Helminth fauna of small mammals (insectivores and rodents) in Doñana (southeastern Iberian Peninsula).

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Abstract: The helminthological study of 312 small mammals (Insectivora: *Crocidura russula* (Hermann, 1780); Rodentia: *Apodemus sylvaticus* Linnaeus, 1758; *Mus spretus*, Lataste, 1883; *Rattus rattus* Linnaeus, 1758 and *Arvicola sapidus* (Miller, 1908) allowed the detection of 28 species of helminth parasites (1 Digenid Trematode, 8 Cestodes and 19 Nematodes). The hosts were captured in different biotopes of Doñana National Park and Nature Park, on the southeastern Iberian Peninsula. This is the first important faunistic study relating to parasites in mammals in this major nature reserve. The absence of water-cycle Digenid Trematodes is the most noteworthy finding, considering the repeated presence of these Platyhelminths in other humid zones on the Iberian Peninsula (Delta del Ebro, the Albufera in Valencia). Certain biotic (populations of Carnivores) and abiotic factors (humidity and temperature in Doñana) influence the qualitative and quantitative configuration of the helminthfaunas.

Key words: Helminthfauna, small mammals, Doñana National Park

Resumen: El estudio helmintológico de 312 micromamíferos (Insectivora: *Crocidura russula* (Hermann, 1780); Rodentia: *Apodemus sylvaticus* Linnaeus, 1758; *Mus spretus*, Lataste, 1883; *Rattus rattus* Linnaeus, 1758 and *Arvicola sapidus* (Miller, 1908), procedentes de diversos biotopos de los Parques Natural y Nacional de Doñana (SO de la Península Ibérica), permitió la detección de 28 helmintos parásitos (1 Trematodo Digénido, 8 Cestodos y 19 Nematodos). Se trata de un estudio helmintológico amplio y novedoso en relación a los helmintos parásitos de mamíferos en Doñana. La ausencia de Trematodos Digénidos de ciclo vital acuático es el aspecto más destacable, sobre todo teniendo en cuenta la reiterada presencia de los mismos en otras zonas húmedas de la Península Ibérica (Delta del Ebro, Albufera de Valencia). Algunos factores bióticos como las poblaciones de Carnívoros y ciertos factores abióticos (humedad y temperatura) ejercen una cierta influencia sobre el espectro cualitativo y cuantitativo de las respectivas helmintofaunas.

Palabras clave: Helmintofauna, micromamíferos, Parque Nacional de Doñana

1. Introduction

The area of Doñana on the southeastern Iberian Peninsula has been described in detail (Valverde, 1958; García *et al.*, 1978). Basically, the area comprises a large extension of marshland limited to the west by an eolic mantle, and to the south by a dune barrier, which isolates it from the sea. The vegetation in the marshland zones varies according to the flood levels, and is dominated by shrubby glasswort (*Arthrocnemun macrostachyum*) (Moric) in the higher lying areas, and by alkali bulrush (*Scirpus maritimus* L.) in the lower zones. A characteristic of the woodland is the presence of rockroses (*Halimium halimifolium* L.), with the appearance mainly of besom heath (*Erica scoparia* L.) and gorse (*Ulex minor* L.) as the soil humidity increases. The tree population is represented by a few hundred centenary cork trees (*Quercus suber*

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L.), associated with the presence of humidity in the soil, and extensive spreads of pine (*Pinus pinea* L.). Important extensions were repopulated in the late nineteenth century with eucalyptus (*Eucaliptus* sp.), though these are presently being removed. Most of the marshland was drained or transformed into rice fields in the sixties, as a result of which only one-tenth of the original natural wetland currently remains. Two large reserves are found within this extensive area: the National and Natural Parks of Doñana, with different management systems and degrees of protection. These two Parks globally comprise one of the largest biological reserves in Western Europe.

