Evaluation of the role of the invasive American raccoon (*Procyon lotor*) as host of pathogens of importance for public and veterinary health

Carlos García Sanjuán

José Ignacio Aguirre & Michael Yabsley

Universidad Complutense de Madrid
Máster Universitario en Zoología

Evaluation of the role of the invasive American raccoon (*Procyon lotor*) as a host of pathogens of importance for public and veterinary health

- Trabajo Fin de Máster -

Carlos García Sanjuán

Departamento de Biodiversidad, Ecología y Evolución,
Universidad Complutense de Madrid

Septiembre 2021

El/La autor/a:

Fdo.:

El/La tutor/a:

Departamento y Centro

El/la tutor/a:

Fdo.:__________________________
Departamento y Centro

El/la tutor/a:

Fdo.:__________________________
Departamento y Centro

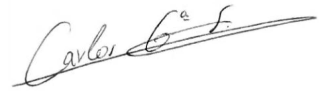
ANEXO I: DECLARACIÓN DE NO PLAGIO

D./Dña. Carlos García Sanjuán con NIF 49390683T , estudiante de Máster en la Facultad de Ciencias Biológicas de la Universidad Complutense de Madrid en el curso 2020-2021 , como autor/a del trabajo de fin de máster titulado Evaluation of the role of the invasive American raccoon (*Procyon lotor*) as a host of pathogens of importance for public and veterinary health y presentado para la obtención del título correspondiente, cuyo/s tutor/ es/son: José Ignacio Aguirre y Michael Yabsley

DECLARO QUE:

El trabajo de fin de máster que presento está elaborado por mí y es original. No copio, ni utilizo ideas, formulaciones, citas integrales e ilustraciones de cualquier obra, artículo, memoria, o documento (en versión impresa o electrónica), sin mencionar de forma clara y estricta su origen, tanto en el cuerpo del texto como en la bibliografía. Así mismo declaro que los datos son veraces y que no he hecho uso de información no autorizada de cualquier fuente escrita de otra persona o de cualquier otra fuente.
De igual manera, soy plenamente consciente de que el hecho de no respetar estos extremos es objeto de sanciones universitarias y/o de otro orden.

En Madrid, a 14 de Septiembre de 2021

Fdo.: 

Esta DECLARACIÓN debe ser insertada en primera página de todos los trabajos fin de máster conducentes a la obtención del Título.

Abstract

The American raccoon (*Procyon lotor*) is an invasive meso-carnivore which has been introduced and established in many European countries. Although the presence of the raccoon in the Iberian Peninsula was confirmed around 20 years ago, there are few data on pathogens of these animals in this region. For this work, 56 American raccoons from two subpopulations in the central region of the Iberian Peninsula were studied. Both ectoparasites and endoparasites were detected. Tick infestation was high (68%), increased during the sampling season, and richness was highest in the Henares subpopulation. Male raccoons also had a higher diversity of ectoparasites. *Rhipicephalus pusillus* was the most frequently found (64%), followed by *R. turanicus* (18%), *Ixodes ventalloi* (4%), and *Dermacentor marginatus* (2%). Regarding the fleas, *Pulex irritans* was the most prevalent (43%) followed by low rates of *Ctenocephalides felis* (4%) and *C. canis* (2%) infestations. In contrast to ticks, flea prevalence decreased throughout the sampling season. Raccoons had a very low prevalence and diversity of gastrointestinal parasites including nematodes (*Strongyloides procyonis*), cestodes (*Dilepis* sp.), trematodes (*Plagiorchis* sp.), and acanthocephalans (*Moniliformis moniliformis)*. Importantly, *Baylisascaris procyonis* was not found. Finally, no subcutaneous nematodes (i.e., *Dracunculus* and *Dirofilaria* spp.) were found. The results of this work show that the American raccoons currently harbor few endoparasites but are commonly infested with ectoparasites, several of which can transmit pathogens relevant for public and veterinary health.

Keywords: raccoon, *Procyon lotor*, Iberian Peninsula, invasive species, ticks, fleas, endoparasites.

Resumen

El mapache norteamericano (*Procyon lotor*) es un mesocarnívoro que ha sido introducido en muchos países de Europa, estableciéndose como especie invasora. A pesar de que la presencia del mapache en la Península Ibérica se confirmó hace unos 20 años, no existe apenas información acerca de los parásitos de estos animales en esta región. Para este trabajo se estudiaron 56 mapaches de dos subpoblaciones en la región central de la Península Ibérica. Se detectaron tanto ectoparásitos como endoparásitos. La infección por garrapatas fue alta (68%), aumentando durante la temporada de muestreo, y la riqueza de especies fue mayor en la subpoblación del Henares. Además, los mapaches macho presentaron una mayor diversidad de ectoparásitos. *Rhipicephalus pusillus* fue la especie más frecuentemente encontrada (64%), seguida de *R. turanicus* (18%), *Ixodes ventalloi* (4%), y *Dermacentor marginatus* (2%). Respecto a las pulgas, *Pulex irritans* fue la especie más prevalente (43%), seguida de bajas tasas de infección por *Ctenocephalides felis* (4%) y *C. canis* (2%). A diferencia de las garrapatas, la prevalencia de pulgas disminuyó a lo largo de la temporada de muestreo. Los mapaches presentaron una prevalencia y diversidad de parásitos intestinales muy bajas, incluyendo nematodos (*Strongyloides procyonis*), cestodos (*Dilepis* sp.), trematodos (*Plagiorchis* sp.), y acantocéfalos (*Moniliformis moniliformis)*. Importantemente, no se detectó *Baylisascaris procyonis*. Finalmente, tampoco se detectaron nematodos subcutáneos (por ejemplo, *Dracunculus* o *Dirofilaria* spp.). Los resultados de este trabajo muestran que los mapaches norteamericanos albergan actualmente pocos endoparásitos, pero son frecuentemente infectados por ectoparásitos, varios de los cuales pueden transmitir patógenos relevantes para la salud pública y veterinaria.

Palabras clave: mapache, *Procyon lotor*, Península Ibérica, especie invasora, garrapatas, pulgas, endoparásitos.

Index

1. Introduction

Invasive species are of importance due to their big negative impact for native ecosystems and species (Sakai *et al.*, 2001). These impacts can either be direct (e.g., predation, parasitism or competence for resources) or indirect (e.g., alterations of habitat or food chains). All of these affects the survival of native species and, therefore, becomes a problem to their conservation (Sakai *et al.*, 2001). On a global scale, 14% of threatened vertebrate species are negatively affected by invasive species, and this percentage is even greater for bird species (Duenas *et al.*, 2021). Of all taxon groups, birds, amphibians and mammals are the most affected by invasive species, with mammals being the most significant group of invasive taxa (Duenas *et al.*, 2021). There are different explanations for the success of the invasive species, generally related with the absence of predators or climatic factors, decrease in native parasites and diseases (enemy release hypothesis), as well as human activity or natural selection of the most adaptive genotypes (Colautti *et al.*, 2004).

The North American raccoon (*Procyon lotor*) is a meso-mammal native to the American continent where it ranges from Central America to Southern Canada (Bartoszewicz, 2011). One of the most important factors for the success of raccoon's ability to invade new areas is their diverse diet which includes both terrestrial and arboreal animals and plants (Bartoszewicz, 2011). Across their native and introduced ranges, raccoon populations can increase rapidly as females can breed as young as 1 year of age and can produce 3-5 kits per litter (Asano *et al.*, 2003).

Raccoons can negatively affect native fauna in numerous ways including direct competition for food or other resources or feeding on a specific type of prey, such as certain bird, crustacean, or other invertebrate species (Vilà *et al.*, 2006). Raccoons have a great plasticity when taking advantage of food resources and occupying different types of habitat (Beasley *et al.*, 2007). Among all the factors related to the presence of raccoons in a specific ecosystem, two are particularly important, the existence of water and wooded areas and areas with dense vegetation (Baldwin *et al.*, 2006). When available, raccoons take advantage of easy food items such as crops (e.g., corn (*Zea mays*) which means that raccoons can cause economic losses for farmers (Beasley *et al.*, 2007; Beasley & Rhodes Jr, 2008; Vilà *et al.*, 2006).

The first records of North American raccoons in Europe date from the 1930s, in the German region of Hessen, where a population of these animals established and multiplied very rapidly (Kauhala, 1996). Since that first introduction and throughout the last century raccoons spread to many other countries in Europe, including France, the Netherlands, Austria, Switzerland or Luxembourg (Frantz *et al.*, 2005; Kauhala, 1996). More recently, raccoons have also been documented in other countries such as Belgium, Italy, and northern and eastern European countries (Canova & Rossi, 2009; Ćirović & Milenković, 2003; Kauhala, 1996). Through a combination of both accidental or deliberate releases, raccoons have now established in at least 27 European countries (Salgado, 2018). Moreover, raccoon populations in central Europe are growing exponentially with an increase in the number of individuals by 300% compared to the 1990s (Salgado, 2018). Besides this, the American raccoon has also been introduced in in other parts of the world such as Japan or Iran (Asano *et al.*, 2003; Farashi & Naderi, 2017).

1.1 The American raccoon in the Iberian Peninsula

The first records of the North American raccoon in Spain were in 2001 from Algaida (Mallorca) (Salgado, 2015). Since then, raccoons has been reported in at least 30 locations on the Iberian Peninsula (García *et al.*, 2012). Currently, the raccoon is distributed throughout practically the entire region, with the populations in Santoña (Cantabria) and Malaga marking the northern and southern limits of distribution, respectively (García *et al.*, 2012). The raccoon population in the central region of the Iberian Peninsula, detected between 2004 and 2008 and which has more than 500 individuals, seems to be the most important within the whole of this territory (García *et al.*, 2012; Salgado, 2015). Over the last 15 years, more than 1,000 records of raccoons have been collected in the Iberian Peninsula, most of them (80%) belonging to observations in the provinces of Madrid and Guadalajara (Valdez, 2020). In 2011, raccoons were detected for the first time in Doñana National Park (Andalusia) (Fernández-Aguilar *et al.*, 2012), which poses a risk for the autochthonous species which inhabit this region. Later, raccoons were confirmed in the communities of Andalusia, Galicia and the Valencian Community (Salgado, 2015). The American raccoon has also been detected in other areas, such as Cantabria, Catalonia, the Basque Country, Murcia, Castilla-La Mancha, Extremadura, the Balearic Islands and the Canary Islands (García *et al.*, 2012; Salgado, 2015; Valdez, 2020; Vilà *et al.*, 2006). In addition, the data from the annual captures of raccoons in the different regions of the Iberian Peninsula suggest that the population densities of these animals are high in many of these places (Salgado, 2015). For this reason, the American raccoon is currently included in the Spanish list of invasive alien species, according to Real Decreto 630/2013 (B.O.E., 2013).

