

Parasite fauna of rodents (Murinae) from El Hierro (Canary Islands, Spain): a multidisciplinary approach

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Abstract

The parasite fauna (protozoa, helminths and insects) of the two most widespread Murinae rodents in El Hierro (Canary Islands, Spain), the black rat (*Rattus rattus*) and the house mouse (*Mus musculus domesticus*) was studied. Faunistic, ecological, ecotoxicological data, as well as information on the biology of some nematode parasites of *R. rattus* are provided. The present work is unprecedented in the Canary Islands, and provides the first data on the parasite biodiversity in Murinae from the archipelago. Concerning to parasitofaunas stands out: a) impoverishment of biodiversity of helminths respect of which have the same hosts in other islands; b) increasing the number of species of Siphonaptera, even compared with flea species that parasitize the same hosts from continental biotopes.

Keywords

Canary Islands, El Hierro, parasites, heavy metals, *Mus musculus domesticus*, *Rattus rattus*

Introduction

The role of the black rat (*Rattus rattus*) and the house mouse (*Mus musculus domesticus*) for public health has been approached in multiple occasions since they are cosmopolitan species that colonize diverse habitats and tend to be peridomestic (Cameron 1949, Gratz 1994, Meyer *et al.* 1995, Battersby and Webster 2001, Singleton *et al.* 2003, etc). In isolated ecosystems most of these studies have been targeting at the parasite helminth fauna and the potential role of rodents as reservoirs of parasitic zoonoses (Casanova *et al.* 1996, Miquel *et al.* 1996, Waugh *et al.* 2006, Milazzo *et al.* 2010).

The present work was carried out in El Hierro (Fig. 1), the most western island of the Canary Archipelago (Spain). Several studies on parasites (protozoan, helminths and arthropods) of *R. rattus* and *M. m. domesticus*, the two most widespread rodents on the island, have permitted to provide the first data about the parasite fauna of both murine species in the Canary

Islands. Previously, similar multidisciplinary studies had been performed only sporadically. In addition to analyzing the parasite biodiversity, their importance relies on the necessity of information on the degree of infections in nature as well as on their sanitary and epidemiological relevance (Webster and Macdonald 1995). This work also includes data on the biology of some helminth species (Nematoda), and about the presence of heavy metals (Cd, Pb, Hg) in hosts. Heavy metals, similarly to other pollutants, are worldwide distributed into the environment and can cause toxic effects in the biota. Information about these effects in wild mammals is limited despite that it may be relevant to predict environmental risk (Hamers *et al.* 2006, Sanchez-Chardi *et al.* 2007). Therefore, biomonitoring pollution through wild animals is useful for the assessment of environmental quality. Nevertheless, few works have been carried out in areas of high ecological interest, which are also subject to other types of anthropogenic chemical stress (Eira *et al.* 2005). Such is the case of El Hierro designated as biosphere reserve by UNESCO in year 2000.

Materials and methods

Samples collection

El Hierro, with an area of 268 km², is located 17°53'–18°09'W and 27°38'–27°50'N and belongs to the Canary Archipelago (Northwest of Africa) (Fig. 1). From this island, a total of 226 rodents (53 *R. rattus* and 173 *M. m. domesticus*) were captured in 2007–2010 and euthanized. The samples were obtained during all the seasons at different altitudes, between 76–1337 m, mainly in the northern part of the island.

Parasites examination

Faeces were obtained from 37 animals (11 rats and 26 mice), homogenized in vials containing 2% (w/v) of aqueous potassium dichromate (K₂Cr₂O₇), and stored at 4°C. In the laboratory, faecal samples were examined for the presence of parasites using different techniques. A flotation method using modified Sheather's sugar solution (sp.g.1.3) was carried out in order to look for *Eimeria* (Sheather 1923).

