al.*, 2009; Varodi *et al.*, 2017). As with *C. vulpis*, the prevalence of *E. aerophilus* was related to altitude, annual temperature and annual rainfall. This parasite has a direct life cycle, and earthworms can act as paratenic hosts

(Anderson, 2000), yet there is limited information on other ecological and epidemiological aspects of *E. aerophilus* infection that could help interpret these associations.

Filaroides spp. has been sporadically described in dogs in Europe (Caro-Vadillo *et al.*, 2005; Cervone *et al.*, 2018) and rarely in wild canids (Magi *et al.*, 2015). Sanchis-Monsonís *et al.* (2013) detected *F. hirthi* in 2% of foxes in southeast Spain. Here we report the first *F. hirthi* infection in wolves worldwide. The rare detection of this parasite in wild canids could be partly due to the fact that *F. hirthi* is a very small, fragile nematode located in the bronchioles and alveoli and, consequently, difficult to detect by direct examination. The absence or low prevalence of *F. hirthi* in wild canids in general should be evaluated with care, as the presence of this parasite in these host species may be underestimated. We have been able to detect *F. hirthi* because the lung parenchyma of each animal was enzymatically digested, which increases the probability of detection, as has been proven in dogs (Bahnemann and Bauer, 1994). This finding highlights the potential role of wolves in the maintenance of the parasite's transmission in nature. Dogs are infected by ingestion of infectious larvae present in saliva (Anderson, 2000). In the case of *Filaroides osleri*, maternal cleanliness is assumed to be an important transmission route in the dog, while regurgitation of food by parents to feed pups may also be an important form of transmission in wild canids (Polley and Creighton, 1977; Clayton and Lindsay, 1979; Dunsmore and Spratt, 1979). In addition, it has been suggested that infection in dogs is possible by coprophagy of fresh faeces (Georgi *et al.*, 1979). Since *F. hirthi* and F. osleri belong to the same genus, it is assumable that the transmission is similar in both parasites and, therefore, the social behavior of the wolf could explain the intraspecific transmission of *F. hirthi*. Further epidemiological studies are needed to better understand the risk factors associated with *F. hirthi* transmission and to assess the role of the wolf as a reservoir of this parasite, as well as the degree of overlap between the domestic and sylvatic epidemiological cycles.

Finally, *Dirofilaria immitis* was not detected in our study, but this was somewhat not surprising, since the northwest Spain is considered an area of low risk of transmission, given that climatic conditions are not ideal for the mosquito vector (Simón *et al.*, 2014). *D. immitis* has been previously detected in Iberian wolves (Segovia *et al.*, 2001) and foxes (Gortázar *et al.*, 1994; Gortázar *et al.*, 1998; Mañas *et al.*, 2005; Eira *et al.*, 2006) in other parts of the Iberian Peninsula, and also in other European areas (Georgieva *et al.*, 2001; Pascucci *et al.*, 2007; Magi *et al.*, 2008; Penezić *et al.*, 2014).

CONCLUSIONS

Northwest Spain is highly endemic for *A. vasorum*, *C. vulpis* and *E. aerophilus,* and prevalence and infection intensity is significantly greater in foxes than in wolves. Differences between host species are probably related to differences in trophic behavior (Buck *et al.*, 2018), with foxes of all ages and juvenile wolves feeding on intermediate and paratenic hosts of the first two nematode species, and adult wolves more on larger prey which not participate of the life cycle of these parasites. We hypothesized that this is an adaptive host-parasite response, so the highest infection risk occurs in the wild canid species (fox) that requires less cardiorespiratory effort to hunt their usual prey (Brose, 2010; Sand *et al.*, 2016). In other words, cardiopulmonary nematodes, although adapted to several host species, have managed to link their life cycle to a type of prey more likely to be consumed by the smaller predator (fox). This prevents the cardiorespiratory capacity of a large predator, located at the top of the trophic chain (mainly adult wolves), to be compromised, which has meant the adoption of a trophic strategy by the wolf that reduces the risk of infection.

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CAPÍTULO 3: Differential contribution of foxes and wolves to gastrointestinal helminth infections in the domestic-wildlife interface

Differential contribution of foxes and wolves to gastrointestinal helminth infections in the domestic-wildlife interface

ABSTRACT

Red fox (*Vulpes vulpes*) and Iberian wolf (*Canis lupus signatus*) are the two species of wild canids that inhabit the Iberian Peninsula, currently their populations are expanding and drift into greater contact with human settlements and domestic animals. Gastrointestinal helminths are parasites frequently described in these wild canids, and many of them also can affect domestic animals and human. The aim of the present study was to describe the gastrointestinal helminthfauna of foxes and wolves in the northwest of the Iberian Peninsula analyzing the epidemiological role that each of these wild canids has in the natural nidality of these parasites. During the period 2008-2014, a total of 257 red foxes and 83 Iberian wolves were necropsied, and the alimentary tract of each animal was examined for parasite morphometrical identification. A total of 13 helminth species were detected (5 nematodes and 8 cestodes). *Uncinaria stenocephala, Toxocara canis, Trichuris vulpis, Taenia hydatigena, Taenia pisiformis, Taenia crassiceps, Mesocestoides spp.* and *Dipylidium caninum* were found in both wild canids, whereas *Ancylostoma caninum* and *Taenia krabbei* was detected only in wolves, and *Toxascaris leonina, Taenia polyacantha* and *Taenia taeniaeformis* were found exclusively in foxes. In addition, a PCR protocol was performed in 94 individual cestodes from 27 wolves for molecular identification. Amplified products were obtained in 63 samples and sequences confirmed the presence of two cestode species, *T. hydatigena* (16/63 sequences) and *T. krabbei* (47/63 sequences). Overall prevalence of gastrointestinal nematodes was higher in wolves (80.7%) than in foxes (61.9%), as well as overall prevalence of cestodes (68.7% and 44.0%, respectively). Also, median intensity of cestodes was significantly higher in wolves (14 cestodes per infected host) than in foxes (7 cestodes per infected host). This is the first report of *T. krabbei* in wolves from the Iberian Peninsula, ascorroborated the molecular analyses. *Echinococcus* was not detected in this study. Most of the helminths identified are transmitted through food webs by intermediary or paratenic hosts. In this sense, foxes were mostly parasitized by helminths harbored by small animals, such as rodents, while wolves were principally affected by parasites transmitted by ungulates. Therefore, we demonstrated that, despite foxes and wolves are theoretically susceptible to the same gastrointestinal helminths, the trophic particularities of these canids favour the presence of a well-differentiated gastrointestinal helminthfauna and, consequently, a different epidemiological role in nidality of these helminths.

INTRODUCTION

The relationships between hosts and their parasites are asymmetrical interactions. A parasite is totally dependent on its host for its reproduction and survival, and the parasite fitness is a compromise between a maximum exploitation of its host and the host responses through behavioral or immunological defenses (Morand, 2015).

Red fox (*Vulpes vulpes*) and wolf (*Canis lupus signatus*) are the only two wild canid species described in the Iberian Peninsula. Wolves can be found both in Spain and Portugal (Torres and Fonseca*,* 2016; Blanco *et al.* 2017). The main population of Portugal is located in the north of river Duero and is contiguous to the main population of Spain, which occupy areas of Galicia, Asturias, Cantabria and north of Castilla y León. On the other hand, foxes are common all over the Iberian Peninsula (López-Martín, 2017).

The proportion of wild and domestic ungulates in the diet of wolves depends on the availability of both kinds of prey. In an area with abundant livestock, principally semi-wild horses, domestic ungulates are the most abundant prey or could be consumed as carrion, while wild ungulates are less consumed. In contrast, where livestock is scarce, roe deer and wild boar are the most frequent preys of wolves (Llaneza *et al.*, 1996). However, Barja (2009) found a predilection of wolves to prey on wild ungulates, principally roe deer, red deer and wild boar, instead of on domestic prey in an area from northwestern Spain where extensive livestock is present. By the other hand, as reviewed in Díaz-Ruíz *et al.* (2013), the red fox is a generalist predator whose trophic patterns can be explained by geographical variables, habitat type and seasonality. The diet of this wild canid showed a latitudinal pattern characterized by higher consumption of lagomorphs and invertebrates in southern areas than in northern ones, where micromammals, fruits and seeds were most frequent as trophic resources.

Wildlife are frequently considered in One Health contexts as reservoir of emerging diseases that threaten human and domestic animals' health (Jenkins *et al.,* 2015). Anthropogenic conversion of natural and semi-natural habitats to urbanized land is one of the main causes of land-use change worldwide (Pickett *et al.*, 2001; Piorr *et al.*, 2011; Šálek *et al.*, 2015) and seriously affects ecosystem structure and function. This process can cause substantial loss, modification and fragmentation of natural habitats that ultimately negatively impact biodiversity conservation (McKinney, 2008; Plumer *et al.*, 2014). However, the urban ecosystem could provide even greater diversity and heterogeneity of potential habitats and ecological niches to wildlife (Červinka *et al.*, 2014). Availability of resources, lack of natural competitors and abiotic factors such as specific urban microclimate conditions are the main factors which favor the presence of wildlife in urban habitats (Shea and Chesson 2002; Šálek et al, 2015). Increased interactions between humans, their domestic animals and wildlife facilitate pathogen transmission from domesticated reservoir populations to sympatric wildlife (spillover) and subsequently, these wildlife populations could act as reservoirs and/or amplifiers of diseases for domestic animals and humans (spillback) (Thompson *et al.*, 2009; Jenkins *et al.,* 2015). We must take into account that the circulation of parasites between wolves, foxes and dogs is possible in shared areas as these canids are closely phylogenetically related species with similar behavioural and physiological patterns (Huang *et al.*, 2014). In particular, Duscher *et al.* (2015) affirmed that foxes play a key role in the interface between wildlife, pets and humans, which could be attributed to the increasing population of foxes and vicinity to human settlements as a consequence of their synanthropic lifestyle or their susceptibility to pathogens. Some of these pathogens are related to ingestion of small mammals which leads to frequent ingestion of intermediate hosts (Wandeler *et al.*, 2003; Deplazes *et al.*, 2004; Duscher *et al.*, 2006). In the last decades, the application of conservation policies and the development of awareness campaigns, but also the abandonment of rural areas has favored the recolonization by wildlife of areas formerly occupied by people ("Pax Romana"; Martínez-Abraín, *et al.*, 2019). This new scenario has favoured the expansion of wildlife, particularly wolves in Spain which can be seen during the day in areas of central and northwestern Spain where human presence is scarce and harmless (Martínez-Abraín, *et al.*, 2019).

The importance of the digestive helminthfauna of these canids lies in the capacity of certain of these parasites to be transmitted to domestic dogs, livestock and also to human (Gortázar *et al.*, 1998; Segovia *et al.*, 2001, 2003; Di Cerbo *et al.*, 2008). Several studies have been conducted in Europe describing gastrointestinal parasites affecting foxes and wolves, but the trend so far has been to study both hosts separately, notwithstanding that these two wild species are susceptible to the same gastrointestinal helminths and, in addition, they share habitat. In this new epidemiological scenery of expansion of these wild canid species and its approaching to anthropized areas, it is important to understand the epidemiological role that these carnivores could play contributing to the spread of pathogens of sanitary interest. So, the aim of this study was to describe the digestive helminthfauna of red foxes and wolves in an area shared by both canids, domestic animals and human population. Also, the epidemiological role that each of these wild canids plays in the natural nidality of these parasites was analyzed.

MATERIAL AND METHODS

Study area

Wild canids analyzed in this study came from the provinces of Asturias, A Coruña, Lugo, Ourense and Pontevedra, located in northwestern Spain. The climate in the study area is predominantly oceanic, although in areas far from the Atlantic coast, the climate can acquire continental or Mediterranean characteristics (south of Ourense). In the south of Asturias, a colder climate is registered due to influence of Cantabrian Range (Valenzuela *et al.*, 2018). In A Coruña, Lugo, Ourense and Pontevedra, the average annual rainfall is 1180 mm, with a homogeneous distribution throughout the year, and the average annual temperature is 13.3°C, with limited yearly variations (Castillo-Rodríguez *et al.*, 2007). In Asturias, values are very similar to those recorded in the abovementioned provinces, with average annual precipitation and temperature range from 960 mm and 13.3°C (lowest areas and near the coast) to 1688 mm and 11.4°C (mountainous areas) (Valenzuela *et al.*, 2018).

Animals and sampling

Red foxes included in this survey (n=257) were hunted as part of the hunting tournaments organized by the Galician Hunting Federation during January and February 2008, in the provinces of A Coruña (n=34), Lugo (n=156) and Pontevedra (n=67) (Figure 10). Necropsies and sampling procedures were carried out by veterinarians *in situ*. On the other hand, Iberian wolves (n=83) were collected in the provinces of Asturias (n=48), A Coruña (n=18), Lugo (n=8), Ourense (n=1) and Pontevedra (n=9) from 2009 to 2014 (Figure 1). Most of the sampled wolves were hunted by authorized hunters as a result of management campaigns, although some of them were found roadkilled. Necropsies and sampling were carried out at the Veterinary Faculty of Lugo (wolves from A Coruña, Lugo, Ourense and Pontevedra) or at the Servicio Regional de Investigación y Desarrollo Agroalimentario (wolves from Asturias). Age of wild canids was determined by tooth wear (Gipson *et al.,* 2000). In both cases, digestive viscera from each animal were separated, stored in individual labelled bags and frozen at -20°C. Subsequently, all samples were dispatched to the Veterinary Faculty of Murcia for their parasitological evaluation.

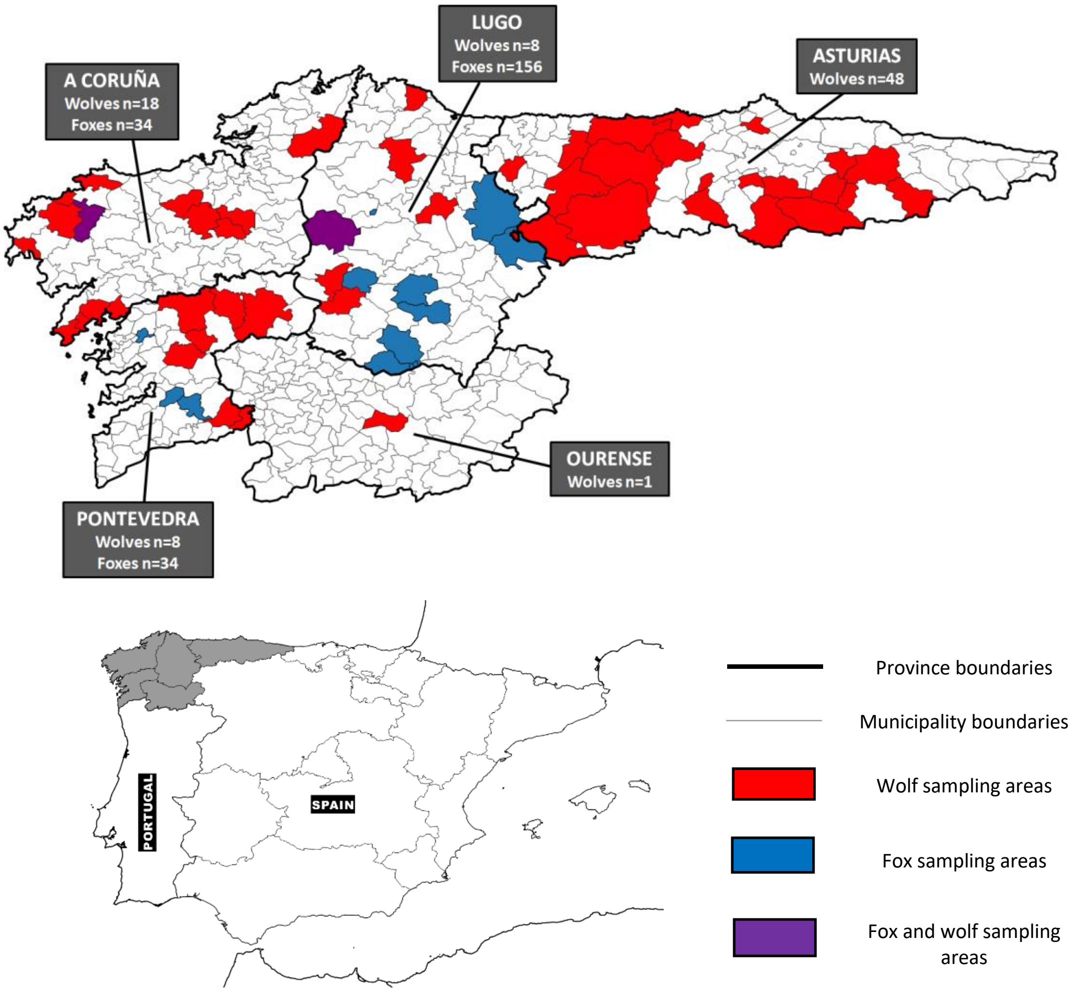

Figura 10 Municipalities where wolves and foxes were sampled to study the gastrointestinal helminthfauna.

Parasitological procedures and identification

The processing of the digestive tract was carried out similarly to described in Martínez-Carrasco *et al.* (2007), and sections of the alimentary tract (stomach, small and large intestine) were analyzed separately. Each part of the digestive tract was cut open longitudinally, scraped and washed over a sieve (mesh size 0.3 mm diameter). Both the filtered content and the digestive mucosa were observed under a stereomicroscope.

Parasites recovered from each animal were washed with distilled water, counted and stored in ethanol 70%. Some proglottids from wolf taenids were selected according to their different shapes and sizes (stating that they could belong to different species of cestodes), preserved in 100% ethanol and stored at 4°C for further genetic analyses. Nematodes were cleared with lactophenol and morphometrically identified attending to morphologic keys as Gibbs (1961), Burrows (1962), Mozgovoia (1968), Soulsby (1987).

Cestodes were stained with Semichon's acetic carmine (Schmidt, 1986) and morphometrically identified attending to morphologic keys as Verster (1962) and Euzéby (1981a, 1981b).

Molecular analyses of wolf cestodes

DNA was extracted from one or more proglottids of each specimen using the QIAamp DNA Mini kit (Qiagen) following manufacturer's instructions, with a final elution in 40 microL of sterile water. For the molecular discrimination of taenid cestodes, a fragment of the cytochrome c oxidase subunit 1 mitochondrial gene (cox1) was amplified by qualitative PCR, using primers JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (5′ TAAAGAAAGAACATAATGAAAATG-3′) amplifying a region of ∼450 bp (Bowles *et al.*,1992). A touchdown PCR protocol was performed under the following conditions: 95°C for 3 m; 10 cycles at 95°C for 40 s, 50°C for 45 s and 72°C for 45 s, 30 cycles at 95°C for 40 s, 48°C for 45 s and 72°C for 45 s; final elongation 72°C for 7 m. PCR products were subsequently gel-purified using the Wizard® SV Gel and PCR Clean-Up System (Promega) and Sanger sequenced.

Statistical analyses

Statistical analyses were carried out using the software R version 3.4.3 (R core Team, 2018). Prevalence, intensity and abundance were calculated for each parasite species according to Bush *et al.* (1997), as well as parasite richness.

Normality of data was analyzed with Shapiro test. Proportions were compared using Yates-corrected chi-squared test while the non-parametric Kruskal-Wallis test was used to compare medians. Logistic regression models were developed in those parasite species with prevalence superior to 10% to investigate the relationship between parasite infection, dependent variables with two possible outcomes, adjusting for host characteristics as host species, sex, age category and, also, environmental variables from the municipality where animals were captured, including, human population density (people per km2), altitude, average annual temperature (ºC) and average annual rainfall (mm). Independent variables were categorized prior to inclusion in the model. Parameters were estimated using the maximum likelihood method p-values were calculated with the chi-squared test and significance was considered at the 5% (P<0.05) level for a two-tailed test.

RESULTS

A total of 13 helminth species were detected. Concretely, *Uncinaria stenocephala, Toxocara canis* and *Trichuris vulpis* were found in wolves and foxes, whereas *Ancylostoma caninum* was detected only in wolves, and *Toxascaris leonina* only in foxes. With regard to cestodes, five species were identified in both wolves and foxes, including *Taenia hydatigena*, *Taenia pisiformis, Taenia crassiceps, Mesocestoides spp.* and *Dipylidium caninum*. *Taenia krabbei* was detected only in wolves, while *Taenia polyacantha* and *Taenia taeniaeformis* were found exclusively in foxes. Prevalence, median intensity and median abundance of these helminths are shown in Table 7.

Tabla 7 Gastrointestinal parasites found in foxes and wolves from northwestern Spain.

	Foxes (n=257)			Wolves (n=83)		
	Prevalence %	Median intensity (Range)	Total helminths	Prevalence %	Median intensity (Range)	Total helminths
NEMATODES						
Uncinaria stenocephala	52 (46-58)	8 (1-49)	1784	78 (69-87)	12 (1-686)	2641
Ancylostoma caninum	0	0 (0)	0	5 (0-10)	1.5 (1-7)	11
Toxocara canis	27 (21-32)	2 (1-57)	351	10 (3-16)	4 (1-15)	45
Toxascaris leonina	11 (7-15)	1 (1-4)	41	0	0 (0)	0
Trichuris vulpis	12 (8-16)	1 (1-8)	62	16 (8-24)	1 (1-17)	41
CESTODES						
Taenia hydatigena	0.4 (0-1)	4 (4)	4	43 (33-54)	3 (1-37)	211
Taenia krabbei	0	0 (0)	0	55 (45-66)	6 (1-122)	743
Taenia pisiformis	1 (0-3)	1 (1-3)	5	5 (0-10)	4 (2-7)	17
Taenia polyacantha	26 (20-31)	6 (1-77)	658	0	0 (0)	0
Taenia crassiceps	26 (21-32)	7 (1-212)	1227	1 (0-4)	1 (1)	1
Taenia taeniaeformis	1 (0-3)	3 (1-3)	7	0	0 (0)	0
Mesocestoides spp.	4 (2-6)	6.5 (1-81)	200	1 (0-4)	1 (1)	1
Dipylidium caninum	2 (1-4)	1 (1-2)	8	1 (0-4)	1 (1)	1

The overall prevalence of gastrointestinal parasites in foxes and wolves (n=340) was 81% (95% confidence limits: 76-85) but was higher in wolves, 93% (87-99), than in foxes, 77% (71-82) ($p<0.05$). Overall prevalence of gastrointestinal nematodes and cestodes in both canids were 67% (61-72) and 50% (45-55) respectively, and also was higher in wolves than in foxes ($p<0.05$). Specifically, prevalences of *U. stenocephala*, *A. caninum*, *T. hydatigena* and *T. krabbei* were significantly higher ($p<0.05$) in wolves than in foxes, while prevalences of *T. canis*, *T. leonina*, *T. polyacantha* and *T. crassiceps* were significantly higher ($p<0.05$) in foxes than in wolves.