Similar to its native range of distribution, rivers and riparian zones in the Iberian Peninsula, which receives between 400 and 1450 mm of annual precipitation, are essential for the survival of raccoons (Baldwin *et al.*, 2006; Salgado, 2015; Valdez, 2020). An analysis of the diet of the raccoons showed that red swamp crayfish (*Procambarus clarkii*) and corn (*Zea mays*) are the preferential food items (Ceballos-Escalera *et al.*, 2013). Vegetable fruits and arthropods are also important elements in the diet (Salgado, 2015). Besides this, the American raccoon could become a grave problem for the native fauna species of the Iberian Peninsula. It has been documented that raccoons predate the Spanish pond turtle (*Mauremys leprosa*) (Alvarez, 2008) and several species of aquatic birds (García *et al.*, 2012). In the Canary Islands, an individual raccoon killed ~100 Scopoli's shearwaters (*Calonectris diomedea*), a protected species (García *et al.*, 2012). In the Balearic Islands, raccoons have been reported to prey on Balearic green toads (*Bufotes balearicus*) and Majorcan midwife toads (*Alytes muletensis*) (Salgado, 2015). Although the effect of raccoons on other species of Iberian meso-carnivores has not been studied, it is likely that these invasive animals could compete with native species for resources (Salgado, 2015).

Within the Iberian Peninsula, the raccoon population of the central region is believed to be the largest in terms of number of individuals, with more than 500 specimens (Alda *et al.*, 2013; García *et al.*, 2012; Salgado, 2015). Genetic characterization of raccoons in this region showed that there are two subpopulations, each of which originated from an introduction event of raccoons along the Henares and Jarama rivers, respectively (Alda *et al.*, 2013). Although raccoons were sporadically reported in the Community of Madrid between 2004 and 2007, it was in 2008 when the existence of a well-established population in this region was confirmed (García *et al.*, 2012). The origin of these raccoons seems to be related to escapes and/or deliberate releases of individuals (García *et al.*, 2012). Currently, the American raccoon is distributed throughout much of the Community of Madrid (Ceballos-Escalera *et al.*, 2013).

At present, the raccoon population occupies around 100 kilometres of territory: ~70% of them are located in the Parque Regional del Sureste, which includes part of the Jarama, Tajuña, Manzanares and Henares rivers; and the remaining 30~ are located following the Henares river to the province of Guadalajara (Ceballos-Escalera *et al.*, 2013). The highest population densities of raccoons in the Community of Madrid occur in the Parque Regional del Sureste, a region with many water courses, swampy areas and riverside forests, as well as irrigated crops, mainly corn (García *et al.*, 2012). The occurrence of raccoons in areas close to waterways confirms that they can act as natural corridors and hence facilitate the dispersal of this species (García *et al.*, 2012; Rodríguez & Miranzo; Valdez, 2020).

1.2 Ectoparasites of the American raccoon

In their native range of distribution, raccoons are hosts for a wide variety of parasites. Among them are lice, such as *Trichodectes octomaculatus*, or mites, such as *Androlaelaps fahrenholzi*, both species widely distributed in North America (Pung *et al.*, 1994; Rainwater *et al.*, 2017; Richardson *et al.*, 1994; Rockett & Johnston, 1988; Whitaker Jr & Goff, 1979). In the case of ticks there is a greater variation in their abundance and diversity depending on the different regions of the American continent. In some areas, species of the genus *Ixodes* (e.g., *I. texanus, I. cookei* or *I. scapularis*) are the predominant ones (Lee *et al.*, 2019; Richardson *et al.*, 1994; Rockett & Johnston, 1988; Smith *et al.*, 2019; Whitaker Jr & Goff, 1979). In other regions, however, there is a clear predominance of other species, such as *Amblyomma americanum* (Ouellette *et al.*, 1997; Pung *et al.*, 1994) or *Dermacentor variabilis* (Durden & Richardson, 2013; Hertz *et al.*, 2017). Another important species recently found in raccoons in the United States is the exotic Asian longhorned tick (*Haemaphysalis longicornis*) (Lee *et al.*, 2019; White *et al.*, 2021). Finally, regarding the fleas there is also a great diversity of species which frequently parasitize raccoons in their native range of distribution. Two of the most abundant species in this context are *Chaetopsylla lotoris* and *Orchopeas howardii* (Richardson *et al.*, 1994; Rockett & Johnston, 1988; Whitaker Jr & Goff, 1979). Furthermore, infestation with *Pulex simulans*, species of the genus *Ctenocephalides* (e.g., *C. felis*), and *Euhoplopsyllus glacialis* are also frequent (Durden & Richardson, 2013; Richardson *et al.*, 1994; Whitaker Jr & Goff, 1979).

Raccoons also act as hosts for different ectoparasite species in those regions where they have been introduced. This fact represents a potential danger to public health, since the invasion of new areas by raccoons may favour the appearance of new parasite-host relationships and, therefore, new routes of transmission for diseases associated with these animals (Doi *et al.*, 2018). In a recent study carried out in Iran, *Rhipicephalus sanguineus* was the most abundant, followed by *Ixodes ricinus*, *Haemaphysalis concinna* and *R. turanicus* (Sharifdini *et al.*, 2021). In that same study, fleas belonging to the species *Pulex irritans* and *Ctenocephalides felis* were also identified. In Japan, where raccoons have also established as an invasive species, they harbored mainly ticks of the genera *Haemaphysalis*, *Amblyomma* and *Ixodes* (Doi *et al.*, 2018; Doi *et al.*, 2021).

In the specific case of the Iberian Peninsula, there are no data on ecto- and endo-parasites of raccoons, although there are studies that show the diversity of ectoparasites that can be found on other species of wild vertebrates. In some of these studies, where different species of wild mammals from the province of Burgos were examined, 83% of individuals had ectoparasites, including ticks such as *Ixodes ricinus*, *Haemaphysalis punctata*, *I. hexagonus* and *Dermacentor reticulatus*, and fleas, mainly *Pulex irritans* (Domínguez-Peñafiel *et al.*, 2011; Dominguez, 2004; Lledó *et al.*, 2010). Other species found in these studies included *I. ventalloi*, *D. marginatus*, *Rhipicephalus pusillus* and *R. turanicus*, as well as *Ctenocephalides felis* and *C. canis*. Similar results were obtained when studying the ectoparasites of wild carnivores from the area of the Basque Country (Sagarna, 2010). In the case of Andalusia, the most frequent tick species found on wild mammals were *R. pusillus* and *R. turanicus*; while in the most common flea species included *P. irritans*, *C. felis* and *C. canis* (Márquez *et al.*, 2009; Millán *et al.*, 2007). Apart from that, in a study carried out in the central region of the Iberian Peninsula, the tick *Hyalomma lusitanicum* was the predominant species, followed by *D. marginatus* and *R. sanguineus* (Toledo *et al.*, 2009). To a lesser extent, specimens of *R. pusillus*, *R. bursa*, *H. marginatum*, *I. ricinus* and *H. hispanica* were also detected.

1.3 Endoparasites of the American raccoon

Due to their omnivorous habits and their great plasticity when adapting to different types of habitat, raccoons are capable of harboring a wide variety of endoparasites (Kresta *et al.*, 2009). Among these species, well studied in their native range of distribution, there are nematodes, (e.g., *Baylisascaris*, *Dracunculus*, *Capillaria*, *Aonchotheca* or *Strongyloides*); cestodes, mainly *Atriotaenia* and *Spirometra* spp.; trematodes; and acanthocephalans belonging to the genus *Macrocanthorhynchus* (Butterworth & Beverley-Burton, 1981; Cole & Shoop, 1987; Kresta *et al.*, 2009; Otranto & Deplazes, 2019; Richardson *et al.*, 1992).

Among the endoparasites, *Baylisascaris procyonis* is especially relevant due to its potential risk for public health. This parasite is a gastrointestinal nematode that occurs very frequently in raccoons, although it is known to infect around 150 other vertebrate species as intermediate hosts (Graeff-Teixeira *et al.*, 2016; Kazacos *et al.*, 2013). Infection by *B. procyonis* is asymptomatic for raccoons, but, in the case of intermediate hosts, which can include humans, it can cause severe disease (Graeff-Teixeira *et al.*, 2016). The danger of the infection by *B. procyonis* is due to the tendency of its larvae to migrate to different host tissues, where it causes an infection which can cause severe morbidity or even fatal (Kazacos *et al.*, 2013; Murray, 2002). The disease caused by this nematode, known as baylisascariasis, is currently recognized as a zoonosis relevant for public health (Graeff-Teixeira *et al.*, 2016). The prevalence of *B. procyonis* infection in North American raccoon populations is high, ranging from 66% to 90% (Graeff-Teixeira *et al.*, 2016; Sorvillo *et al.*, 2002). More than 50 cases of baylisascariasis have been reported in humans, although this number is underrepresented due to the difficulty of diagnosing this specific disease (Sapp *et al.*, 2016). Furthermore, it has been shown that dogs can act as intermediate or definitive hosts for *B. procyonis*, with the consequent risk of infection for humans who live with them (Sapp *et al.*, 2020; Yabsley & Sapp, 2017). It has also been documented that this nematode can seriously affect populations of native species, such as *Neotoma magister* in eastern North America (Logiudice, 2003; Page, 2013).

In Europe, invasive raccoons have led to the spread of *B. procyonis* throughout the continent. This parasitic nematode has been detected in several countries, including the Czech Republic, Slovakia, Germany, Norway and Poland, as well as in areas of Asia, such as Japan (Kazacos, 2001; Kazacos *et al.*, 2013). In the specific case of Germany, *B. procyonis* is widely distributed in its central zone, and infection prevalences of up to 80% have been detected in raccoons (Heddergott *et al.*, 2020; Hohmann *et al.*, 2002). Recently, *B. procyonis* has also been found in raccoons from Denmark (Al-Sabi *et al.*, 2015) and the Netherlands (Maas *et al.*, 2021). This nematode has not been found in wild raccoons in Spain. However, two specimens of white-headed lemur (*Eulemur albifrons*) from a zoological facility in Lugo tested positive for *B. procyonis* after being in close contact with captive raccoons (Martinez *et al.*, 2015).

Apart from *B. procyonis*, another nematode species that often appears on raccoons and is important for veterinary health is *Dracunculus insignis*. This species can infect a wide variety of wildlife, and infection prevalences of 30% and 70% have been detected in raccoons from USA and Canada, respectively (Cleveland *et al.*, 2020; Cleveland *et al.*, 2018). Recently, an infection by a species of the genus *Dracunculus* was detected in a dog from Toledo (Spain), being the first record of these nematodes in a mammal in Europe, and suggesting that the origin of this parasite could be in a raccoon present in the zone (Diekmann *et al.*, 2020).

The American raccoon can also be a host for endoparasites in those areas where it has been introduced. In Poland, several different species of nematodes (e.g., *Baylisascaris procyonis*, *Strongyloides procyonis*, *Ancylostoma* spp. and *Spirocerca lupi*) were identified in raccoons (Bartoszewicz *et al.*, 2008; Popiołek *et al.*, 2011). Recently, in Italy, nematodes (*S. procyonis* and *Aonchotheca putorii*), trematodes of the genus *Plagiorchis,* and cestodes in the genus *Dilepis* were reported (Romeo *et al.*, 2021). Importantly, *B. procyonis* was not found in the Italian raccoons. In Japan, *S. procyonis* was also detected in the digestive tract of the raccoons (Sato *et al.*, 2006). In this region, other species of nematodes (e.g., *Toxocara* sp. and *A. putorii*) were also found; as well as cestodes (e.g., *Taenia* and *Mesocestoides*) and several species of acanthocephalans and trematodes (Matoba *et al.*, 2006; Sato & Suzuki, 2006).