The special ecological characteristics and biodiversity of the area constitute an ideal setting for faunistic, ecological and biological studies, with an evident added interest represented by the animal populations of the zone. Although zoological studies associated with Vertebrates and Invertebrates have been very abundant in Doñana, the same cannot be said of the parasite populations. Taking as reference hosts pertaining to the Order Mammalia, the publications addressing parasites have been few. In this sense, Hernández *et al.* (1992) detected a new species of Apicomplexa in deer in Doñana, while Pérez *et al.* (1990) cited certain faunistic and taxonomic aspects of Phthiraptera arthropods parasitizing Carnivores. Blasco *et al.* (1996) and Torres *et al.* (1998) included hosts captured in Doñana in their references to helminths of peninsular *Oryctolagus cuniculus* (Linnaeus, 1758) and *Lynx pardinus* (Temminck, 1827), respectively. Previously, Aymerich *et al.* (1984) had done the same with the Nematode *Skrjabingylus nasicola* (Leuckart, 1842) (Nematoda: Skrjabingylidae), a species distributed throughout much of the Iberian Peninsula, where it parasitizes Mustelidae carnivores.

Studies were started in late 2000 of the helminth parasites of small mammals (Insectivores and Rodents) in Doñana, with a series of objectives. The work comprised both basic research (of a faunistic, taxonomic, systematic, and ecological nature) and more applied studies (helminths as bioindicators of environmental contamination, for example). This first study addresses the helminth ecological data in relation to these host species, and represents the first important investigation of the mammal parasite communities in Doñana. Knowledge of this parasitic fauna should serve as a complement to the numerous studies carried out in other humid regions on the Iberian Peninsula (Delta del Ebro, Delta del Llobregat, the Albufera in Valencia). In these zones the populations of helminths have been shown to possess basic faunistic, biological and ecological peculiarities (Gallego *et al.*, 1984; Feliu *et al.*, 1985; Gracenea *et al.*, 1987; Montoliu *et al.*, 1987; Portoles *et al.*, 1996 and 2000; Toledo *et al.*, 1998; Esteban *et al.*, 1997).

2. Material and Methods

The number of small mammals subjected to helminthological study was 312. In November 2000, a total of 102 Algerian mouse, *Mus spretus* Lataste 1883, were captured. Another two field expeditions were made (October 2001 and January 2002), yielding 179 specimens of different hosts from several biotopes: greater white-toothed shrew, *Crocidura russula* (Hermann, 1780) (7 specimens); long-tailed field mouse, *Apodemus sylvaticus* Linnaeus, 1758 (28); black rat, *Rattus rattus* Linnaeus, 1758 (45); and the abovementioned *Mus spretus* (99).

The parasitological study of the southern water vole, *Arvicola sapidus* (Miller, 1908) was carried out in two ways. Searching for and identifying eggs and larvae of helminths in fresh feces collected from live hosts, with a total of 153 samples analyzed, and studying dead animals (n=31), in exactly the same way as in other mammals. The water vole material originated from temporary lagoons distributed throughout the woodlands in both the "*Abalario*" and the Biological Reserve, and from rushes located on the marshland shores.

The organs and viscera of each dead host were analyzed for the presence of helminths. The isolated parasites were processed for specific identification according to the procedures applicable in general Helminthology.

