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Parasites (viruses, coccidia and helminths) of the wild rabbit (*Oryctolagus cuniculus*) introduced to Canary Islands from Iberian Peninsula

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Abstract

The presence of viruses (myxomatosis and haemorrhagic fever), helminth parasites and coccidia were studied in European wild rabbit (*Oryctolagus cuniculus*) introduced from the Iberian Peninsula to Tenerife Island (Canary Islands). Rabbits were captured between 1998 and 2000 in four geographic zones in Tenerife Island. Blood samples were taken from rabbits before dissection and faeces were obtained from the rectum of the individuals for the purposes of the protozoological study. In sera samples, Elisa test was carried out to detect antibodies for myxomatosis and haemorrhagic fever. Helminths were removed from the digestive tract and sporulated coccidia were obtained in potassium dichromate. Significant differences in the prevalence of the myxomatosis between years, seasons and zones were found. In all zones, there were no statistically significant differences between rabbits infected with haemorrhagic fever. Furthermore, no significant differences in prevalence between years and seasons were observed for the haemorrhagic fever. Five helminth species were found: *Taenia pisiformis* (larvae), *Mosgovoyia ctenoides*, *Andrya cuniculi*, *Trichostrongylus retortaeformis* and *Passalurus ambiguus*. No significant seasonal differences in the prevalence for any helminth species were detected. There were significant differences for the mean intensities of *T. retortaeformis* between the spring and the autumn 1999 and between the autumn and the winter 1999. Seasonal significant differences in the prevalence for *Eimeria* spp. were detected. A significant correlation between the myxomatosis and the mean intensity of *T. pisiformis* was found. Haemorrhagic fever and coccidian prevalence showed no significant relationship. The obtained results on parasites (viruses, coccidia and helminths) relationship in different zones, years and seasons are discussed in light of their transmission in Tenerife Island.

Key words

Wild rabbit, viruses, coccidia, helminths, Canary Islands

Introduction

The wild rabbit, *Oryctolagus cuniculus* (L.), was introduced to the Canary Islands at least 500 years ago, due to the common transporting of couples by sailors in those days to provide food in the islands on their way to America. This practice was boosted by the new colonists because of the hunting interest at this species. In Tenerife, the wild rabbit had successfully colonized most habitats on the island, with a total population estimated at 300,000 individuals. Both hunting and cuniculture became an important economical resource for the island being an established element in traditional Canary culture. The introduction of the rabbit implied that protozoan and helminth species were able to colonize the islands where they found optimal conditions to their establishment (Foronda *et al*. 2000). The wild rabbit populations of Tenerife had not come into contact with diseases like myxomatosis or haemorrhagic fever (HF) until 1996 when the first cases were found. Both diseases reduced the rabbit population and decreased the profitability of hunting and cuniculture.

The aim of this work was to study the distribution of the myxomatosis and the haemorrhagic fever and its relationship with the distribution of the coccidia and helminth parasites of rabbits.

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Materials and methods

The Canary Archipelago is located off NW Africa, between 13°23´ and 18°8´W and 27°37´and 29°24´N. Between 1998 and 2000, the Canary government allowed the capture of wild rabbits in four geographic zones of Tenerife (north, south-east, south-west and central) (Fig. 1). During these three years of sampling, 244 individuals were studied.

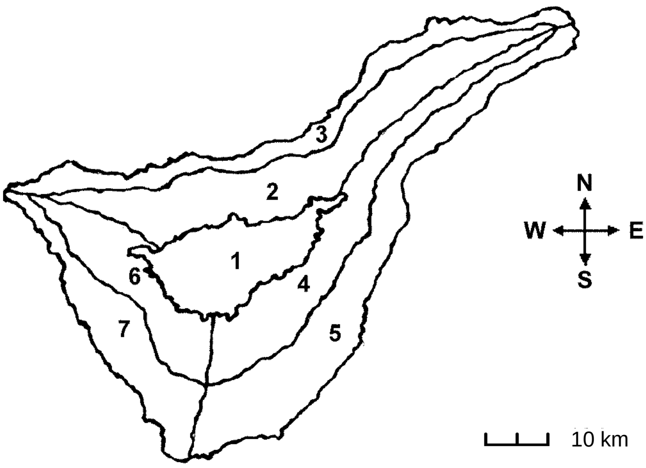

Fig. 1. Localization of the biogeographical zones considered in Tenerife Island: **1** – central, **2** – high north, **3** – low north, **4** – high south-eastern, **5** – low south-eastern, **6** – high south-western, **7** – low south-western

To determine viral diseases, sera samples were taken from blood extracted from rabbits before their dissection. Elisa test was carried out to detect the antibodies using the commercial kits "CIVTEST^{TM} Cuni Myxomatosis" and "INGENZIM Rabbit 1.7. RHDK. K.1. Ingenasa S.A." for myxomatosis and haemorrhagic fever, respectively.