Thirty faecal samples (14 rats and 16 mice) were concentrated using a modification of the Ritchie's formaldehyde-ether method in sterile conditions (Ritchie 1948). Part of the sediment was fixed with 10% formaldehyde and analysed individually for the presence of *Giardia* cysts using direct observation with iodine solution (Golvan and Drouhet 1977). Thin smears were made from concentrated faecal samples of nine rats and 26 mice, and screened for *Cryptosporidium* oocysts by using the modified Ziehl-Nielsen stain (Henriksen and Pohlenz 1981). Protozoan

cysts were studied using a Provis AX 70 (Olympus, New York, USA) microscope.

Helminth and arthropods parasites were collected and processed for morphological analysis. All material was preserved in 70% ethanol. Cestodes and acanthocephalans were stained in ferrum-acetocarmine and mounted in Canada balsam. Nematodes were cleared in Amann lactophenol. Fleas were treated with potash prior to be mounted in Canada balsam.

Intermediate hosts

In order to look for the intermediate hosts of the detected helminths, five invertebrate species, 97 *Pimelia laevigata costipennis*, 150 *Hegeter amaroides*, 28 *Alloxantha ochracea* (Coleoptera), 2 *Cydnius aterrimus* (Hemiptera) and 4 *Canari-labis maxima* (Dermaptera) from the location called Guinea (27°46'29"N; 17°59'55"W) were dissected and analyzed.

Heavy metals

Twenty rats from Guinea and 20 mice from Frontera forest site (27°45'00"N; 17°59'09"W), were used to analyze levels of metals (Cd, Hg and Pb) in their tissues. Samples of kidneys, liver and muscle were taken using stainless-steel instruments and frozen at –20°C until being processed. Around 100 mg of each sample was mineralized in Teflon vessels with 2 ml HNO₃ (Merck, Suprapur, Darmstadt, Germany) and 1 ml H₂O₂ (Panreac, Barcelona, Spain) overnight in an oven at 90°C. All process was standardized in the Centres Científics i Tecnològics de la Universitat de Barcelona (detection limits and accuracy of results). All concentrations were determined as ng g^{–1} wet weight.

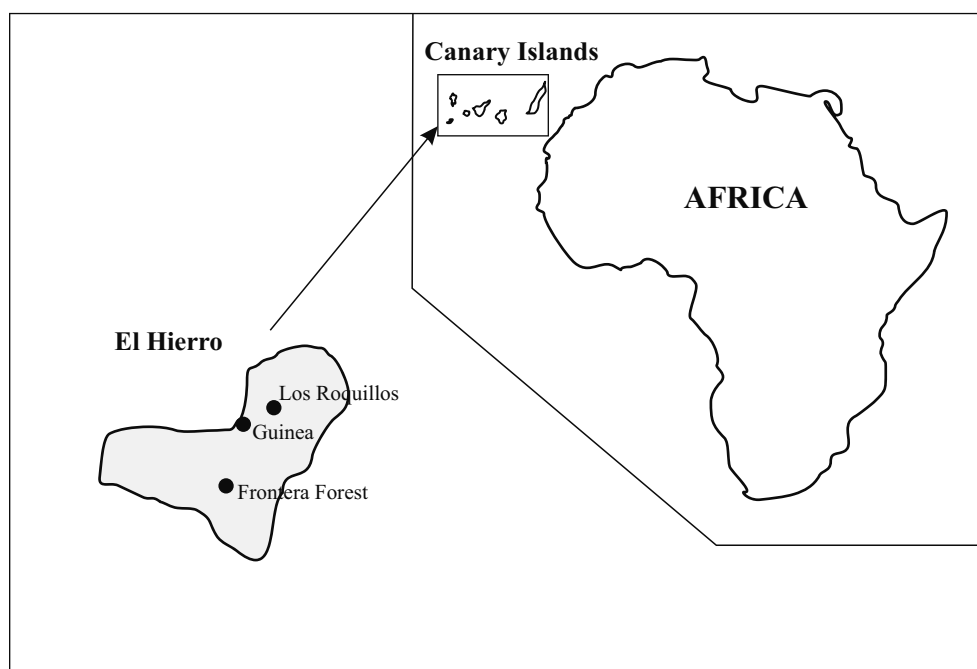


Fig. 1. Location of El Hierro (Canary Islands, Spain) and the studied places

Data analysis

The ecological terminology follows Bush *et al.* (1997). Several statistical tests, such as χ^2 , Kruskal-Wallis and Mann-Whitney, were used to evaluate significance in the comparisons of prevalences and abundances. Non parametric Spearman's correlation was used to test relationship between abundance of helminths and altitude.