2. Objectives and main hypothesis

The main objective of this work was the identification of the parasite species harbored by the American raccoons of the central region of the Iberian Peninsula. Although there is wide knowledge about raccoon parasites in their native range of distribution, there are hardly any previous studies that cover this topic in the specific context of the Iberian Peninsula. Thus, the results obtained in this work will be very useful to know which species of both ectoparasites and endoparasites appear in raccoons of this region. In addition, these data will also be useful to know whether any of them may represent a potential risk to public and/or veterinary health. In the specific case of endoparasites, one of the main objectives of this work was the detection of *Baylisascaris procyonis*, a parasitic nematode that is especially relevant due to its implications for public health.

Given the mentioned lack of information about this topic in the Iberian Peninsula, the main hypothesis of this work is that the parasites of American raccoons in the central region of Spain are similar to those existing in their native range of distribution. These similarities between the two regions would be reflected both in the parasite species richness and in the specific composition of these species.

3. Materials and methods

3.1 Collection of samples

Raccoons were captured as part of the raccoon population control and eradication campaign carried out by the Community of Madrid. Individuals were transferred to the Centro de Recuperación de Animales Silvestres (CRAS) Madrid-Viñuelas for humane euthanasia and sampling. From March to June 2021, 56 raccoons were captured from 9 localities of Madrid (Table 1; Figure 1). At total of 30 raccoons were from the Henares subpopulation and 26 individuals were from the Jarama subpopulation (as defined by Alda *et al.* (2013)). 35 specimens were males while 21 were females. All animal capture and sampling methods were approved by the Dirección General de Biodiversidad y Recursos Naturales de la Comunidad de Madrid (document verification code: 1276516383071600270582).

Each raccoon was carefully examined for ectoparasites in their fur and/or on skin. All ectoparasites were preserved in 70% ethanol. Necropsies were performed to test for subcutaneous parasites (e.g., *Dracunculus* or *Dirofilaria*). The entire gastrointestinal tract (stomach to colon) was collected and frozen until laboratory analysis.

Table 1. Characterization of the raccoons captured in Madrid localities.

Locality	Subpopulation	No. of raccoons	Sex [1]	Age
San Fernando de Henares	Henares	18	12M, 6F	Adults
Mejorada del Campo	Henares	6	5M, 1F	Adults
Alcalá de Henares	Henares	6	4M, 2F	Adults
Rivas-Vaciamadrid	Jarama	10	4M, 6F	Adults
Velilla de San Antonio	Jarama	9	5M, 4F	Adults
Coslada	Jarama	3	1M, 2F	Juveniles
Madrid-Barajas	Jarama	2	2M	Adults
Alcobendas	Jarama	1	1M	Adults
Fuenlabrada	Jarama	1	1M	Adults
Total		56	35M, 21F	53 adults, 3 juveniles

[1] M = males, F = females.

Figure 1. Locations where raccoons were captured.

3.2 Preparation and processing of samples

Once in the laboratory, the ectoparasite samples continued to be preserved in 70% ethanol and at ambient temperature for later identification. In the same way, the digestive tract samples were kept frozen until they were processed. For the preparation of the digestive tract samples, the first step was to unfreeze them to allow their manipulation. Next, the stomach and intestines were linearized and opened with the help of laboratory scissors, running its entire length from one end to the other. During this process, a visual examination of the interior of the intestine was performed in order to detect the possible endoparasites that could appear in this area. The whole process described so far for the digestive tract samples, as well as the following steps, were carried out on a laboratory tray, so that the entire intestinal content was collected in it. Thus, once the presence of endoparasites on the intestine had been visually verified, the sample was washed. For this purpose, water was applied throughout the entire intestine, with special care that its interior area was clean and, therefore, all the intestinal content remained in the laboratory tray. Subsequently, the content of the tray was passed through a 0.1 mm sieve. The material retained by the screen were preserved in 70% ethanol. The containers were kept at ambient temperature until the moment of their examination for endoparasite search. All this procedure, consisting of opening, washing the interior and conserving the content, was carried out in the same way, and simultaneously, in the case of the stomach.

Once the intestinal and stomach content samples were prepared, they were examined to verify the presence of endoparasites. For this purpose, small volumes of content were deposited in a Petri dish and, with the help of a dissecting scope, a meticulous inspection throughout the sample was performed. By doing this, it was possible to visually detect the endoparasites present in it. In the case of the finding of some type of endoparasite, it was collected with the help of a Pasteur pipette and deposited in a small tube with 70% ethanol. By this manner, the found specimens were preserved for later identification. All this procedure was carried out for every one of the intestinal and stomach content samples, verifying in all of them the existence of endoparasites in the digestive tract of the corresponding raccoon specimen.

3.3 Identification of parasites

Parasites were morphologically identified with the aid of a dissecting scope and an optical microscope. Ectoparasites were identified using published keys and descriptions (Estrada-Peña *et al.*, 2018a, 2018b, 2018c; Pratt, 1956; Walker, 2003). Endoparasites were identified to at the lowest least genus based on published keys and descriptions and to species, when possible (Amin, 1987; Amin *et al.*, 2016; Khalil, 1994; Little, 1966a; Van Cleave, 1923).

3.4 Statistical analyses

Statistical analyses and creation of figures were carried out using *R* and *Excel* programs. The species richness for both ectoparasites and endoparasites was calculated. In addition, the prevalence, mean intensity and relative abundance values for each species were calculated as described by Margolis *et al.* (1982). Prevalence was defined as the percentage of individuals infected by a specific parasite species, with respect to the total number of hosts studied. Mean intensity refers to the mean number of individuals of a particular parasite species per infected host. Finally, relative abundance refers to the mean number of individuals of a specific parasite species per host studied.

In addition, the possible differences between individuals in the richness, abundance and prevalence of the different parasites were studied. For this purpose, General Linear Models (GLM) were used. The following independent variables were included: sex of the individuals (male or female), age (adult or juvenile), subpopulation to which they belong (Henares or Jarama) and date (transformed to Julian date). Thus, a GLM was performed for each of the following dependent variables: parasite richness, abundance and prevalence. For ectoparasites, overall richness and abundance were examined, while prevalences were studied separately for ticks and fleas. Statistical analyses were not conducted for endoparasites because of their very low prevalences.

The distinction of two subpopulations of raccoons is supported by the results obtained by Alda *et al.* (2013), which indicate the existence of two subpopulations of raccoons in the central region of the Iberian Peninsula, related to the Henares and Jarama rivers, respectively.

4. Results

A total of 47 of 56 raccoons (83.9%) were infected by at least one parasite species (Figure 2A). In total, 332 parasites were recovered from the raccoons and the majority (96%) were ectoparasites (Figure 2B).

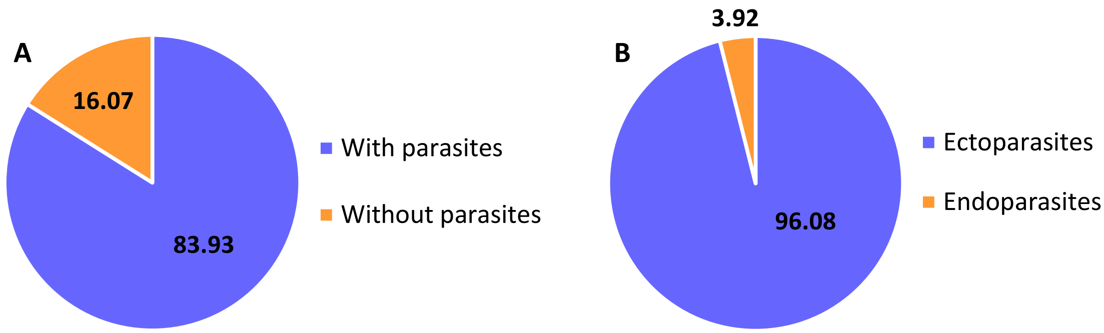


Figure 2. Proportion of **(A)** raccoons that presented some type of parasite and **(B)** ecto and endoparasites with respect to the total number of found parasites.

4.1 Ectoparasites

Fleas and ticks were the only two ectoparasite groups detected. More raccoons were infested with ticks (67.9%, 38 raccoons) compared with fleas (42.9%, 24 raccoons). Coinfection with fleas and ticks were observed for 15 raccoons (26.8%).

Four tick species were identified. The most frequently found species was *Rhipicephalus pusillus* (89.74% of all ticks found), followed by *Rhipicephalus turanicus* (9.16%), *Ixodes ventalloi* (0.73%) and *Dermacentor marginatus* (0.37%) (Figure 3). All specimens were adults. Three flea species were detected. Most were *Pulex irritans* (93.48% of all fleas), followed by *Ctenocephalides felis* (4.35%) and *Ctenocephalides canis* (2.17%) (Figure 4).

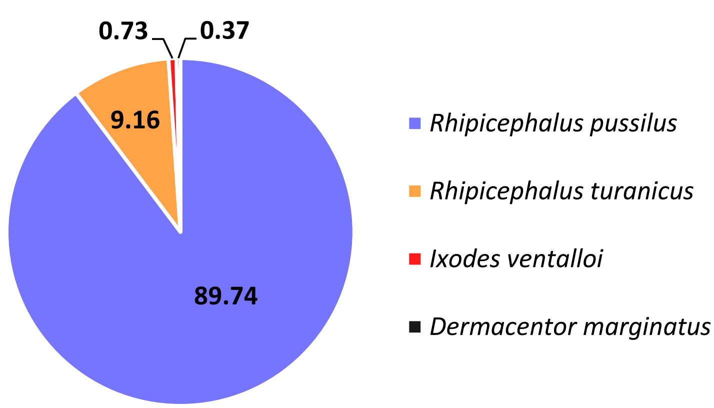


Figure 3. Proportion of the different tick species with respect to the total number of found ticks.

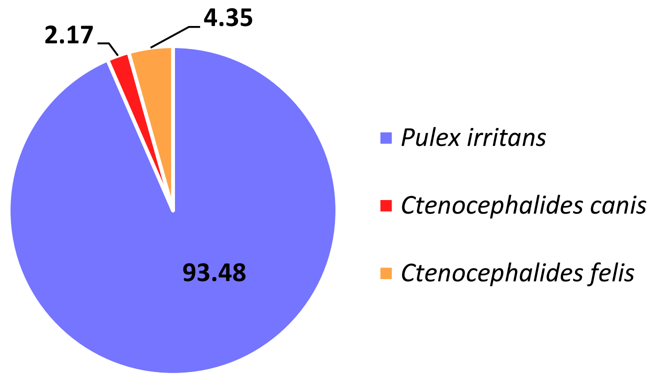


Figure 4. Proportion of the different flea species with respect to the total number of found fleas.

For fleas, the highest prevalence of infestation was for *R. pusillus* (64%) followed by *R. turanicus* on 17.9% of raccoons (Table 2). *Pulex irritans* was the most prevalent flea species (42.9%) (Table 2). Relative few ticks and fleas of other species were found and overall intensities and burdens were low (Table 2).

Table 2. Data on the different ectoparasite species detected on raccoons. For each of the species is shown: number of infected raccoons, number of parasites found, prevalence (%), mean intensity (no. of parasites per host) and relative abundance (no. of parasites per host).