Faeces obtained from rectum of the individuals were maintained in potassium dichromate. One month later, sporulated oocysts of coccidia were identified under light microscope on the basis of the work of COST 89/820 (1995).

Hosts were dissected and the helminth parasites were removed and preserved in 70% ethanol. For species determination under light microscope, platyhelminths were stained with Semichon's acid carmine, sequentially dehydrated in ethanol, cleared in xylene and mounted in Canada balsam. Nematodes were cleared in Amann's lactophenol. Helminth identification was based on the previous works by Skryabin *et al*. (1954), Verster (1969), Beveridge (1978), Tenora and Murai (1978), Tenora *et al*. (1981/82) and Hugot *et al*. (1983). Statistical chi-square and ANOVA tests were used to test for differences in the prevalence and the mean intensity of the helminth species (as defined by Bush *et al*. 1997) between the zones studied. Helminth counts were log transformed to normalize the distribution.

Results

The general prevalence of the myxomatosis in Tenerife was 27%, with infected rabbits in all studied zones. The prevalence over a three-year period is shown in Table I. Significant differences were observed between 1998 and 2000 (χ^2 = 12.87; p<0.05) and between 1999 and 2000 (χ^2 = 4.11; p<0.05). Seasonally, the prevalence in winter was significantly higher than in summer (χ^2 = 7.04; p<0.05) and autumn (χ^2 = 5.06; p<0.05) (Table I). Between zones, the prevalence ranged between 12% in the central and 40% in the south-western (Table I), with significant differences between them (χ^2 = 6.02; p<0.05).

The general prevalence of the haemorrhagic fever in Tenerife was 14% with infected rabbits in all studied zones. The prevalence over three years is shown in Table I, without significant differences. No seasonal or geographic differences were found (Table I).

The oocysts found in faeces belonged to the genus *Eimeria*. Their prevalence was 51%. Significant differences were found between summer 1998 and autumn 1998, winter 1998–1999 and summer 1999 (χ^2 = 4, p<0.05; χ^2 = 4.4, p<0.05; χ^2 = 4.6, p<0.05).

Five helminth species were found in the studied seasons from the summer 1998 to the winter 1999–2000 (Table II). Voucher specimens were deposited in the Natural History Museum of Tenerife: *Mosgovoyia ctenoides* (accession no. TFMCCS/0004), *Trichostrongylus retortaeformis* (TFMCNA/0001) and *Passalurus ambiguus* (TFMCNA/0002) were found in all seasons, with prevalence higher than 10% in all cases except *M. ctenoides* in winter 1998–1999. The general prevalence of these species was 29% for *M. ctenoides*, 43% for *T. retortaeformis* and 48% for *P. ambiguus*. *Taenia pisiformis* cisticercus (TFMCCS/0005), with a general prevalence of 16%, was only detected between winter 1998–1999 and summer 1999. *Andrya cuniculi* (TFMCCS/0003) was the species with the lowest prevalence and it did not appear

Table I. Prevalences (P) of myxomatosis (MX) and haemorrhagic fever (HF) in Tenerife

		N	MX P (%)	HF P (%)
Zone	north	132	24	11
	south-eastern	60	30	12
	south-western	42	40	14
	central	40	12	10
Year	1998	83	13	15
	1999	103	25	16
	2000	87	41	14
Season	spring	51	25	13
	summer	155	23	15
	autumn	33	18	15
	winter	34	50	18

N – number of studied individuals in each sample.