Regarding parasite richness, 34% (66/197) of foxes were affected by only one parasite species, 36% (72/197) by two helminths and 21% (41/197) by three helminths, while the remaining 9% (18/197) of foxes harbored 4-7 helminths species. By the other hand, 29% of wolves (22/77) were affected by only one parasite species, 22% (17/77) were parasitized by two helminths, 40% (31/77) by three parasites, while the remaining 9% (7/77) were parasitized by four or five helminths.

Gastrointestinal helminth distribution in infected foxes and wolves were widely variable and not normally distributed. The overall median intensity of digestive parasites in wolves and foxes ($n=340$) was 16.5 helminths per infected host, with a range of 1 to687 helminths, and the median abundance was 10 helminths. The overall median intensity of digestive parasites was higher in wolves than in foxes, 29 helminths per infected wolf and 13 helminths per infected fox ($p<0.05$). The overall median intensity of nematodes in wolves and foxes ($n=340$) was 9 nematodes per infected host, ranging from 1 to 687 nematodes, and the median abundance was 3 nematodes. No differences were found in median intensity of nematodes between foxes (9 nematodes per infected host) and wolves (12 nematodes per infected host). On the other hand, overall median intensity of cestodes in both canids was 8.5 cestodes per infected host, with a range of 1 to 275 specimens, while median abundance was 0.5 cestodes. Median intensity of cestodes was significantly higher in wolves (14 cestodes per infected host) than in foxes (7 cestodes per infected host) ($p<0.05$). No differences were found between median intensity of each parasite species and host species.

The bivariate relationship between parasite infection and abundance, and demographic and environmental variables are shown in table 8 for foxes ($n=257$) and table 9 for wolves ($n=83$). Prevalence of *U. stenocephala* was higher in juvenile male foxes, coinciding also with higher median intensity in male foxes. Prevalence of this nematode was also higher in areas of medium human population density, annual temperature and in areas of lowest altitude. Prevalence of both ascarids was higher in male foxes, moreover, higher median intensity of *T. canis* was found these hosts. Prevalence of *T. crassiceps* and *T. polyacantha* in foxes were higher in areas of lowest human population density, annual temperature, and in areas of highest altitude. By the other hand, wolves showed a higher prevalence of *T. vulpis* in areas of highest annual temperature and rainfall. The prevalence of T. krabbei in wolves was higher in areas of highest altitude and in areas of lower population density, annual rainfall and annual temperature, in this areas of lower annual temperature higher median intensity for T. krabbei was also found.

Multivariate relationship between infection and demographic and environmental variables are shown in table 10 and table 11 for foxes (n=257) and wolves (n=83) respectively. Only the helminths which its prevalence exceeded 10% were selected for this study. In this sense, models for *U. stenocephala, T. canis, T. leonina, T. vulpis, T. polyacantha* and *T. crassiceps* were developed for foxes while *U. stenocephala, T. canis, T. vulpis T. hydatigena* and *T. krabbei* were modelled for wolves.

We performed PCR protocol on 94 individual cestodes from 27 wolves. We obtained amplified products in 63 samples (67.0%). The sequences confirmed the presence of two cestode species, *T. hydatigena* and *T. krabbei*: sixteen sequences of 63 (25.4%) were identified as *T. hydatigena*, while the remaining 47 sequences (74.6%) were identified as *T. krabbei*.

Tabla 8 Percentage of positive foxes (n=257) and intensity of each parasite species according to host and environmental features.

Variable	Levels	N	*U. stenocephala*		*T. canis*		*T. leonina*		*T. vulpis*		*T. polyacantha*		*T. crassiceps*	
			% p[a]	Median (Range)	% p	Median (Range)	% p	Median (Range)	% p	Median (Range)	% p	Median (Range)	% p	Median (Range)
Age	Adult	186	47	8 (1-49)	26	2 (1-36)	10	1 (1-3)	9	1 (1-8)	25	5.5 (1-77)	23	5 (1-212)
	Juvenile	71	63*	11 (1-38)	30	2 (1-57)	14	1 (1-4)	18	2 (1-6)	27	7 (1-54)	34	7 (1-97)
Sex	Female	108	43	6 (1-36)	12	1 (1-5)	5	1 (1-2)	13	1 (1-6)	23	7.5 (1-54)	21	5 (1-56)
	Male	149	58*	10 (1-49)*	38*	2 (1-57)*	15*	1 (1-4)	11	2 (1-8)	28	5 (1-77)	30	8 (1-212)
Human population density (people/km2)	3.1-13.3	105	51	9 (1-45)	28	2 (1-57)	11	1 (1-4)	14	2 (1-8)	44*	7 (1-77)	40*	7 (1-212)
	13.4-54.6	71	63*	6 (1-38)	35	2 (1-36)	10	1 (1-3)	14	1 (1-3)	7	10 (2-16)	16	9 (1-92)
	54.7-344	81	42	12.5 (1-49)	19	2 (1-14)	12	1 (1-3)	6	1 (1-5)	17	4 (1-21)	17	2 (1-56)
Altitude (m)	0-316	130	59*	10 (1-49)	32	3 (1-17)	12	1 (1-3)	11	1 (1-5)	10	4 (1-36)	15	13 (1-92)
	317-633	98	48	8 (1-45)	20	7.5 (1-36)	9	1 (1-4)	12	2 (1-8)	37	7 (1-27)	37	7 (1-212)
	634-950	29	31	3 (1-42)	28	4.5 (1-57)	10	1 (1-3)	14	1 (1-3)	55*	5.5 (1-77)	41*	6 (1-32)

Tabla 8 continución Percentage of positive foxes (n=257) and intensity of each parasite species according to host and environmental features.

Variable	Levels	N	*U. stenocephala*		*T. canis*		*T. leonina*		*T. vulpis*		*T. polyacantha*		*T. crassiceps*	
			% p[a]	Median (Range)	% p	Median (Range)	% p	Median (Range)	% p	Median (Range)	% p	Median (Range)	% p	Median (Range)
Annual temperature (°C)	8.89-10.7	44	25	3 (1-42)	18	1.5 (1-57)	7	1 (1-3)	11	1 (1-3)	46*	5 (1-77)	43*	2 (1-32)
	10.8-12.6	111	65*	9.5 (1-45)	31	2 (1-17)	9	1 (1-4)	14	2 (1-8)	27	7 (1-36)	32	9.5 (1-212)
	12.7-14.4	102	49	10.5 (1-49)	27	2 (1-36)	15	1 (1-3)	9	1 (1-5)	15	5 (1-21)	12	11 (1-56)*
Annual rainfall (mm)	893-1270	114	44	6.5 (1-45)	25	2.5 (1-36)	11	1 (1-4)	11	2 (1-5)	40*	6 (1-77)	29	7 (1-97)
	1271-1660	76	59	6 (1-36)	24	1 (1-57)	7	1 (1)	15	1 (1-8)	24	4.5 (1-27)	38*	5 (1-212)
	1661-2040	67	57	13.0 (1-49)	34	2 (1-14)	16	1 (1-3)	10	1 (1-3)	3	9 (1-16)	8	13 (1-50)

* Significant differences between levels of the variable. Asterisk placed in level with highest value.

[a] % p Percentage of positive animals (pos/n).

Tabla 9 Percentage of positive foxes (n=83) and intensity of each parasite species according to host and environmental features.

Variable	Levels	n	*U. stenocephala*		*T. canis*		*T. vulpis*		*T. hydatigena*		*T. krabbei*	
			% p[a]	Median (Range)	% p	Median (Range)	% p	Median (Range)	% p	Median (Range)	% p	Median (Range)
Age	Adult	36	86	14 (1-686)	6	3 (1-5)	19	1 (1-17)	50	4 (1-37)	56	8 (1-122)
	Juvenile	47	72	8 (1-222)	13	4.5 (1-15)	13	1 (1-4)	38	2 (1-17)	55	3.5 (1-113)
Sex	Female	45	84	15.5 (1-222)	13	2 (1-15)	16	2 (1-17)*	42	4 (1-20)	51	7 (1-122)
	Male	38	71	8 (1-686)	5	9.5 (6-13)	16	1 (1)	45	2 (1-37)	61	4 (1-113)
Human population density (people/km^2)	3.1-13.3	23	83	6 (1-126)	9	9 (3-15)	4	1 (1)	52	3.5 (1-20)	83*	7 (1-56)
	13.4-54.6	44	77	18.5 (1-686)*	7	1 (1)	21	1 (1-17)	41	3.5 (1-37)	50	5 (1-122)
	54.7-344	16	75	10 (1-122)	19	6 (5-13)	19	1 (1-2)	38	1 (1-9)	31	2 (1-11)
Altitude (m)	0-316	40	75	15.5 (1-686)	10	5.5 (1-13)	23	1 (1-9)	40	2 (1-13)	38	4 (1-56)
	317-633	37	84	8 (1-211)	11	2 (1-15)	11	1 (1-17)	43	3.5 (1-37)	70	6 (1-122)
	634-950	6	67	21 (6-40)	0	0 (0)	0	0 (0)	67	4.5 (1-14)	83*	9 (1-19)
Annual temperature (°C)	8.89-10.7	26	85	5.5 (1-211)	8	8 (1-15)	8	9 (1-17)	62	4.5 (1-20)	89*	8 (1-122)*
	10.8-12.6	30	87	20 (1-126)	10	1 (1-3)	10	1 (1-9)	37	3 (1-37)	50	2 (1-32)
	12.7-14.4	27	63	21 (3-686)	11	6 (5-13)	30*	1 (1-4)	33	2 (1-6)	30	3.5 (1-36)
Annual rainfall (mm)	893-1270	22	86	11 (1-82)	9	1 (1)	9	1 (1)	64	3.5 (1-14)	68*	6 (1-122)
	1271-1660	48	77	10 (1-211)	6	3 (1-15)	13	2.5 (1-17)	38	2.5 (1-37)	60	6 (1-56)
	1661-2040	13	69	16 (3-686)	23	6 (5-13)	39*	1 (1-2)	31	3 (1-6)	15	6 (2-10)

* Significant differences between levels of the variable. Asterisk placed in level with highest value.

[a] % p Percentage of positive animals (pos/n).

Tabla 10 Estimates of logistic regression models investigating the effect of host dependent variables (sex, age) and environmental variables (altitude, average annual temperature, average annual rainfall and human population density) over the prevalence of *U stenocephala, T canis, T. vulpis, T. leonina, T. polyacantha* and *T. crassiceps* in foxes (n=257).

Variable	Levels	Estimate	Std. Error	p value
1) Model for prevalence of *U. stenocephala*				
Sex	Female			
	Male	0.821	0.278	0.003
Annual temperature	8.89-10.7	0.000		
	10.7-12.6	2.014	0.430	0.000
	12.6-14.4	1.144	0.574	0.046
Annual rainfall	893_1270			
	1271-1660	0.801	0.368	0.029
	1661-2040	0.840	0.441	0.057
2) Model for prevalence of *T. canis*				
Sex	Female			
	Male	1.639	0.356	0.000
Altitude	0-316			
	317-633	-1.563	0.565	0.005
	634-950	-1.513	0.726	0.037
Human population density	03.1-13.3			
	13.4-54.6	-0.677	0.601	0.259
	54.7-344	-1.755	0.614	0.004
3) Model for prevalence of *T. vulpis*				
Age	Adult			
	Juvenile	0.801	0.398	0.044
4) Model for prevalence of *T. leonina*				
Sex	Female			
	Male	1.324	0.511	0.009
5) Model for prevalence of *T. polyacantha*				
Altitude	0-316			
	317-633	0.899	0.398	0.024
	634-950	1.676	0.504	0.000
Annual rainfall	893_1270			
	1271-1660	-0.599	0.345	0.082
	1661-2040	-2.222	0.806	0.005

Tabla 10 continuación Estimates of logistic regression models investigating the effect of host dependent variables (sex, age) and environmental variables (altitude, average annual temperature, average annual rainfall and human population density) over the prevalence of *U stenocephala, T canis, T. vulpis, T. leonina, T. polyacantha* and *T. crassiceps* in foxes (n=257).

Variable	Levels	Estimate	Std. Error	p value
6) Model for prevalence of *T. crassiceps*				
Altitude	0-316			
	317-633	-0.9402	0.715	0.189
	634-950	-4.081	2.032	0.044
Annual temperature	8.89-10.7			
	10.7-12.6	-3.155	1.433	0.027
	12.6-14.4	-1.000	1.004	0.319
Annual rainfall	893_1270			
	1271-1660	1.147	0.466	0.013
	1661-2040	-1.543	0.716	0.031
Human population density	03.1-13.3			
	13.4-54.6	-2.850	0.927	0.002
	54.7-344	-3.660	1.385	0.008

Tabla 11 Estimates of logistic regression models investigating the effect of host dependent variables (sex, age) and environmental variables (altitude, average annual temperature, average annual rainfall and human population density) over the prevalence of *T. vulpis, T. hydatigena and T. krabbei* in wolves (n=83).

Variable	Levels	Estimate	Std. Error	p value
1) Model for prevalence of *T. vulpis*				
Annual rainfall	893_1270			
	1271-1660	0.356	0.860	0.678
	1661-2040	1.832	0.935	0.050
2) Model for prevalence of *T. hydatigena*				
Annual temperature	8.89-10.7			
	10.7-12.6	-1.1595	0.5856	0.047
	12.6-14.4	-0.8140	0.6849	0.234
Annual rainfall	893_1270			
	1271-1660	-1.1671	0.5838	0.045
	1661-2040	-1.2483	0.9373	0.182
3) Model for prevalence of *T. krabbei*				
Annual temperature	8.89-10.7			
	10.7-12.6	-2.0369	0.714	0.004
	12.6-14.4	-2.9019	0.744	0.000

DISCUSSION

Gastrointestinal helminth species found in the present study have been reported previously in other studies of these wild canids from the Iberian Peninsula and other European areas. A total of eleven species of nematodes and cestodes were recovered from foxes in this study. This result was similar to records in other European studies (Gortázar *et al.*, 1998; Eira *et al.,* 2006; Saeed *et al.,* 2006; Martínez-Carrasco *et al.*, 2007; Vergles-Rataj *et al.,* 2013; Fiocchi *et al.,* 2016). Many of them include the same species recovered in the present study in addition to other helminths as *Pterygodermatites affinis* or *Joyeuxiella paqualei*, which are frequent in semiarid areas (Martínez-Carrasco *et al.*, 2007). By the other hand, a total of ten species of nematodes and cestodes were identified in wolves of the present study. The number of species found is consistent with other European studies (Torres *et al.,* 2000; Segovia *et al.,* 2001, 2003; Moks *et al.,* 2006; Bagrade *et al.,* 2009), However, the absence of trematodes and acanthocephalans in the present study is significant as some species have been reported in many of the abovementioned surveys. *Alaria alata* or *Pseudamphistomum truncatum* are some of the trematodes previously described, although usually with very low prevalence (Shimalov and Shimalov, 2000; Segovia *et al.*, 2001; Segovia *et al.*, 2004; Saeed *et al.*, 2006). The absence of trematodes in our study could be due to the aquatic way of living of the intermediate hosts of these helminths (fish and amphibians), which are only used as an alternative food source by foxes and wolves (Segovia *et al.,* 2004). Similarly, the absence of Acanthocephalans, as *Macracanthorynchus catulinus* (Gortázar *et al.,* 1998; Martínez-Carrasco *et al.,* 2007) suggests that their intermediary (arthropods, e.g. beetles) or paratenic (reptiles and amphibians) hosts (Kennedy, 2006) are not part of these wild carnivores' diet in the study area.

Uncinaria stenocephala was the most prevalent helminth in our study, both in foxes (52%) and wolves (78%). This result is consistent with previous studies carried out in the Iberian Peninsula, although prevalence of this parasite varies considerably in foxes. In this sense, our result in foxes was similar to the one recorded by Criado-Fornelio *et al.* (2000) in central Spain, but lower than values showed in Segovia *et al.* (2004) in several provinces of Spain, central Portugal and Andorra, and Eira *et al.* (2006) in central Portugal. However, it is higher than results obtained by Gortázar *et al.* (1998) in northeastern Spain, or Martínez-Carrasco *et al.* (2007) in southeastern Spain. In other European studies, *U. stenocephala* is also frequentin foxes, and prevalence varies considerably between study areas. In this sense, similar prevalences than in our study can be found in Great Britain, Italy and Slovenia (Smith *et al.,* 2003; Di Cerbo *et al.,* 2008; Vergles-Rataj *et al.,* 2013) or even higher in Denmark, Switzerland and Italy (Saeed *et al.,* 2006; Reperant *et al.,* 2007; Fiocchi *et al.,* 2016). By the other hand, the prevalence of

U. stenocephala in wolves from northwest of Spain was higher than previous records from Iberian Peninsula (Torres *et al.*, 2000; Segovia *et al.,* 2001; 2003). In other European countries, prevalence of this nematode varied widely, but remarkably lower prevalence than in our study can be found in Belarus and Latvia (Shimalov and Shimalov, 2000; Bagrade *et al.,* 2009), as well as similar records in Estonia and Italy (Mosk *et al.,* 2006; Fiocchi *et al.,* 2016), or even higher by Al-Sabi *et al.* (2018) in Denmark.

Canid hosts get infected by the ingestion of L3 of *U. stenocephala* present in environment (Anderson, 2000; Otranto *et al.*, 2015). Therefore, environmental parameters are critical for survival of infective larvae: humid climates or the presence of irrigated areas favors the survival of the parasite (Gortázar *et al.*, 1998; Criado-Fornelio *et al.*, 2000; Fiocchi *et al.*, 2016), while its prevalence is low in semi-arid climates (Martínez-Carrasco *et al.*, 2007). Also, soil moisture and humidity are thought to be important for the successful development of these geohelminths, and vegetation may be a useful proxy for soil moisture since a large amount of vegetation tends to prevent evaporation and conserve soil moisture (Brooker and Michael, 2000). The climate in our study area gather these conditions, and favors the high prevalence of *U. stenocephala* in foxes and wolves from our study area. In our study, prevalence of *U. stenocephala* was higher in low and medium altitudes. According to Brooker and Michael (2000) altitude is usually a limiting factor in the distribution of geohelminths, since at higher altitudes temperature, humidity conditions and soil types hinder the survival of infective forms. the distribution of *Uncinaria* in our study meets areas where environmental conditions are milder, and favors the presence of crop areas and vegetation cover which prevents freezing or desiccation of the infective forms. Also, higher prevalence of *U. stenocephala* was described in areas of medium density of human population, probably due to the coincidence of these areas with semi-urban, rural environments or abandoned agricultural areas, which favor the expansion of these wild canids. In the present study, prevalence of this nematode was higher in wolves than in foxes, probably as consequence of the direct transmission of this nematode, therefore, the infection would be favored in wolves as they live in herds composed of several individuals. Similar to our results, Stuart *et al.* (2013), in a study with foxes from Ireland, found higher prevalence of *U. stenocephala* in young animals than in adults. In fact, young foxes are exposed to *U. stenocephala* when they explore the terrain surrounding the burrow transited by adults (Richards *et al.*, 1995; Stuart *et al.*, 2013). Moreover, when foxes reach adulthood, they have acquired immunity which protects them from reinfection (Stuart *et al.*, 2013). On the other hand, we found higher prevalence of *U. stenocephala* in male foxes than in females. This is consistent with the results reported by Eira *et al.* (2006), who associated these discrepancies to differences in behavior of male foxes during the reproduction season, when they are more exposed to parasites due to their dispersion movements. Also, males have greater interaction with contaminated feces as they take an important role in territory marking. This interaction may be linked to coprophagy in wolves, coyotes or dogs, and becomes a risk factor for transmission of parasites between canids (Boze, 2008). On the other hand, females consume the feces of the offspring to keep the nest clean and undetectable by predators (Houpt, 1982; Boze, 2008). Hart *et al.* (2018) hypothesized that coprophagy in dogs could be linked to an ancestral behavior inherited from wolves to keep the den resting area free of accumulating feces. It has been described fecal coprophagy between different animal species, which could increase the risk for transmission of parasites between dogs and wild canids (Boze, 2008). In this sense, it must be emphatized the risk of parasite flow between wild and domestic fauna in anthropized environments (Thomson, 2013), which is enhanced by the increasing expansion of both wild canids.

We found *A. caninum* in wolves (5%) but not in foxes, as happened in the abovementioned studies in foxes from Iberian Peninsula, except Segovia *et al.* (2004), in the same terms, in other European countries *A. caninum* is described punctually in foxes and with low prevalence (Saeed *et al.,* 2006; Fiocchi *et al.,* 2016). In general, the prevalence of this parasite in wolves from the Iberian Peninsula and other European countries are low (Shimalov and Shimalov, 2000; Torres *et al.,* 2000; Segovia *et al.,* 2001, 2003; Bagrade *et al.,* 2009).

Our results showed a significantly lower prevalence of *A. caninum* than of *U. stenocephala*. Segovia *et al.* (2003) suggested that this difference in wolves and the absence of *A. caninum* in foxes could be related to differences in the host´s behavior, concretely, transmission of *A. caninum* would be favored in canids which live in large groups. However, Spanish wolves usually gather in packs of fewer than 10 individuals because they are forced to consume quite small preys (Blanco *et al.* 1992). The small size of packs due to forced diet adaptations may have hindered the development of the biological cycle of *A. caninum*, while favoring the prevalence of *U. stenocephala*, whose larvae and eggs are more resistant in the external environment.

Ancylostoma caninum is a well-known zoonotic agent; the migration of *A. caninum* larvae through the skin of human hosts may cause cutaneous larva migrans (CLM). Cases of CLM have been reported in several European countries during the last decade while the role of *U. stenocephala* in CLM remains unclear (Bowman *et al.*, 2010; Otranto *et al.*, 2015). In this sense, one case in the UK was attributed to *U. stenocephala* due to the absence of autochthonous *A. caninum* infections (Beattie and Fleming, 2002; Otranto *et al.*, 2015). The higher risk of infection with these nematodes should be taken into account in view of the approximation of wolves and foxes to humanized areas, where both dogs and human could get infected with faeces of wild canids.