Generalised linear models with SPSS Statistics 17.0 were used to evaluate factors affecting abundance of helminth species. Negative binomial error terms were specified for this purpose. Abundance of the most prevalent species was screened as dependent variable. The abundance of the remaining helminth species, total abundance and richness of the communities, host-sex, season and kind of biotope where the hosts were trapped were regarded as independent variables. The influence of parasitism on the weight of the rats was assessed by a log-normal regression. In that case, abundance of all helminths and flea species, as well as biotope, were included as predictors. Several pairwise two-way interactions between the above specified variables were also considered in the preliminary models. The minimal acceptable model was derived by backward stepwise deletion from a preliminary model that included all variables and interactions considered. The cut-off for keeping a variable in the final model was set to $p < 0.05$.

Results

Three protozoan, 18 helminths, five siphonapteran and one anopluran species were identified (Table I).

Both rodent species were found infested by *Giardia muris*, *Eimeria* sp. and *Cryptosporidium parvum*, with general prevalences 16.6%, 67.6% and 48.6%, respectively. There were no significant differences between hosts, neither between biotopes (Table I).

The helminth fauna of *M. m. domesticus* is composed of two cestode and six nematode species, plus acanthocephalan larvae of one species. The oioxenous species *Rodentolepis microstoma* and *Syphacia obvelata* had the highest prevalences and mean intensities (Table I). Among the oligoxenous/euxenous, *Trichuris muris* and *Mastophorus muris* were the most frequently detected species, while *Protospirura* sp., *Rictularia* sp., *Heterakis spumosa* and *Prosthorrhynchus cylindraceus* larvae were occasional. The most remarkable absence belonged to digenean.

The general infection by helminths in *M. m. domesticus* was not high (60.7%) and it was altitudinally concentrated, especially for *R. microstoma* and *T. muris*. In fact, mice parasitized by both helminth species have mainly been found in the zone of the highest altitude, which corresponds to Frontera forest place (KW = 18.34, $p < 0.01$).

Considering the mice with helminths, most of them appeared infested by few species (46.2% with one, 11.6% and 2.9% with two and three, respectively).

The helminth fauna of *R. rattus* was constituted of four cestode, 10 nematode, and one acanthocephalan species. The composition of this helminth fauna, from qualitative and quantitative points of view, was surprising, due to the atypical presence of four nematode species isolated from stomach (*M. muris*, *Protospirura* sp., *Streptopharagus greenbergi* and *Gongylonema neoplasticum*) and *Moniliformis moniliformis* in the intestine, with a notable prevalence and intensity of parasitism (Table I). These species, except *M. muris*, were mainly found in semiarid and close to sea level biotopes. This fact was relevant in Guinea, where appeared the last five cited species. *Streptopharagus greenbergi* and *M. muris* were the most prevalent species (35.8% and 34%, respectively) and *M. moniliformis* had the highest mean intensity (55.9). *Rodentolepis fraterna*, *Mesocestoides* sp. larvae, *Calodium hepaticum*, *Rictularia* sp., and *H. spumosa* were lacking of importance in rats studied. Again, the absence of digenean occurred.

Polyparasitism was significantly higher in *R. rattus* compared to *M. m. domesticus* (U = 2433.0, $p < 0.01$). The 79.1% of the studied rats presented helminth species, where 20.7% had one species, 22.6% and 15.1% showed two and three, respectively; and 11.3% hosted four to six.