Table II. Prevalences (P), mean intensities (MI), standard deviations (SD) and range (min-max) of the coccidia and helminth species in Tenerife

Season Year	*Eimeria* spp. P (%)	*T. pisiformis* P (%) NMI ± SD (min-max)	*M. ctenoides* P (%) MI ± SD (min-max)	*A. cuniculi* P (%) MI ± SD (min-max)	*T. retortaeformis* P (%) MI ± SD (min-max)	*P. ambiguus* P (%) MI ± SD (min-max)
Summer 1998 N = 12	92	–	25 1 ± 0 (1)	17 9 ± 11.3 (1–17)	67 157.1 ± 165.1 (1–500)	50 1145.3 ± 896.2 (2–2225)
Autumn 1998 N = 9	22	–	44 1.25 ± 0.5 (1–2)	–	11 20 ± 0 (20)	78 764 ± 886.3 (11–2260)
Winter 1998–1999 N = 14	29	43 20.3 ± 35.4 (1–90)	5 1.3 ± 0.6 (1–2)	–	36 44.5 ± 31.8 (6–73)	21 370 ± 268.7 (180–560)
Spring 1999 N = 19	74	26 4.8 ± 3.1 (1–8)	26 1.2 ± 0.4 (1–2)	–	42 39.6 ± 32.8 (12–106)	26 759.8 ± 1152.6 (3–2750)
Summer 1999 N = 23	4	28 6.2 ± 10.0 (1–24)	30 1.3 ± 0.5 (1–2)	26 1.7 ± 0.8 (1–3)	48 42.4 ± 101.0 (2–360)	65 442.3 ± 1094.4 (2–3620)
Autumn 1999 N = 13	38	–	38 1.2 ± 0.4 (1–2)	15 2 ± 0 (2)	54 11.6 ± 11.6 (2–34)	61 489.7 ± 667 (5–1890)
Winter 1999–2000 N = 9	78	–	22 1.5 ± 0.7 (1–2)	11 3 ± 0 (3)	22 89 ± 22.6 (73–105)	33 480.3 ± 754.5 (1–1350)

N – number of individuals studied in each sample.

between the autumn 1998 and the spring 1999. There were no significant seasonal differences in the prevalence for any helminth species. Comparing the mean intensities, significant differences were found for *T. retortaeformis* between the spring and the autumn 1999 ($U = 8.0$, $p<0.05$) and between the autumn and the winter 1999 ($U = 0.1$, $p<0.05$).

The obtained results for coccidia and helminth parasites in infected and in non-infected rabbits by myxomatosis and haemorrhagic fever viruses were compared. In the total sample, the hosts with myxomatosis showed a lower mean intensity for *T. pisiformis* ($U = 889.5$; $p<0.035$). If the haemorrhagic fever is more prevalent the coccidian prevalence decreases.

Discussion

The wild rabbit *O. cuniculus* has an Iberian origin and a wide distribution in Europe. It is one of the best small colonizers of island ecosystems (Rowe 1981, Amstrong 1982, Wodzicki and Taylor 1984). The population of *O. cuniculus* in the Canary Islands recently arose by human transport along the sea. The constant reintroduction of rabbit individuals from Iberian Peninsula resulted in the introduction of several helminth and coccidian species in these islands. Although, the helminth richness found in this host in Tenerife was very low compared with the Iberian Peninsula helminth fauna. This fact has been widely explained in Foronda *et al*. (2003b). In that work, the explanation of why some parasite species colonized Tenerife and others were not successfully discussed. The qualitative and quantitative distributions of these species at the island are influenced by biotic and abiotic factors (Foronda *et al*. 2003a). The helminth species mentioned in the latter paper are invading species for Tenerife, but they do not affect any of the local species. It is due to *O. cuniculus* which is the only lagomorpha species in Tenerife, and these helminth species are very specific. *Taenia pisiformis* also parasite of dogs, but it has been reported for this host in Tenerife since 1985 (Foronda *et al*. 2003a). More recently, viral diseases such as the myxomatosis and HF were also introduced. The first records of myxomatosis and HF in Iberian Peninsula were in 1953 and 1988, respectively. The introduction of both diseases in Canary Islands is very recent and it is due to the repeated introduction of rabbits for hunting activities. The pathology of both diseases is well known and their effects on the wild rabbit populations are well documented. The clinical forms of myxomatosis depend on the strain virulence and all of them could be mortal (Joubert *et al*. 1973). The use of myxomatosis to control the wild rabbit populations in Australia and Coll Island (Scotland) is well known; this disease drastically reduced their population density (Boag 1987). The clinical forms of the HF are related to the degree of infection and their effects on wild rabbit population are similar to those of the myxomatosis. Martins (1993) reported that HF in 1990 wiped out rabbit populations in most of Azores Archipelago islands. The impact of both diseases on the Canary Islands wild rabbits is not evaluated due to their recent introduction. In several zones of Europe, myxomatosis shows seasonal dynamics with an epidemic phase in summer and autumn due to the ecology of the vectors (Parer and Korn 1989). In Tenerife, we observed an increase of the disease between 1998 and 2000 with peaks during the winter. This could be explained by the fact that the high temperatures make the transmission of the virus difficult as it was demonstrated in other studies (Parer and Korn 1989). This fact also explains the low prevalence of the myxomatosis recorded by us in the rabbit population of the central zone of the island where the climate is dry and cold along the year whereas the remaining zones, characterised by lower altitudes, exhibit higher degree of humidity and moderate temperatures.