3. Results

Twenty-eight species of helminths were detected, of which one constituted a Digenid Trematode. The rest of species comprised 8 Cestodes (3 in larval stage) and 19 Nematodes. The parasites identified were: *Brachylaima* sp. (Brachylaimidae, Trematoda); the Cestodes: *Taenia taenuicollis larvae* (Rudolphi, 1819) and *Taenia parva larvae* Baer, 1926 (Taeniidae); *Mesocestoides* sp. *larvae* (Mesocestoididae); *Hymenolepis scalaris* (Dujardin, 1845), *Hymenolepis tiara* (Dujardin, 1845), *Hymenolepis diminuta* (Rudolphi, 1819), *Hymenolepis* sp. and *Pseudhymenolepis redonica* Joyeux et Baer, 1935 (Hymenolepididae); and the Nematodes: *Trichuris arvicolae* Feliu *et al.*, 2000, *Aonchotheca europaea* Mas-Coma et Galán-Puchades, 1985, *Eucoleus gastricus* (Baylis, 1926) and *Trichosomoides crassicauda* (Bellingham, 1845) (Trichuridae); *Mastophorus muris* (Gmelin, 1790) (Spirocercidae); *Gongylonema neoplasticum* (Fibiger et Ditlevsen, 1914) (Gongylonematidae); *Porrocaecum* sp. *larvae* (Ascarididae); *Angiostrongylus* sp. (Angiostrongylidae); *Carolinensis minutus* (Dujardin, 1845), *Nippostrongylus brasiliensis* (Travassos, 1914), *Longistriata confusa* Desportes et Chabaud, 1961 and *Longistriata* sp. (Heligmonellidae); *Heligmosomoides polygyrus* (Dujardin, 1845) and *Heligmosomoides* sp. (Heligmosomidae); *Syphacia obvelata* (Rudolphi, 1802), *Syphacia stroma* (Linstow, 1884), *Syphacia frederici* Roman, 1945, *Syphacia muris* (Yamaguti, 1935) and *Syphacia nigeriana* Baylis, 1928 (Oxyuridae).

The distribution of these helminths in the host species is reflected in Table 1, which also summarizes the data relating to prevalence and the intensity of parasitation. The absence of specific determination of some helminths is attributable to different reasons. In the cases of *Hymenolepis* sp. and *Longistriata* sp., as well as in *Heligmosomoides* sp., the reason was that these species are new to science and are currently in the publication phase. As to the pulmonary nematode *Angiostrongylus* sp., it should be pointed out that certain morphometric data of the limited materials available diverge from the species *A. dujardini* Drozdz et Doby, 1970, as a result of which we are very probably dealing with a new species; however, new material under good conditions for morphological evaluation are needed. No description has been possible for the Digenid *Brachylaima* sp., since its life cycle is not known (Gracenea and Gónzalez-Moreno, 2002); the same applies to the larval stages of *Mesocestoides* and *Porrocaecum* (Galán–Puchades, 1986; Portoles, 1997).

4. Discussion

In qualitative terms the results of this study are comparable to those obtained in other areas of the Iberian Peninsula where similar ecological conditions are found, with the exception of the Digenid Trematodes. One of the most complete studies in this sense (Torres, 1988), involving a much larger number of hosts (n=2057), identified structurally very similar helminthfaunas. Indeed, even on analyzing the parasitic fauna of concrete host species (*C. russula* and *A. sylvaticus*), analogy was observed in the configuration of the corresponding helminthfaunas (Portoles *et al.*, 1996 and 2000), despite the limited number of hosts dissected in our study. Another very patent example is the water vole, *A. sapidus*, for which the three Nematode species found in Doñana coincide with the Nematode fauna of the rodent detected in the Ebro Delta (Torres, 1988).

The absence in our study of water-cycle Digenid Trematodes is surprising, considering the environmental context, the specificity of the Platyhelminths and the constant presence of Digenids in small mammals in habitats of this nature, even in very reduced areas such as the delta of the Llobregat river (Gracenea and Montoliu, 1992). To date in Insectivores and Rodents of the wetlands on the Iberian Peninsula a total of 12 species of water-cycle Digenid Trematodes have been identified: *Echinostoma friedi* Toledo *et al.*, 2000; *Echinostoma echinatum* (Zeder, 1803) (= *E. lindoense*); *Echinoparyphium recurvatum* (von Linstow, 1873); *Euparyphium albuferensis* Esteban *et al.*, 1997; and *Hypoderaeum conoideum* (Block, 1782) (Echinostomatidae); *Psilotrema spiculigerum* (Mühling, 1898) (Psilostomidae); *Maritrema feliui* Gracenea *et al.*, 1993 and *Levinseniella* sp.