We found two ascarid species, *Toxocara canis* and *Toxascaris leonina*, although the second one was only detected in foxes. Prevalence of *T. canis* in this study (27%) was higher than prevalence of *T. leonina* (11%) in foxes, as reported Segovia *et al.* (2004), Martínez-Carrasco *et al.* (2007) or Figueiredo *et al.* (2017). However, Gortázar *et al.* (1998) and Criado-Fornelio *et al.* (2000) described the opposite. Prevalence of *T. canis* in foxes from our study was lower than the one in other European studies (Smith *et al.,* 2003; Saeed *et al.,* 2006; Reperant *et al.,* 2007; Di Cerbo *et al.,* 2008; Vergles-Rataj *et al.,* 2013; Fiocchi *et al.,* 2016); however, prevalence of *T. leonina* were lower than the present study. By the other hand, prevalence of *T. canis* was low in wolves from the present study (10%) and only slightly higher than results reported in Torres *et al.* (2000) and Segovia *et al.* (2001; 2003). Also, these studies showed a low prevalence of *T. leonina.* In other surveys with wolves from European countries, reported prevalences of *T. canis* are similar to our results (Moks *et al.,* 2006; Bagrade *et al.,* 2009; Ćirović *et al.,* 2015), with the exception of Shimalov and Shimalov (2000). In contrast to our results in wolves, Shimalov and Shimalov (2000), Kloch *et al.* (2005) and Moks *et al.* (2006) found *T. leonina*.

Animals’ behavior such as territory marking or coprophagy must suppose a risk of transmission of *T. canis* and *T. leonina* between canids, although the vertical transmission of *T. canis* is probably the most important infection route. In this sense, Szabova *et al.* (2007) described higher prevalence of *T. canis* in dog pups up to 6 months (53.2%) while it was significantly lower (18.8%) in dogs over 1 year of age. The transmission of *T. canis* larvae through the placenta leads to a higher prevalence of fox cubs infection in spring and summer, while values decrease in winter as foxes grow (Saeed and Kapel, 2006; Okulewicz *et al.*, 2012). The lower prevalence of *T. leonina* when compared with *T. canis* could be derived from the horizontal transmission as the only option for this parasite. Studies of soil contamination with ascarid eggs (Ruiz de Ybáñez *et al.,* 2001; Aydenizöz-Ozkayhan, 2006), showed that *Toxascaris leonina* eggs were less frequent than *Toxocara* spp*.* ones, so infection with this helminth is less common. Soil contamination with *Toxocara* eggs was higher in urban than in rural or peri-urban areas (Reperant *et al.*, 2007). Although this fact used to be associated with the higher density of domestic carnivores, particularly in parks and green areas, the current dispersion of foxes towards humanized areas, and the high prevalence rates of *T. canis* found in this host cannot be discarded as a risk factor for infection with ascarids (Reperant *et al.*, 2007). We found higher prevalence of *T. canis* and *T. leonina* in male foxes, consistently with Segovia *et al.* (2004) as male foxes could be more exposed to the parasite due to the greater size of their home range (Torres *et al.*, 2006). The presence of *T. canis* in the study area could be also linked to the important presence of rodents in fox’ diet in north Spain (Díaz-Ruíz *et al.*, 2013), which can act as paratenic hosts (Strube *et al.,* 2013). Our results suggest that the role of foxes in maintaining the sylvatic cycles of *T. canis* is more significant than the role played by wolves. For this reason, the increasing expansion of the fox, which favors its presence in anthropized areas, must be taken into account as a source of transmission of this parasite, also able to infect both human (larva migrans) and domestic dogs (Overgaaw and Knapen, 2013; Morgan *et al.,* 2013; Otranto *et al.,* 2015).

We found a low prevalence of *Trichuris vulpis* in foxes from our study area (12%), according to Gortázar *et al.* (1998), Segovia *et al.* (2004), Eira *et al.* (2006), Martínez-Carrasco *et al.* (2007) and Figueiredo *et al.* (2017), but not with the high prevalence reported by Criado-Fornelio *et al.* (2000) (38.8%). Prevalence of this helminth in foxes from other European countries showed lower values than ours (Smith *et al.,* 2003; Saeed *et al.,* 2006; Di Cerbo *et al.,* 2008; Vergles-Rataj *et al.,* 2013). Similarly, the presence of *T. vulpis* in Iberian wolves was also scarce, with prevalence slightly lower (10%) than ours (16%)in Spain (Torres *et al.*, 2000), Segovia *et al.,*2001; 2003) and Belarus (Shimalov and Shimalov, 2000) but lower than results found by Kloch *et al.* (2005) in Poland. The relatively constant occurrence of *T. vulpis* throughout different areas of the Iberian Peninsula may be due to the high resistance of its eggs in the environment, which remain viables and infective for years, either in humid environments or in semiarid habitats Traversa (2011). The zoonotic potential of *T. vulpis* is being debated. There are registered cases of *T. vulpis* visceral larva migrans syndrome and patent intestinal infections in humans, but further definitive evidence is needed to conclude that *T. vulpis* is a zoonotic worm (Traversa, 2011).

The cestode *T. hydatigena* has been described in foxes from the Iberian Peninsula only once and showing a low prevalence (2%) (Segovia *et al.,* 2004), as happened in our study (0.4%) and in other European studies (Saeed *et al.,* 2006; Di Cerbo *et al.,* 2008), where the presence of *T. hydatigena* in foxes was only testimonial. However, *T. hydatigena* is one of the most prevalent taenid species described in wolves from Iberian Peninsula. In our study, prevalence of this cestode was high (43%), as described by Segovia *et al.* (2001), but even less frequent than Segovia *et al.* (2003) and Torres *et al.* (2000). However, in other European studies, prevalence of this cestode showed lower values (Shimalov and Shimalov, 2000; Moks *et al.,* 2006; Ćirović *et al.,* 2015; Al-Sabi *et al.,* 2018), except forBagrade *et al.* (2009), who found a similar prevalence.

Our results are consistent with the feeding habits of wolves in northwestern Spain, which include goats, wild boars, roe deer, and mainly young horses as preys (Llaneza *et al.*, 1996; Segovia *et al.*, 2001), species known to be intermediate hosts of *T. hydatigena* (Cordero del Campillo *et al.*, 1994; Segovia *et al.*, 2001). The low prevalence of *T. hydatigena* in foxes could be due to consumption of carrion from ungulates since foxes do not use to prey actively on them. Although there are references about foxes hunting young roe deer o chamois they have not been able to develop the larval stage of the parasite yet, and, hence, they are not an appropriate source of infection for canid hosts.

The implementation of the PCR technique enabled us to confirm the presence of *T. krabbei* in the wolves sampled in the present study. The morphometric identification of this cestode is very difficult because it is nearly indistinguishable from *T. ovis* (Lavikainen *et al.*, 2008) or even from *T. multiceps*. So, it is necessary to have detailed and updated taxonomic keys when molecular biology techniques cannot be accessed. The high prevalence of *T. krabbei* (55%) contrast with previous literature to the point that this is the first report of this cestode in the Iberian Peninsula. It has been recently reported in other European countries, usually with lower prevalences than in our study (Gori *et al.,* 2015; Poglayen *et al.,* 2017; Al-Sabi *et al.,* 2018), with the exception of Lesniak *et al.* (2017), who found a prevalence even higher than ours. *Taenia multiceps* is frequent in wolves from the Iberian Peninsula, whose prevalence range from 28% to 40% (Torres *et al.*, 2000; Segovia *et al.*, 2001; 2003), however we could not confirm its presence in our study, neither by PCR nor morphometrically. Previous studies identified *T. multiceps* only by morphometricaly, and in the author's opinion, molecular analyses are necessary to achieve a correct differentiation between *T. multiceps* and *T. krabbei* due to its morphological similarity.

According to Formenti *et al.* (2018), wild cervids are the main intermediate host for *T. krabbei,* while cattle, goats, sheep and pigs are refractory to that parasite (Al-Sabi *et al.*, 2013; Lavikainen *et al.*, 2013). This confirms the preference of wolves to prey on wild ungulates in our study area, probably due to the low density of domestic ungulates, as was remarked in Llaneza *et al.* (1996). Moreover, cysticerci of *T. krabbei* are located in the skeletal muscle (Al-Sabi *et al.* 2013), and so they are easily ingested when the wolf feeds. According to Stahler *et al.* (2006), wolves initially consume internal organs of thoracic and abdominal cavities and subsequently large muscular masses, but they left parts difficult to consume, as skull, bones and hide. Zimmermann *et al.* (2015) affirmed that small packs of wolves in areas where preys are abundant tend to hunt more preys and consume only the most nutritious parts of the food patch since finding a new food patch requires a relatively low energy cost. Partial prey consumption has been suggested as an optimal foraging strategy for wolves on Isle Royale, in the United States (Vucetich *et al.*, 2012; Zimmermann *et al.,* 2015). As optimal foragers and risk minimizers, wolves may reduce their risk of detection by humans by decreasing the time spent at new kills after devouring the most nutritious parts of a carcass (Stahler *et al.,* 2006; Zimmermann *et al.*, 2015). So, this foraging strategy could favor the transmission of *T. krabbei* instead of *T. multiceps*, since its coenuri are located in the brain of the intermediary hosts, which has a difficult access to wolves. Prevalence of *T. krabbei* in our study was higher in areas of medium and high altitude, with low annual temperature and reduced human population density. These areas favor the presence of wild ungulates (intermediate hosts) far from human populations. In this sense, roe deer is one of the most frequent prey of wolves in northwestern Spain (Llaneza *et al.,* 2000; Barja, 2009) and the presence of this ungulate is associated to forest areas (Mateos-Quesada, 2017) with low density of human population (Hewison *et al.,* 2001; Acevedo *et al.*, 2005).

We found *T. crassiceps* and *T. polyacantha*, both frequently described in foxes, with prevalences of 26%. Other reports of these parasites in the Iberian Peninsula are scarce; Segovia *et al.* (2004) found both cestode species and Gortázar *et al.* (1998) described only *T. polyacantha* while in other European studies both cestodes have been reported (Di Cerbo *et al.,* 2008; Vergles-Rataj *et al.,* 2013; Fiocchi *et al.,* 2016). These cestodes have been punctually described in Iberian wolves, as Guerra *et al.* (2013), who only found *T. polyacantha*. However, in other European studies, both taenids have previously been described (Shimalov and Shimalov, 2000; Bagrade *et al.,* 2009).

The larval stage of *T. crassiceps* and *T. polyacantha* is developed in different rodents, mainly microtines (Barabási *et al.* 2010). The prevalence of both cestodes described in our study confirms that rodents are the predominant components of foxes' diet in northwestern Spain, which contrasts with the lower prevalences in the abovementioned studies from Iberian Peninsula, probably due to differences in food availability since foxes from Mediterranean areas of Iberian Peninsula, preferably, feed on other food sources as lagomorphs (Díaz-Ruíz *et al.*, 2013). In wolves from our study area, the prevalence of *T. crassiceps* was very low in comparison to the high prevalence of *T. hydatigena* and *T. krabbei*, indicating the clear preference of wolves for preying on big ungulates. *Taenia crassiceps* was detected only in one juvenile wolf, probably as a consequence of the preference of adult wolves for preying on big mammals, while juvenile wolves are simply scavengers or free riders within the social predator group, as observed in other carnivore species (Scheel and Packer, 1991; Carbone *et al.,* 1997; Zimmermann *et al.*, 2015). We found higher prevalences of *T. polyacantha* and *T. crassiceps* in areas of high altitude, low and medium annual temperature and annual rainfall. These are the climatic conditions that favor the presence of forest areas, which provide refuge to highly common rodents in northwestern Spain (Palomo *et al.,* 2007). These kind of areas are located far from the main human settlements, coinciding with the greater prevalence of both cestodes recovered in areas of lower human density in this study.

Taenia crassiceps infection in humans is extremely rare, with only 10 confirmed cases of cysticercosis, principally subcutaneous and intraocular, and associated to immunocompromised patients (Lescano and Zunt, 2013). However, foxes increasingly approaching peri-urban areas should be considered as a potential risk to domestic animals and humans.

Taenia pisiformis has been previously described in foxes and wolves in Iberian Peninsula usually showing low prevalence, as happened in the present study (1% and 5% respectively). In foxes, slightly higher prevalences than in our study have been reported (Gortázar *et al.* 1998; Criado-Fornelio *et al.,* 2000; Segovia *et al.,* 2004; Eira *et al.,* 2006; Martínez-Carrasco *et al.,* 2007; Guerra *et al.,* 2013). However, lower prevalences have also been reported (Smith *et al.,* 2003; Saeed *et al.,* 2006; Vergles-Rataj *et al.,* 2013; Fiocchi *et al.,* 2016). In wolves, Segovia *et al.* (2003) found a similar prevalence of *T. pisiformis*, but records in Torres *et al.* (2000) were higher. Prevalences in other European studies are lower than 10% (Shimalov and Shimalov, 2000; Ćirović *et al.,* 2015), with an exception in Bagrade *et al.* (2009), who found a significantly higher prevalence (20%). Lagomorphs (rabbits, hares) host the larval forms of this tapeworm, mainly in the liver and the peritoneal cavity (Fiocchi *et al.*, 2016). European rabbit is present in practically the whole Iberian Peninsula but it is uncommon in northern Spain (Villafuerte, 2002; 2007). For this reason, the broom hare (*Lepus castroviejoi*), found in our area of study, should be involved in the cycle of *T. pisiformis*. However, the low prevalence of this cestode suggest a minor role of this lagomorphs in the diet of both foxes and wolves.

Mesocestoides have been reported in foxes from the Iberian Peninsula with significantly higher prevalences than in our study (4%) (Gortázar *et al.* 1998; Segovia *et al.,* 2004; Eira *et al.,* 2006; Martínez-Carrasco *et al.*, 2007). Only Criado-Fornelio *et al.* (2000) described a lower prevalence of this cestode than the one in this study. This tendency is confirmed in other European studies, where prevalences of *Mesocestoides spp.* are significantly higher (Saeed *et al.,* 2006; Di Cerbo *et al.,* 2008; Vergles-Rataj *et al.,* 2013; Fiocchi *et al.,* 2016). In wolves, prevalences were slightly higher in other studies from the Iberian Peninsula (Torres *et al.,* 2000; Segovia *et al.,* 2001; 2003) and other European countries (Shimalov and Shimalov, 2000; Moks *et al.,* 2006; Bagrade *et al.,* 2009; Al-sabi *et al.,* 2018) than in our results (1%). Transmission of *Mesocestoides spp.* requires different kinds of vertebrates, including amphibians, reptiles, birds and mammals (e.g. rodents) as second intermediate hosts (Fiocchi *et al.*, 2016). According to Martínez-Carrasco *et al.* (2007), *Mesocestoides* spp. are usually associated to semiarid areas, where foxes' diet probably includes reptiles, birds and, to a lesser extent, rodents (since *T. polyacantha* was not recovered in their study). However, in northwestern Spain the scenery seems to be the opposite, and rodents could be a significantly greater proportion of fox's diet, instead of reptile and birds. On the other hand, *Mesocestoides spp.* was recovered from one juvenile wolf, suggesting that juvenile wolves are not experienced hunters of large preys yet and they complete their diet with preys easily to hunt (Zimmermann *et al.*, 2015).

We found the cestode *D. caninum* in our foxes and wolves with very low prevalence (2% and 1% respectively). According to literature, the presence of this parasite is sporadic, and prevalence is low in foxes (Gortázar *et al.,* 1998; Martínez-Carrasco *et al.,* 2007) and wolves (Torres *et al.,* 2000; Segovia *et al.,* 2001; 2003). Similarly, in other European countries the prevalence of this cestode in foxes was low (Smith *et al.,* 2003; Reperant *et al.,* 2007; Vergles-Rataj *et al.,* 2013; Fiocchi *et al.,* 2016) likewise in wolves (Shimalov and Shimalov, 2000; Kloch *et al.,* 2005). The life cycle of *D. caninum* requires an arthropod (fleas and lice) as intermediate host (Jiang *et al.*, 2017). The prevalence of *D. caninum* is usually low in wildlife, while in domestic animals a high prevalence of this cestode has been described (Benito *et al.,* 2003; Xhaxhiu *et al.,* 2010; Kmpel *et al.,* 2010), since domestic animals typically inhabit urban or semi-urban environments where high densities of definite hosts can be reached and, simultaneously, they are less exposed to adverse environmental conditions, which favors the presence and transmission of the intermediate hosts and the helminth. On the contrary, the population density of wild canids is usually lower, which difficult the contact with intermediate hosts. In addition, eggs of *D. caninum* are more exposed to adverse environmental conditions in these rural areas, hindering the infection with this cestode. The low infection in wildlife could also be due to the use of dens by wild canids, as dens are the places that would allow the concentration of fleas and their larvae and, therefore, transmission of this cestode to intermediary and final host. In this sense both foxes and wolves stay in well protected burrows during the breeding season but the rest of the cycle they stay in places that provide refuge for a short time (Theuerkauf *et al.,* 2003; Carter *et al.,* 2012; Bassi *et al.,* 2015), therefore, these wild canids would be less exposed to fleas that harbor the parasite when they are not in breeding season. Although *D. caninum* is recognized as zoonotic, the risk of human infection is low, mainly affecting children due to their playing habits and proximity to pets (Jiang *et al.*, 2017). Human cases are often asymptomatic but sings as anal pruritus, diarrhea, mild abdominal pain or decrease in appetite may be seen (Ramana *et al.*, 2011; García-Agudo *et al.*, 2014; Jiang *et al.*, 2017).

We did not find *Echinococcus* spp. in our foxes and wolves. The main species described in Europe, *E. granulosus* and *E. multilocularis*, are well-known zoonotic agents. Patent *E. granulosus* infections have been detected in wolves in Italy (Guberti *et al.*, 2004; Gori *et al.*, 2015) and in four wolves (15% prevalence) in Spain (Sobrino *et al.*, 2006). In addition, this cestode have been found in foxes from Spain, although with a very low prevalence (0.2%) (Segovia *et al.,* 2004). By the other hand, *E. multilocularis* has been described in several countries of Central Europe and it is expanding across Europe with the growing red fox populations, favoring the progressive urbanisation of this parasite life cycle (Liccioli *et al.*, 2015; Otranto *et al.*, 2015).

Cestodes are transmitted in predator-prey cycles. Some of the abovementioned species are linked to a specific group of prey species, for example *T. krabbei* to cervids or *T. crassiceps* and *T. polyacantha* to rodents. Trophically-transmitted parasites that use phylogenetically distant animals as intermediate and definitive hosts are well known to have a crucial importance in food web connectance, as well as for host population and community ecology (Lafferty *et al.,* 2006; Thieltges *et al.*, 2013; Stephens *et al.,* 2019). Information obtained in this study should be consideredas a source of information to interpret trophic interactions that occur in ecosystems. As can be inferred from these results, the role played by foxes in sustaining the biological cycles of *T. crassiceps* and *T. polyacantha* in the environment is more important than the one of wolves, while concerning *T. hydatigena* and *T. krabbei* wolves are the key part in the maintenance of their sylvatic cycles.

The present study demonstrated that, despite foxes and wolves are theoretically susceptible to same gastrointestinal helminths, the ecological particularities, mainly trophic habits, of these canids favour the presence of a well-differentiated gastrointestinal helminthfauna and, consequently, they play a different epidemiological role in the nidality of these helminths.

Wolf and fox populations are currently expanding, and both canines are easy to find in anthropized environments. Several helminths described in this study, as *A. caninum*, *T. canis* or *T. crassiceps*, have the potential to be transmitted between wild and domestic canids, livestock and even humans. Therefore, it is highly important to become aware of the health status of wild canine populations, especially foxes, since they settle very close to human areas.

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DISCUSIÓN GLOBAL

Según Stephens *et al.* (2019), las interacciones ecológicas, como por ejemplo la competencia, el mutualismo y la depredación, también pueden crear oportunidades para compartir parásitos. Las interacciones entre predadores y presas se encuentran entre las interacciones ecológicas directas mejor caracterizadas, y pueden proporcionar importantes vías para que los parásitos invadan nuevas especies hospedadoras, como por ejemplo a través del contacto directo con el pelo, la piel y los fluidos corporales, así como a través de la ingestión de los propios parásitos. Algunas de las interacciones depredador-presa mejor estudiadas que ocurren entre mamíferos son entre carnívoros (orden: Carnivora) y ungulados. Como consecuencia, los parásitos encontrados en algunas especies de carnívoros pueden haber sido transmitidos por la ingestión de ungulados.

Para los parásitos de transmisión trófica, la predación y la transmisión están intrínsecamente unidas, ya que dependen de la predación de sus hospedadores intermediarios para infectar a los hospedadores definitivos y explotar así los vínculos predador-presa (Marcogliese y Cone, 1997; Lafferty, 1999; Thieltges *et al.*, 2013). Los predadores generalistas podrían infectarse con más especies de parásitos de transmisión trófica en comparación con depredadores especializados (Chen *et al.* 2008, Rossiter y Sukhdeo 2011).