Species	No. of infested raccoons	No. of parasites	Prevalence	Mean intensity	Relative abundance
Rhipicephalus pusillus	36	245	64.29	6.81	4.375
Rhipicephalus turanicus	10	25	17.86	2.5	0.446
Ixodes ventalloi	2	2	3.57	1	0.036
Dermacentor marginatus	1	1	1.79	1	0.018
Pulex irritans	24	43	42.86	1.79	0.768
Ctenocephalides canis	1	1	1.79	1	0.018
Ctenocephalides felis	2	2	3.57	1	0.036

The maximum number of ectoparasite species observed on one individual was three. Most of the raccoons (41.1%) were only infested with one ectoparasite species, followed by raccoons infested with two species (33.93%) and three species (8.93%), respectively.

The GLM results (Table 3) showed that ectoparasite species richness was significantly greater in the Henares subpopulation compared to the Jarama subpopulation (p=0.0051) (Figure 5). Furthermore, species richness also differed significantly between the sexes with males having a richer community compared with females (p=0.0432)

(Figure 6). No significant differences were found concerning the date or the age of the individuals.

Table 3. Results of the GLM for the ectoparasite richness.

Ectoparasite richness	Estimate	Std. error	t value	p value
SubpopulationJarama	-0.6359	0.2170	-2.9300	0.0051
Date	0.0053	0.0034	1.5460	0.1284
AgeYoung	-0.1211	0.4995	-0.2420	0.8094
SexMale	0.4518	0.2178	2.0740	0.0432

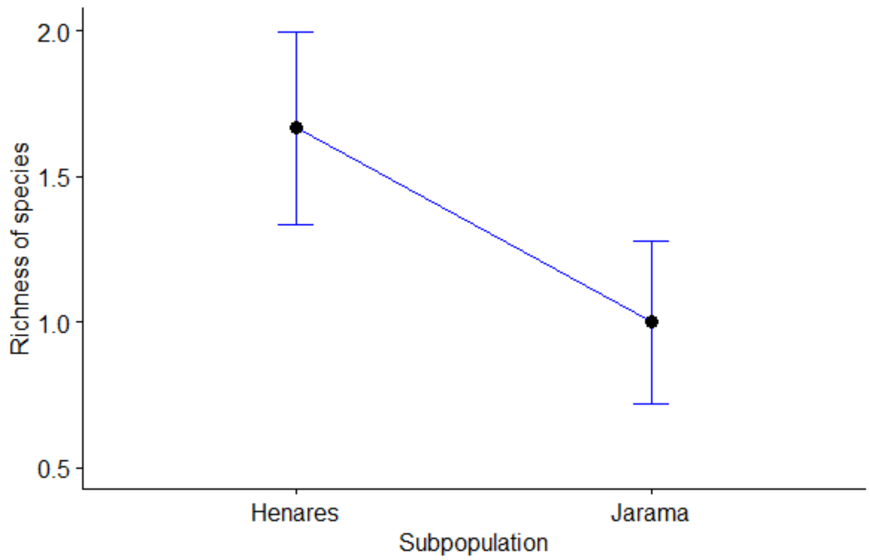


Figure 5. Ectoparasite species richness in two subpopulations of raccoons (*Procyon lotor*) in Madrid, Spain.

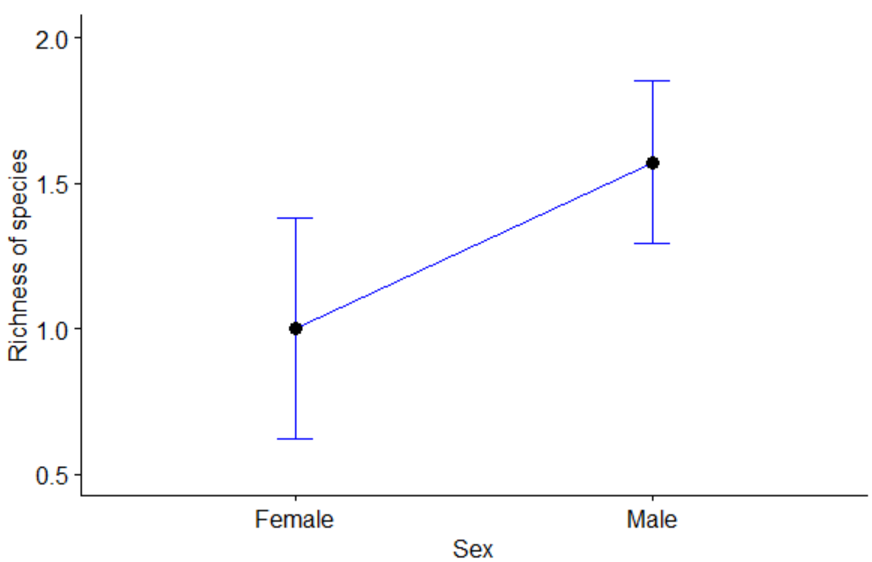


Figure 6. Ectoparasite species richness in male and female raccoons (*Procyon lotor*).

The abundance of ectoparasites significantly increased during the sampling season (p=0.0001) (Table 4; Figure 7). No significant differences were found for the remaining variables (Table 4).

Table 4. Results of the GLM for the ectoparasite abundance.

Ectoparasite abundance	Estimate	Std. error	t value	p value
SubpopulationJarama	-3.0781	1.9102	-1.6110	0.1133
Date	0.1236	0.0302	4.0990	0.0001
AgeYoung	-8.5032	4.3966	-1.9340	0.0587
SexMale	1.2549	1.9175	0.6540	0.5158

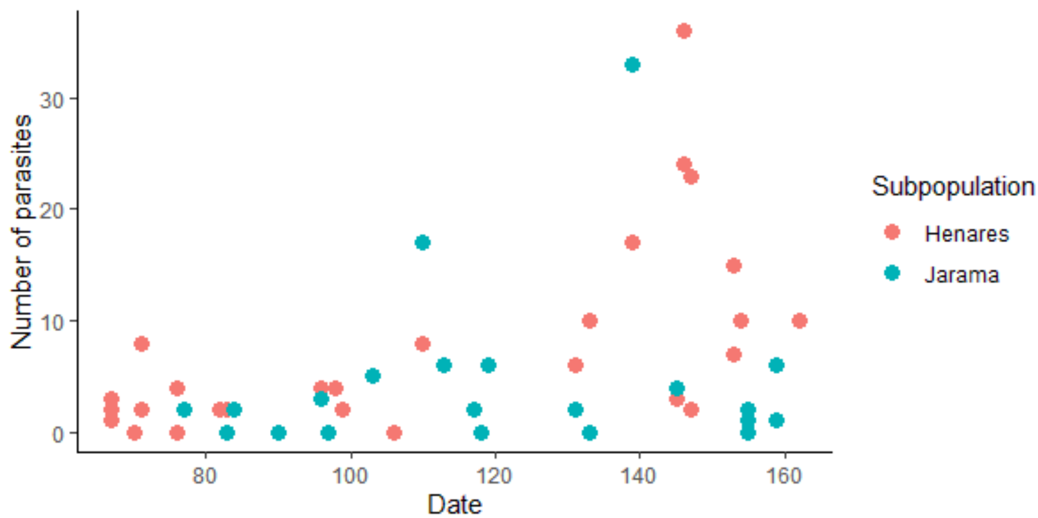


Figure 7. Representation of the relationship between the number of ectoparasites in raccoons (*Procyon lotor*) and the date. Red points correspond to raccoons of the Henares subpopulation while blue points correspond to raccoons of the Jarama subpopulation.

Regarding ticks, the prevalence of infestation was significantly greater in raccoons from the Henares subpopulation compared to the Jarama subpopulation (p=0.006) (Table 5; Figure 8). This prevalence was also greater as the season advanced (p=0.0039) (Table 5; Figure 9). No significant relationship was found for sex or age of the individuals (Table 5).

Table 5. Results of the GLM for the tick prevalence.

Tick prevalence	Estimate	Std. error	z value	p value
SubpopulationJarama	-2.8131	1.0230	-2.7500	0.0060
Date	0.0582	0.0202	2.8870	0.0039
AgeYoung	-3.1144	1.6362	-1.9030	0.0570
SexMale	0.9950	0.7502	1.3260	0.1847

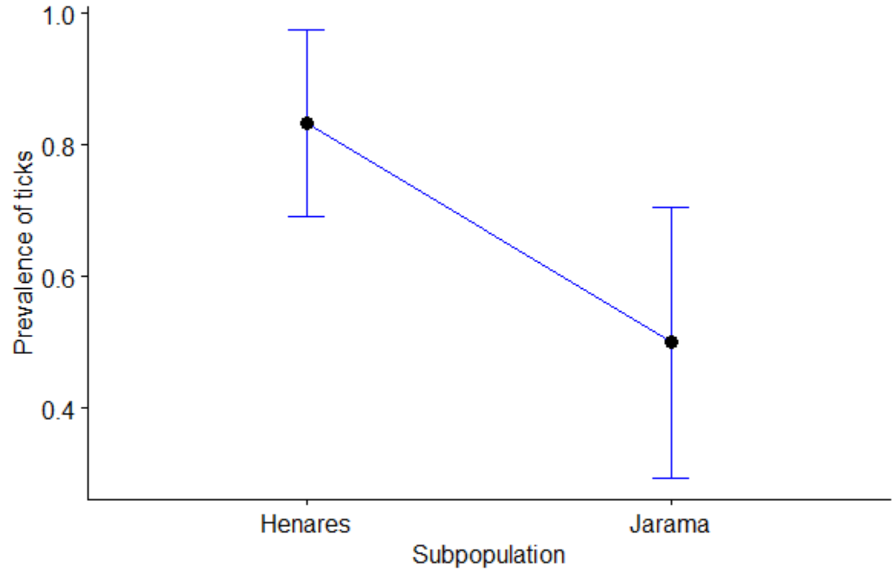


Figure 8. Prevalence of ticks in two subpopulations of raccoons (*Procyon lotor*) in Madrid, Spain.

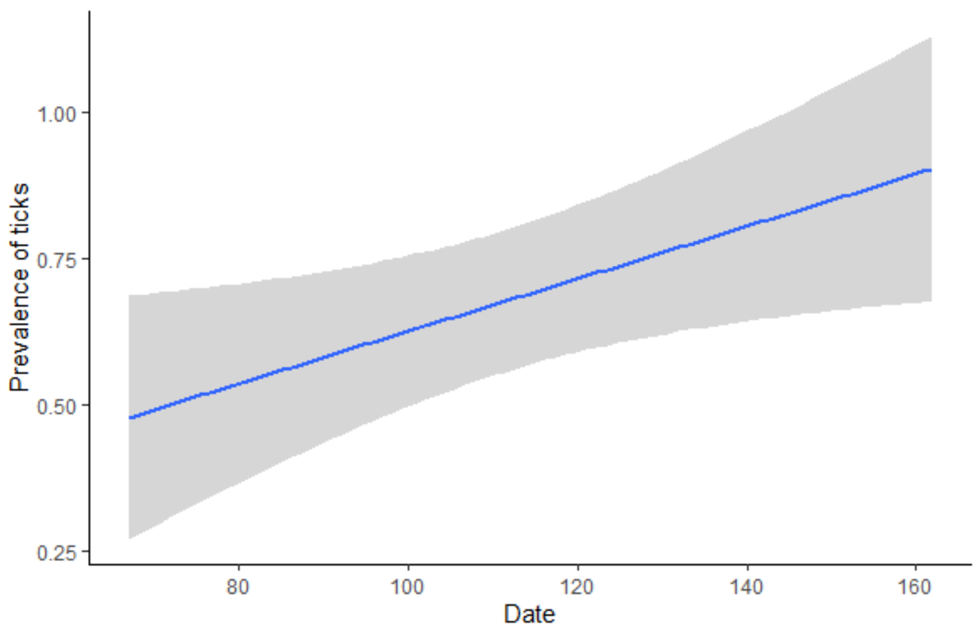


Figure 9. Representation of the relationship between the prevalence of tick infection in raccoons (*Procyon lotor*) and the date. The darker zone represents the 95% confidence interval.

