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Helminth fauna of wild rabbit *Oryctolagus cuniculus* in the Canary Islands, Spain

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Summary

Helminths were examined in 292 wild rabbits (*Oryctolagus cuniculus*) collected in Tenerife, Fuerteventura, La Palma and El Hierro islands, and La Graciosa islet (Canary Islands, Spain), from 2008 to 2012. Three species of cestodes, *Taenia pisiformis* (Taeniidae), *Andrya cuniculi* and *Neoctenotaenia ctenoides* (Anoplocephalidae), and three species of nematodes *Trichostrongylus retortaeformis* (Trichostrongylidae), *Passalurus ambiguus* (Oxyuridae) and *Calodium hepaticum* (Capillariidae), were identified. Only 2 species (*N. ctenoides* and *P. ambiguus*) were regularly present in all the islands, with an average prevalence above 50 %. Possible causes of the present distribution of helminths are commented. Insular and founder effects on parasite species in isolated ecosystems are demonstrated. The finding of the zoonotic species *C. hepaticum* solely in La Palma, represents a novel identification of this nematode in *O. cuniculus* in the Canary Islands.

Keywords: *Oryctolagus cuniculus*; Canary Islands; helminth; *Calodium hepaticum*

Introduction

In the 15th century, the Canary Islands were conquered by the Spanish Empire and some species of small mammals, including the wild rabbit (*Oryctolagus cuniculus*) (Lagomorpha: Leporidae), were introduced (Foronda *et al*., 2003a). This species has colonized successfully most of the habitats of the main islands, achieving a stable population of individuals. Populations were also present in La Graciosa, Alegranza and the Lobos islets, but not in Montaña Clara, where it has been eradicated (Nogales *et al*., 2006). This is a very important species for hunting and cuniculture sectors, with a strong tradition in the Archipelago (Foronda *et al*., 2005). However, it has negative ecological effect on islands, as they affect numerous endemic plants and their associated fauna (Nogales *et al*., 2006; Delibes-Mateos *et al*., 2009).

The density of rabbits depends on several factors, i.e. seasonal changes (Cabrera-Rodríguez, 2008) or epidemics such as rabbit hemorrhagic disease (RHD) and myxomatosis (Foronda *et al*., 2000, 2005). However, it has been demonstrated that rabbit populations are able of recover slower or quicker after a catastrophe (Villafuerte *et al*., 1995).

At present, two subspecies genetically differentiated are well known (Hardy *et al*., 1995) and separated geographically. While *Oryctolagus cuniculus algirus* is restricted to the southwestern of the Iberian Peninsula, North Africa, Mediterranean and Portuguese Atlantic islands, *Oryctolagus cuniculus cuniculus* is present in the rest of mainland Spain and Europe, and in those places where they have been introduced by humans (Blasco *et al*., 1996; Branco *et al*., 2000). In the case of the Canary Islands, the introduced fauna exhibit marked European influences mostly from the Iberian Peninsula, where *O. cuniculus* is native (Juan *et al*., 2000). Similarly, species of coccidia and helminths that parasitize this animal have found optimal conditions for their establishment in the Archipelago (Foronda *et al*., 2000).

The only available data on the occurrence of helminths in wild rabbits in the Canary Islands have been reported on Tenerife island (Foronda *et al*., 2000, 2003a, 2003b, 2005) and Alegranza islet (Foronda *et al*., 2003b). So far, the species identified in Tenerife have been three cestodes: *Taenia pisiformis* (Bloch, 1780) larvae (Taeniidae), *Andrya cuniculi* (Blanchard, 1891) and *Neoctenotaenia ctenoides* (Railliet, 1890) (Anoplocephalidae); and two nematodes: *Trichostrongylus retortaeformis* (Zeder, 1800) (Trichostrongylidae) and *Passalurus ambiguus* (Rudolphi, 1819) (Oxyuridae); while in Alegranza only *N. ctenoides* and *P. ambiguus* have been found.

Therefore, the aim of the present study was to widen the existing helminthological data of the wild rabbit population in the Canary Islands, describing the helminth parasite fauna present in Fuerteventura, El Hierro and La Palma islands, and La Graciosa islet, where no previous data were available, and extending the previous information for Tenerife.