Related to ectoparasites, the louse *Polyplax spinulosa* was present in 4.4% of rats. All five siphonapteran species detected in this study parasitized *M. m. domesticus* but only three of them were detected on rats (Table I). *Rattus rattus* and *M. m. domesticus* showed similar overall flea prevalence (42.2% and 43.1%, respectively; $\chi^2 = 1.40$, $p = 0.24$). However, significant differences were found in prevalence of *Xenopsylla cheopis* ($\chi^2 = 5.85$, $p < 0.05$) and *Leptopsylla segnis* ($\chi^2 = 10.12$, $p < 0.01$) with respect to host species. In contrast, parasitic burdens were not significantly different for the three common flea species detected in both rodent species.

In mice, the most prevalent flea species was *S. t. tripectinata* (28.8%). The prevalence of *N. barbarus* and *L. segnis* ranged from 9.4–7.5%. Concerning *Echidnophaga murina* and *Xenopsylla cheopis*, both fleas showed the lowest prevalence (5.0–3.8%). In the same host, a negative correlation was found between abundance of *E. murina* and host weight (B = -0.166; Wald = 5.68; $p < 0.05$). Every siphonapteran species, except *S. t. tripectinata*, displayed similar mean intensity. Most of *M. m. domesticus* parasitized by fleas harboured only one flea species (52 individuals), some of them (16 individuals) carried out two flea species and only one mouse was parasitized by three species (*L. segnis*, *S. t. tripectinata* and *N. barbarus*). The most prevalent flea association was *S. t. tripectinata*-*N. barbarus* (6 of 16 individuals, 37.5%).

In rats, *L. segnis* and *X. cheopis* were the most prevalent siphonapteran species (24.4% and 13.3%, respectively). The other flea species (*N. barbarus*) showed 8.9% of prevalence (Table I). Ten rats were parasitized by one flea species, four by two and one carried three species (*L. segnis*, *X. cheopis* and *N. barbarus*). In this murinae species, the most prevalent two-flea association was *L. segnis*-*N. barbarus* (3 of 4 individu-

Table 1. Infection site, prevalence (P), mean intensity (MI) and the variance (Var)/mean ratio (MR) of protozoans, helminths and ectoparasites of *Mus musculus domesticus* (n = 173) and *Rattus rattus* (n = 53) from El Hierro, Canary Islands

Parasite group	Parasite species	Infection site	Host taxon			<i>Rattus rattus</i>		
			<i>Mus musculus domesticus</i>	<i>Mus musculus domesticus</i>	<i>Mus musculus domesticus</i>	MI	Var/MR	Var/MR
			MI (CI 95%)	Var/MR	P (%)	(CI 95%)		
Protozoa	<i>Giardia muris</i>	Intestine			12.5 (n = 16)			
	<i>Eimeria</i> sp.	Intestine			69.2 (n = 26)			
	<i>Cryptosporidium parvum</i>	Intestine			38.5 (n = 26)			
Cestoda	<i>Rodentolepis microstoma</i>	Liver	4.6 (3.5–5.8)	7.2	27.2			
	<i>Taenia taeniaeformis</i> larvae	Liver	1.1 (1.0–1.3)	1.1	5.8			
	<i>Hymenolepis diminuta</i>	Small intestine						
	<i>Rodentolepis fraterna</i>	Small intestine						
	<i>Mesocestoides</i> sp. larvae	Peritoneal cavity						
Nematoda	<i>Calodium hepaticum</i>	Liver						
	<i>Trichosomoides crassicauda</i>	Bladder						
	<i>Gongylonema neoplasticum</i>	Stomach						
	<i>Mastophorus muris</i>	Stomach						
	<i>Protospirura</i> sp.	Stomach						
	<i>Streptopharagus greenbergi</i>	Stomach						
	<i>Rictularia</i> sp.	Small intestine						
	<i>Heterakis spumosa</i>	Caecum						
	<i>Syphacia muris</i>	Caecum						
	<i>Syphacia obvelata</i>	Caecum						
	<i>Trichuris muris</i>	Caecum						
Acanthocephala	<i>Moniliformis moniliformis</i>	Small intestine						
	<i>Prosthynchus cylindraceus</i> larvae	Peritoneal cavity						
Siphonaptera	<i>Echidnophaga murina</i>	Fur						
	<i>Leptopsylla segnis</i>	Fur						
	<i>Nosopsyllus barbarus</i>	Fur						
	<i>Stenoponia tripectinata tripectinata</i>	Fur						
	<i>Xenopsylla cheopis</i>	Fur						
Anoplura	<i>Polyplox spinulosa</i>	Fur						