In the case of the fleas, the prevalence of infection was significantly lower as the season advanced (p=0.017) (Table 6; Figure 10). No significant differences were found for the remaining variables (Table 6).

Table 6. Results of the GLM for the flea prevalence.

Flea prevalence	Estimate	Std. error	z value	p value
SubpopulationJarama	0.4160	0.6151	0.6760	0.4990
Date	-0.0250	0.0105	-2.3870	0.0170
AgeYoung	2.2152	1.4179	1.5620	0.1180
SexMale	0.6064	0.6250	0.9700	0.3320

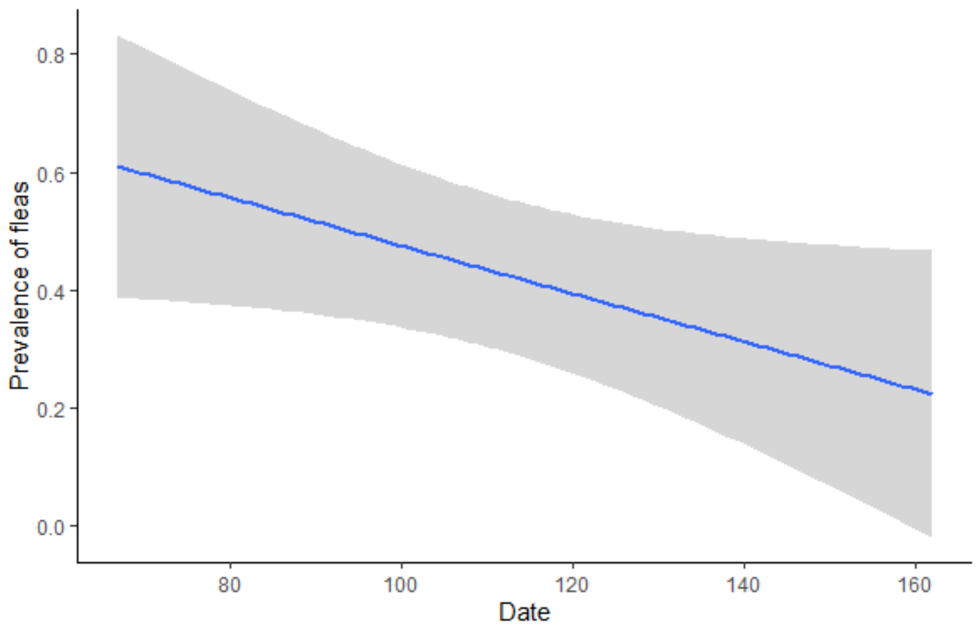


Figure 10. Representation of the relationship between the prevalence of flea infection in raccoons (*Procyon lotor*) and the date. The darker zone represents the 95% confidence interval.

4.2 Endoparasites

Five species of endoparasites were observed in the digestive tract of the raccoons. Most of the raccoons (83.9%) were negative for parasites; low numbers of raccoons were infected with one (14.29%) or three (1.79%) endoparasite species, respectively.

Overall, the prevalence, mean intensity and relative abundance were low for all the endoparasites (Table 7). More than half of these parasites (53.84%) were nematodes, some of which were *Strongyloides procyonis*, which accounts for 23.1% of the total endoparasites (Figure 11), and the remaining were unidentified nematodes in 4 raccoons. In addition to nematodes, a small number of cestodes (*Dilepis* sp. (23.08%)), acanthocephalans (*Moniliformis moniliformis)* (15.38%) and trematodes (*Plagiorchis* sp.) (7.7%) were detected (Figure 11). No subcutaneous nematodes (i.e., *Dracunculus* and *Dirofilaria* spp.) were found.

Table 7. Data on the different endoparasite species detected in raccoons. For each of the species is shown: number of infected raccoons, number of parasites found, prevalence (%), mean intensity (no. of parasites per host) and relative abundance (no. of parasites per host).

Species	No. of infected racoons	Total no. of parasites	Prevalence	Mean intensity	Relative abundance
Plagiorchis sp.	1	1	1.79	1	0.018
Dilepis sp.	3	3	5.36	1	0.054
Unidentified Nematoda	4	4	7.14	1	0.071
Strongyloides procyonis	2	3	3.57	1.5	0.054
Moniliformis moniliformis	1	2	1.79	2	0.036

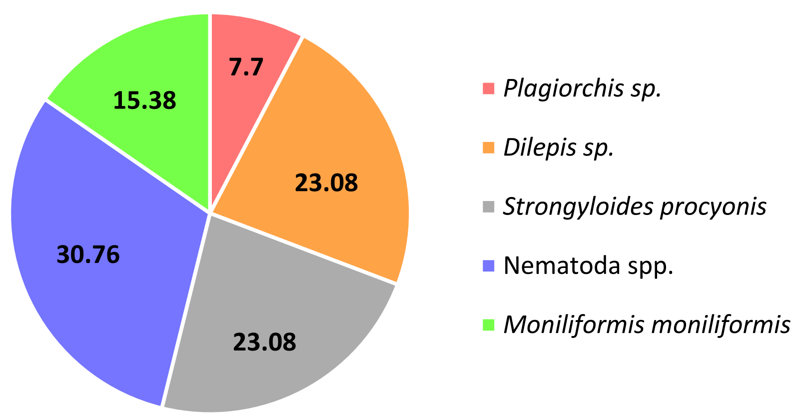


Figure 11. Proportion of the different endoparasite species with respect to the total number of found endoparasites.

5. Discussion

5.1 Overall findings

The overall prevalence of parasite infection of raccoons from the Madrid province in Spain was high (~84%) with the majority (>96%) being ectoparasites. Gastrointesinal parasites were rare with only 16% of raccoons being infected with low numbers of parasites. No raccoons were positive for *Baylisascaris* and *Dracunculus* spp.

5.2 Ectoparasites

The high prevalences of ectoparasites observed in this study are similar to studies on ectoparasites on raccoons in their native range (often >90%) as well as in some other introduced regions (e.g., Japan) (Doi *et al.*, 2018; Doi *et al.*, 2021; Durden & Richardson, 2013; Hertz *et al.*, 2017; Lee *et al.*, 2019; Monello & Gompper, 2010). However, prevalence rates can also be low in some native ranges, especially during certain seasons, or in some introduced regions (e.g., Iran) (Sharifdini *et al.*, 2021). Although this is the first study to examine raccoons in Spain, similar high prevalences of ectoparasite on other wild mammals of the Iberian Peninsula, such as red foxes (*Vulpes vulpes*), wildcats (*Felis silvestris*), Egyptian mongooses (*Herpestes ichneumon*) and European badgers (*Meles meles*) have been reported (Dominguez, 2004; Millán *et al.*, 2007).

Among the species of ectoparasites, ticks were the predominant group, infesting >67% of raccoons. This predominance of ticks over fleas has also been reported for raccoons in their native range of distribution (Durden & Richardson, 2013; Monello & Gompper, 2010; Richardson *et al.*, 1994). The overall prevalence of flea infestation observed in this study (~43%) was slightly higher than those found in previous studies, although it is highly dependent on different factors, such as seasonality (Monello & Gompper, 2009).

A significant difference in ectoparasite infestation was noted between the two subpopulations of raccoons in our sampled region of Spain. Raccoons from the Henares subpopulation had a greater ectoparasite species richness than those from Jarama subpopulation. The life cycle of ectoparasites, including their behaviour, reproduction and probability of survival in the absence of the host, is determined not only by the environmental characteristics but also by those related with their host ecology (Monello & Gompper, 2010). Thus, the results of the present work suggest that there could be differences between the two subpopulations of raccoons in one or more of these factors.

It was also observed that the sex of the individual raccoons was an important factor related to species richness, with a higher diversity on males. The territory required by male raccoons has been found to be 2-3 times larger than that required by females (Hohmann *et al.*, 2000). In addition, once males reach maturity they disperse to new territories (Bartoszewicz, 2011). These factors could mean a greater exposure of males to ectoparasites, derived from the mobility over larger extensions of territory, which would explain that these animals present a greater ectoparasite richness than that which appears in females.

Finally, results also showed the existence of a significant relationship between the abundance of ectoparasites and the date, so that the number of ectoparasites was higher as the season advanced. The explanation to this finding is provided in the next point (see 5.2.1). No significant sex- or age-related differences were found, which agrees with what can be observed in other wild carnivores of the Iberian Peninsula (e.g., red fox, Iberian lynx, European genet and Eurasian badger) (Millán *et al.*, 2007).

5.2.1 Ticks

The four tick species that were identified on raccoons are native to the Mediterranean region (Estrada-Peña *et al.*, 2004; Estrada-Peña *et al.*, 2018d) and none of the more common tick species on raccoons in North America (e.g., *D. variabilis*, *I. texanus* or *Amblyomma americanum*, depending on the geographic region) were found (Hertz *et al.*, 2017; Lee *et al.*, 2019; Smith *et al.*, 2019). Similar results were reported from Japan where raccoons were infested with native tick species in the genera *Haemaphysalis*, *Amblyomma* and *Ixodes* (Doi *et al.*, 2021), and Iran where only native tick species of *Rhipicephalus*, *Ixodes* and *Haemaphysalis* were reported (Sharifdini et al., 2021). Of note, no *Hyalomma* species were detected on raccoons despite being very abundant in the central region of the Iberian Peninsula (Toledo et al., 2009) and reported on other wild mammals, mainly domestic and wild ungulates and less frequently carnivores or insectivores (Estrada-Peña *et al.*, 2004; Estrada-Peña *et al.*, 2018d).

Similar to the results of ectoparasite diversity, the prevalence of ticks was higher for the Henares subpopulation. Furthermore, it was observed that the tick infection prevalence increased later in the summer season. *Rhipicephalus pusillus*, the most predominant species on the raccoons in this study, has been reported to have a peak of abundance between the months of February and June (Estrada-Peña *et al.*, 2018d; Millán *et al.*, 2007), which agrees with our results. This fact also explains the relationship observed between the ectoparasite abundance and the date.

5.2.2 Fleas

The three flea species that were found in the raccoons of this work are cosmopolitan and infest a large diversity of vertebrate hosts (Lewis, 1993). Of them, *Pulex irritans* was the predominant species on the raccoons, followed by *Ctenocephalides felis* and *C. canis*. Although all three of these flea species occur in the native range of raccoons, the most common flea species reported include *Orchopeas howardii*, *Chaetopsylla lotoris* and *Pulex simulans* (Durden & Richardson, 2013; Monello & Gompper, 2010; Richardson et al., 1994). Interestingly, a recent study of invasive raccoons in Iran found very similar results, with *P. irritans* and *C. felis* being found on examined raccoons (Sharifdini et al., 2021). The three flea species detected on raccoons in this study are commonly documented on other wild mammals of the Iberian Peninsula including the red fox, the wildcat, and the European badger (Dominguez, 2004; Márquez *et al.*, 2009; Millán *et al.*, 2007).