Materials and methods

Study area

The Canary Archipelago presents volcanic origin and is located about 100 km from the NW coast of Africa, between 13º 23´and 18º 8´W and 27º 37´and 29º 24´N, in the Macaronesian region. It is comprised of seven main islands and several islets (Fig. 1).

The western (La Gomera, La Palma and El Hierro) and middle (Gran Canaria and Tenerife) islands have mountain ranges of remarkable altitude, which enable the formation of the sea of clouds on the windward slopes, (approximately 1000 m a.s.l.), by the accumulation of the upper hot dry air and by the lower humid trade winds. Different from the rough middle and western islands, eastern islands (Fuerteventura and Lanzarote) and islets display lower altitudes low rainfall, and are influenced by dry winds blowing from the close Sahara Desert (Juan *et al*., 2000).

According to altitude and orientation, a variety of habitats or bioclimatic areas displaying differential also marked by vegetation features can be found. In the eastern islands and low zones of the central and western islands, vegetation is characterized by dry xerophytic shrub, while in the rest of the areas in the western and central islands, the habitats – ranging from low to high altitudes- are mixed wood, humid evergreen cloud forest (laurel forest), pine forest and shrub layer dominated by leguminous genera (Nogales *et al*., 2006). Mean temperature and annual precipitation range from about 21 ºC and 100 – 300 mm in coastal zones, and about 9 ºC and 500 – 800 mm at higher altitudes, respectively (Table 1).

Host and parasite sampling

Between 2008 and 2012, a total of 292 wild rabbit were collected from La Palma (708 km^2), El Hierro (278 Km^2), Tenerife (2036 km^2), Fuerteventura (1659 km^2) and La Graciosa (29 km^2) (Table 2), thanks to the contributions of hunters, and with the permission of *Excmo. Cabildo Insular* of all the islands studied.

Information on capture date, location and age, were recorded. Rabbits were dissected so as to examine both helminth species and burdens. The abdominal cavity was opened and the contents removed. The liver, mesenteries, pelvic area and body cavity were examined. The stomach contents were examined dry (without saline solution) in a large glass Petri dish. The small and large intestines were initially examined directly in sections and their contents filtered through a 100 mesh (125mm) sieve.

The total number of cestodes and nematodes were counted for each rabbit, and stored in 70 % ethanol before microscopic study. For species determination under light microscope, cestodes were stained with Semichon's acid carmine, sequentially dehydrated in ethanol, cleared in xylene and mounted in Canada balsam. Nematodes were cleared