CI – confidence interval; * occasional presence.

Table II. Prevalence (%) of larval helminths in invertebrate intermediate hosts

Intermediate host species	Parasite genera		
	<i>Streptopharagus</i>	<i>Gongylonema</i>	<i>Mastophorus</i>
<i>Pimelia laevigata costipennis</i>	16.5	4.1	2.0
<i>Hegeter amaroides</i>	7.3	2.6	–
<i>Alloxantha ochracea</i>	–	–	–
<i>Cydnius aterrinus</i>	–	–	–
<i>Canarilabis maxima</i>	–	–	–

als, 75%). The overall prevalence of ectoparasites was similar in both host species (42.8%).

After eliminating in a backward stepwise manner non significant explanatory variables or interactions from the preliminary saturated model, weight of the rats was found to be negatively correlated with abundance of *M. muris* ($B = -0.018$, Wald = 7.21, $p < 0.01$); *S. greenbergi* ($B = -0.025$, Wald = 12.47, $p < 0.01$); *M. moniliformis* ($B = -0.006$, Wald = 9.53, $p < 0.01$) and the flea *L. segnis* ($B = -0.294$, Wald = 7.75, $p < 0.01$). On the other hand, significant positive correlations were found between abundance of *Protospirura* sp. ($B = 0.021$, Wald = 12.14, $p < 0.01$); *G. neoplasticum* ($B = 0.050$, Wald = 4.66, $p < 0.05$); *T. muris* ($B = 0.994$, Wald = 23.60, $p < 0.01$) as well as coinfection *M. muris*-*L. segnis* ($B = 0.026$, Wald = 5.32, $p < 0.05$) and weight of rats.

Concerning to the identification of the larval stages found in the intermediate hosts, it was based on the articles with detailed larval morphology (Desportes *et al.* 1949; Chabaud 1954 a, b; Quentin 1970; Quentin and Seureau 1978; Quentin *et al.* 1986) (Table II).

The mean concentration of Cd in kidneys, liver and muscle of *R. rattus* were 96.3, 19.1 and 1.3 ng g⁻¹, respectively, whereas in *M. m. domesticus* were 36.8, 12.6 and 1.4 ng g⁻¹, respectively. Concerning Pb, the respective obtained values were 111.6, 24.6 and 14.6 ng g⁻¹ in rats and 10.3, 14.9, and 10.1 in mice. It was possible to quantify the level on Hg in kidneys of rats only (70.4 ng g⁻¹) while the hepatic and the muscular levels were under the limit of detection. Renal, hepatic and muscular levels of Hg from mice were 44.5, 25.6 and 11.1 ng g⁻¹ respectively. The highest individual levels of Cd, Pb and Hg were found in the kidneys of individuals of *R. rattus* (752.6, 452.2 and 245.1 ng g⁻¹, respectively).

Discussion

The presence of protozoans in Murinae rodents has been revealed several times and with varying percentages (Franjola *et al.* 1995, Webster and Macdonald 1995, Chalmers *et al.* 1997, Torres *et al.* 2000, van Keulen *et al.* 2002, Sturdee *et al.* 2003, Lv *et al.* 2009). The general prevalences of *Giardia* and *Eimeria* detected in the present work were similar to that found in murids by other authors (Higgs and Nowell 2000, Bajer 2008). Relative to *Cryptosporidium*, the prevalence was higher than

found for murids by other authors, where values were around 10% (Torres *et al.* 2000, Foo *et al.* 2007).