A lo largo de los tres capítulos que componen este trabajo se han descrito en los tres carnívoros hospedadores numerosas especies de parásitos cuya transmisión está directamente vinculada a la interacción entre estos predadores y sus presas, pudiendo estas actuar como hospedadores intermediarios imprescindibles para el desarrollo de las fases larvarias de los parásitos (por ejemplo, los cestodos que han sido descritos en lobo y zorro en el Capítulo 3 o los nematodos del género *Angiostrongylus* descritos en los tres hospedadores en los Capítulos 1 y 2) o como hospedadores paraténicos que acumulan fases infectivas en sus tejidos sin que en ellos exista maduración del parásito (por ejemplo, los nematodos *A. putorii* descrito en el Capítulo 1 o *T. canis* descrito en el Capítulo 3). La amplia variedad de especies parasitarias de transmisión trófica descritas implica también diversidad en los hospedadores intermediarios y paraténicos que las albergan. En este sentido, se han descrito parásitos transmitidos por el consumo de invertebrados como gasterópodos (nematodos de los géneros *Angiostrongylus* y *Crenosoma*, en los Capítulos 1 y 2) y lombrices terrestres (nematodos *A. putorii* y *E. aerophilus*, citados en los Capítulos 1 y 2), pero también helmintos en cuyos ciclos intervienen vertebrados como los roedores (cestodos *T. crassiceps* y *T. polyacantha*, mencionados en el Capítulo 3) y los ungulados (cestodos *T. hydatigena* y *T. krabbei*, citados en el Capítulo 3). La presencia de estos parásitos aporta información muy valiosa sobre la ecología trófica de estos tres carnívoros, y demuestra la capacidad que poseen estas especies de adaptar su dieta en función de las necesidades o los recursos disponibles (Friesen y Roth, 2016). Por ejemplo, los resultados de los Capítulos 2 y 3 han demostrado que el zorro es una especie marcadamente generalista con una dieta diversa, en la que tanto los invertebrados como los roedores y otros pequeños vertebrados son parte fundamental. Sin embargo, en los resultados obtenidos en los lobos en el Capítulos 3 se aprecia un marcado contraste, ya que se ha demostrado una clara predilección por la predación sobre ungulados en lugar de pequeños vertebrados; incluso en el Capítulo 2 se ha demostrado la diferencia con los zorros, ya que el lobo recurre en menor medida al consumo de invertebrados pese a que, en vista de los resultados, no se debe menospreciar su importancia. De hecho, el consumo de presas alternativas más pequeñas puede beneficiar a los lobos más allá de ser una fuente de nutrición cuando las presas más grandes son escasas. El consumo de estas presas alternativas se considera que, aunque no son recursos habituales en una especie de carnívoro concreta, forman parte de la dieta cuando no se dispone de las presas principales; este cambio de dieta, que puede ser pasajero o permanente, puede afectar sustancialmente a la dinámica de la población de predadores y de las presas pero, además, influye sobre los parásitos que afectan a ambos tipos de hospedadores, el cazador y la presa (Roth *et al.* 2007; Adams *et al.* 2010; Latham *et al.* 2011; Friesen y Roth, 2016).

En el Capítulo 1 de esta Tesis Doctoral se estudió la helmintofauna del visón americano en Galicia. Gracias a este trabajo se pudo contribuir a un mayor conocimiento sobre el papel epidemiológico que este mustélido exótico desempeña en los ecosistemas del noroeste de España. Los resultados demostraron que el visón americano actua como hospedador de varias especies de helmintos propias de otros mustélidos autóctonos, como el visón europeo o el tejón; es el caso de *M. patens*, *A. putorii* y *C. melesi*. Por otra parte, nuestro estudio ha permitido identificar, por primera vez en el visón americano, el nematodo *A. daskalovi*. Además, se demostró la capacidad de adaptación del nematodo *A. annulosa* a un hospedador totalmente distinto de los habituales (roedores). El hallazgo del trematodo *T. acutum* tuvo gran relevancia ya que se comprobó que el visón americano interviene activamente en el mantenimiento del ciclo biológico de este parásito, que posee un marcado potencial patógeno en los hospedadores; además, fue hallado en una ubicación ectópica, diferente a la habitual, por lo que las consecuencias patológicas podrían resultar más graves de las que ya se conocían.

La introducción y propagación de especies no autóctonas amenaza la biodiversidad nativa y, además, la función de los ecosistemas y de las comunidades que los habitan (Jenkins, 2003; Clavero y García-Bertou, 2005; Kelly *et al.*, 2009). Tradicionalmente se ha considerado que el impacto de las especies exóticas invasoras sobre las autóctonas se debe principalmente a la predación y la competencia (D'Antonio y Kark 2002, Sax *et al.*, 2002; Kelly *et al.*, 2009), pero ahora también existen pruebas concluyentes de que las especies invasoras pueden ser una pieza clave en la transmisión de enfermedades (Daszak *et al.*, 2000; Cleaveland *et al.*, 2002; Ladeau *et al.* 2007; Kelly *et al.*, 2009). Junto con un hospedador exótico puede producirse la introducción de agentes infecciosos. Estos agentes pueden afectar a las comunidades nativas, especialmente cuando los hospedadores introducidos actúan como reservorios desde los cuales se puede producir el fenómeno del "spillover" hacia las especies nativas. Además, los hospedadores exóticos también pueden intervenir en los ciclos biológicos de agentes infecciosos nativos, pudiendo ser transmisores de estos patógenos hacia hospedadores autóctonos, produciéndose el fenómeno denominado "spillback" (Daszak *et al.*, 2000; Tompkins y Poulin, 2006; Kelly *et al.*, 2009). Así, según Torchin *et al.* (2003), cuando se introduce un hospedador exótico tiende a perder, al menos, parte de sus parásitos naturales, pero pueden adquirir parásitos generalistas de la fauna local (Poulin y Mouillot 2003). En el caso de los visones americanos incluidos en este estudio, no se encontró la presencia de ningún agente parasitario exótico introducido en los ecosistemas del noroeste de España junto a este mustélido, pero sí se pudo confirmar la pérdida de muchas especies de parásitos descritas en su área originaria de distribución, así como la adaptación a helmintos generalistas propios de otras especies de mustélidos autóctonos de la península ibérica.

Por tanto, los resultados obtenidos en el Capítulo 1 demuestran que el visón americano es una especie invasora que puede adquirir parásitos de especies autóctonas con las que comparte hábitat y, por lo tanto, puede lograr un papel epidemiológico relevante en el anidamiento natural de estos parásitos. Esto supone un riesgo sanitario para otras especies de mustélidos que habitan en la misma zona, lo que debería ser tenido en cuenta a la hora de controlar las poblaciones de visón americano, sobre todo en áreas de simpatría con otros mustélidos autóctonos.

La mayoría de los parásitos identificados en este estudio presentan el potencial de transmitirse a distintos hábitats, mostrando una considerable ubicuidad. Esta característica está directamente condicionada por la propia plasticidad de las especies hospedadoras que los albergan. En este sentido, puede implicar el tránsito de estos agentes desde ambientes silvestres hasta áreas antropizadas, como zonas rurales con tierras de cultivo abandonadas, ambientes semiurbanos (Recio *et al.*, 2015; Martínez-Abraín *et al.*, 2019) o incluso núcleos urbanos, aunque de momento no se ha demostrado la presencia de poblaciones perfectamente establecidas de estos carnívoros en núcleos poblacionales españoles (Segovia *et al.*, 2004), a diferencia de lo que se ha descrito en otros países europeos como Suiza (Reperant *et al.*, 2007) o Bélgica (Brochier *et al.*, 2007).

El flujo de estos parásitos entre lobos y zorros es muy posible debido a que son dos cánidos que pueden compartir hábitat y están filogenéticamente muy próximos (Huang *et al.*, 2014; Stephens *et al.*, 2019). Además, las crecientes poblaciones de estos cánidos silvestres y acercamiento a zonas con presencia humana puede dar lugar a interacciones con animales domésticos como el perro (Maestas *et al.*, 2003), por ejemplo, a través de las heces de estos animales silvestres (Boze, 2008) o incluso mediante la intervención de hospedadores intermediarios o parátenicos que también poseen esta capacidad de ocupar la interfaz silvestres-domésticos, como pueden ser los roedores (Froeschke y Matthee, 2014). En este caso, el papel que puede llegar a desempeñar el perro puede ser clave en la transmisión de estos helmintos, puesto que puede actuar como nexo entre los ciclos biológicos silvestres y ciclos domésticos de estos parásitos. Es decir, se podría producirse un fenómeno de "spillover" de agentes patógenos silvestres hacía los animales domésticos. No obstante, también puede ocurrir el caso opuesto, en que un patógeno que está presente de forma natural entre animales domésticos pueda acabar utilizando como reservorios a los cánidos silvestres (Thompson, 2013) y estos, a su vez, pueden volver a ser fuente de transmisión para los domésticos (Jenkins *et al.*, 2015). A este ciclo de transmisión de patógenos entre fauna silvestre y doméstica se une el ser humano, ya que algunos de los parásitos descritos en los Capítulos 2 y 3 son potenciales agentes zoonósicos, como *T. canis*, *A. caninum* o *E. aerophilus*, por tanto, esta interacción también puede tener importantes implicaciones desde el punto de vista de la Sanidad Pública.

Entre los agentes zoonósicos detectados cabe destacar el papel de *T. canis*. Según Otranto y Deplazes (2019), las infecciones por *T. canis* en humanos se adquieren predominantemente por la ingestión de huevos embrionados por contacto orofecal después de actividades en ambientes contaminados tales como areneros, parques u otros lugares donde los carnívoros han defecado. Aunque se desconoce el verdadero papel de la fauna silvestre en la transmisión de este nematodo, diversos estudios indican que se puede asumir que los zorros pueden desempeñar un papel importante en el mantenimiento del ciclo biológico silvestre de *T. canis*, así como en el fortalecimiento de una transmisión continua a humanos y animales domésticos debido a su presencia en zonas antropizadas y su comportamiento de marcar el territorio con excrementos (Guislain *et al.*, 2007; Morgan *et al.*, 2013; Otranto y Deplazes, 2019).

Por el contrario, el papel que desempeña el visón americano en la transmisión de los parásitos es distinto al zorro y lobo, puesto que no se ha descubierto, hasta el momento, ningún parásito de este mustélido que sea potencialmente zoonósico. Por otra parte, la posibilidad de transferencia a animales domésticos es muy baja, al ser parásitos que muestran una alta especificidad por los mustélidos. No obstante, sí ha quedado demostrada en el Capítulo 1 la importancia del visón americano como reservorio y posible transmisor de estos helmintos en ambientes silvestres hacia especies de mustélidos autóctonos, entre los cuales se encuentra el visón europeo, una especie en peligro de extinción, pudiendo suponer una amenaza desde el punto de vista sanitario para la conservación de estas especies.

La identificación de los parásitos hallados en este estudio se realizó en base a las características morfométricas de los mismos con la ayuda de claves taxonómicas. No obstante, para la correcta identificación de algunas especies fue necesario el uso de técnicas de biología molecular complementarias. La identificación morfométrica presenta como inconvenientes el tiempo requerido para su realización, además de la necesidad de una alta especialización por parte de los investigadores (Besansky *et al.*, 2003). En general, para una correcta identificación morfométrica es necesario el acceso a claves taxonómicas precisas y detalladas, que no siempre están disponibles o son excesivamente antiguas; además, existen especies de parásitos que presentan características morfológicas tan similares que son muy difíciles de distinguir entre sí (Fahrion *et al.*, 2011). Durante el desarrollo del Capítulo 3 se realizó la identificación morfométrica de los cestodos de zorro y lobo, un proceso arduo que requiere un gran esfuerzo y tiempo, ya que es necesaria una tinción previa para la visualización de las estructuras que permiten diferenciar las distintas especies. Esta identificación resulta complicada en el caso de los cestodos que presentan una morfología muy similar, como en algunas especies del género *Taenia* (Lavikainen *et al.*, 2008). La identificación de las especies de este género que fueron encontradas en los zorros presentaban características morfológicas que permitían discriminar entre las distintas especies con ayuda de claves morfológicas con mayor facilidad que las que se hallaron en los lobos. Por este motivo, en el caso del estudio de los cestodos del lobo ibérico se optó por la realización de una PCR clásica en varios especímenes de cestodos. Los productos amplificados fueron purificados y secuenciados para, finalmente, demostrar la presencia de al menos dos especies de cestodos, *T. hydatigena* y *T. krabbei*. Gracias a la confirmación de estas especies mediante técnicas moleculares se pudo utilizar como complemento a la identificación morfométrica. De esta forma, quedó demostrada la utilidad de las técnicas de biología molecular como un medio de diagnóstico en Parasitología cuando se trabaja con parásitos muy difíciles de identificar mediante las técnicas morfométricas clásicas.

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CONCLUSIONES/CONCLUSIONS

CONCLUSIONES DEL CAPÍTULO I

PRIMERA: El visón americano (*Neovison vison*) del noroeste de la península ibérica presenta bajas intensidades de parasitación por helmintos gastrointestinales y cardiorrespiratorios. No obstante, la elevada prevalencia de animales parasitados, así como el hecho de que la mayoría de las especies de parásitos encontradas sean compartidas por otras especies de mustélidos autóctonos de la misma área de estudio, demuestra que el visón americano ha adquirido un papel epidemiológico en el mantenimiento y dispersión de estos parásitos en el hábitat que ocupa y, en consecuencia, su presencia debe ser tenida en cuenta como un factor de riesgo para otras especies de mesocarnívoros autóctonos.

SEGUNDA: Es la primera vez que se cita en el visón americano la presencia de los nematodos *Aonchotheca annulosa* y *Angiostrongylus daskalovi*, siendo la prueba de que este mustélido alóctono ha sufrido un proceso de adaptación parasitaria, integrándose en el ciclo biológico de estos helmintos que presentan anidamiento natural en las áreas de expansión de dicho mamífero.

TERCERA: Es la primera vez que se describe la presencia del trematodo *Troglotrema acutum* en el pulmón del visón americano, cuando la ubicación habitualmente descrita era exclusivamente en los senos nasolacrimales. Esta localización anatómica demuestra que las posibles repercusiones para la salud del visón europeo y de otros mustélidos en los que ha sido descrito este parásito, son mayores de las que se suponía hasta ahora.

CONCLUSIONES DEL CAPÍTULO II

PRIMERA: Las prevalencias de *Angiostrongylus vasorum*, *Crenosoma vulpis* y *Eucoleus aerophilus* en zorros (*Vulpes vulpes*) del noroeste de la península ibérica son mayores que las encontradas en el lobo ibérico (*Canis lupus signatus*), lo que indica que el zorro tiene un papel epidemiológico más importante que el lobo en el mantenimiento del ciclo biológico selvático de estos nematodos cardiopulmonares.

SEGUNDA: Este contraste posiblemente se deba a comportamientos tróficos diferentes entre ambos tipos de cánidos silvestres. En el caso del zorro, la mayor prevalencia de parasitación de *Angiostrongylus vasorum* y *Crenosoma vulpis* sugiere que su dieta se sustenta, en mayor medida que la del lobo, en la ingestión de presas de pequeño tamaño que actúan como hospedadores intermediarios o paraténicos de estos parásitos cardiopulmonares.

TERCERA: Es la primera vez que se cita la presencia de *Filaroides hirthi* en el lobo a nivel mundial. Su detección probablemente ha sido posible gracias al empleo de la técnica de digestión enzimática del parénquima pulmonar. Teniendo en cuenta el pequeño tamaño de *F. hirthi*, así como su ubicación en el parénquima pulmonar, es aconsejable el empleo de esta técnica para evitar falsos negativos y, en consecuencia, la subestima de su prevalencia en cánidos silvestres.

CONCLUSIONES DEL CAPÍTULO III

PRIMERA: El zorro (*Vulpes vulpes*) y el lobo ibérico (*Canis lupus signatus*) del noroeste de la península ibérica presentan una variada helmintofauna gastrointestinal que, en términos generales, es compartida por ambos hospedadores, lo que evidencia que participan en el mantenimiento de estos parásitos en el medio natural. No obstante, existen diferencias respecto a especies de cestodos concretas, posiblemente debido a la ecología trófica que caracteriza a cada uno de estos cánidos. En concreto, el papel epidemiológico del zorro es más destacado en el caso de los cestodos transmitidos por el consumo de presas pequeñas (*Taenia crassiceps* y *Taenia polyacantha*), en tanto que el del lobo está mayoritariamente asociado a cestodos cuyos hospedadores intermediarios son grandes presas, como los ungulados (*Taenia hydatigena* y *Taenia krabbei*).

SEGUNDA: La detección de nematodos que son zoonósicos (*Toxocara canis* y *Ancylostoma caninum*), indica que el zorro y el lobo deben ser tenidos en cuenta a la hora de explicar la epidemiología de estas helmintosis en la interfaz doméstico-silvestre-humano.

TERCERA: El uso de técnicas moleculares para la identificación de cestodos de los cánidos silvestres es fundamental para descartar identificaciones erróneas basadas exclusivamente en las características morfométricas del parásito, ya que permite discriminar entre especies de cestodos que presentan una morfología muy similar. De hecho, gracias a esta técnica se ha podido confirmar la presencia de *Taenia krabbei* en lobos, siendo la primera vez que se cita en la península ibérica.

CONCLUSIONS OF CHAPTER I

FIRST: American mink (*Neovison vison*) from the northwest of the Iberian Peninsula shows low parasite intensities by gastrointestinal and cardiorespiratory helminths. However, the high prevalence of parasitized animals, as well as the fact that most of the parasite species found can be shared by other native mustelid species to the same study area, demonstrates that the American mink has acquired an epidemiological role in the maintenance and dispersion of these parasites in the invaded habitat and, consequently, their presence must be taken into account as a risk factor for other autochthonous mesocarnivores.

SECOND: This is the first report of the nematodes *Aonchotheca annulosa* and *Angiostrongylus daskalovi* in the American mink, being the proof that this allochthonous mustelid has undergone a process of parasitic adaptation, integrating itself in the life cycles of these helminthes which present natural nesting in the areas of expansion of this mammal.

THIRD: This is the first description of the trematode *Troglotrema acutum* in the lung of the American mink, when the location that is usually described is exclusively the nasolacrimal sinuses. This anatomical location demonstrates that the possible health consequences for the European mink and other mustelids in which this parasite has been described can be greater than previously assumed.

CONCLUSIONS OF CHAPTER II

FIRST: Prevalences of *Angiostrongylus vasorum*, *Crenosoma vulpis* and *Eucoleus aerophilus* in foxes (*Vulpes vulpes*) from the Northwest of the Iberian Peninsula are higher than those found in the Iberian wolf (*Canis lupus signatus*), indicating that the fox plays a more significant epidemiological role than the wolf in the sylvatic life cycle of these cardiopulmonary nematodes.

SECOND: This contrast is possibly due to different trophic behaviors between the two wild canid species. In the case of the fox, the greater prevalence of *A. vasorum* and *C. vulpis* suggests that its diet is based, to a greater extent than wolves, on the ingestion of small preys which act as intermediate or paratenic hosts of these cardiopulmonary parasites.

THIRD: This is the first time that *Filaroides hirthi* in the wolf has been cited worldwide. Its detection has probably been achieved thanks to the use of the enzymatic digestion technique of the lung parenchyma. Considering the small size of *F. hirthi*, as well as its location in the pulmonary parenchyma, it is advisable to use this technique to avoid false negatives and, consequently, the underestimation of its prevalence in wild canids.

CONCLUSIONS OF CHAPTER III

FIRST: The fox (*Vulpes vulpes*) and the Iberian wolf (*Canis lupus signatus*) from the northwestern Iberian Peninsula present a varied gastrointestinal helminthfauna that, in broad terms, is shared by both hosts, indicating that they participate in the maintenance of these parasites in the natural environment. However, there are differences regarding specific cestode species, possibly due to the trophic ecology of each of these host canids. Specifically, the epidemiological role of foxes is more prominent in the case of cestodes transmitted by consumption of small prey (*Taenia crassiceps* and *Taenia polyacantha*), while that of the wolves is mostly associated with cestodes whose intermediate hosts are large prey, such as ungulates (*Taenia hydatigena* and *Taenia krabbei*).

SECOND: The detection of zoonotic nematodes (*Toxocara canis* and *Ancylostoma caninum*) indicates that foxes and wolves should be taken into account when explaining the epidemiology of these helminths in the domestic-wild-human interface.

THIRD: The use of molecular techniques for the identification of cestodes of wild canids is essential to avoid wrong identifications based exclusively on the parasite morphometric characteristics, since it allows to differentiate between cestode species having a very similar morphology. In fact, the presence of *Taenia krabbei* in wolves has been confirmed by this technique, being the first time that this cestode has been cited in the Iberian Peninsula.

ANEXOS

Anexo 1 Fotografías del material y metodología de estudio

Figura 11 Visones americanos antes de la necropsia.

Figura 12 Zorro antes de la necropsia.

Figura 13 Lobo antes de la necropsia.

Figura 14 Paquete digestivo de visón americano.

Figura 15 Paquete intestinal de lobo.

Anexo 1 continuación Fotografías del material y metodología de estudio


Figura 16 Paquete cardiorrespiratorio de lobo.


Figura 18 Procesado de intestino de visón americano.


Figura 17 Procesado de pulmón de lobo.

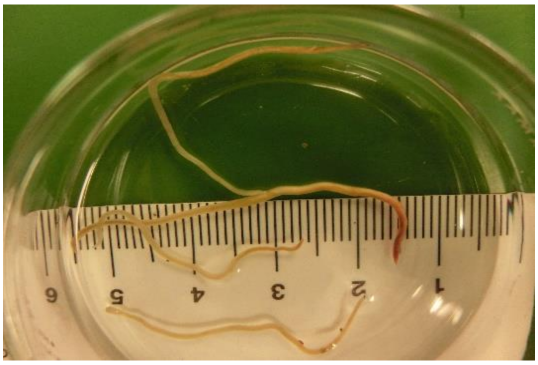


Figura 19 *Toxocara canis* extraídos de intestino de zorro.