In myxomatosis, the direct contact is less significant for the transmission compared to the vectorial transmission by fleas and mosquitoes (Joubert *et al*. 1973). Rabbit fleas are one of the principal vectors. With the introduction of the wild rabbits to the Canaries, different species of fleas were introduced (Cordero del Campillo *et al*. 1994). In our study about parasites of the wild rabbits in Tenerife, the only one detected flea species is *Ctenocephaloides canis* (Foronda, unpubl. data), which has dogs and cats as usual hosts. The presence of *C. canis* in the wild *O. cuniculus* is not surprising due to the considerable hunting activities in Tenerife. *C. canis* is probably involved in the transmission of myxomatosis, due to that the vectorial capacity of other flea species has been demonstrated earlier (Joubert *et al*. 1973). The biology of the flea species allows to maintain their vectorial capacity in wide geographical ranges with diverse climatic conditions.

The mosquitoes of the family Culicidae play a main role in the transmission of the myxomatosis. The species of *Anopheles* and *Aedes* are the most important vectors in the transmission of this disease. In the Canary Islands, several species of these genera have been reported (Baez 1987, Cordero del Campillo *et al*. 1994). Due to the climatic conditions on Tenerife Island, the mosquito species can be present in every season and maintain the infection in the wild rabbits. The biogeographical conditions in several zones in Tenerife influence the density of mosquitoes. This fact could explain the seasonal character of the myxomatosis but no statistical differences were found in the prevalence in the four zones studied in the course of the present investigations.

The most important transmission way of HF is the aerial because the virus is strongly resistant to abiotical factors (Du *et al*. 1991). The indirect transmission is also possible by the high resistance of the virus in the secretions of the wild rabbits in the burrows. This disease could appear in any season although several studies revealed a seasonal incidence in spring and autumn. In our study of HF, no significant differences were found between years, seasons or geographical zones. The high temperatures seem not to be in favour for the transmission of the viruses (Rosell *et al*. 1990). In Tenerife, the prevalence of HF is homogeneous in the studied zones, though their different climatic conditions.

Unclear relationship exists between the presence of the myxomatosis and the helminth parasites. The only available data of the influence of this virus on the helminth fauna are reported in the experimental work by Mykytowycz (1959) and, under natural conditions, by Boag (1985) in Scotland and Molina *et al*. (1998) in Iberian Peninsula. Mykytowycz (1959) reported an increase in the number of *Graphidium strigosum* in myxomatous rabbits. The results of Boag (1988) suggested that the myxomatosis reduces the resistance of rabbits to the infection by several parasite agents and causes an increase of the helminth burdens in cases of *T. retortaeformis* and *P. ambiguus*. Molina *et al*. (1998) found that *G. strigosum* and *T. retortaeformis* had a significant lower infection rate in hosts suffering from the myxomatosis than in the healthy ones. No data are available about the effects of the myxomatosis on coccidian infections. Our results show that there is not a statistically significant relationship between coccidian infections and the myxomatosis. Moreover, no significant differences in the prevalence and the mean intensities were found for any of the helminth species in the infected rabbits.

The effect of the HF disease on the coccidia and the helminth fauna of the wild rabbit was never studied. Our results demonstrated that HF has no influence on the prevalence of coccidia and helminths and on the mean intensities of helminths in the studied zones from Tenerife.

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