From the helminthological point of view, the parasite fauna of *M. m. domesticus* and *R. rattus* were structurally similar to that found in the same hosts in other Atlantic/Mediterranean islands, although with less richness (Jimenez 1992; Casanova *et al.* 1996; Mas-Coma *et al.* 2000; Milazzo *et al.* 2003, 2010). The studied hosts did not show notable infections, with the exception of *R. microstoma* in *M. m. domesticus* trapped in Frontera forest (a laurel forest located at 1305 m asl), and *R. rattus* with stomach nematodes and *M. moniliformis* in Guinea and Los Roquillos (27°49'12"N; 17°57'49"W)(semi-arid isoclimatic zones located at 76 m and 392 m asl, respectively).

The fact that *R. microstoma* was the most prevalent species (27.2%) in *M. m. domesticus* from El Hierro could be due to the high number of mice studied ($n = 75$) from Frontera forest place, where this cestode was preferentially located, opposite to that occur in other Canary islands where *R. microstoma* is highly spread (Foronda *et al.* 2011). However, there is a clear seasonal influence in these results (KW = 25.9, $p < 0.01$). At the beginning of autumn, a high percentage of infested mice (57.7%) was observed, with a mean intensity of 4.4. The mean weight of infested mice was 12.5 g in this season ($n = 52$).

In the same area, no infection by *R. microstoma* was found in middle winter, and the mean weight was higher (14.5 g, $n = 16$). However, no relationship was found between *R. microstoma* and weight, neither considering only these two parameters, nor considering the coinfection with other helminth species, especially *T. muris*. It is important to take into account that the hepatic site of *R. microstoma* and its big size imply several physiological and digestive pathologies previously described (Simpson and Gleason 1975, Pappas and Schroeder 1977, Evans *et al.* 1985, Novak *et al.* 1985, Novak and Nombrado 1988, etc).

In rats from Guinea and Los Roquillos, *M. muris*, *S. greenbergi*, *Protospirura* sp., *G. neoplasticum* (stomach) and *M. moniliformis* (duodenum-small intestine) were detected at digestive level and showed notable prevalences. This poly-parasitism is uncommon in *R. rattus*, following previous studies about this host in insular and continental ecosystems (Jimenez 1992, Feliu *et al.* 1997). Only in the North of Africa the presence of several stomach nematodes seems usual

(Jimenez 1992). The characteristics of the helminth fauna in the isolated ecosystems (Magnanou and Morand 2006) and the physiography of the island, with biotopes similar to North Africa, could explain these results. In this case, the seasonal incidence was again evident (KW = 16.53, $p < 0.01$), appearing the highest prevalence of infested rats (91.3%) in autumn (with a mean of 2.14 species in stomach/duodenum). This was notably lower in winter (14.2%, with a mean of one species), and increased in summer (50%, mean 2.25). There were not enough animals captured in spring to be compared. A relationship between polyparasitism and weight of the rats is observed. In the periods when the infections was higher, the rats were juveniles and sub-adult (101.1 g mean weight in autumn; 78.2 g in summer) while in the period of low infections, rats were adult (140.1 g mean weight in winter) (< 100 g, juveniles; > 130 g, adults; following Delattre and Le Louar 1981).

The negative correlation between *M. muris* and rat weight could be related to the occupancy of the typical site (stomach) by the nematode, which could cause digestive disturbance, and consequently weight loss.