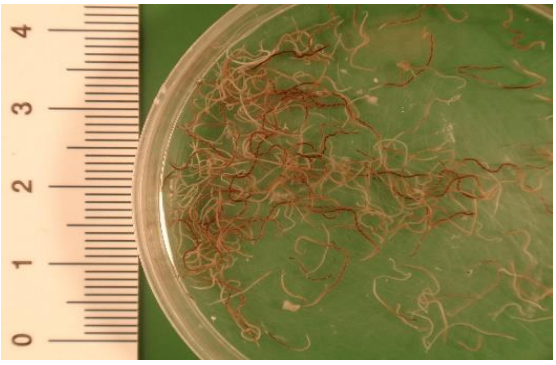
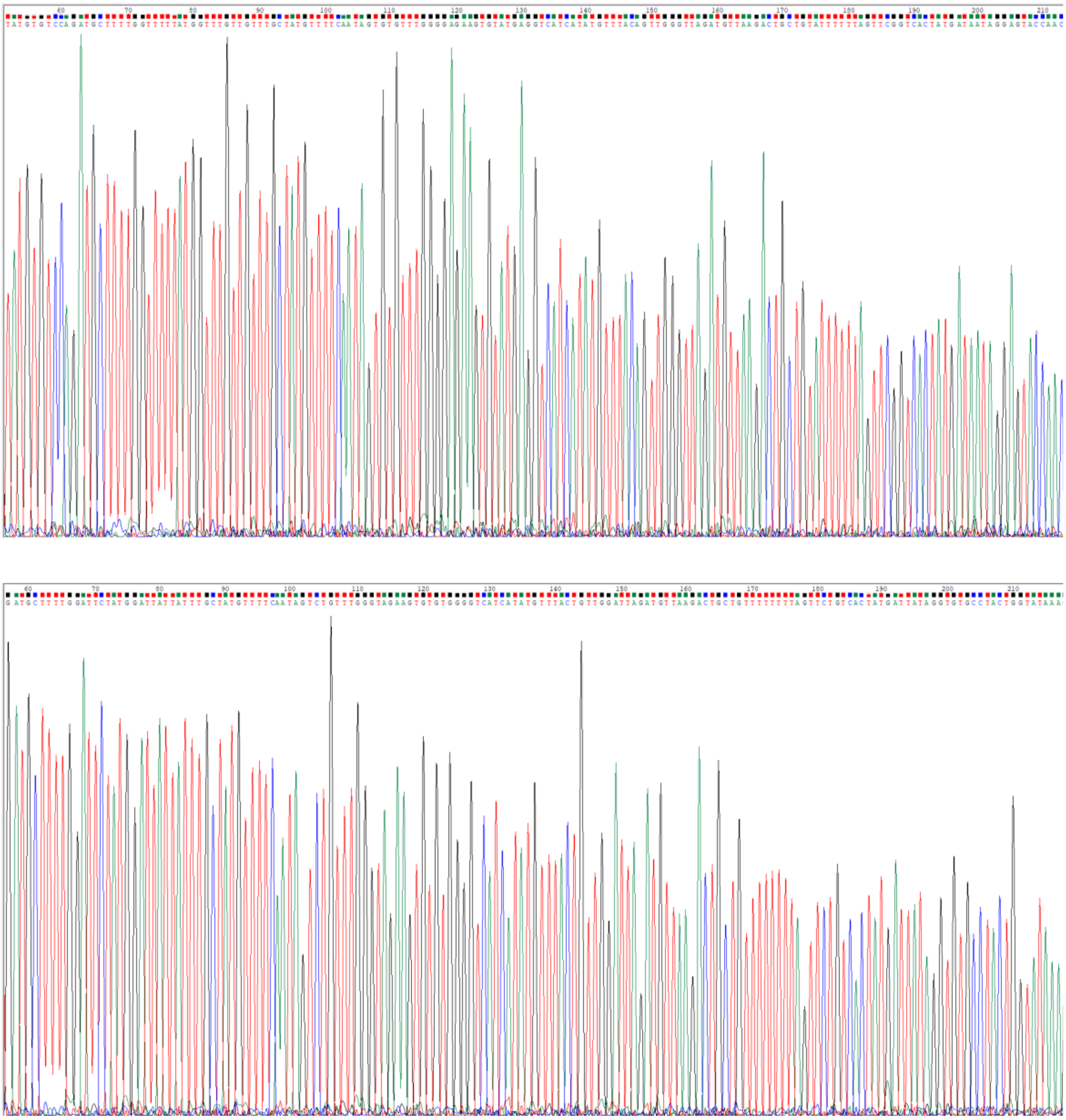


Figura 20 *Angiostrongylus vasorum* extraídos de pulmón de zorro.


Figura 21 Cestodos extraídos de intestino de lobo.

Anexo 2 Ejemplo de secuencias ampliadas mediante el primer JB3 de *T. krabbei* (superior) y *T. hydatigena* (inferior) del mismo fragmento de nucleótidos (60-220 aproximadamente) en el que se aprecia la diferente composición del material genético de ambos cestodos.



Anexo 3 Fotografías de las principals especies de helmintos encontradas

Figura 22 *Crenosoma vulpis* (zorro).

Figura 23 *Angiostrongylus vasorum*, bolsa copulatriz (zorro).

Figura 24 *Crenosoma melesi*, bolsa copulatriz (visón americano).

Figura 25 *Angiostrongylus daskalovi*, bolsa copulatriz (visón americano).

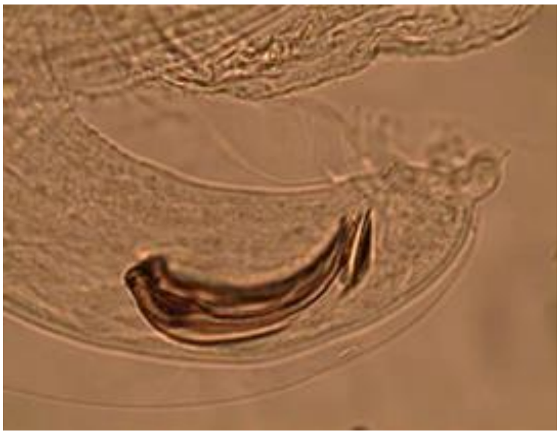


Figura 26 *Aelurostrongylus* spp., bolsa copulatriz (visón americano).

Figura 27 *Molineus patens*, bolsa copulatriz (visón americano).

Anexo 3 continuación Fotografías de las principals especies de helmintos encontradas

Figura 28 *Aonchotheca putorii*, región vulvar (visón americano).

Figura 29 *Aonchotheca annulosa*, región vulvar (visón americano).

Figura 30 *Uncinaria stenocephala*, cápsula bucal (lobo).

Figura 31 *Toxascaris leonina*, detalle de las espículas (zorro).

Figura 32 Escólex de *Taenia crassiceps* (zorro).

Figura 33 Escólex de *Taenia crassiceps* (zorro).

Anexo 3 continuación Fotografías de las principals especies de helmintos encontradas

Figura 34 Escólex de *Taenia krabbei* (lobo).

Figura 35 Escólex de *Taenia hydatigena* (lobo).

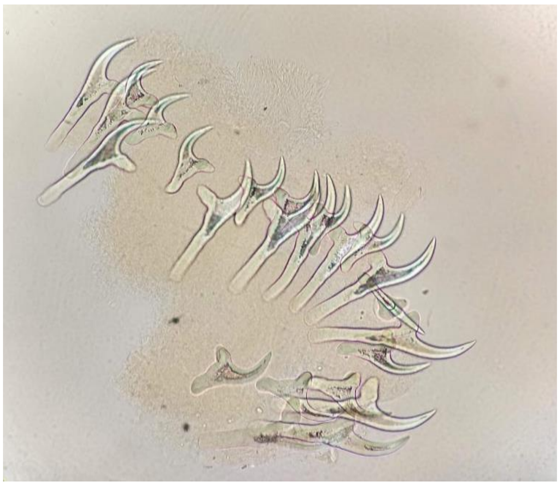


Figura 36 Ganchos de *Taenia pisiformis* (lobo).

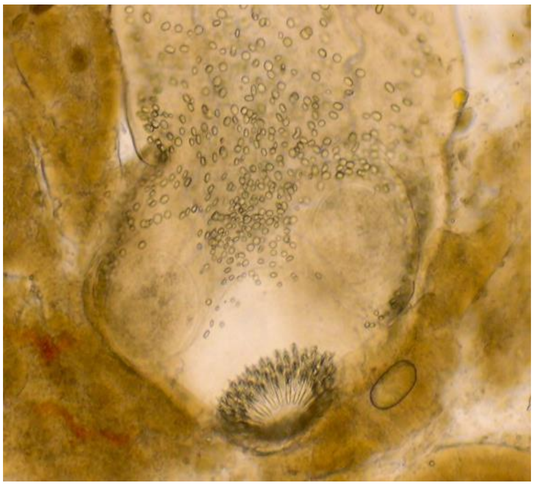


Figura 37 Escólex de *Dipylidium caninum* (zorro).

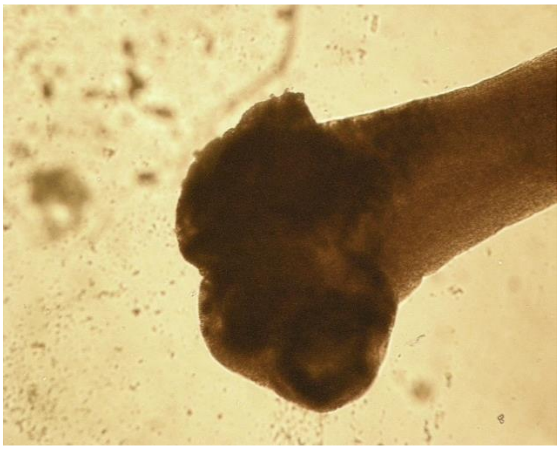


Figura 38 Escólex de *Mesocestoides* spp. (zorro).

Anexo 4 Principales especies de helmintos descritas en el zorro en Europa

ESPECIE	**ÁREA DE ESTUDIO**	**REFERENCIA BIBLIOGRÁFICA**
NEMATODOS		
Angiostrongylus vasorum	España	Mañas *et al.*, 2005
	Portugal	Eira *et al.*, 2006
	Dinamarca	Al-Sabi *et al.*, 2014
	Italia	Magi *et al.*, 2015
	Rumanía	Deak *et al.*, 2017
	Alemana	Schug *et al.*, 2018
Ancylostoma caninum	España	Segovia *et al.*, 2004
	Dinamarca	Saeed *et al.*, 2006
	Italia	Fiocchi *et al.*, 2016
Crenosoma vulpis	España	Mañas *et al.*, 2005
	Dinamarca	Al-Sabi *et al.*, 2014
	Italia	Magi *et al.*, 2015
	Alemana	Schug *et al.*, 2018
Dirofilaria immitis	España	Gortázar *et al.*, 1998
	Portugal	Eira *et al.*, 2006
	Italia	Fiocchi *et al.*, 2016
Eucoleus aerophilus	España	Martínez-Carrasco *et al.*, 2007
	Dinamarca	Al-Sabi *et al.*, 2014
	Italia	Magi *et al.*, 2015
	Alemana	Schug *et al.*, 2018
Molineus patens	Portugal	Eira *et al.*, 2006
	Eslovenia	Vergles Rataj *et al.*, 2013
Oxynema crassispiculum	España	Martínez-Carrasco *et al.*, 2007
	Italia	Di Cerbo *et al.*, 2008
Pearsonema plica	España	Gortázar *et al.*, 1998
	Dinamarca	Saeed *et al.*, 2006
	Italia	Magi *et al.*, 2015
Pterygodermatites affinis	Portugal	Eira *et al.*, 2006
	España	Martínez-Carrasco *et al.*, 2007
	Italia	Fiocchi *et al.*, 2016
Spirocerca lupi	España	Gortázar *et al.*, 1998
	Portugal	Eira *et al.*, 2006
	Italia	Magi *et al.*, 2015
Toxascaris leonina	Dinamarca	Saeed *et al.*, 2006
	Portugal	Eira *et al.*, 2006
	Suiza	Reperant *et al.*, 2007
	España	Martínez-Carrasco *et al.*, 2007
	Italia	Di Cerbo *et al.*, 2008
	Eslovenia	Vergles Rataj *et al.*, 2013
Toxocara canis	Dinamarca	Saeed *et al.*, 2006
	Portugal	Eira *et al.*, 2006
	Suiza	Reperant *et al.*, 2007
	España	Martínez-Carrasco *et al.*, 2007
	Italia	Di Cerbo *et al.*, 2008
	Eslovenia	Vergles Rataj *et al.*, 2013
Toxocara cati	España	Martínez-Carrasco *et al.*, 2007
Trichuris vulpis	España	Gortázar *et al.*, 1998
	Dinamarca	Saeed *et al.*, 2006
	Portugal	Eira *et al.*, 2006
	Suiza	Reperant *et al.*, 2007
	Italia	Di Cerbo *et al.*, 2008
	Eslovenia	Vergles Rataj *et al.*, 2013

Anexo 4 continuación Principales especies de helmintos descritas en el zorro en Europa

ESPECIE	ÁREA DE ESTUDIO	REFERENCIA BIBLIOGRÁFICA
Uncinaria stenocephala	España	Segovia *et al.,* 2004
	Dinamarca	Saeed *et al.*, 2006
	Suiza	Reperant *et al.*, 2007
	Italia	Di Cerbo *et al.*, 2008
	Eslovenia	Vergles Rataj *et al.*, 2013
CESTODOS		
Dipylidium caninum	España	Martínez-Carrasco *et al.*, 2007
	Suiza	Reperant *et al.*, 2007
	Eslovenia	Vergles Rataj *et al.*,2013
	Italia	Fiocchi *et al.*, 2016
Echinococcus granulosus	España	Segovia *et al.,* 2004
Echinococcus multilocularis	Italia	Di Cerbo *et al.*, 2008
Hymenolepis nana	Eslovenia	Vergles Rataj *et al.*,2013
Joyeuxiella pasqualei	España	Martínez-Carrasco *et al.*, 2007
Mesocestoides spp.	Dinamarca	Saeed *et al.*, 2006
	España	Martínez-Carrasco *et al.*, 2007
	Italia	Di Cerbo *et al.*, 2008
	Eslovenia	Vergles Rataj *et al.*, 2013
Taenia crassiceps	España	Segovia *et al.,* 2004
	Italia	Di Cerbo *et al.*, 2008
	Eslovenia	Vergles Rataj *et al.*, 2013
Taenia hydatigena	España	Segovia *et al.,* 2004
	Dinamarca	Saeed *et al.*, 2006
	Italia	Di Cerbo *et al.*, 2008
Taenia pisiformis	Portugal	Eira *et al.*, 2006
	Dinamarca	Saeed *et al.*, 2006
	España	Martínez-Carrasco *et al.*, 2007
	Italia	Fiocchi *et al.*, 2016
Taenia polyacantha	España	Segovia *et al.,* 2004
	Italia	Di Cerbo *et al.*, 2008
	Eslovenia	Vergles Rataj *et al.*, 2013
Taenia taeniaeformis	España	Segovia *et al.,* 2004
	Dinamarca	Saeed *et al.*, 2006
	Italia	Di Cerbo *et al.*, 2008
TREMATODOS		
Alaria alata	España	Segovia *et al.*, 2004
	Portugal	Eira *et al.*, 2006
	Dinamarca	Saeed *et al.*, 2006
	Italia	Fiocchi *et al.*, 2016
Ascocotyle longa	Portugal	Eira *et al.*, 2006
Brachylaima spp.	Italia	Fiocchi *et al.*, 2016
Cryptocotyle lingua	Dinamarca	Saeed *et al.*, 2006
Echinochasmus perfoliatus	Dinamarca	Saeed *et al.*, 2006
Heterophyes heterophyes	Eslovenia	Vergles Rataj *et al.*, 2013
Metagonimus yokogawai	Eslovenia	Vergles Rataj *et al.*, 2013
Metorchis bilis	España	Segovia *et al.*, 2004
Opistorchis felineus	España	Segovia *et al.*, 2004
Prohemistomum appendiculatum	Eslovenia	Vergles Rataj *et al.*, 2013
Pseudamphistomum truncatum	Portugal	Eira *et al.*, 2006
	Dinamarca	Saeed *et al.*, 2006
Rossicotrema donicum	Eslovenia	Vergles Rataj *et al.*, 2013

Anexo 5 Principales especies de helmintos descritas en el lobo en Europa

ESPECIE	ÁREA DE ESTUDIO	REFERENCIA BIBLIOGRÁFICA
NEMATODOS		
Ancylostoma caninum	España	Torres *et al.*, 2000
	Bielorrusia	Shimalov y Shimalov, 2000
	Letonia	Bagrade *et al.*, 2009
Angiostrongylus vasorum	España	Segovia *et al.*, 2001
	Italia	Eleni *et al.*, 2014
Crenosoma vulpis	Bielorrusia	Shimalov y Shimalov, 2000
	España	Balmori *et al.*, 2000
	Letonia	Bagrade *et al.*, 2009
Dirofilaria immitis	España	Segovia *et al.*, 2001
Eucoleus aerophilus	España	Torres *et al.*, 2000
	Bielorrusia	Shimalov y Shimalov, 2000
	Letonia	Bagrade *et al.*, 2009
Pearsonema plica	Bielorrusia	Shimalov y Shimalov, 2000
	España	Segovia *et al.*, 2001
	Letonia	Bagrade *et al.*, 2009
Spirocerca lupi	Bielorrusia	Shimalov y Shimalov, 2000
	España	Domínguez y De la Torre, 2002
Toxascaris leonina	España	Torres *et al.*, 2000
	Bielorrusia	Shimalov y Shimalov, 2000
	Polonia	Kloch *et al.*, 2005
	Estonia	Mosk *et al.*, 2006
Toxocara canis	España	Torres *et al.*, 2000
	Bielorrusia	Shimalov y Shimalov, 2000
	Polonia	Kloch *et al.*, 2005
	Estonia	Mosk *et al.*, 2006
	Letonia	Bagrade *et al.*, 2009
	Serbia	Ćirović *et al.*, 2015
Trichuris vulpis	Bielorrusia	Shimalov y Shimalov, 2000
	España	Segovia *et al.*, 2003
	Polonia	Kloch *et al.*, 2005
Uncinaria stenocephala	Bielorrusia	Shimalov y Shimalov, 2000
	España	Segovia *et al.*, 2001
	Estonia	Mosk *et al.*, 2006
	Letonia	Bagrade *et al.*, 2009
	Dinamarca	Al-Sabi *et al.*, 2018
CESTODOS		
Dipylidium caninum	Bielorrusia	Shimalov y Shimalov, 2000
	España	Segovia *et al.*, 2003
	Polonia	Kloch *et al.*, 2005
Echinoccocus granulosus	Bielorrusia	Shimalov y Shimalov, 2000
	España	Sobrino *et al.*, 2006
Mesocestoides spp.	Bielorrusia	Shimalov y Shimalov, 2000
	España	Segovia *et al.*, 2001
	Estonia	Mosk *et al.*, 2006
	Letonia	Bagrade *et al.*, 2009
	Dinamarca	Al-Sabi *et al.*, 2018
Spirometra erinacei	Bielorrusia	Shimalov y Shimalov, 2000

Anexo 5 continuación Principales especies de helmintos descritas en el lobo en Europa

ESPECIE	ÁREA DE ESTUDIO	REFERENCIA BIBLIOGRÁFICA
Taenia crassiceps	Bielorrusia	Shimalov y Shimalov, 2000
	Letonia	Bagrade *et al.*, 2009
Taenia hydatigena	Bielorrusia	Shimalov y Shimalov, 2000
	España	Segovia *et al.*, 2001
	Estonia	Mosk *et al.*, 2006
	Letonia	Bagrade *et al.*, 2009
	Serbia	Ćirović *et al.*, 2015
	Dinamarca	Al-Sabi *et al.*, 2018
Taenia krabbei	Italia	Poglayen *et al.*, 2017
	Alemania	Lesniak *et al.*, 2017
	Dinamarca	Al-Sabi *et al.*, 2018
Taenia multiceps	España	Torres *et al.*, 2000
	Bielorrusia	Shimalov y Shimalov, 2000
Taenia pisiformis	Bielorrusia	Shimalov y Shimalov, 2000
	España	Segovia *et al.*, 2003
	Letonia	Bagrade *et al.*, 2009
	Serbia	Ćirović *et al.*, 2015
Taenia polyacantha	Bielorrusia	Shimalov y Shimalov, 2000
	Letonia	Bagrade *et al.*, 2009
	Portugal	Guerra *et al.*, 2013
Taenia serialis	España	Segovia *et al.*, 2001
TREMATODOS		
Alaria alata	Bielorrusia	Shimalov y Shimalov, 2000
	España	Segovia *et al.*, 2003
	Alemania	Lesniak *et al.*, 2017
Isthmiophora melis	Bielorrusia	Shimalov y Shimalov, 2000
Opisthorchis felineus	Bielorrusia	Shimalov y Shimalov, 2000
Pseudamphistomum truncatum	Bielorrusia	Shimalov y Shimalov, 2000

Anexo 6 Principales especies de helmintos descritas en el visón americano en Europa

ESPECIE	ÁREA DE ESTUDIO	REFERENCIA BIBLIOGRÁFICA
NEMATODOS		
Aonchotheca putorii	Bielorrusia	Shimalov y Shimalov,2001
	España	Torres *et al.*, 2003
	Francia	Torres *et al.*, 2008
Ascaris devosi	Bielorrusia	Shimalov y Shimalov, 2001
Crenosoma melesi	España	Torres *et al.*, 2003
Crenosoma taiga	Bielorrusia	Shimalov y Shimalov,2001
Eucoleus aerophilus	Francia	Torres *et al.*, 2008
Filaroides martis	Bielorrusia	Shimalov y Shimalov, 2001
Molineus patens	Bielorrusia	Shimalov y Shimalov, 2001
	España	Torres *et al.*, 2003
	Francia	Torres *et al.*, 2008
Mustelivingylus skrjabini	Bielorrusia	Shimalov y Shimalov,2001
Pearsonema mucronata	Bielorrusia	Shimalov y Shimalov,2001
Pearsonema plica	Francia	Torres *et al.*, 2008
Skrjabingylus nasicola	Bielorrusia	Shimalov y Shimalov, 2001
	España	Torres *et al.*, 2006
	Francia	Torres *et al.*, 2008
Strongyloides martis	Bielorrusia	Shimalov y Shimalov, 2001
	Francia	Torres *et al.*, 2008
CESTODOS		
Taenia martis	España	Torres *et al.*, 2003
Taenia tenuicollis	Bielorrusia	Shimalov y Shimalov, 2001
	España	Torres *et al.*, 2003
Spirometra erinacei	Bielorrusia	Shimalov y Shimalov, 2001
TREMATODOS		
Alaria alata	Bielorrusia	Shimalov y Shimalov, 2001
Apophallus donicus	Bielorrusia	Shimalov y Shimalov, 2001
Ascocotyle spp.	Francia	Torres *et al.*, 2008
Euparyphium melis	Francia	Torres *et al.*, 2008
Euryhelmis squamula	España	Torres *et al.*, 2003
	Francia	Torres *et al.*, 2008
Isthmiophora melis	Bielorrusia	Shimalov y Shimalov, 2001
Metorchis bilis	Bielorrusia	Shimalov y Shimalov, 2001
Opistorchis felineus	Bielorrusia	Shimalov y Shimalov, 2001
Pseudamphistomum truncatum	Bielorrusia	Shimalov y Shimalov, 2001
	Irlanda	Hawkins *et al.*,2010
Troglotrema acutum	España	Torres *et al.*, 2006
	Francia	Torres *et al.*, 2008

Anexo 7 Comunicaciones a congresos derivadas de esta Tesis Doctoral

Título: Helmintos gastrointestinales del visón americano (*Neovison vison*) en Galicia: un ejemplo del riesgo sanitario asociado a la presencia de un hospedador alóctono.
Tipo de participación: Comunicación oral.
Nombre del congreso: I Jornadas Doctorales de la Universidad de Murcia.
Lugar de celebración: Murcia (Región de Murcia), España.
Fecha de celebración: 28/04/2015 - 29/04/2015.
Entidad organizadora: Escuela Internacional de Doctorado de la Universidad de Murcia.
Autores: F.J. Martínez Rondán, A.M López Beceiro, M.R. Ruiz de Ybáñez, L.E. Fidalgo, P. Tizzani, C. Martínez-Carrasco.