In contrast to the findings with ticks, flea infestation prevalence decreased throughout the sampling period. In general, mild temperatures and high relative humidity promote increases in flea populations (Durden & Hinkle, 2019). On the Iberian Peninsula, summers are very dry and hot which would explain a decrease in fleas during the summer. Previous studies on wild carnivores of the Iberian Peninsula also noted that fleas were more abundant in winter than in spring (Millán *et al.*, 2007). Moreover, *Pulex irritans*, the most frequently species found on the raccoons, has been reported to have a peak abundance on dogs in autumn (Gálvez *et al.*, 2017).

5.2.3 Importance for public and veterinary health

Rhipicephalus pusillus typically infests the European rabbit (*Oryctolagus cuniculus*), but it can be found in a great diversity of wild fauna, including rodents, ungulates and carnivores (Estrada-Peña *et al.*, 2018d). It is an important species for public health, since it can act as vector of human rickettsial pathogens such as *Rickettsia sibirica mongolitimonae, R. massiliae* and *Anaplasma* spp. (Estrada-Peña *et al.*, 2018d; Toledo *et al.*, 2009). *Rhipicephalus turanicus* also infests a wide range of domestic and wild species, including birds and mammals (Estrada-Peña *et al.*, 2004). It is also a vector of *R. aeschlimannii* and *R. massiliae* which are important human pathogens (Toledo *et al.*, 2009).

Dermacentor marginatus was rarely found on raccoons, but this tick species is important vector of pathogens of medical and veterinary concern. This tick species can also infest a large diversity of hosts, including carnivores, ungulates or insectivore mammals (Estrada-Peña *et al.*, 2018d). Among the most important human pathogens that are associated with this tick species are *Rickettsia conori* (Boutonneuse fever or Mediterranean spotted fever), *R. slovaca* (TIBOLA, *tick-borne lymphadenopathy*), Crimean-Congo haemorrhagic fever virus, Omsk haemorrhagic fever virus, and Tick-borne encephalitis virus (Estrada-Peña *et al.*, 2004; Estrada-Peña *et al.*, 2018d). Of veterinary concern, this tick can transmit *Babesia canis*, causative agent of babesiosis in domestic dogs (Estrada-Peña *et al.*, 2004). Other pathogens associated with this tick include *Francisella tularensis*, causative agent of tularemia; *Borrelia burgdorferi* (Lyme disease) and *Anaplasma* spp. (Merino *et al.*, 2005; Toledo *et al.*, 2009).

Ixodes ventalloi, also known as a rabbit tick, typically infests lagomorphs, although it can infest a wide diversity of wild fauna, including rodents, meso-mammals and birds (Estrada-Peña *et al.*, 2018d). This tick species can also transmit pathogens such as *Rickettsia helvetica*, *Rickettsia* sp. IRS3, *Coxiella burnetii*, and Eyach and Erve viruses (Estrada-Peña *et al.*, 2018d).

The three flea species that were found in the raccoons in this study (*Pulex irritans*, *Ctenocephalides felis* and *C. canis*) are intermediate hosts of *Dipylidium caninum*, a cestode which can parasitize domestic animals and, accidentally, humans (Lewis, 1993). *Bartonella* spp., which are the causative agents of different diseases in humans, domestic animals and wildlife (including raccoons (Fenton *et al.*, 2021)), have also been found in these three flea species (Márquez et al., 2009). These *Bartonella* spp. can cause endocarditis, bacillary angiomatosis or persistent bacteraemia, among others (Márquez et al., 2009). In the specific case of *Ctenocephalides felis*, it is also vector of *Rickettsia felis* (Lledó *et al.*, 2010). From a veterinary point of view, *C. felis* is the most relevant species, since it is the flea that most frequently parasitizes domestic dogs in Spain (Gracia *et al.*, 2008).

5.3 Endoparasites

A very low prevalence and diversity of gastrointestinal parasites were found in raccoons in this study. Among the total of 56 examined raccoons, only 9 (~16%) were infected and only five parasite species were detected. These findings contrast with what can be found in raccoons in their native range of distribution, where studies show that these animals can harbor more than 20 different species of endoparasites, many of them, in addition, with quite high infection prevalence (Cole & Shoop, 1987; Kresta et al., 2009; Richardson et al., 1992). However, the low diversity of endoparasites detected in the raccoons in this work is similar to results obtained in other regions where American raccoons have been introduced, such as raccoons from European countries such as Poland (Popiołek et al., 2011) and Italy (Romeo et al., 2021). In Japan, endoparasite species richness is greater than in Spain and other studies mentioned above, but is still lower than that observed in North America (Matoba et al., 2006; Sato & Suzuki, 2006; Sato et al., 2006). In a general way, it is known that invasive species harbored around half the number of parasite species in those areas where they have been introduced compared to their native range of distribution (Torchin *et al.*, 2003). This seems to be especially true in the case of raccoons and their endoparasites, in accordance with previous studies, and as shown by the results of this work.

In the present work were found, in first place, different species of nematodes. Three of these specimens could be identified as *Strongyloides procyonis*. These nematodes are gastrointestinal parasites which infect a great diversity of vertebrate species, including humans (Ko *et al.*, 2020). In the case of the Iberian Peninsula, they have been detected in other wild mammals such as the European badger (*Meles meles*) (Torres *et al.*, 2001). *Strongyloides procyonis*, which typically appears in raccoons, was found for the first time in 1966 in the intestine of one of these animals (Little, 1966b). However, this nematode species is not restricted to the raccoons of North America, but it has also been detected in established raccoons in other world regions. Thus, close to 30% infection prevalences have been observed in raccoons from Japan (Sato et al., 2006) and Italy (Romeo et al., 2021), and a lower prevalence was reported in Poland (Popiołek et al., 2011).

The remaining endoparasites found in raccoons belonged to unidentified species of nematodes, trematodes (*Plagiorchis* sp.), cestodes of the genus *Dilepis* and acanthocephalans identified as *Moniliformis moniliformis*. The present results are, therefore, similar to those obtained in Italian raccoons (Romeo et al., 2021).

It is also important to highlight the absence of nematodes of *Baylisascaris* and *Dracunculus* genera in the raccoons from the central region of the Iberian Peninsula. The infection prevalence by *B. procyonis* is high (between 66% and 90%) in North American populations (Graeff-Teixeira et al., 2016; Sorvillo et al., 2002). Furthermore, this parasitic nematode has been detected in the raccoons that were introduced in many European countries, as well as in Japan (Kazacos, 2001; Kazacos et al., 2013). In some of these cases, the infection prevalences by *B. procyonis* are very high, as occurs in Germany, where they can reach up to 80% (Hohmann et al., 2002). Besides this, recently prevalence over 60% has been documented in the raccoons from the Netherlands (Maas et al., 2021). Considering all these previous findings, the presence of *B. procyonis* in the raccoons of this work was expected. However, this parasitic nematode was not detected in any of the examined raccoons what agrees, however, with the recent results from the raccoons of Italy (Romeo et al., 2021).

5.4 Future implications

For this work the raccoons from the population of the central region of the Iberian Peninsula were studied. Therefore, the obtained results are only valid to the specific case of this region. Further studies are needed to check if there are differences in the parasites harbored by raccoons in other areas of the Iberian Peninsula. Moreover, the study of the presence of *Baylisascaris procyonis* is especially important due to its great relevance for public health. Although this parasitic nematode was not found in this work, it is necessary to check if it is present in other areas of the Iberian Peninsula or if, on the contrary, its absence is generalizable to the whole of this region. Population growth theory predicts that raccoon populations will stabilize upon reaching the carrying capacity of ecosystems. However, for the moment that limit has not been reached, so the effects on native species derived from the constant increase in the number of raccoons continue to be dangerous and uncertain (Salgado, 2018). A study carried out in 2019 showed that, mainly due to climate change, the favourable areas for the establishment of raccoons are predicted to increase, which would allow the colonization of new regions by these animals (Louppe *et al.*, 2019). The expansion of the raccoon through different countries in Europe and other world regions is an important problem, not only for the native species, but also for humans, since these animals can transmit new and potentially dangerous diseases (Beltrán-Beck *et al.*, 2012; Maas *et al.*, 2021). All this makes future and periodic studies necessary to control both the expansion of raccoons throughout the territory and the parasites which they harbor, as well as their potential importance for public and veterinary health.

6. Conclusion

In this work it has been proved that the American raccoon can act as a host for different parasite species, some of them relevant for public and veterinary health. Among the tick species, *Rhipicephalus pusillus* was the most frequently found in the raccoons, followed by *R. turanicus*, *Dermacentor marginatus* and *Ixodes ventalloi*. Regarding the fleas, *Pulex irritans* was the most abundant, also detecting *Ctenocephalides felis* and *C. canis*. Furthermore, significant differences were found between individuals in aspects as ectoparasite species richness, ectoparasite abundance and infection prevalence as a function of sex, date and subpopulation to which the raccoons belonged. Besides this, the raccoons of this work also harbored few types of endoparasites with very low infection prevalences. Among them, nematodes, some of them belonging the species *Strongyloides procyonis*, were identified, as well as cestodes of the genus *Dilepis*, trematodes in the genus *Plagiorchis*, and acanthocephalans of the species *Moniliformis moniliformis*. They were not found, however, nematodes of the species *Baylisascaris procyonis*, which is relevant from a public health point of view.

7. Acknowledgements

I would like to thank my tutors José Ignacio Aguirre and Michael Yabsley for all the help they have offered me during the course of this project. It has been a real pleasure to be able to work and learn about such an interesting and exciting topic. I also want to thank the CRAS staff in Madrid for their collaboration in taking and transporting the samples for this work. Last but not least, I thank my family, girlfriend and friends for all the support shown during this time. It would not have been possible without them.

8. References

Al-Sabi, M. N. S., Chriél, M., Hansen, M. S., & Enemark, H. L. (2015). Baylisascaris procyonis in wild raccoons (Procyon lotor) in Denmark. *Veterinary Parasitology: Regional Studies and Reports, 1*, 55-58.

Alda, F., Ruiz-López, M. J., García, F. J., Gompper, M. E., Eggert, L. S., & García, J. T. (2013). Genetic evidence for multiple introduction events of raccoons (Procyon lotor) in Spain. *Biological invasions, 15*(3), 687-698.

Alvarez, A. (2008). Predation of Spanish terrapin Mauremys leprosa clutches by raccoons. *Quercus, 269*, 49.

Amin, O. M. (1987). Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). *The Journal of parasitology*, 1216-1219.

Amin, O. M., Heckmann, R. A., Mohammed, O., & Evans, R. P. (2016). Morphological and molecular descriptions of Moniliformis saudi sp. n.(Acanthocephala: Moniliformidae) from the desert hedgehog, Paraechinus aethiopicus (Ehrenberg) in Saudi Arabia, with a key to species and notes on histopathology. *Folia Parasitologica, 63*, 1.