A histopathological study of the microhabitat (duodenum) of the helminth with the highest mean intensity (*M. moniliformis*) was carried out. Histological lesions were observed. These lesions included mild catarrhal enteritis and occasional dilatation of the intestinal crypts. Parasitic forms compatible with acanthocephalans were also observed associated with the luminal surface. Taking into account that the detected pathology is compatible with a weight loss (Stephenson *et al.* 2000) and the fact that bacterial co-infection are often present in severe infections by acanthocephalans (Taraschewski 2000), it is highly probable that the parasitic burden that harbour the rats affect the growth, or even cause the host death (Crompton and Nickol 1985). It is more probable in the common cases where *M. moniliformis* share host with other stomach species. At this point, it is important to remember the negative correlation between *S. greenbergi*, *M. muris* and *M. moniliformis* and rat weight. Furthermore, diverse physiopathological effects caused by *Mastophorus* and *Protophysura* have been described (Krauss 1977, Mafiana *et al.* 1997, Lowrie *et al.* 2004). On the other hand, the capacity of *G. neoplasticum* to cause carcinoma in rats have been questioned and related with avitaminoses (Petithory *et al.* 1997, Anderson 2000).

The fact that different helminths coexisted at the stomach of rats from Guinea was an indication that these worms could have the same intermediate host (Anderson 2000). This led us to found the nematode larvae of the majority of the stomach species (*Streptopharagus*, *Gongylonema* and *Mastophorus*) in the two darkling beetles species endemic to El Hierro *P. l. costipennis* and *H. amaroides*. Guinea represents an exclusive endemiotope of the island, which would explain the singularity of the stomach polyparasitism in *R. rattus* at this enclave, although there is no information on the intermediate host of *Protophysura* sp.

The only louse species found in the present work is specific of rats. Concerning siphonapterans, both host species

showed quite similar overall prevalence. *Mus m. domesticus* displayed more richness than *R. rattus* (Index of Margalef = 0.75; 0.51, respectively). Mice from El Hierro were richer in flea species than from Iberian Peninsula, opposite to that occurs for helminths (Gomez *et al.* 1988). In this study, as in Beaucournu *et al.* (1989), neither *S. t. tripectinata* nor *E. murina* were detected on *R. rattus*, although both had been previously reported on this host (Beaucournu and Launay 1990). Among the five species found in El Hierro, the most prevalent and abundant was *S. t. tripectinata*. *Xenopsylla cheopis* was detected in both hosts, but with greater frequency and abundance in rats, its usual host.

Nevertheless the most prevalent flea association in rats was *L. segnis*-*N. barbarus*. These two species have already been reported in mice and rats (Beaucournu and Launay 1990) but results of our study showed that *L. segnis* seems to have a predilection for *R. rattus*. On the other hand, *N. barbarus* was present in both hosts with quite similar prevalence. It should be pointed out that the association between *L. segnis* and *M. muris* could be explained on the life-cycle context of the nematode, in which the flea can be involved as intermediate host (Beaucournu and Chabaud 1963).

In the ecotoxicological aspect, comparing the present results concerning Cd and Pb from insular habitats with those from continental areas reported by Torres *et al.* (2004, 2006) in Catalonia (NE Spain) and using the wood mouse (*Apodemus sylvaticus*), it is worth to emphasize that in Catalonia the detected levels of Cd were higher (around 645 ng g⁻¹ wet weight in kidney) than those found in El Hierro. Relative to Pb, similar values were obtained and no available data exist for Hg in the study performed in Catalonia. On the basis of the present data it can be confirmed that *M. m. domesticus* and *R. rattus* are suitable species to be used as bioindicators of environmental pollution by heavy metals. In addition, the present data may be used to compare this kind of pollution among other islands, especially those of the Canary Archipelago.

In absence of data related to the biology of the most of the detected parasites and respect to the effects of the parasites and the heavy metals on murine hosts in nature, all seem to indicate that, at least in certain periods of the year, the parasite burden could cause an important imbalance on the physiological conditions of these hosts at El Hierro. The specific ecological characteristics of the island (geographical location, physiography, area) could allow the presence of particular endemiotopes like Guinea and Frontera forest. This phenomenon, not denounced until present in other Atlantic islands and unique in Canaries (pers. obs.), could be the beginning of next parasitological, physiopathological and/or toxicological studies on wild fauna from isolated ecosystems.

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