Título: El visón americano (*Neovison vison*) como portador de helmintos en Galicia (Noroeste de España): un ejemplo del riesgo sanitario asociado a la presencia de un hospedador alóctono.
Tipo de participación: Comunicación oral.
Nombre del congreso: 35º Reencuentros del Grupo de Ecopatología de la Fauna salvaje de Montaña (G.E.E.F.S.M.).
Lugar de celebración: Balme (Turín), Italia.
Fecha de celebración: 24/05/2015 - 25/05/2015.
Entidad organizadora: Groupe d'Etudes sur l'Ecopathologie de la Faune Sauvage de Montagne (GEEFSM).
Autores: F.J. Martínez Rondán, A.M López Beceiro, P. Tizzani, L.E. Fidalgo, M.R. Ruiz de Ybáñez, C. Martínez-Carrasco.

Título: Cestodos del zorro rojo (*Vulpes vulpes*) en el Noroeste de la península ibérica.
Tipo de participación: Comunicación oral.
Nombre del congreso: II Jornadas Doctorales Escuela Internacional de Doctorado de la Universidad de Murcia (EIDUM).
Lugar de celebración: Murcia (Región de Murcia), España.
Fecha de celebración: 31/05/2016 – 2/06/2016.
Entidad organizadora: Escuela Internacional de Doctorado de la Universidad de Murcia.
Autores: I. Arcenillas Hernández, F. M. Macías, M.R. Ruiz de Ybáñez, A. M. López Beceiro, L. E. Fidalgo, P. Tizzani, F. J. Martínez Rondán, C. Martínez-Carrasco.

Título: Especies de ixódidos presentes en visones americanos (*Neovison vison*) capturados en la provincia de Lugo (Galicia, N.O. España).
Tipo de participación: Póster.
Nombre del congreso: III Jornadas Doctorales Escuela Internacional de Doctorado de la Universidad de Murcia (EIDUM).
Lugar de celebración: Murcia (Región de Murcia), España.
Fecha de celebración: 30/05/2017 - 1/06/2017.
Entidad organizadora: Escuela Internacional de Doctorado de la Universidad de Murcia.
Autores: F.J. Martínez Rondán, A.M López Beceiro, D. Mosteiro Díaz, M.R. Ruiz de Ybáñez, L.E. Fidalgo, C. Martínez-Carrasco.

Título: Resultados preliminares de la presencia de *Thelazia callipaeda* en zorros (*Vulpes vulpes*) de ambientes semiáridos de la península ibérica.
Tipo de participación: Comunicación oral.
Nombre del congreso: III Jornadas Doctorales Escuela Internacional de Doctorado de la Universidad de Murcia.
Lugar de celebración: Murcia (Región de Murcia), España.
Fecha de celebración: 30/05/2017 - 1/06/2017.
Entidad organizadora: Escuela Internacional de Doctorado de la Universidad de Murcia.
Autores: I. Arcenillas Hernández, F.J. Martínez Rondán, M. Gonzálvez, M.R. Ruiz de Ybáñez, R. Escribano, C. Martínez-Carrasco.

Título: Gastrointestinal helminths in wolves (*Canis lupus signatus*) from the Northwestern Spain.
Tipo de participación: Póster.
Nombre del congreso: XX Congreso de la Sociedad Española de Parasitología y XV Meeting del European Veterinary Parasitology College.
Lugar de celebración: San Cristóbal de la Laguna (Tenerife), España.
Fecha de celebración: 19/07/2017 - 21/07/2017.
Entidad organizadora: Sociedad Española de Parasitología (SOCEPA) y European Veterinary Parasitology College (EVPC).
Autores: F.J. Martínez Rondán, A. Huertas, A.M López Beceiro, M.R. Ruiz de Ybáñez, J.L. Marcos, A. Oleaga, C. Martínez-Carrasco.

Título: Nematodos broncopulmonares del zorro rojo (*Vulpes vulpes*) en ambientes semiáridos de la península ibérica.
Tipo de participación: Comunicación oral.
Nombre del congreso: IV Jornadas Doctorales de la Universidad de Murcia.
Lugar de celebración: Murcia (Región de Murcia), España.
Fecha de celebración: 29/05/2018 - 31/05/2018.
Entidad organizadora: Escuela Internacional de Doctorado de la Universidad de Murcia.
Autores: I. Arcenillas Hernández, M.R. Ruiz de Ybáñez, F.J. Martínez Rondán, C. Martínez-Carrasco.

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Research paper

The American mink (*Neovison vison*) is a competent host for native European parasites

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ABSTRACT

The American mink (*Neovison vison*) is a mustelid native to North America that was introduced in Europe and the former USSR for fur farming. Throughout the last century, accidental or deliberate escapes of mink from farms caused the establishment of stable feral populations. In fact, the American mink is considered an invasive alien species in 28 European countries. The present study evaluates the gastrointestinal and cardiopulmonary helminth fauna of the American mink in Galicia (NW Spain) to understand its role as a potential reservoir for parasites affecting other autochthonous mustelids. In the period 2008–2014, fifty American mink (35 males and 15 females) of different ages (22 immature and 28 adults) from the provinces of Lugo, Ourense and Pontevedra were captured and sacrificed. Eight parasite species were found (6 nematodes and 2 trematodes) with the following prevalences: *Molineus patens* (68%), *Aonchotheca putorii* (54%), *Crenosoma melesi* (10%), *Aonchotheca annulosa* (8%), *Angiostrongylus daskalovi* (6%), *Aelurostrongylus* spp. (2%), *Troglotrema acutum* (2%) and an unidentified trematode (2%). Eighty-two per cent of the mink harboured helminths, including 15 animals (30%) infected by only one parasite species, 19 (38%) by two species, 5 (10%) by three species and 2 mink (4%) by four species. All helminth species identified are native to European mustelids. Statistical models were used to evaluate if animal characteristics (age, sex and weight), date and capture area influenced the prevalence, intensity or parasite richness. Statistical differences were detected only in models for intensity of *M. patens*, *A. putorii* and *C. melesi*. This is the first report of *Angiostrongylus daskalovi*, a cardiopulmonary nematode, and *A. annulosa*, a gastrointestinal nematode specific of rodents, in American mink. Moreover, although the fluke *T. acutum* has already been cited in American mink, to our knowledge, the present study represents the first report of this trematode in the lung.

1. Introduction

The American mink (*Neovison vison* Schreber, 1777) is a semi-aquatic mustelid native to North America, widespread in this area with the exception of the Arctic Circle, the southern of United States and Mexico (Banfield, 1974; Linscombe et al., 1982; Melero and Palazón, 2011). It was introduced in Europe and the former USSR in the early 20s of last century for fur industry purposes (Vidal-Figueroa and Delibes, 1987; Dunstone, 1993; Melero and Palazón, 2011). In Spain, first fur farms were established during the late 1950s (Bravo and Bueno, 1992).

Accidental or deliberate releases consecutive to accidents (fires, windstorms, etc.), limited security measures and/or cessation of farm's activity led to the establishment of feral populations (Vidal-Figueroa and Delibes, 1987; Palazón and Ruíz-Olmo, 1997). In fact, the American mink is considered an invasive species in 28 European countries (Bonesi and Palazón, 2007). In Spain, the first feral mink was reported in Central Spain (Segovia) in 1978 (Delibes and Amores, 1978) and, since then, this mustelid has progressively colonized Southwest of Galicia (Vidal-Figueroa and Delibes, 1987), Northeastern Catalonia (Ruíz-Olmo, 1987) and Central Spain (Bueno and Bravo, 1990). Currently, three other core populations are established in Cantabria, North of Galicia and Teruel-Castellón (Ruíz-Olmo et al., 1997).

American mink is a generalist and opportunistic species which consumes a wide spectrum of both aquatic and terrestrial preys. Its diet varies depending on the habitat, the prey availability and the presence of other competitor species (Bonesi et al., 2004). Fragmented landscapes, with a wide variety of habitats and food resources, favor its

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presence (Rodríguez and Zuberogoitia, 2011). This mustelid preferably inhabits territories with well-structured riparian vegetation (Zuberogoitia et al., 2006; Zabala et al., 2007; Melero et al., 2008b; Melero and Palazón, 2011).

American mink can cause adverse effects on autochthonous prey populations (Bonesi and Palazón, 2007), such as European crayfish (*Austropotamobius pallipes*), Pyrenean desman (*Galemys pyrenaicus*), water vole (*Arvicola sapidus*) and Mediterranean water shrew (*Neomys anomalus*) (Palazón and Ruíz-Olmo, 1997; Bergmans and Blom, 2001; Palomo and Gisbert, 2002; García-Díaz et al., 2013). Moreover, it could have a negative impact on the populations of other carnivores, such as the European mink (*Mustela lutreola*), the otter (*Lutra lutra*) and the European polecat (*Mustela putorius*), due to the competition for space or food resources (Sidorovich et al., 1999; Melero et al., 2012). Furthermore, the introduction of an invasive species can have unpredictable epidemiological consequences, because it can carry alien agents potentially pathogenic for native host species or, alternatively, the same alien species can act as a new reservoir for autochthonous infectious agents (Sepúlveda et al., 2014; Sherrard-Smith et al., 2015). For all these reasons, a national specific regulation was published in Spain, and the Ministry of Agriculture, Food and Environment developed the program "Management strategy, control and eradication of the American mink in Spain".

The American mink's control programs in mainland areas are quite complex and, moreover, the area from which the species is removed can be easily recolonized (Bryce et al., 2011). For these reasons, this carnivore is likely to persist in Spain over a long period of time. Therefore, it is important to acquire further information about the epidemiological role of this mustelid in the maintenance and diffusion of infectious pathogens. The aim of this study was to describe the gastrointestinal and cardiorespiratory macroparasites of the American mink in Galicia (NW Spain), an area where the species is present since the 1980s (Fig. 1).

Table 1
Distribution of the American mink (n = 50) by month of capture, sex and age.

Month	Number of mink				
	Sex		Age		Total
	Male	Female	Immature	Adult	
January	1	0	0	1	1
February	10	2	5	7	12
March	6	2	3	5	8
April	1	0	1	0	1
May	4	2	2	4	6
June	0	2	2	0	2
July	3	4	5	2	7
August	2	0	0	2	2
September	2	0	0	2	2
October	1	0	0	1	1
November	3	2	2	3	5
December	2	1	1	2	3
Total	35	15	21	29	50

2. Material and methods

2.1. Sampled animals

American mink included in this study were caught in river basins from Lugo, Ourense and Pontevedra provinces (Galicia, northwest Spain). The climate in Galicia is predominantly oceanic. The average annual rainfall is 1180 mm, with a homogeneous distribution throughout the year, and the average annual temperature is 13.3 °C, with limited yearly variations (Castillo-Rodríguez et al., 2007).

Between 2008 and 2014, fifty American mink (the sample distribution by sex, age and month of capture is shown in Table 1) were captured during the official control plan implemented by the Galician administrative authority (Consellería de Medio Ambiente, e Ordenación do Territorio).

Captured mink were sent to the Wildlife Recovery Centers of O Veral (Lugo), O Rodicio (Ourense) and Cotorredondo (Pontevedra),

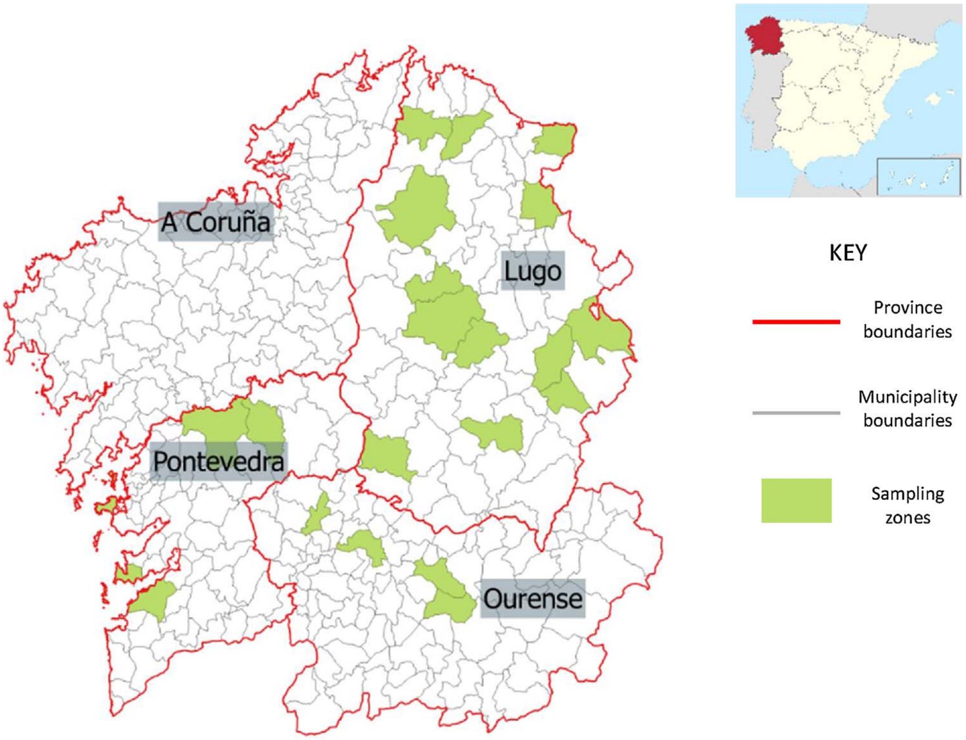

Fig. 1. Map showing the sampling zones in Galicia (northwestern Spain).

Table 2
Gastrointestinal (*) and cardiorespiratory (**) parasites recovered from 50 American mink captured in Galicia, Spain.

	Prevalence% (95% C.I.)					Range	Median intensity ± SD	Median abundance ± SD
	Total	Sex		Age				
		Male	Female	Immature	Adult			
*Molineus patens**	68 (54.6–81.3)	77 (62.5–91.7)	46.6 (18.0–75.2)	59.0 (36.7–81.4)	75.0 (57.9–92.0)	1–193	8.0 ± 33.6	5.0 ± 28.8
*Aonchotheca putorii**	54 (39.6–68.3)	57.1 (39.8–74.3)	46.6 (18.0–75.2)	50.0 (27.3–72.6)	57.1 (37.6–76.6)	1–83	5.0 ± 21.7	1.0 ± 17.1
*Crenosoma melesi***	10 (1.3–18.6)	11.4 (0.3–22.5)	6.6 (0.0–20.9)	9.0 (0.0–22.1)	10.7 (0.0–22.9)	1–42	1.0 ± 18.2	0.0 ± 5.9
*Aonchotheca annulosa**	8 (2–15.7)	8.5 (0.0–18.3)	6.6 (0.0–20.9)	4.5 (0.0–13.9)	10.7 (0.0–22.9)	2–29	10.5 ± 11.7	0.0 ± 4.6
*Angiostrongylus daskalovi***	6 (0–12)	8.5 (0.0–18.3)	0	9.0 (0.0–22.1)	3.5 (0.0–10.8)	1–3	2.0 ± 1.0	0.0 ± 0.52
Aelurostrongylus spp.**	2 (0–6)	2.8 (0.0–8.6)	0	0	3.5 (0.0–10.8)	1	–	–
*Troglotrema acutum***	2 (0–6)	2.8 (0.0–8.6)	0	4.5 (0.0–13.9)	0	7	–	–
Trematode spp.*[a]	2 (0–6)	2.8 (0.0–8.6)	0	4.5 (0.0–13.9)	0	27	–	–

[a] Genus was not determined due to the poor preservation status of these specimens.

where animals were euthanized by the veterinary staff, under current Spanish and European Animal Welfare Legislation.

2.2. Laboratory procedures

Digestive and cardiorespiratory tracts of the necropsied mink were removed and individually stored in labelled plastic bags. All samples were frozen (−20 °C) and sent to the Veterinary Faculty of Murcia for parasitological evaluation.

The digestive viscera (stomach, small and large intestine, liver and esophagus) were analyzed. Sections of the alimentary tract were longitudinally opened and scraped, the content was washed over a sieve (mesh size 0.3 mm diameter), and both content and mucosa were observed under a stereomicroscope to detect helminth specimens. Liver parenchyma was sliced and bile ducts and gallbladder were opened and also examined using a stereomicroscope. Heart, pulmonary veins and pulmonary arteries were carefully separated from trachea and lungs. Trachea, bronchial tree and pulmonary blood vessels were longitudinally opened, washed over a sieve 0.3 mm diameter and, subsequently, the retained material was observed by stereomicroscope. To investigate the presence of lung larvae, the filtrated liquid was collected and examined using the Baermann larval migration technique. Once completed the examination of the lung parenchyma by stereomicroscope, this tissue was also analyzed with the same technique. In cases where bronchopulmonary larvae were detected, lung parenchyma was cut into small stripes (approximately 0.5 cm diameter) and, subsequently, enzymatically digested in freshly prepared 1.5% (w/v) pepsin (1:10,000 activity) and 1.5% (w/v) chlorhydric acid in distilled water in order to isolate adult nematodes. Digestion was carried out at 40 °C for 1 h under gentle shaking, after which the undigested remains were filtered through a sieve of 63 µm diameter to collect the nematodes. Finally, the heart chambers and the major blood vessels were carefully opened, washed, and the resultant liquid was filtered through a 63 µm sieve before stereomicroscopic examination of the retained material.

2.3. Parasite identification

Parasites collected from each American mink were washed in distilled water, counted and stored in tubes with 70% ethanol for further examination. For taxonomic identification purposes, nematodes were cleared with lactophenol, while flukes were stained with Semichon's acetic carmine (Schmidt, 1986). Parasites were morphometrically identified attending Skrjabin (1964), Skrjabin and Petrow (1928), Yamaguti (1971), Butterworth and Beverley-Burton (1980), Janchev and Genov (1988), Mascato et al. (1993), Popiolek et al. (2009) and Gherman et al. (2016).

2.4. Statistical analyses

Prevalence, intensity and abundance were calculated for each parasite genus or species according to Bush et al. (1997), as well as parasite richness (number of parasite species per host). Data obtained from parasitological analysis (prevalence, intensity, richness—considered as dependent variables) and host characteristics (sex, age, weight, capture area, month and season of trapping—considered as risk factors) were analyzed using a Generalized Linear Models (GLM).

Different distribution families were used for the GLM analysis, according to the distribution of the dependent variable. A binomial distribution was used to analyse prevalence, both Poisson and negative binomial distributions were tested for intensity and, finally, a Poisson distribution was used for richness. The multivariate analysis for prevalence was carried out only for the species whose prevalence was higher than 10%, while the analysis of intensity and richness considered all parasite species. A stepwise approach and the "Akaike information criterion (AIC) were used to select the best model" (Akaike, 1974). Significant level was considered for $p < 0.05$

Statistical analysis was performed using the software R 3.1.0 (R Core Team, 2014). The package MASS was used to apply the negative binomial GLM.

3. Results

Six nematode and two trematode species were recovered. Eighty-two percent of mink (41/50, C.I. 95% = 70.9–93) harboured at least one helminth species, 52% (26/50, C.I. 95% = 37.6–66.3) were infected with two or more species, and a maximum of four species were detected in 4% of mink (2/50, C.I. 95% = 0–9.6%).

Prevalence, median intensity and median abundance of these helminths are shown in Table 2.

Angiostrongylus daskalovi, *Aelurostrongylus* spp., *Troglotrema acutum* and the unidentified trematode were found only in male mink. *Aelurostrongylus* spp. was found only in one adult mink, whereas *T. acutum* and the unidentified trematode were only found in one immature animal, respectively.

Statistically significant influence of some risk factors were detected in models only for intensity of *Molineus patens* and, *Aonchotheca* (*Capillaria*) *putorii*. Concretely, the best model explaining the intensity of *M. patens* selected the sex and season (AIC = 253.6; explained variance = 46.6%), with males and spring showing a lower *M. patens* intensity. Regarding *A. putorii*, the best models selected the age and season, detecting the lower intensity in subadult mink and in winter (AIC = 192.34, explained variance = 31.5%); specifically, mink captured in July showed higher intensity of this nematode species

($p < 0.001$).

No significant effect of the studied factors was observed for prevalence and parasite richness.

4. Discussion

The overall prevalence of helminths in our study is similar to the one described in American mink from Southwest of France (81%) (Torres et al., 2008) and Belarus (78%) (Shimalov and Shimalov, 2001). However, it should be noted that the number of parasitized animals in our study differs considerably from the results described by Torres et al. (2003) in American mink from 12 Spanish provinces; these authors divided their sample in two groups: one including American mink from areas where European mink was present, and the other group with American mink captured in areas where the European mink was absent. In this study, the prevalence was significantly lower than in our study (respectively 35.5% and 48.0%).

Moreover, parasite richness (eight species) and helminths identified in our study differ from the values recorded in other European areas. Concretely, Shimalov and Shimalov (2001) found higher parasite richness (19 species) in Belarus. In the same country, Sidorovich and Anisimova (1997) found a higher richness in American mink captured in less polluted areas than in those from contaminated ones (17 and 12 species, respectively). Our results are closer to those described in Torres et al. (2008) in France (richness of nine species) and to the seven species that had been previously described in American mink captured in Spain (Torres et al., 2003). According to these authors, a greater number of species may be associated to an earlier host adaptation to the area. In this sense, American mink has been described as invasive species in Belarus since the 50 s of last century (Sidorovich, 1993) and, therefore, this nonindigenous mustelid has shared habitat for a longer time with the native parasite fauna than the American mink in Galicia. However, this wide parasite richness also includes some helminths that could have been introduced in Belarus carried by the American mink from its natural distribution area, as may happen with *Baylisascaris devosi*, *Capillaria mustelorum*, *Metorchis albidus* and *Apophallus donicus* (Anisimova, 2004). Furthermore, differences in parasite richness between Galicia and Belarus could be due to the wide expansion of American mink in Belarus, since parasite richness tends to increase when the host species is distributed along greater territories (Morand, 2015). In this sense, according to Bonesi and Palazón (2007), American mink is much more spread in Belarus than in Spain. So, it is reasonable to assume that, if American mink continues its expansion across the Iberian Peninsula, it could increase its parasite richness, acquiring a new epidemiological role in the maintenance and diffusion of parasites shared by other autochthonous mustelids.