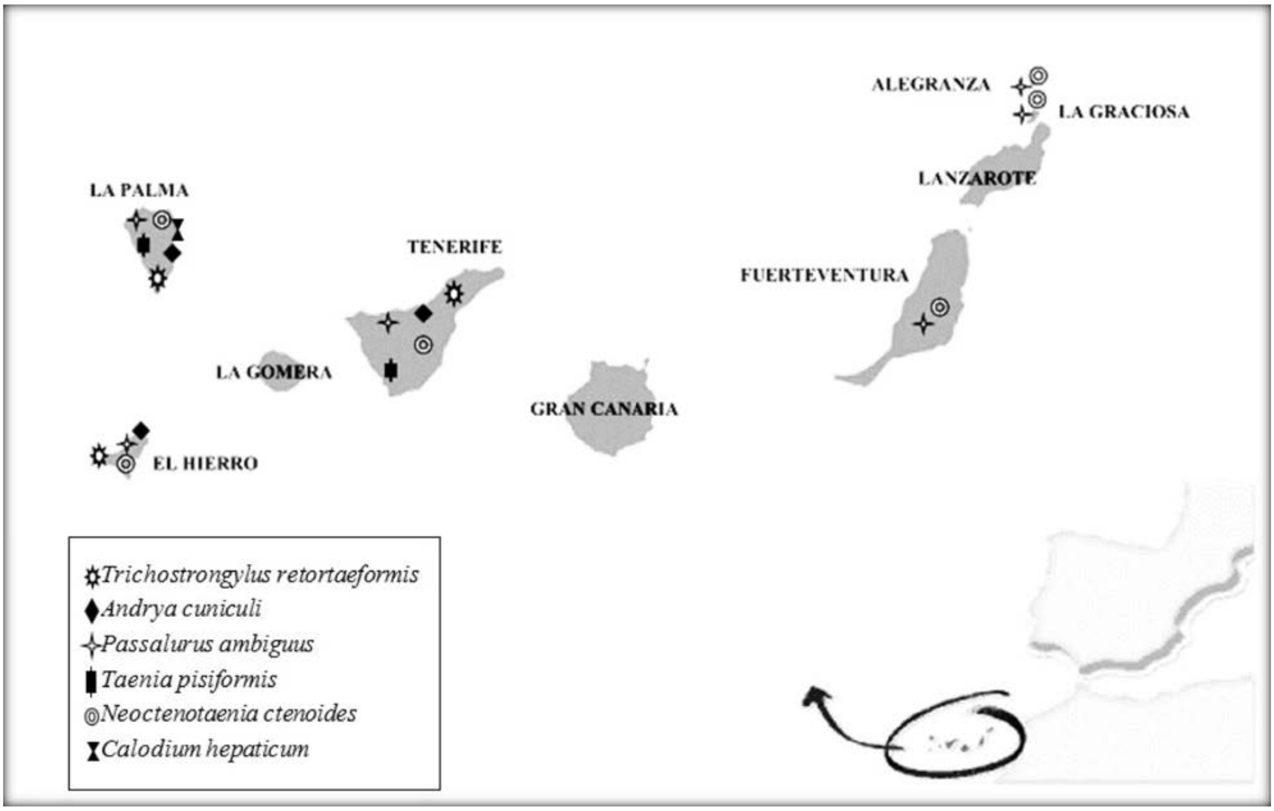

Fig. 1. Distribution of the helminth species detected in the Canary Islands. Data for Alegranza obtained from Foronda *et al*. (2003b)

Table 1. Range of values on altitude and climate in the different islands of the Canary Islands (data obtained from *AEMET*, State Meteorological Agency of Spain)

Island	Altitude (m)	Relative humidity (%)	Annual rainfall (mm)	Temperature (ºC)
La Palma	0 – 2426	60 – 78	150 – 1050	8 – 22
El Hierro	0 – 1501	70 – 82	50 – 850	12 – 22
Tenerife	0 – 3718	38 – 82	50 – 950	4 – 22
Fuerteventura	0 – 807	64 – 74	50 – 250	18 – 22
La Graciosa	0 – 266	66 – 68	50 – 150	18 – 20

in Amann's lactophenol. Helminth identification was performed based on previous works by Skrjabin *et al*. (1950, 1954, 1970), Verster (1969), Beveridge (1978), Tenora and Murai (1978), Hugot *et al*. (1983) and Tenora *et al*. (2002).

Data analysis

The statistical chi-square and ANOVA tests were used to determine differences of the helminth species in prevalence and mean intensity, respectively (Bush *et al*., 1997), among the different islands. Helminth counts of *P. ambiguus* and *T. retortaeformis* were log transformed to normalize the distribution. Significance level was p = 0.05 in both tests.

Results

Six helminth species were detected in the rabbits, cestodes *A. cuniculi*, *N. ctenoides* and *T. pisiformis* larvae; and nematodes *P. ambiguus*, *T. retortaeformis* and *Calodium hepaticum* (Bancroft, 1893) [syn. *Capillaria hepatica*] (Capillariidae).

The statistical analyses showed that the helminth species were irregularly distributed in the Archipelago (Fig. 1). Only two species, *N. ctenoides* and *P. ambiguus*, were regularly found throughout the Archipelago, with 50 % and 58 % prevalence, respectively. The rest of the parasites were characteristic of several islands. La Palma was the only island harboring all six species. In Tenerife, all the species were present except *C. hepaticum*, whilst in El Hierro, only *C. hepaticum* and *T. pisiformis* were absent. Differently, in both Fuerteventura and La Graciosa only two species *N. ctenoides* and *P. ambiguus* were found. Values of the prevalence, mean intensity and range of individual helminths are given in Table 2.