Asano, M., Matoba, Y., Ikeda, T., Suzuki, M., Asakawa, M., & Ohtaishi, N. (2003). Reproductive characteristics of the feral raccoon (Procyon lotor) in Hokkaido, Japan. *Journal of veterinary medical science, 65*(3), 369-373.

Real Decreto 630/2013, de 2 de agosto, por el que se regula el Catálogo español de especies exóticas invasoras. *Boletín Oficial del Estado*. 3 de agosto de 2013, núm. 185, pp. 56764-56786., (2013).

Baldwin, R. A., Houston, A. E., Kennedy, M. L., & Liu, P. S. (2006). Predicting raccoon, Procyon lotor, occurrence through the use of microhabitat variables. *The Canadian Field-Naturalist, 120*(2), 225-231.

Bartoszewicz, M. (2011). NOBANIS – Invasive Alien Species Fact Sheet – Procyon lotor.

Bartoszewicz, M., Okarma, H., Zalewski, A., & Szczęsna, J. (2008). *Ecology of the raccoon (Procyon lotor) from western Poland.* Paper presented at the Annales Zoologici Fennici.

Beasley, J. C., DeVault, T. L., Retamosa, M. I., & Rhodes Jr, O. E. (2007). A hierarchical analysis of habitat selection by raccoons in northern Indiana. *The Journal of Wildlife Management, 71*(4), 1125-1133.

Beasley, J. C., & Rhodes Jr, O. E. (2008). Relationship between raccoon abundance and crop damage. *Human-Wildlife Conflicts, 2*(2), 248-259.

Beltrán-Beck, B., García, F. J., & Gortázar, C. (2012). Raccoons in Europe: disease hazards due to the establishment of an invasive species. *European Journal of Wildlife Research, 58*(1), 5-15.

Butterworth, E. W., & Beverley-Burton, M. (1981). Observations on the prevalence and intensity of Capillaria spp.(Nematoda: Trichuroidea) in wild carnivora from Ontario, Canada. *Proceedings of the Helminthological Society of Washington, 48*(1), 24-37.

Canova, L., & Rossi, S. (2009). First records of the northern raccoon Procyon lotor in Italy. *Hystrix, the Italian Journal of Mammalogy, 19*(2).

Ceballos-Escalera, J., Lara, J., Montoro, J., García-Román, L., Herrera, J., Gonzáles, J., García, F., López-nieva, P., Aramburu, M., & Ortega, M. (2013). *Gestión de la* *población de mapache (Procyon lotor L.) en la Comunidad de Madrid.* Paper presented at the Sexto Congreso Forestal Español. Sociedad española de Ciencias Forest.

Ćirović, D., & Milenković, M. (2003). The first record of the free-ranging raccoon (Procyon lotor Linnaeus, 1758) in Yugoslavia. *Mammalian Biology, 68*(2), 116-117.

Cleveland, C. A., Eberhard, M. L., Garrett, K. B., Thompson, A. T., Swanepoel, L., Miller, E. A., Stephens, O. L., & Yabsley, M. J. (2020). Dracunculus species in meso-mammals from Georgia, United States, and implications for the Guinea Worm Eradication Program in Chad, Africa. *The Journal of parasitology, 106*(5), 616-622.

Cleveland, C. A., Garrett, K. B., Cozad, R. A., Williams, B. M., Murray, M. H., & Yabsley, M. J. (2018). The wild world of Guinea Worms: A review of the genus Dracunculus in wildlife. *International Journal for Parasitology: Parasites and Wildlife, 7*(3), 289-300.

Colautti, R. I., Ricciardi, A., Grigorovich, I. A., & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology letters, 7*(8), 721-733.

Cole, R. A., & Shoop, W. L. (1987). Helminths of the raccoon (Procyon lotor) in western Kentucky. *The Journal of parasitology*, 762-768.

Diekmann, I., Alnassan, A. A., Globokar, M., Pantchev, N., Kurzrock, L., Hernandez, L., Lopez, J., Ruano, R., Herrero, S., & von Samson-Himmelstjerna, G. (2020). Canine Dracunculus Nematode Infection, Toledo, Spain. *Emerging infectious diseases, 26*(8), 1860.

Doi, K., Kato, T., & Hayama, S.-i. (2018). Infestation of introduced raccoons (Procyon lotor) with indigenous ixodid ticks on the Miura Peninsula, Kanagawa Prefecture, Japan. *International Journal for Parasitology: Parasites and Wildlife, 7*(3), 355-359.

Doi, K., Kono, M., Kato, T., & Hayama, S.-i. (2021). Ecological traps and boosters of ixodid ticks: The differing ecological roles of two sympatric introduced mammals. *Ticks and tick-borne diseases, 12*(3), 101687.

Domínguez-Peñafiel, G., Giménez-Pardo, C., Gegúndez, M., & Lledó, L. (2011). Prevalence of ectoparasitic arthropods on wild animals and cattle in the Las Merindades area (Burgos, Spain). *Parasite: journal de la Société Française de Parasitologie, 18*(3), 251.

Dominguez, G. (2004). North Spain (Burgos) wild mammals ectoparasites. *Parasite, 11*(3), 267-272.

Duenas, M.-A., Hemming, D. J., Roberts, A., & Diaz-Soltero, H. (2021). The threat of invasive species to IUCN-listed critically endangered species: A systematic review. *Global Ecology and Conservation*, e01476.

Durden, L. A., & Hinkle, N. C. (2019). Fleas (siphonaptera). In *Medical and veterinary entomology* (pp. 145-169): Elsevier.

Durden, L. A., & Richardson, D. J. (2013). Ectoparasites of the Virginia opossum (Didelphis virginiana), raccoon (Procyon lotor), and striped skunk (Mephitis mephitis) from Keith County, Nebraska.

Estrada-Peña, Bouattour, J-L, & Walker. (2004). *Ticks of Domestic Animals in the Mediterranean Region. A Guide to Identification of Species*.

Estrada-Peña, Mihalca, & Petney. (2018a). Genus *Dermacentor* Koch, 1844. In Estrada-Peña, Mihalca, & Petney (Eds.), *Ticks of Europe and North Africa: a guide to species identification* (pp. 279-292): Springer.

Estrada-Peña, Mihalca, & Petney. (2018b). Genus *Ixodes* Latreille, 1795. In Estrada-Peña, Mihalca, & Petney (Eds.), *Ticks of Europe and North Africa: a guide to species identification* (pp. 79-224): Springer.

Estrada-Peña, Mihalca, & Petney. (2018c). Genus *Rhipicephalus* Koch, 1844. In Estrada-Peña, Mihalca, & Petney (Eds.), *Ticks of Europe and North Africa: a guide to species identification* (pp. 293-342): Springer.

Estrada-Peña, Mihalca, A. D., & Petney, T. N. (2018d). *Ticks of Europe and North Africa: a guide to species identification*: Springer.

Farashi, A., & Naderi, M. (2017). Predicting invasion risk of raccoon Procyon lotor in Iran using environmental niche models. *Landscape and Ecological Engineering, 13*(2), 229-236.

Fenton, H., McBurney, S., Elsmo, E. J., Cleveland, C. A., & Yabsley, M. J. (2021). Lesions associated with Bartonella taylorii–like bacterium infection in a free-ranging, young-of-the-year raccoon from Prince Edward Island, Canada. *Journal of Veterinary Diagnostic Investigation, 33*(2), 362-365.

Fernández-Aguilar, X., Molina-Vacas, G., Ramiro, V., Carro, F. A., Barasosa, J. Á., Vicente, J., & Gutiérrez, C. (2012). Presence of raccoon (Procyon lotor) in Doñana National Park and its surroundings. *Galemys, 24*, 76-79.

Frantz, Cyriacks, P., & Schley, L. (2005). Spatial behaviour of a female raccoon (Procyon lotor) at the edge of the species' European distribution range. *European Journal of Wildlife Research, 51*(2), 126-130.

Gálvez, R., Musella, V., Descalzo, M. A., Montoya, A., Checa, R., Marino, V., Martín, O., Cringoli, G., Rinaldi, L., & Miró, G. (2017). Modelling the current distribution and predicted spread of the flea species Ctenocephalides felis infesting outdoor dogs in Spain. *Parasites & Vectors, 10*(1), 1-8.

García, J. T., García, F. J., Alda, F., González, J. L., Aramburu, M. J., Cortés, Y., Prieto, B., Pliego, B., Pérez, M., & Herrera, J. (2012). Recent invasion and status of the raccoon (Procyon lotor) in Spain. *Biological invasions, 14*(7), 1305-1310.

Gracia, M., Calvete, C., Estrada, R., Castillo, J., Peribanez, M., & Lucientes, J. (2008). Fleas parasitizing domestic dogs in Spain. *Veterinary parasitology, 151*(2-4), 312-319.

Graeff-Teixeira, C., Morassutti, A. L., & Kazacos, K. R. (2016). Update on baylisascariasis, a highly pathogenic zoonotic infection. *Clinical Microbiology Reviews, 29*(2), 375-399.

Heddergott, M., Steinbach, P., Schwarz, S., Anheyer-Behmenburg, H. E., Sutor, A., Schliephake, A., Jeschke, D., Striese, M., Müller, F., & Meyer-Kayser, E. (2020). Geographic distribution of raccoon roundworm, Baylisascaris procyonis, Germany and Luxembourg. *Emerging infectious diseases, 26*(4), 821.

Hertz, J. C., Clemons, B. C. F., Lord, C. C., Allan, S. A., & Kaufman, P. E. (2017). Distribution and host associations of ixodid ticks collected from wildlife in Florida, USA. *Experimental and Applied Acarology, 73*(2), 223-236.

Hohmann, Gerhard, R., & Kasper, M. (2000). Home range size of adult raccoons (Procyon lotor) in Germany. *Zeitschrift fur Saugetierkunde, 65*(2), 124-127.

Hohmann, Voigt, S., & Andreas, U. (2002). Raccoons take the offensive. A current assessment. *Biologische Invasionen. Herausforderung zum Handeln, 1*, 191-192.

Kauhala, K. (1996). Introduced carnivores in Europe with special reference to central and northern Europe. *Wildlife biology, 2*(3), 197-204.

Kazacos, K. R. (2001). Baylisascaris procyonis and related species. *Parasitic diseases of wild mammals, 2*, 301-341.

Kazacos, K. R., Jelicks, L. A., & Tanowitz, H. B. (2013). Baylisascaris larva migrans. *Handbook of clinical neurology, 114*, 251-262.

Khalil, L. F. (1994). *Keys to the cestode parasites of vertebrates*.

Ko, P. P., Suzuki, K., Canales-Ramos, M., Htwe, M. P. P. T. H., Htike, W. W., Yoshida, A., Montes, M., Morishita, K., Gotuzzo, E., & Maruyama, H. (2020). Phylogenetic relationships of Strongyloides species in carnivore hosts. *Parasitology International, 78*, 102151.

Kresta, A. E., Henke, S. E., & Pence, D. B. (2009). Gastrointestinal helminths in raccoons in Texas. *Journal of Wildlife Diseases, 45*(1), 1-13.

Lee, X., Murphy, D. S., Hoang Johnson, D., & Paskewitz, S. M. (2019). Passive animal surveillance to identify ticks in Wisconsin, 2011–2017. *Insects, 10*(9), 289.

Lewis, R. E. (1993). Fleas (Siphonaptera). In *Medical insects and arachnids* (pp. 529-575): Springer.