So far, all the helminth species found in American mink captured in Spain are native from European mustelids (Torres et al., 2003, 2006), as also in our study. These findings suggest that American mink lost their original helminths during its adaptation to Iberian environments (Torchin et al., 2003) or, as the feral populations comes from animals raised in fur farms, it is possible that these American mink were treated against parasites, eliminating their specific parasites. It is well known that, in the case of alien invasive species released without previous antiparasitic treatment, it is possible the parasite adaptation to new habitats and autochthonous hosts; for example, Tizzani et al. (2011) found *Obeliscoides cuniculi* in European brown hare, probably introduced by the invasive Eastern cottontail rabbit (*Sylvilagus floridanus*) in areas where both lagomorphs live in sympatry (Tizzani et al., 2011, 2014). Our results suggest that American mink from Galicia is currently playing an epidemiological role in the maintenance of the life cycle of native European parasites, but fortunately not of alien parasites whose establishment in the invaded areas could have unpredictable consequences on European mustelids, with the risk of changes in the composition of host-parasite communities, and the incidence of polyparasitism within individual hosts (Polley and Thompson, 2015).

Molineus patens was the most prevalent parasite in our study (68%). This gastrointestinal nematode is frequently found in Iberian mustelids (Feliú et al., 1991 Torres et al., 1997). In the authors knowledge, this is the greatest record described in European studies to date: 50% in France (Torres et al., 2008), 9.7% in Spanish areas where American mink share habitat with European mink and 36% in the rest of Spain (Torres et al., 2003), and 8% in Belarus (Shimalov and Shimalov, 2001). According to Poulin and Mouillot (2003) and Sherrard-Smith et al. (2015), nonindigenous host species that have lost their natural parasites tend to acquire generalist parasites from other local host species. Our results indicate that American mink is a competent host for *M. patens*, a parasite common to Palearctic mustelids, contributing to its transmission and natural nidality in Galicia. In our study, males showed a significantly lower *M. patens* intensity than females, and spring was the season with a lower intensity. However, the heterogeneity of the sample not allow to establish any conclusion, and therefore further studies are necessary in order to improve the knowledge of these and other epidemiological aspects.

We also found the gastric nematode *A. putorii*, one of the most common parasite of mustelids worldwide. Although host's infection is usually associated to the direct ingestion of this parasite's eggs, it has also been described the role of earthworms as paratenic hosts (Anderson, 2000). Prevalence of *A. putorii* recorded in Galicia was considerably higher than the ones observed in other European areas: 25.8% in American mink captured in shared areas with European mink in Spain, and 12% in other Spanish territories (Torres et al., 2003); 20% in Belarus (Shimalov and Shimalov, 2001); and 18.42% in France (Torres et al., 2008). As in the case of *M. patens*, the high prevalence of *A. putorii* indicates that American mink is a well host involved in the natural nidality of this parasite in Galicia. In our study, the intensity of parasitic infection by *A. putorii* was significantly lower in subadult mink and in winter but, as in the above-mentioned case of *M. patens*, we have no sound arguments to justify these results that should be contrasted with future studies.

Crenosoma melesi is the third more prevalent (10%) parasite found in American mink from Galicia. Until now, this cardiorespiratory nematode has been cited only in American mink captured in Europe (prevalence of 2%) by Torres et al. (2003), but not in America. It has also been described in badger and other Iberian mustelids (Torres et al., 1997, 2001). This parasite has an indirect life cycle with slugs and snails as intermediate hosts (Anderson, 2000), indicating that these gastropods are part of the trophic resources of American mink in Galicia (Melero et al., 2008a). The remarkable prevalence found in our study suggests that American mink is a competent host for *C. melesi*, and provides evidence that this alien mustelid could impact on native mustelid populations by means of a spillback effect (Kelly et al., 2009).

To our knowledge, this is the first report of a nematode belonging to the genus *Angiostrongylus* in American mink. The enzymatic digestion of lung parenchyma has probably facilitated the isolation of this cardiorespiratory nematode since it is frequently found into the small vessels of lungs. In addition, this procedure is highly useful to avoid the underestimation of mustelid's respiratory helminths due to the scarce amount of pulmonary tissue usually obtained, allowing the detection of most of these small nematodes. The morphometric characteristics do not adjust to those of *A. vasorum*, a parasite recovered from domestic and wild canids worldwide (Segovia et al., 2001; Conboy, 2004; Bourque et al., 2008; Morgan et al., 2008; Gerrikagoitia et al., 2010; Eleni et al., 2013), and also described in Eurasian badger (*Meles meles*) from Spain (Miquel et al., 1993; Feliú et al., 1996; Torres et al., 2001). Specimens isolated in our study were identified as *A. daskalovi* according to Janchev and Genov (1988) and Gherman et al. (2016). This nematode has been previously cited in pine marten (*Martes martes*), stone marten (*Martes foina*) and badger from Bulgaria (Janchev and Genov, 1988), and in badgers from Spain and Romania (Gerrikagoitia et al., 2010; Gherman et al., 2016). Our findings are consistent with the hypothesis that an alien host species can be a competent host for native parasites, increasing the risk of transmission to other indigenous mustelids by parasite spillback (Kelly et al., 2009). In other words, American mink is a new host that participates in the indirect life cycle of *A. daskalovi*, acting as reservoir host that could change the epidemiological dynamics of this parasite and, consequently, producing a negative impact on native mustelids.

We have also identified *Aelurostrongylus* spp. in one adult American mink. Morphometric characteristics of the unique specimen found do not match with *Aelurostrongylus pridhami*, the species previously described in Eurasian badger (Torres et al., 2001) and European mink (Torres et al., 2003). Unfortunately, the nematode was cleared in lactophenol for the morphometric exam and it was not possible to analyze by PCR. *Aelurostrongylus* shows an indirect life cycle with slugs and snails as intermediate hosts (Anderson, 2000). As for *A. daskalovi* and *C. melesi*, the presence of this respiratory nematode in American mink indicates that it is a well-adapted host for native metastrongyloid species present in Galicia, participating in its life cycle owing to the abundance of gastropods in riparian areas, which probably form part of the mink's diet.

In our study, *A. annulosa* was isolated from the small intestine of four mink, even though this capillarid nematode is usually located in the small intestine of various species of rodents (Mascato et al., 1993; Feliu et al., 1997). Taking into account that rodents are part of the diet of American mink (Melero et al., 2008a; Morales et al., 2010), the finding of *A. annulosa* in this mustelid could be considered as a pseudoparasite due to the ingestion of infected rodents. However, nematodes in our study did not show any kind of degradation attributable to the digestion process, and all specimens were found in the small intestine (the usual microhabitat of *A. annulosa*), but not in stomach or large intestine; moreover, fertile female nematodes (with eggs inside) were recovered, as a proof of the species viability and its reproductive capacity into the small intestine of the American mink. In fact, *A. annulosa* has been previously described in other mammals different to rodents; in this sense, Umur et al. (2012) found *A. annulosa* in a Hamadryas baboon (*Papio hamadryas*) from a zoo in northern Turkey, attributing the infection to the ingestion of infective eggs expelled in the feces by a rodent. In our opinion, the finding of *A. annulosa* in the American mink indicates that this mustelid could have acquired the infection through the ingestion of embryonated eggs or invertebrates infected with third-stage larvae (Anderson, 2000). It could be interpreted as further evidence that native parasites are able to take advantage of the mink's presence to acquire a new host in the invaded habitat.

This is the first time that *T. acutum* is recorded in American mink from the Northwest of the Iberian Peninsula. This trematode was detected in one immature mink. Considering that we did not have the opportunity to examine the head of mink, our results probably underestimate the prevalence of *T. acutum* in Galicia, because its usual anatomical location are the nasolacrimal sinuses (Koubek et al., 2004; Torres et al., 2006). According to Koubek et al. (2004), the European polecat is the main definitive host, although other mustelid species could act as hosts and may contribute to the maintenance of this cranial fluke in the environment (Ribas et al., 2012), whose life cycle includes prosobranch snails and amphibians as intermediate hosts (Vogel and Voelker, 1978; Koubek et al., 2004). This parasite causes suppurative osteomyelitis and severe injuries to the skull bones (Jubb and Kennedy, 1963; Koubek et al., 2004; Torres et al., 2008). In Europe, the highest prevalences of *T. acutum* in American mink have been recorded in France (33%) by Torres et al. (2008) and in Álava (North of Spain), where the prevalence was 30.4% (Torres et al., 2006). In our study, we found seven specimens of *T. acutum*, all of them collected from the lung parenchyma. Since three of these trematodes were mature specimens, this finding indicates that *T. acutum* is able to originate ectopic foci and, moreover, to complete its development in the lung parenchyma of the American mink. This anatomical location could drive to even more severe pathological consequences than those assumed so far; unfortunately, the histopathological study to confirm the tissue damage could not be carried out as lung tissue was not preserved after dissection.

We found only one species of digestive trematode in our study. Concretely, twenty-seven immature specimens were isolated from the liver and the small intestine of a mink but, unfortunately, the morphometric identification was not completed due to their poor preservation status. In Europe, *Ascocotyle* spp., *Euparyphium melis*, *Pseudamphistomum truncatum* (whose intermediate hosts are fish) and *Euryhelmis squamula* (with amphibians as intermediate hosts) have been detected in American mink (Shimalov and Shimalov, 2001; Torres et al., 2008; Hawkins et al., 2010). In Spain, *E. squamula* is the only trematode species cited in American mink, showing a low prevalence (2–3.2%) (Torres et al., 2003). Despite fish and amphibians have been described as a main component of the diet of American mink in Galicia (Vidal-Figueroa and Delibes, 1987), recent studies (Romero, 2013, 2015) have demonstrated that its diet is focused mainly on rodents and other terrestrial animals, reducing the ingestion of aquatic and riparian preys in habitats where otter populations are expanding (Bonesi et al., 2004). So, the scarcity of digestive flukes in American mink from Galicia could be due to the diet of this allochthonous mustelid.

Any of the analyzed American mink was parasitized by cestodes. However, *Taenia martis* and *Taenia tenuicollis* have been previously cited in this mustelid from Spain with a prevalence of 2% and 3.2%, respectively (Torres et al., 2003). In France, Torres et al. (2008) found *T. tenuicollis* in European polecat (12.1%), but not in American mink. In Belarus, Shimalov and Shimalov (2001) detected *T. mustelae* (synonym *T. martis*) with a low prevalence (4%), and *Spirometra erinacei* larvae (10% prevalence) in American mink. According to Vidal-Figueroa and Delibes (1987) and Romero (2013, 2015), the diet of the American mink in Galicia includes small mammals (the intermediate hosts of all these tapeworms) but, as mentioned above, these helminths were not detected in our study. These results could indicate that cestodes are not frequent in the mustelid species present in Galicia.

As shown in the present study, American mink is an invasive species that can acquire parasites from the indigenous host species with which this mustelid shares habitat, and thus, may achieve a relevant epidemiological role on these parasites' natural nidality. Moreover, our results pointed to an unpredictable environmental impact of these adaptive phenomena, mainly in other mustelid species that inhabit the same area, since parasites from different host species are included, as happens with *A. daskalovi*. The American mink can contribute to the dispersion of these parasites among populations of native mustelids in Spain. This can turn into a severe conservation challenge for endangered species such as the European mink. In this sense, we recommend the parasite monitoring of American mink populations in habitats where this allochthonous mustelid are present, mainly in new areas of invasion.

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Cardiopulmonary nematode infections in wild canids: Does the key lie on host-prey-parasite evolution?

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ABSTRACT

Cardiopulmonary nematodes are among the most pathogenic parasites of domestic and wild canids. The aim of this study was to describe the species diversity, prevalence and infection intensity of these parasites in the Iberian wolf (*Canis lupus signatus*) and the red fox (*Vulpes vulpes*) in the northwest of the Iberian Peninsula. 257 foxes and 74 wolves were necropsied between 2008 and 2014. Four nematode species were identified: *Angiostrongylus vasorum*, *Eucoleus aerophilus*, *Crenosoma vulpis* and *Filaroides hirthi*. This last species was only found in wolves, being the first time that is cited worldwide in this wild canid. The overall parasite prevalence was significantly higher in foxes (70%) than in wolves (28%). Specifically, prevalences in foxes and wolves were, respectively, 43% and 22% for *A. vasorum*, 33% and 5% for *E. aerophilus*, and 30% and 9% for *C. vulpis*. The prevalence of *F. hirthi* was 16%. The *A. vasorum* intensity was significantly higher in foxes than in wolves. Differences between host species in the risk of infection would be associated to diverging feeding behavior, and possibly reflects a parasite-host adaptation related to host's hunting strategies and cardiorespiratory requirements. This study revealed an association between infection and environmental factors, and highlighted a wide variation in the spatial distribution of *A. vasorum*. Our results indicate that cardiopulmonary parasites are widespread in wild canids in northwest Spain, and further agrees with other studies indicating the expansion of *A. vasorum* in Europe and, therefore, the urgent need to investigate infection in dogs in sympatric areas.

1. Introduction

The Iberian wolf (*Canis lupus signatus*) and the red fox (*Vulpes vulpes*) are the only two wild canid species present in the Iberian Peninsula. There are around 2000 wolves in Spain, with the main population located in the northwest (Blanco and Cortés, 2012). In contrast, red fox can be found throughout the Iberian Peninsula (Gortázar, 2007). Both species occupy a wide variety of habitats, including anthropogenic landscapes (Blanco, 2017; Harris and Smith, 1987; Gloor et al., 2001; López-Martín, 2017).

Parasite distributions in wildlife are highly conditioned by the nature of their life-cycle. Specifically, in the case of trophic transmitted parasites of carnivores, the kind of prey affects both predator-prey and host-parasite interactions and, thus, potentially influence the dynamics and trophic web structure (Friesen and Roth, 2016). In this regard, it has been suggested that vertebrate carnivores are able to balance their nutrient intake through selective predation or feeding to obtain specific nutrients (Kohl et al., 2015; Potter et al., 2018). On the other hand, the size of the prey caught by carnivores and, consequently, the foraging strategy are influenced by the ecological niches available to them and the encounter rate with prey. In fact, large terrestrial mammalian carnivores over 21.5 kg usually prey on large-sized animals to maximize their energetic intake while minimizing their energy expenditure (Carbone et al., 1999; Carbone et al., 2007).

There are remarkable differences between the diet of the wolf and the fox; the wolf prefers to prey on domestic and wild ungulates (Llaneza et al., 2012; Torres et al., 2015; López-Martín, 2017), whereas the fox is a generalist predator with a high ecological plasticity

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(Dell'Arte et al., 2007) that feeds on a broad trophic spectrum, including vegetables, small prey, carrion and garbage (Díaz-Ruiz et al., 2013; López-Martín, 2017).

Cardiopulmonary nematodes are among the most important parasites of canids, specially *Angiostrongylus vasorum* and *Dirofilaria immitis*, which are highly pathogenic and emerging parasites that cause congestive heart failure, severe dyspnea and even death (Traversa et al., 2010). The impact of these and other cardiopulmonary parasites as *Crenosoma vulpis* and *Eucoleus aerophilus* on wild canids has not been analyzed but, based on the evolutionary proximity of wolves, foxes and dog, we could assume a comparable pathogenicity in all species. Moreover, considering that wolves and, to a lesser extent, foxes require optimal cardiorespiratory capacity for successful predatory activity, it is reasonable to infer that cardiopulmonary parasites could have a significant impact on the species survival and especially on individuals with high parasite loads and co-infections. The trend so far has been to study cardiopulmonary nematodes in wolves and foxes separately, without considering that both species share habitats and are susceptible to the same parasites. For all these reasons, the objective of this study was to describe the cardiopulmonary parasites of wolves and foxes in the northwestern Iberian Peninsula, discussing the epidemiological role that each of these wild canids have in the natural nidality of these parasites.

2. Material and methods

The study was performed on Iberian wolves and red foxes from the regions of Asturias and Galicia (northwest Spain), covering an area of approximately 11,000 km^2 and 30,000 km^2, respectively. Galicia includes the provinces of A Coruña, Lugo, Ourense and Pontevedra (Fig. 1). Climate is predominantly oceanic with average annual rainfall and temperature ranging 960–1688 mm and 11.4–13.3 °C (Castillo-Rodríguez et al., 2006; Valenzuela et al., 2018). The study was performed in 257 red foxes hunted in January and February 2008 in municipalities of A Coruña (34), Lugo (156) and Pontevedra (67), in the context of official hunting tournaments, and 74 Iberian wolves from Asturias (40)A Coruña (18), Lugo (8), Ourense (1) and Pontevedra (7), mostly shot in officially authorized hunts between 2009 and 2014, and some found dead due to road traffic accidents.

Animals were necropsied, and the trachea, lungs and heart were removed and stored frozen at −20 °C until examined for parasites. To this effect, following defrosting, the heart chambers and its great vessels were carefully dissected, washed and filtered through a 62 μm mesh sieve. Trachea, bronchial tree and pulmonary blood vessels were longitudinally opened and observed under a stereomicroscope to collect parasites. Subsequently, in order to remove nematodes that may have remained in the bronchi and of smaller diameter vessels, the lungs were washed through a sieve and the retained material was examined under a stereomicroscope. Finally, to ensure that no nematode was left undetected, pulmonary parenchyma was enzymatically digested in pepsin and chlorhydric acid, according to Martínez-Rondán et al. (2017).

Nematodes were cleared with lactophenol and identified according to Georgi (1979), Butterworth and Beverley-Burton (1980), Costa et al. (2003) and Popiolek et al. (2009). Prevalence (percentage of infected animals), parasite intensity (number of parasites in infected animals) and species richness (number of species in infected animals) were calculated (Bush et al., 1997) and Yate's-corrected chi-square test and the non-parametric Kruskal-Wallis test were used to compare proportions and medians, respectively. Mixed logistic regression models were developed to investigate the relationship between infection with a particular parasite species (outcome variable) and host species (fox or wolf), adjusted for sex, age and environmental variables of the area were animals were collected, and including municipality as a random effect to allow for spatial clustering of infection (Agresti, 2013). Environmental variables, included the human population density, average annual temperature and average annual rainfall, were categorized prior to inclusion in the model. Parameters were estimated using the maximum likelihood method, *p*-values were calculated with the chi-squared test and significance was considered at the 5% ($p < .05$) level for a two-tailed test. Analyses were carried out using R 3.4.3 software (R core Team, 2018).

3. Results

Four nematode species were identified: *Angiostrongylus vasorum*, *Eucoleus aerophilus*, *Crenosoma vulpis* and *Filaroides hirthi* (Table 1). Parasite prevalence (95% CI) was 70% (65–76) in foxes and 28% (18–39) in wolves ($p < .05$). The percentage of foxes infected with one, two or three nematode species were 54% (98/181), 40% (73/181) and 6% (10/181), respectively. These same prevalences for wolves were 62% (13/21), 24% (5/21), and 5% (1/21), and 10% (2/21) of wolves were infected with the four nematode species.

The parasite distribution in infected foxes and wolves was widely variable and positively skewed, with most animals having low parasite burdens. Foxes had the largest number of *A. vasorum*, *C. vulpis* and *E. aerophilus*, and median *A. vasorum* and *C. vulpis* intensity were higher ($p < .05$) and marginally higher (p-value = .06), respectively, in this host species compared to wolves. It was not possible to estimate the median intensity of *F. hirthi*, because most nematodes were fragmented or damaged after the enzymatic digestion of the pulmonary parenchyma required to detect this small nematode species.

The prevalence and median intensity of *A. vasorum*, *C. vulpis* and *E. aerophilus* in wolves and foxes varied according to some of the environmental variables, but they did not follow an increasing or decreasing trend with any of the variables analyzed except for *E. aerophilus* prevalence in foxes, which decreased with increasing annual rainfall (Tables 2 and 3).

The logistic regression models confirmed the significantly higher risk of foxes being infected with cardiopulmonary parasites compared to wolves, and the association between *A. vasorum* infection and areas with medium human population density, *C. vulpis* and juvenile hosts, and *E. aerophilus* and low temperature and precipitation ($p < .05$). Moreover, *A. vasorum* models revealed additional, unexplained variation in the risk of infection between municipalities, highlighting the strong spatial aggregation of this parasite species (Table 4).

4. Discussion

Angiostrongylus vasorum has been described in canids worldwide (Spratt, 2015). However, until a few decades ago, its geographical distribution in Europe was localized to a few endemic foci (Morgan et al., 2009). The study confirms that *A. vasorum* is endemic in northwest Spain with higher prevalence in foxes (43%) than elsewhere in the Iberian Peninsula (16–36%) (Gortázar et al., 1998; Segovia et al., 2004; Mañas et al., 2005; Eira et al., 2006; Gerrikagoitia et al., 2010). Likewise, prevalence of this species in wolves (22%) was higher than in previous surveys in northwest Spain (2–5%) (Torres et al., 2000; Segovia et al., 2001; Segovia et al., 2007a, 2007b; Garrido-Castañé et al., 2015). The sensitivity of detection of *A. vasorum* in foxes by dissection of the heart and pulmonary arteries is estimated at 84% (Houpin et al., 2016). We additionally used enzymatic digestion of lung parenchyma to reduce the number of false-negative cases. It is likely that the prevalence of *A. vasorum* was underestimated in other studies in which enzymatic digestion was not used.