In general, all species, except *C. hepaticum* and *T. pisiformis* on La Palma, and *A. cuniculi* on El Hierro and La Palma, displayed overall prevalence higher than 10 %.

Considering the cestode species, the highest prevalence

Table 2. Prevalence (P %), mean intensity (MI) and range (in parentheses) of infection for the helminth species found in *Oryctolagus cuniculus* in the Canary Islands. (*Ac*: *Andrya cuniculi, Nc*: *Neoctenotaenia ctenoides, Tp*: *Taenia pisiformis, Pa*: *Passalurus ambiguus, Tr*: *Trichostrongylus retortaeformis, Ch*: *Calodium hepaticum*)

	Ac	*Nc*	*Tp*	*Pa*	*Tr*	*Ch*
Island	P (%) MI ± SD (range)	P (%) MI ± SD (range)	P (%) MI ± SD (range)	P (%) MI ± SD (range)	P (%) MI ± SD (range)	P (%) MI ± SD (range)
La Palma n = 106	5 1.8 ± 1.1 (1 – 4)	56 1.8 ± 1.5 (1 – 8)	7 3 ± 1.8 (1 – 5)	55 492.7 ± 1251.9 (2 – 9000)	18 42 ± 50 (2 – 200)	0.9 - (6)
Tenerife n = 115	20 4.5 ± 4.1 (1 – 17)	35 1.3 ± 0.5 (1 – 2)	23 8.7 ± 18 (1 – 90)	52 819.7 ± 1154.3 (1 – 5680)	50 93.8 ± 130.9 (1 – 500)	0 - -
El Hierro n = 50	2 3 ± 0 (3)	78 1.8 ± 0.6 (1 – 4)	0 - -	70 461.8 ± 517.2 (2 – 2000)	28 51.6 ± 60.6 (4 – 236)	0 - -
La Graciosa n = 15	0 - -	33 1.2 ± 0.4 (1 – 2)	0 - -	87 1176.8 ± 1764 (1 – 4820)	0 - -	0 - -
Fuerteventura n = 6	0 - -	67 1 ± 0 (1)	0 - -	67 772 ± 1286.5 (14 – 3000)	0 - -	0 - -
General n = 292	10 3.9 ± 3.8 (1 – 17)	50 1.6 ± 1.1 (1 – 8)	12 8.6 ± 19.1 (1 – 90)	58 660.7 ± 1176.6 (1 – 9000)	31 76.5 ± 112 (1 – 500)	0.34 - (6)

was found for *N. ctenoides* in El Hierro (78 %), while in the group of the nematodes, *P. ambiguus* showed the highest prevalence, specifically in La Graciosa islet (87 %). Comparative analysis of prevalence of the parasite species demonstrated the existence of significant differences in most of them. Attending to the general prevalence of cestodes and nematodes, tapeworms are significantly higher than nematodes ($\chi^2 = 7.8$; $p < 0.05$).

Regarding to the distribution of each helminth species, *A. cuniculi* showed higher prevalence in Tenerife than in La Palma ($\chi^2 = 11.6$; $p < 0.05$) and El Hierro ($\chi^2 = 9.1$; $p < 0.05$), and *T. pisiformis* was more prevalent in Tenerife compared to La Palma ($\chi^2 = 12.1$; $p < 0.05$). However, *N. ctenoides* was more frequent in Tenerife than in La Palma ($\chi^2 = 9.7$; $p < 0.05$) and El Hierro ($\chi^2 = 17.0$; $p < 0.05$); and El Hierro compared to La Graciosa ($\chi^2 = 10.5$; $p < 0.05$) and La Palma ($\chi^2 = 7.3$; $p < 0.05$).

With respect to nematodes, *P. ambiguus* was more prevalent in Tenerife than in El Hierro ($\chi^2 = 4.5$; $p < 0.05$) and La Graciosa ($\chi^2 = 6.4$; $p < 0.05$); and La Palma compared to La Graciosa ($\chi^2 = 5.5$; $p < 0.05$); *T. retortaeformis* showed higher values in Tenerife than in La Palma ($\chi^2 = 25.7$; $p < 0.05$) and El Hierro ($\chi^2 = 7.1$; $p < 0.05$).