(Microphallidae); *Postorchigenes gymnesicus* Mas-Coma *et al.*, 1981 (Lecithodendriidae); *Notocotylus neyrai* Gonzalez Castro, 1945 and *Notocotylus gonzalezi* Simon Vicente *et al.*, 1985 (Notocotylidae); and *Plagiorchis* sp. (Plagiorchiidae) (Gracenea *et al.*, 1987 and 1993; Montoliu *et al.*, 1987; Torres, 1988; Cordero del Campillo *et al.*, 1994; Esteban *et al.*, 1997; Toledo *et al.*, 2000). Based on the data currently available, we are unable to account for this result. Personal research conducted in autumn and winter (the two seasons prospected in Doñana) has revealed a very low population density for aquatic gasteropods or amphibians (only a few specimens of Physidae having been collected only in autumn). However, considering that drought is more apparent in the summer season, it does not seem logical to think of a seasonal phenomenon. In certain delta areas (Torres, 1988) slight salinity of certain lagoons has been reported in an attempt to account for the absence of Digenid species distributed throughout much of the environment. This phenomenon likewise does not seem applicable to Doñana. Moreover, Montes (in Castroviejo, 1993) reported the disappearance of two aquatic gasteropod species, *Lymnaea peregra* (Muller, 1774) and *L. stagnalis* (Linnaeus, 1758) (Gastropoda: Lymnaeidae) present in Doñana, as a consequence of the population increase of the American crab, which has had an impact upon both the plant substrate and on the gasteropod population.

Undoubtedly, regarding biodiversity, the absence of water-cycle Trematodes configures atypical helminthfaunas in the host species in Doñana. These Platyhelminths always appear in the larger wetlands of Spain. Another qualitative observation exclusive to Doñana is the presence of larval stages of Cestodes (Tenids and Mesocestoidides), that undoubtedly should be related to the wild Carnivore populations. The detection of the larva of *Taenia parva* in the field mouse is common, though not so infestation with *Taenia tenuicollis* and *Mesocestoides* sp. in Muridae rodents (Feliu, 1980; Cordero del Campillo *et al.*, 1994). The high prevalence in our study in the case of *T. parva* (60,7%) is highly significant, since in the past percentage infestation has always been under 5% (Feliu, 1980; Feliu *et al.*, 1992). It should furthermore be taken into account that infestation of the definitive host of this Tenid, *Genetta genetta* Linnaeus, 1758 exhibits its lowest values in Andalucía with respect to the rest of the Iberian Peninsula (Casanova *et al.*, 2000).

The coprological study conducted in the case of *A. sapidus* confirms the observations of other authors (Torres *et al.*, 2001) in the sense that coprological findings can be used to establish the helminthfauna of a host. However, significant differences continue to appear on assessing the parasite populations. In all cases the values of the coprological analysis are inferior to those obtained by dissection of the host (table 1).

The habitual presence in our study of monoxenous geohelminth or pseudogeohelminth Nematodes (*T. arvicolae, C. minutus, N. brasiliensis, Longistriata* spp., *Heligmosomoides* spp.) confirms the existence of certain humidity conditions in Doñana which are absolutely essential for the life cycles of these Nematodes. This observation does not coincide with the results of other studies carried out in other meridian Iberian areas (Sierra de Filabres, Sierra Nevada, the coast of Málaga), where the presence of such helminths is seen to be much more limited (Feliu "com. pers."). Another observation pointing in the same direction is the detection in Doñana of a Nematode of the genus A*ngiostrongyl*us Kamensky, 1905, since these parasites conclude their biological cycles in slugs and water snails (Anderson, 2000), though the low prevalence of detection (0.5%) could be related, as in the case of the Digenid Trematodes, to the low population densities of the gasteropodos.