The prevalence of *A. vasorum* was higher in foxes than in wolves, coinciding in general terms with the literature mentioned above. This result reflects dietary differences between species, with foxes feeding more on intermediate and paratenic hosts of this parasite than wolves, including a wide variety of gastropod species (Ferdushy and Hasan, 2010), and frogs and birds (Bolt et al., 1993; Elsheikha et al., 2014; Mozzer and Lima, 2015), respectively. Moreover, the similar prevalence and *A. vasorum* intensity in juvenile and adult foxes indicates that these

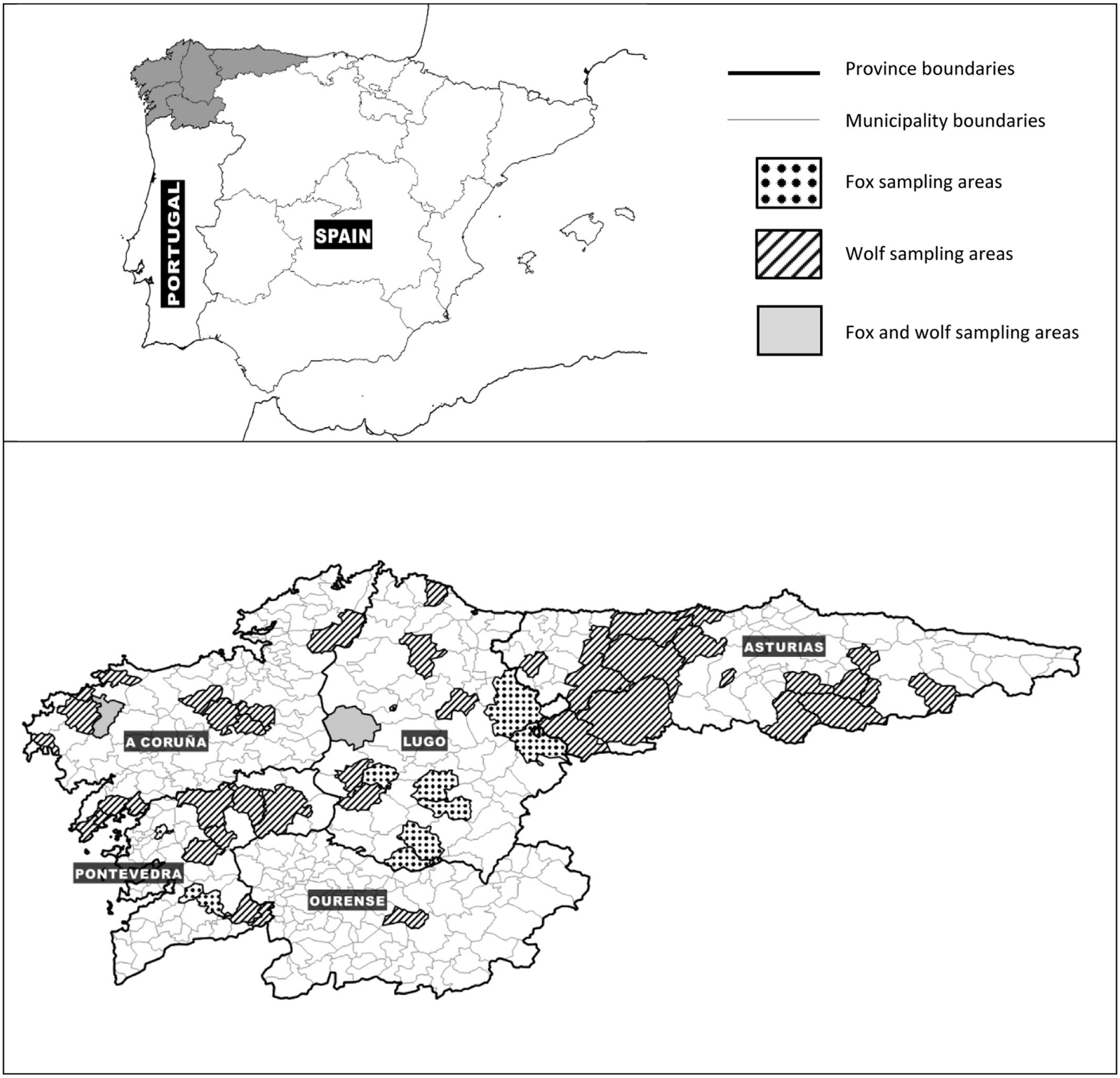

Fig. 1. Municipalities in northwestern Spain where wolves and foxes were sampled to study the cardiopulmonary nematode species.

Table 1

Prevalence (95% CI), median intensity and range of cardiopulmonary nematode species detected in foxes and wolves from northwestern Spain ($n = 331$).

Parasite species	Foxes ($n = 257$)			Wolves ($n = 74$)			All (n = 331)		
	Prevalence (%)	Median intensity	Range	Prevalence (%)	Median intensity	Range	Prevalence (%)	Median intensity	Range
Angiostrongylus vasorum	43.2 (37.0–49.2)	7.0	1–422	21.6 (12.0–31.2)	1.5	1–48	38.4 (33.1–43.6)	6.0	1–422
Crenosoma vulpis	30 (24.3–35.6)	3.0	1–140	9.4 (2.6–16.2)	1.0	1–8	25.4 (20.6–30.1)	3.0	1–140
Eucoleus aerophilus	33.4 (27.6–39.2)	4.0	1–36	5.4 (0.1–10.6)	8.5	1–14	27.2 (22.4–32.0)	4.0	1–36
Filaroides hirthi	0	–	–	16.2 (7.6–24.8)	ND	ND	3.6 (1.6–5.6)	ND	ND

Prevalence refers to the percentage of parasitized animals, and intensity to the number of nematode specimens in parasitized individuals; ND: not determined.

intermediate and paratenic hosts are food sources for foxes throughout their life, and that the host's immune response tolerates or is unable to eliminate this parasite; this finding is in agreement with Gillis-Germitsch et al. (2017), who showed that previously exposed adult foxes can be reinfected and suffer persistent infections. However, the fact that a substantial number of wolves were also infected with *A. vasorum* emphasizes the potentially important epidemiological role of wolves in maintaining its life cycle. In contrast to foxes, *A. vasorum*

Table 2
Prevalence and median intensity of cardiopulmonary nematode species found in foxes (n = 257) according to host and environmental features.

Variable	Levels	n	*Angiostrongylus vasorum*					*Crenosoma vulpis*					*Eucoleus aerophilus*				
			% p (pos/n)[a]	*P* value	Median	Maximum	P value	% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value	Median	Maximum	P value
Age	Adult	186	44.1 (82/186)	0.743	7.0	163	0.677	24.2 (45/186)	0.002	3.0	110	0.623	36.6 (68/186)	0.12	5.0	36	0.034
	Juvenile	71	40.8 (29/71)		6.0	433		45.1 (32/71)		3.0	140		25.4 (18/71)		2.0	15	
Sex	Female	108	48.1 (52/108)	0.216	7.5	53	0.631	27.8 (30/108)	0.608	3.5	92	0.627	32.4 (35/108)	0.864	2.0	30	0.021
	Male	149	39.6 (59 /149)		6.0	422		31.5 (47/149)		3.0	140		34.2 (51/149)		5.0	36	
Human population density (people/km^2)	3.1–13.3	105	28.6 (30/105)	0.000	4.0	163	0.013	41.9 (44/105)	0.001	3.0	140	0.173	48.6 (51/105)	0.000	3.0	30	0.439
	13.4–54.6	71	73.2 (52/71)		10.0	422		26.8 (19/71)		5.0	65		14.1 (10/71)		2.0	12	
	54.7–294	81	35.8 (29/81)		6.0	50		17.3 (14/81)		2.0	15		30.9 (25/81)		5.0	36	
Altitude (m)	0–316	130	53.8 (70/130)	0.000	9.0	422	0.024	20.0 (26/130)	0.000	3.5	65	0.731	18.5 (24/130)	0.000	6.0	36	0.020
	317–633	98	28.6 (28/98)		3.5	51		44.9 (44/98)		3.0	140		37.8 (37/98)		2.0	30	
	634–950	29	44.8 (13/29)		5.0	163		24.1 (7/29)		3.0	12		86.2 (25/29)		5.0	21	
Annual temperature (°C)	8.8–10.7	44	40.9 (18/44)	0.440	5.5	163	0.238	27.3 (12/44)	0.000	3.0	12	0.484	72.7 (32/44)	0.000	4.0	21	0.043
	10.8–12.6	111	39.6 (44/111)		12.5	78		44.1 (49/111)		3.0	140		26.1 (29/111)		2.0	30	
	12.7–14.4	102	48.0 (49/102)		6.0	422		15.7 (16/102)		3.0	65		24.5 (25/102)		5.0	36	
Annual rainfall (mm)	894–1270	114	40.4 (46/114)	0.506	5.0	163	0.025	33.3 (38/114)	0.017	3.0	140	0.405	54.4 (62/114)	0.000	5.0	36	0.034
	1271–1660	76	48.7 (37/76)		13.0	78		36.8 (28/76)		2.0	110		26.3 (20/76)		2.5	13	
	1661–2040	67	41.8 (28/67)		6.0	422		16.4 (11/67)		3.0	65		6.0 (4/67)		1.5	4	

[a] % p (pos/n): % prevalence (positive animals/studied animals).

prevalence and median intensity was higher in juvenile compared to adult wolves, supporting that gastropods and paratenic hosts of *A. vasorum* constitute part of the diet of juveniles during the developmental period until they become accomplished adult hunters of large prey (MacNulty et al., 2012; Zimmermann et al., 2015). It is important to point out, moreover, that carnivores modify their nutrient intake through selective predation or feeding in order to obtain a balanced diet (Kohl et al., 2015). This could partly explain that 19% of adult wolves were parasitized by *A. vasorum*; that is, although adults usually hunt large animals, they also eat smaller prey.

The geographic distribution of *A. vasorum* is characterized by stable foci of high prevalence (Morgan et al., 2009), mostly in areas with mild and wet climates, as is our study area, since the invertebrate hosts are highly sensitive to temperature and moisture (Jeffery et al., 2004; Ferdushy and Hasan, 2010). In Europe, the distribution of *A. vasorum* has increased in recent decades, probably due to the fox's expansion (Van Doorn et al., 2009; Al-Sabi et al., 2013, Al-Sabi et al. 2014). Although the pathogenicity of *A. vasorum* has been described in foxes (Morgan et al., 2008) and wolves (Eleni et al., 2014; De Liberato et al., 2017), the impact on the wider population is unknown. Also, the role of foxes and wolves in the epidemiology of infection in dogs living in close-by areas is also discussed (McCarthy et al., 2016; Schug et al., 2018; Lange et al., 2018). Although few studies compared *A. vasorum* prevalence in synanthropic wild and domestic canids, a wide molecular study of strains from dogs and foxes revealed no evidence of genetic segregation suggesting a common transmission cycle (Jefferies et al., 2010). In our study, prevalence was highest in rural areas with medium human density, as well as the highest intensities in foxes. We hypothesize that, in these rural areas, there are probably more dogs that are allowed to roam freely and, therefore, have the possibility of ingesting intermediate hosts, playing a summative role in the local *A. vasorum* life cycle involving domestic and wild canids.

Crenosoma vulpis has been described in canids from temperate regions worldwide (Shimalov and Shimalov, 2000; Latrofa et al., 2015; Figueiredo et al., 2016; Maksimov et al., 2017), including the Iberian Peninsula, in both foxes (Gortázar et al., 1998; Segovia et al., 2004; Mañas et al., 2005; Eira et al., 2006; Garrido-Castañé et al., 2015) and wolves (Segovia et al., 2007a, 2007b). Like *A. vasorum*, gastropods are the intermediate hosts of this nematode species (Jeffery et al., 2004). The higher *C. vulpis* prevalence in foxes compared to wolves indicates than this host species plays a more prominent role than wolves in the epidemiological dynamics of this parasite. Similar to what has been argued in *A. vasorum*, this result suggests that both canids prey on gastropods, but more notably in foxes (Colella et al., 2016; Lange et al., 2018). *C. vulpis* was more prevalent in juvenile foxes than in adults, and was only found in juvenile wolves. This could be due to differences in trophic behavior and a more effective immune response in adults (Jeffery et al., 2004; Davidson et al., 2006; Hodžić et al., 2016). Notwithstanding this, some adult foxes had a high parasite burden which, although not fatal, would reduce the host's respiratory capacity significantly (Traversa et al., 2010).

Eucoleus aerophilus has been reported in wild and domestic canids (Torres et al., 2000; Morgan et al., 2008; Conboy, 2009; Traversa et al., 2009; Di Cesare et al., 2014). The prevalence in foxes was similar to those reported in other Iberian areas (Gortázar et al., 1998; Segovia et al., 2004; Mañas et al., 2005), contrasting with the low prevalence described by other authors (Eira et al., 2006; Martínez-Carrasco et al., 2007). High prevalences (65–84%) have been described in foxes in other European countries (Davidson et al., 2006; Saeed et al., 2006; Lalošević et al., 2013; Al-Sabi et al., 2014; Hodžić et al., 2016; Schug et al., 2018). In our study, *E. aerophilus* was the least prevalent cardiopulmonary nematode in wolves; in other European countries, the prevalence in wolves (8–36%) is usually lower than that described in foxes (Shimalov and Shimalov, 2000; Popiołek et al., 2007; Bagrade et al., 2009; Varodi et al., 2017). As with *C. vulpis*, the prevalence of *E. aerophilus* was related to altitude, annual temperature and annual

Table 3
Prevalence and median intensity of cardiopulmonary nematode species found in wolves (n = 74) according to host and environmental features.

Variable	Levels	n	*Angiostrongylus vasorum*					*Crenosoma vulpis*					*Eucoleus aerophilus*					*Filaroides hirthi*	
			% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value	Median	Maximum	P value	% p (pos/n)[a]	P value
Age	Adult	31	19.4 (6/31)	0.908	1.0	2	0.035	0.0 (0/31)	0.050	–	–	–	3.2 (1/31)	0.855	2.0	2	0.180	9.7 (3/31)	0.329
	Juvenile	43	23.3 (10/43)		2.0	48		16.3 (7/43)		1.0	8		7.0 (3/43)		9.0	14		21.0 (9/43)	
Sex	Female	38	18.4 (7/38)	0.686	1.0	4	0.424	10.5 (4/38)	1	1.0	5	0.659	5.3 (2/38)	1	11.0	14	0.439	18.4 (7/38)	0.831
	Male	36	25.0 (9/36)		2.0	48		8.3 (3/36)		1.0	8		5.6 (2/36)		5.5	9		13.9 (5/36)	
Human population density (people/km^2)	3.1–13.3	18	5.6 (1/18)	0.014	4.0	4	0.349	11.1 (2/18)	0.906	1.0	1	0.417	0.0 (0/18)	0.182	–	–	–	11.1 (2/18)	0.310
	13.4–54.6	41	34.1 (14/41)		1.5	48		9.8 (4/41)		3.0	8		9.8 (4/41)		8.5	14		22.0 (9/41)	
	54.7–294	15	6.7 (1/15)		1.0	1		6.7 (1/15)		1.0	1		0.0 (0/15)		–	–		6.7 (1/15)	
Altitude (m)	0–316	38	21.1 (8/38)	0.515	2.0	48	0.213	13.2 (5/38)	0.494	1.0	8	0.629	7.9 (3/38)	0.602	9.0	14	0.655	18.4 (7/38)	0.632
	317–633	32	25.0 (8/32)		1.0	4		6.3 (2/32)		3.0	5		3.1 (1/32)		8.0	8		15.6 (5/32)	
	634–950	4	0.0 (0/4)		–	–		0.0 (0/4)		–	–		0.0 (0/4)		–	–		0.0 (0/4)	
Annual temperature (°C)	8.8–10.7	20	10.0 (2/20)	0.331	1.5	2	0.224	5.0 (1/20)	0.426	1.0	1	0.779	5.0 (1/20)	0.796	8.0	8	0.259	5.0 (1/20)	0.127
	10.8–12.6	28	25.0 (7/28)		1.0	4		7.1 (2/28)		3.0	5		3.6 (1/28)		2.0	2		14.3 (4/28)	
	12.7–14.4	26	26.9 (7/26)		2.0	48		15.4 (4/26)		1.0	8		7.7 (2/26)		11.5	14		26.9 (7/26)	
Annual rainfall (mm)	894–1270	16	18.8 (3/16)	0.267	1.0	1	0.213	6.3 (1/16)	0.792	5.0	5	0.420	6.3 (1/16)	0.608	2.0	2	0.180	18.8 (3/16)	0.186
	1271–1660	44	27.3 (12/44)		2.0	48		11.4 (5/44)		1.0	8		6.8 (3/44)		9.0	14		20.5 (9/44)	
	1661–2040	14	7.1 (1/14)		2.0	2		7.1 (1/14)		1.0	1		0.0 (0/14)		–	–		0.0 (0/14)	

[a] % p(pos/n): % prevalence (positive animals/studied animals).

Table 4
Estimates of logistic regression models investigating the effect of host dependent variables (sex, age and canid species) and environmental variables (average annual temperature, average annual rainfall and human population density) over the prevalence of *A. vasorum*, *C. vulpis* and *E. aerophilus* (n = 331).

Variable	Levels	*Angiostrongylus vasorum*			*Crenosoma vulpis*			*Eucoleus aerophilus*		
		Estimate	Std. Error	P value	Estimate	Std. Error	P value	Estimate	Std. Error	P value
Fixed components										
Intercept		−2.5633	0.6245	0.0000	−3.0612	0.6089	0.0000	−0.7097	0.6979	0.3092
Sex	Female	0.0000			0.0000			0.0000		
	Male	−0.1210	0.2595	0.6410	0.1666	0.2791	0.5504	0.0532	0.3135	0.8653
Age	Adult	0.0000			0.0000			0.0000		
	Juvenile	−0.0324	0.2843	0.9094	0.9878	0.2945	0.0008	−0.2148	0.3499	0.5392
Host species	Wolf	0.0000			0.0000			0.0000		
	Fox	1.9509	0.4373	0.0000	1.7527	0.4690	0.0002	2.4230	0.6387	0.0001
Annual temperature (°C)	8.89–10.7	0.0000			0.0000			0.0000		
	10.8–12.6	−0.2429	0.4657	0.6020	0.6141	0.3983	0.1231	−2.1265	0.4558	0.0000
	12.7–14.4	0.2558	0.5788	0.6585	0.2894	0.6411	0.6518	−0.3551	0.6731	0.5978
Annual rainfall (mm)	894–1270	0.0000			0.0000			0.0000		
	1271–1660	−0.1021	0.4008	0.7990	0.3263	0.3785	0.3886	−1.0007	0.4415	0.0234
	1661–2040	−0.9809	0.5018	0.0506	−0.2696	0.5078	0.5954	−3.3379	0.6197	0.0000
Human density (people/km^2)	3.1–13.3	0.0000			0.0000			0.0000		
	13.4–54.6	2.1026	0.5008	0.0000	−0.6111	0.4324	0.1575	−0.8051	0.5472	0.1412
	54.7–294	0.4406	0.5108	0.3884	−0.8072	0.5052	0.1101	−0.7544	0.5527	0.1723
Random effect	Standard deviation			Standard deviation				Standard deviation		
Municipality	0.3404			< 0.0001				< 0.0001		

rainfall. This parasite has a direct life cycle, and earthworms can act as paratenic hosts (Anderson, 2000), yet there is limited information on other ecological and epidemiological aspects of *E. aerophilus* infection that could help interpret these associations.

Filaroides spp. has been sporadically described in dogs in Europe (Caro-Vadillo et al., 2005; Cervone et al., 2018) and rarely in wild canids (Magi et al., 2015). Sanchis-Monsonís et al. (2013) detected *F. hirthi* in 2% of foxes in southeast Spain. Here we report the first *F. hirthi* infection in wolves worldwide. The rare detection of this parasite in wild canids could be partly due to the fact that *F. hirthi* is a very small, fragile nematode located in the bronchioles and alveoli and, consequently, difficult to detect by direct examination. The absence or low prevalence of *F. hirthi* in wild canids in general should be evaluated with care, as the presence of this parasite in these host species may be underestimated. We have been able to detect *F. hirthi* because the lung parenchyma of each animal was enzymatically digested, which increases the probability of detection, as has been proven in dogs (Bahnemann and Bauer, 1994). This finding highlights the potential role of wolves in the maintenance of the parasite's transmission in nature. Dogs are infected by ingestion of infectious larvae present in saliva (Anderson, 2000). In the case of *Filaroides osleri*, maternal cleanliness is assumed to be an important transmission route in the dog, while regurgitation of food by parents to feed pups may also be an important form of transmission in wild canids (Polley and Creighton, 1977; Clayton and Lindsay, 1979; Dunsmore and Spratt, 1979). In addition, it has been suggested that infection in dogs is possible by coprophagy of fresh faeces (Georgi et al., 1979). Since *F. hirthi* and *F. osleri* belong to the same genus, it is assumable that the transmission is similar in both parasites and, therefore, the social behavior of the wolf could explain the intraspecific transmission of *F. hirthi*. Further epidemiological studies are needed to better understand the risk factors associated with *F. hirthi* transmission and to assess the role of the wolf as a reservoir of this parasite, as well as the degree of overlap between the domestic and sylvatic epidemiological cycles.

Finally, *Dirofilaria immitis* was not detected in our study, but this was somewhat not surprising, since the northwest Spain is considered an area of low risk of transmission, given that climatic conditions are not ideal for the mosquito vector (Simón et al., 2014). *D. immitis* has been previously detected in Iberian wolves (Segovia et al., 2001) and foxes (Gortázar et al., 1994; Gortázar et al., 1998; Mañas et al., 2005; Eira et al., 2006) in other parts of the Iberian Peninsula, and also in other European areas (Georgieva et al., 2001; Pascucci et al., 2007; Magi et al., 2008; Penezić et al., 2014).

5. Conclusions

Northwest Spain is highly endemic for *A. vasorum*, *C. vulpis* and *E. aerophilus*, and prevalence and infection intensity is significantly greater in foxes than in wolves. Differences between host species are probably related to differences in trophic behavior (Buck et al., 2018), with foxes of all ages and juvenile wolves feeding on intermediate and paratenic hosts of the first two nematode species, and adult wolves more on larger prey which not participate of the life cycle of these parasites. We hypothesized that this is an adaptive host-parasite response, so the highest infection risk occurs in the wild canid species (fox) that requires less cardiorespiratory effort to hunt their usual prey (Brose, 2010; Sand et al., 2016). In other words, cardiopulmonary nematodes, although adapted to several host species, have managed to link their life cycle to a type of prey more likely to be consumed by the smaller predator (fox). This prevents the cardiorespiratory capacity of a large predator, located at the top of the trophic chain (mainly adult wolves), to be compromised, which has meant the adoption of a trophic strategy by the wolf that reduces the risk of infection.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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