Little, M. (1966a). Comparative morphology of six species of Strongyloides (Nematoda) and redefinition of the genus. *The Journal of parasitology*, 69-84.

Little, M. (1966b). Seven new species of Strongyloides (Nematoda) from Louisiana. *The Journal of parasitology*, 85-97.

Lledó, L., Giménez-Pardo, C., Domínguez-Peñafiel, G., Sousa, R., Gegúndez, M. I., Casado, N., & Criado, A. (2010). Molecular detection of hemoprotozoa and Rickettsia species in arthropods collected from wild animals in the Burgos Province, Spain. *Vector-Borne and Zoonotic Diseases, 10*(8), 735-738.

Logiudice, K. (2003). Trophically transmitted parasites and the conservation of small populations: raccoon roundworm and the imperiled Allegheny woodrat. *Conservation Biology, 17*(1), 258-266.

Louppe, V., Leroy, B., Herrel, A., & Veron, G. (2019). Current and future climatic regions favourable for a globally introduced wild carnivore, the raccoon Procyon lotor. *Scientific reports, 9*(1), 1-13.

Maas, M., Tatem-Dokter, R., Rijks, J. M., Dam-Deisz, C., Franssen, F., van Bolhuis, H., Heddergott, M., Schleimer, A., Schockert, V., & Lambinet, C. (2021). Population genetics, invasion pathways and public health risks of the raccoon and its roundworm Baylisascaris procyonis in northwestern Europe. *Transboundary and Emerging Diseases*.

Margolis, L., Esch, G., Holmes, J., Kuris, A., & Schad, G. (1982). The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *The Journal of parasitology, 68*(1), 131-133.

Márquez, F., Millán, J., Rodriguez-Liebana, J., Garcia-Egea, I., & Muniain, M. (2009). Detection and identification of Bartonella sp. in fleas from carnivorous mammals in Andalusia, Spain. *Medical and veterinary entomology, 23*(4), 393-398.

Martinez, M.-A. J., Cano, E. V., & Rois, J. L. (2015). Baylisascaris procyonis larva migrans in two white-headed lemurs (Eulemur albifrons) in Spain and response to treatment derived from a human pediatric protocol. *Veterinary parasitology, 210*(3-4), 246-249.

Matoba, Y., Yamada, D., Asano, M., Oku, Y., Kitaura, K., Yagi, K., Tenora, F., & Asakawa, M. (2006). Parasitic helminths from feral raccoons (Procyon lotor) in Japan. *Helminthologia, 43*(3), 139-146.

Merino, F. J., Nebreda, T., Serrano, J. L., Fernández-Soto, P., Encinas, A., & Pérez-Sánchez, R. (2005). Tick species and tick-borne infections identified in population from a rural area of Spain. *Epidemiology & Infection, 133*(5), 943-949.

Millán, J., Ruiz-Fons, F., Márquez, F., Viota, M., López-Bao, J., & Paz Martín-Mateo, M. (2007). Ectoparasites of the endangered Iberian lynx Lynx pardinus and sympatric wild and domestic carnivores in Spain. *Medical and veterinary entomology, 21*(3), 248-254.

Monello, R. J., & Gompper, M. E. (2009). Relative importance of demographics, locale, and seasonality underlying louse and flea parasitism of raccoons (Procyon lotor). *Journal of Parasitology, 95*(1), 56-62.

Monello, R. J., & Gompper, M. E. (2010). Differential effects of experimental increases in sociality on ectoparasites of free-ranging raccoons. *Journal of Animal Ecology, 79*(3), 602-609.

Murray, W. J. (2002). Human infections caused by the raccoon roundworm, Baylisascaris procyonis. *Clinical Microbiology Newsletter, 24*(1), 1-7.

Otranto, D., & Deplazes, P. (2019). Zoonotic nematodes of wild carnivores. *International Journal for Parasitology: Parasites and Wildlife, 9*, 370-383.

Ouellette, J., Apperson, C. S., Howard, P., Evans, T. L., & Levine, J. F. (1997). Tick-raccoon associations and the potential for Lyme disease spirochete transmission in the coastal plain of North Carolina. *Journal of Wildlife Diseases, 33*(1), 28-39.

Page, L. K. (2013). Parasites and the conservation of small populations: The case of Baylisascaris procyonis. *International Journal for Parasitology: Parasites and Wildlife, 2*, 203-210.

Popiołek, M., Szczęsna-Staśkiewicz, J., Bartoszewicz, M., Okarma, H., Smalec, B., & Zalewski, A. (2011). Helminth parasites of an introduced invasive carnivore species, the raccoon (Procyon lotor L.), from the Warta Mouth National Park (Poland). *The Journal of parasitology, 97*(2), 357-360.

Pratt, H. D. (1956). Fleas: pictorial key to some common species in the United States. In.

Pung, O. J., Durden, L. A., Banks, C. W., & Jones, D. N. (1994). Ectoparasites of opossums and raccoons in southeastern Georgia. *Journal of Medical Entomology, 31*(6), 915-919.

Rainwater, K. L., Marchese, K., Slavinski, S., Humberg, L. A., Dubovi, E. J., Jarvis, J. A., McAloose, D., & Calle, P. P. (2017). Health survey of free-ranging raccoons (Procyon lotor) in Central Park, New York, New York, USA: implications for human and domestic animal health. *Journal of Wildlife Diseases, 53*(2), 272-284.

Richardson, D. J., Durden, L. A., & Snyder, D. E. (1994). Ectoparasites of the raccoon (Procyon lotor) from north-central Arkansas. *Journal of the Kansas Entomological Society*, 208-212.

Richardson, D. J., Owen, W. B., & Snyder, D. E. (1992). Helminth parasites of the raccoon (Procyon lotor) from north-central Arkansas. *The Journal of parasitology*, 163-166.

Rockett, C. L., & Johnston, S. (1988). Ectoparasitic Arthropods Collected From Some Northern Ohio Mammals. *Great Lakes Entomologist, 21*, 2.

Rodríguez, A. M., & Miranzo, B. M. Caracterización y selección de Hábitat del mapache (*Procyon loto*r L.) en el Parque Regional del Sureste, Madrid.

Romeo, C., Cafiso, A., Fesce, E., Martínez-Rondán, F. J., Panzeri, M., Martinoli, A., Cappai, N., Defilippis, G., & Ferrari, N. (2021). Lost and found: Helminths infecting invasive raccoons introduced to Italy. *Parasitology International*, 102354.

Sagarna, X. G. (2010). *Los carnívoros silvestres como reservorios de enfermedades de interés en sanidad animal y salud pública.* Universidad de Castilla-La Mancha,

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., & Ellstrand, N. C. (2001). The population biology of invasive species. *Annual review of ecology and systematics, 32*(1), 305-332.

Salgado, I. (2015). Mapache–Procyon lotor (Linnaeus, 1758).

Salgado, I. (2018). Is the raccoon (Procyon lotor) out of control in Europe? *Biodiversity and Conservation, 27*(9), 2243-2256.

Sapp, S. G., Elsemore, D. A., Hanna, R., & Yabsley, M. J. (2020). Experimental comparison of Baylisascaris procyonis definitive host competence between domestic dogs and raccoons (Procyon lotor). *Parasitology, 147*(12), 1344-1351.

Sapp, S. G., Rascoe, L. N., Wilkins, P. P., Handali, S., Gray, E. B., Eberhard, M., Woodhall, D. M., Montgomery, S. P., Bailey, K. L., & Lankau, E. W. (2016). Baylisascaris procyonis roundworm seroprevalence among wildlife rehabilitators, United States and Canada, 2012–2015. *Emerging infectious diseases, 22*(12), 2128.

Sato, H., & Suzuki, K. (2006). Gastrointestinal helminths of feral raccoons (Procyon lotor) in Wakayama Prefecture, Japan. *Journal of veterinary medical science, 68*(4), 311-318.

Sato, H., Suzuki, K., Osanai, A., Kamiya, H., & Furuoka, H. (2006). Identification and characterization of the threadworm, Strongyloides procyonis, from feral raccoons (Procyon lotor) in Japan. *Journal of Parasitology, 92*(1), 63-68.

Sharifdini, M., Norouzi, B., Azari-Hamidian, S., & Karamzadeh, N. (2021). The first record of ectoparasites of raccoons (Procyon lotor)(Carnivora, Procyonidae) in Iran. *Persian Journal of Acarology, 10*(1), 41-54.

Smith, K. A., Oesterle, P. T., Jardine, C. M., Dibernardo, A., Huynh, C., Lindsay, R., Pearl, D. L., & Nemeth, N. M. (2019). Tick infestations of wildlife and companion animals in Ontario, Canada, with detection of human pathogens in Ixodes scapularis ticks. *Ticks and tick-borne diseases, 10*(1), 72-76.

Sorvillo, F., Ash, L. R., Berlin, O., Yatabe, J., Degiorgio, C., & Morse, S. A. (2002). Baylisascaris procyonis: an emerging helminthic zoonosis. *Emerging infectious diseases, 8*(4), 355.

Toledo, Á., Olmeda, A. S., Escudero, R., Jado, I., Valcárcel, F., Casado-Nistal, M. A., Rodríguez-Vargas, M., Gil, H., & Anda, P. (2009). Tick-borne zoonotic bacteria in ticks collected from central Spain. *The American journal of tropical medicine and hygiene, 81*(1), 67-74.

Torchin, M. E., Lafferty, K. D., Dobson, A. P., McKenzie, V. J., & Kuris, A. M. (2003). Introduced species and their missing parasites. *Nature, 421*(6923), 628-630.

Torres, J., Miquel, J., & Motjé, M. (2001). Helminth parasites of the Eurasian badger (Meles meles L.) in Spain: a biogeographic approach. *Parasitology research, 87*(4), 259-263.

Valdez, V. F. d. C. (2020). *Raccoon (Procyon lotor) in Portugal: forecasting pathways for an invasive carnivore.*

Van Cleave, H. J. (1923). A key to the genera of Acanthocephala. *Transactions of the American Microscopical Society, 42*(4), 184-191.

Vilà, M., Bacher, S., Hulme, P., Kenis, M., Kobelt, M., Nentwig, W., Sol, D., & Solarz, W. (2006). Impactos ecológicos de las invasiones de plantas y vertebrados terrestres en Europa. *Ecosistemas, 15*(2).

Walker, A. R. (2003). *Ticks of domestic animals in Africa: a guide to identification of species*: Bioscience Reports Edinburgh.

Whitaker Jr, J. O., & Goff, R. (1979). Ectoparasites of wild carnivora of Indiana. *Journal of Medical Entomology, 15*(5-6), 425-430.

White, S. A., Bevins, S. N., Ruder, M. G., Shaw, D., Vigil, S. L., Randall, A., Deliberto, T. J., Dominguez, K., Thompson, A. T., & Mertins, J. W. (2021). Surveys for ticks on wildlife hosts and in the environment at Asian longhorned tick (Haemaphysalis longicornis)-positive sites in Virginia and New Jersey, 2018. *Transboundary and Emerging Diseases, 68*(2), 605-614.

Yabsley, M. J., & Sapp, S. G. (2017). Prevalence of Baylisascaris in domestic dog coprological examinations in the United States, 2013–2016. *Veterinary Parasitology: Regional Studies and Reports, 9*, 65-69.