Discussion

The helminth fauna of *O. cuniculus* in the Canary Islands appears qualitatively impoverished and restricted to six species (3 cestodes and 3 nematodes). Majority of these species, except of *T. retortaeformis* and *C. hepaticum*, show a clear specificity regarding to Leporidae species (Blasco, 1996).

The species that are present in all the examined islands, *N. ctenoides* and *P. ambiguus*, complete their life cycles primarily in the rabbit as definitive host and show a co-evolution process and biogeography closely related to this host (Blasco, 1996).

The loss of helminth species in *O. cuniculus* in the Macaronesian islands has been demonstrated previously in the context of the Palearctic Region (Foronda *et al*., 2003b). This process can be explained by the general characteristics of the parasitofauna in isolated ecosystems (Mas-Coma & Feliu, 1984; Magnanou & Morand, 2006). Similarly, the reduction in the number of parasite species in Fuerteventura, La Graciosa and Alegranza has been noticed (see Foronda *et al*., 2003b for Alegranza). This could be explained basically by three aspects. First, the bioclimatic differences, especially in temperature and vegetation, between the western (Tenerife, La Palma, La Gomera and El Hierro) and the eastern islands situated closer to Africa (Gran Canaria, Fuerteventura, Lanzarote, La Graciosa and Alegranza, see Table 1). Second, the small size of La Graciosa and Alegranza, means lower surface and more biodiversity loss (Mas-Coma & Feliu, 1984, Mas-Coma *et al*., 2000), limiting species with life cycles dependent of the external environment (monoxenous geohelminth nematodes as *T. retortaeformis,* and platyhelminthes, as *A. cuniculi* and *T. pisiformis*). These two last species has been found with no homogenous distribution previously (Foronda *et al*., 2003a). It could be due to an irregular distribution of the intermediate hosts for *A. cuniculi,* and the adult forms in its definitive hosts for *T. pisiformis* (see Foronda *et al*., 2003a). Although the life cycle for *A. cuniculi* is still unknown, it is likely similar to other Anoplocephalidae species, with oribatid mites as intermediate hosts. On the other hand, the irregular presence of *T. pisiformis* could be explained by the same distribution found for the adult forms in Canaries, where dogs (*Canis familiaris*) act as definitive hosts (see Foronda *et al*., 2003a).

The last, third aspect may concern the relatively low number of hosts examined in the eastern islands.

In relation to these processes, the analyses of rabbits from La Gomera, Gran Canaria and Lanzarote would be of high interest since it could confirm these three hypotheses. The three concerns are almost confirmed with the results obtained in the present work. In this sense, one of the most highlighted result is rather high prevalence of *P. ambiguus* in the small islets (87 %), presumable due to the direct life cycle without free-living larval stages, and because of the limited territory that implies more frequent contact between rabbits (Magnanou & Morand, 2006); this is in accordance with the fact that in continental regions the prevalence found for *P. ambiguus* is rather lower (12 % in *Lepus europaeus* from Slovakia) (Dubinský *et al*., 2010).

Further remarkable observation is the higher prevalence of *T. retortaeformis* in Tenerife (58 %), comparing with the rest of western islands. This result fits with the larger size of Tenerife and therefore the smaller impact on the prevalence of parasitic species, including those with dependent cycles of environmental factors. The same fact might explain the prevalence of *A. cuniculi* in Tenerife and El Hierro.

Finally, *C. hepaticum* is a cosmopolitan zoonotic nematode that parasitizes the liver of mammal species, particularly rats and mice, although the list of host species is exceptionally long, including wild lagomorphs (Moreira *et al*., 2013). Cases of human infections with *C. hepaticum* have been reported in different continents and many of them have proved to be fatal (see Mowat *et al*., 2009). The focal distribution of *C. hepaticum* was reported in rodents from La Palma, with prevalence of 100 % in *Rattus norvegicus*, 37.5 % in *Rattus rattus* and 22.5 % in *Mus musculus domesticus* but the parasite was rare or absent in the rest of the islands (Sanchez, 2013). This fact could explain the finding of this nematode in wild rabbit only in La Palma but the current result represents a novel identification of this nematode in *O. cuniculus* in the Canary Islands.

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