Regarding the quantitative data, the general infestation percentages of each host species are similar to those reported by other studies on the Iberian Peninsula (Feliu, 1980; Torres, 1988). Moreover, the species of helminths of greatest prevalence in each host species (*Longistriata* sp., *Heligmosomoides polygyrus, Syphacia obvelata, Mastophorus muris* and *Carolinensis minutus*) in most cases exhibit strict specificity. The exception would be *Mastophorus muris*, a nematode which

Tabla 1. Qualitative and quantitative worm spectrum of the small mammals in Doñana. N = number of parasitized hosts; % = prevalence; R = parasitation range;

	Crocidura russula (n=7)				*Apodemus sylvaticus* (=28)				*Mus spretus* (n=201)				*Rattus rattus* (n=45)				*Arvicola sapidus* (n=31)				
	N	%	R	I m	N	%	R	I m	N	%	R	I m	N	%	R	I m	N	%	R	I m	(%)*
TREMATODA									3	1.5			2	4.4							
Brachylaima sp.									3	1.5	1-13	5.3	2	4.4	1-2	1.5					
CESTODA	7	100			17	60.7			19	9.5			7	15.5							
Taenia tenuicollis larvae									2	1.0	1	1.0	2	4.4	1	1.0					
Taenia parva larvae					17	60.7	1-5	1.6													
Mesocestoides sp. *larvae*													1	2.2	1	1.0					
Hymenolepis scalaris	3	42.8	5-130	47.7																	
Hymenolepis tiara	4	80	1-2	1.3																	
Hymenolepis diminuta													4	8.9	1-2	1.5					
Hymenolepis sp.									19	9.5	1-4	1.7									
Pseudhymenolepis redonica	3	42.8	2-9	5.0																	
NEMATODA	4	57.1			23	82.1			95	47.3			26	57.8			27	87.1			
Trichuris arvicolae																	1	3.2	1	1.0	0.6
Aonchotheca europaea	2	28.6	16-18	17.0																	
Eucoleus gastricus									1	0.5	2	2.0									
Trichosomoides crassicauda													1	2.2	1	1.0					
Mastophorus muris					2	7.1	1	1	1	0.5	1	1	12	26.7	1-10	3.4					
Gongylonema neoplasticum									2	1.0	1-2	1.5									
Porrocaecum sp. larvae	1	14.3	2	2.0																	
Angiostrongylus sp.									1	0.5	8	8									
Carolinensis minutus																	26	83.9	1-32	5.1	32.7
Nippostrongylus brasiliensis													11	24.4	1-60	11.5					
Longistriata confusa	1	14.3	38	38.0																	
Longistriata sp.	4	57.1	3-46	14.2																	
Heligmosomoides polygyrus					20	71.4	1-128	19.7													
Heligmosomoides sp.									24	11.9	1-3	1.9									
Syphacia obvelata									52	25.9	1-148	12.6									
Syphacia stroma					7	25.0	3-300	75.4													
Syphacia frederici					13	46.2	2-100	15.5													
Syphacia muris													9	20.0	4-79	20.0					
Syphacia nigeriana																	17	54.8	1-50	6.5	7.2
PARASITACION TOTAL		100				92.8				51.7				57.7				87.1			

Im = mean intensity; (%)* refers to the 153 feces of *A. sapidus* analyzed.

evolves in numerous species of Rodents and Carnivores as definitive hosts (Casanova, 1993); thereby again pointing to the important populations of Carnivores in Doñana as an influencing factor in this result. On evaluating the mean intensities, the parasites exhibiting higher loads (*Hymenolepis scalaris, Syphacia stroma, Syphacia obvelata, Syphacia muris, Carolinensis minutus)* are oioxenous or stenoxenous species (Galán-Puchades, 1986; Feliu *et al.*, 1997). The limited number of specimens studied in most of the host species, and the fact that evaluation of the entire reserve of Doñana has not yet been completed, precludes the drawing of firm conclusions regarding the factors conditioning the helminthfaunas. Nevertheless, and taking as reference the Iberian Peninsula, Doñana is seen to present singular helminthfaunas. Certain abiotic (humidity, temperature, solar exposure) and biotic factors (principally the populations of Carnivores) seem to be the decisive conditioning aspects Unfortunately, the non-availability of information on parasites of other Vertebrates (birds, amphibians, reptiles) affords no useful information in this aspect.

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