THE HELMINTHFAUNA OF *ORYCTOLAGUS CUNICULUS* (LINNAEUS, 1758) IN THE IBERIAN PENINSULA. FAUNISTIC AND ECOLOGICAL CONSIDERATIONS

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Summary :

Faunistic and ecological analysis of the helminthfauna of the wild rabbit, *Oryctolagus cuniculus* (Linnaeus, 1758), was undertaken in the Iberian Peninsula. For the first time the helminthfaunas of the two iberian subspecies (*O. c. cuniculus* and *O. c. algirus*) are detailed. Fifteen parasite species (1 Digenetic Trematode, 7 Cestodes and 7 Nematodes) were detected. The presence of *Leporidotaenia* spp. (Cestoda: Anoplocephalidae) is the distinguishable feature between both helminthfaunas. Moreover, qualitative and quantitative differences exist between the two hosts subspecies, often statistically significant. The effect of host sex on the helminthfauna of *O. cuniculus* in both populations seems to be low.

KEY WORDS : Helminthfauna, *Oryctolagus cuniculus*, Iberian Peninsula.

Résumé : L'HELMINTHOFAUNE D'*ORYCTOLAGUS CUNICULUS* (LINNAEUS, 1758) DANS LA PÉNINSULE IBÉRIQUE. CONSIDÉRATIONS FAUNISTIQUES ET ÉCOLOGIQUES

L'analyse faunistique et écologique de l'helminthofaune du lapin sauvage, *Oryctolagus cuniculus* (Linnaeus, 1758), de la péninsule ibérique a été effectuée. Cette étude est la première entreprise sur les helminthofaunes d'*O. c. cuniculus* et *O. c. algirus*, deux sous-espèces de la péninsule ibérique. Quinze espèces de parasites (1 Trématode, 7 Cestodes et 7 Nématodes) ont été trouvées. La détection de *Leporidotaenia* spp. est la caractéristique qui permet la différenciation des helminthofaunes entre les deux sous-espèces d'*O. cuniculus*. De plus, il existe des différences qualitatives et quantitatives, parfois statistiquement significatives, parmi ces helminthofaunes. L'influence du sexe de l'hôte sur l'helminthofaune de *O. cuniculus* semble peu importante dans les deux sous-espèces hôtes.

MOTS CLÉS : Helminthofaune, *Oryctolagus cuniculus*, péninsule ibérique.

INTRODUCTION

The wild rabbit, *Oryctolagus cuniculus* (Linnaeus, 1758) (Lagomorpha: Leporidae), is a native mammal from the Iberian Peninsula. Actually, two subspecies are recognized: *O. cuniculus algirus*, restricted to the southwestern of the Iberian Peninsula, and *O. cuniculus cuniculus* in the rest of Spain, Europe and Australia. Both subspecies are genetically differentiated (Monnerot *et al.*, 1994; Ferrand, 1995; Hardy *et al.*, 1995). According to Ferrand (1995) this differentiation happened over the Quaternary glaciations. In Europe, there are numerous studies about the helminthfauna of this host (Boag, 1972 and 1987, Mead-Briggs & Page, 1975 and Boag & Iason, 1986 in Great Britain; Butler, 1994 in Ireland; Haupt & Hartung, 1980 and 1984 in Germany; Arru *et al.*, 1968 in

Italy; Hörning, 1974 in Switzerland; Tenora and Barus, 1957 in the old Czechoslovakia; Sugar *et al.*, 1978 in Hungary; Theodoridis, 1979 in Greece; etc.). Several studies have also been performed in other continents (Bernard, 1965 in Africa; Courtin *et al.*, 1979 and Rubilar & Merello, 1987 in South America; Bull, 1960 and 1964 and Dunsmore, 1978 in Australia; etc.). Numerous helminth species have been reported in *Oryctolagus* in the Iberian Peninsula (Cordero del Campillo *et al.*, 1994) as a result of several studies performed in different iberian areas (Carvalho Varela, 1967; Simon Vicente, 1969; Mendonça and Varela, 1971; Romero-Rodriguez *et al.*, 1973; Tenora *et al.*, 1981-82; Afonso-Roque & Barata, 1984; Reina *et al.*, 1987; etc.).

The present study is the first in analyzing and comparing, from a faunistic and ecological point of view, the helminthfauna of the two iberian subspecies. Moreover, it is the first study in Continental Europe in which a characteristic of host (sex) was considered. Ecological studies have only been described in Great Britain (Mead-Briggs and Vaughan, 1973; Boag, 1985; Boag and Kolb, 1989), Ireland (Butler, 1994) and Australia (Bull, 1959 and 1964; Dudzinski and Mykutowycz, 1963; Dunsmore, 1966a, b, c; Dunsmore & Dudzinski, 1968).

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MATERIALS AND METHODS

Two hundred and fifteen iberian rabbits from two geographically distant areas were helminthologically studied. One hundred and fifty-three specimens were collected between winter 1991 and winter 1993 in Catalonia (North-eastern Iberian Peninsula; Barcelona, Tarragona, Lerida and Gerona provinces), where *O. cuniculus cuniculus* lives; sixty-two were caught during February and April 1993 in Andalusia (South-western Peninsula; Huelva, Sevilla and Cadiz provinces), where *O. cuniculus algirus* is present. The number of hosts per province is shown in Figure 1.

Hosts were kindly supplied by the Catalanian Hunting Federation and Doñana Biological Station (Andalusia). The available data were the location of capture and the sex of the host. This material arrived to the laboratory preserved by freezing. Only the digestive tract was studied from the Andalusian hosts. The abdominal cavities were examined in the laboratory and the presence of any cysts recorded. The lungs, spleens and livers were studied separately. Alimentary tract was separated into three regions, stomach, small intestine and large intestine before being analysed. Viscera were

examined under stereoscopic microscop and the removed helminths counted, preserved in 70° alcohol and processed following general helminthology methods.

Identification of the parasite species was performed based on previous specific descriptions. Anoplocephalid Cestodes were identified following descriptions from Tenora *et al.*, (1981-82), Beveridge (1978), Tenora & Murai (1978) and Genov *et al.*, (1990). Species belonging to the genus *Leporidotaenia* were classified following the description of Genov *et al.*, (1990). *Leporidotaenia cf. wimerosa* was identified according to the large size of the strobila (Genov *et al.*, 1990). Recent new descriptions were used to classify some Nematodes: Durette-Desset & Denke (1978) for *Graphidium strigosum*; Durette-Desset (1979) for *Nematodiroides zembrae*, and Babos (1961) for *Protostrongylus cuniculorum*. *Dermatoxys hispaniensis* was identified following the unique descriptions of Simon Vicente (1969) and Afonso-Roque and Barata (1984).

Two different tests were used to perform statistical analysis of parasite populations. The χ^2 test was used, together with the Yates continuity correction, to carry out a comparative study of prevalences. The Mann-Whitney « U » statistical was used to compare the inten-

Fig. 1. — Number of hosts studied per province in the Iberian Peninsula (B : Barcelona, GE : Gerona, L : Lérida, T : Tarragona, CA: Cádiz, H : Huelva, SE : Sevilla).

sity. Significance level was $\alpha = 0.05$ in both tests. Due to the absence of information on sex in hosts sampled, statistic study on sex influence on infection was done on a smaller sample. Therefore, a hundred and seventy-five individuals have been studied: one hundred and fourteen rabbits from Catalonia (fifty-one males and sixty-three females) and sixty-one rabbits from Andalusia (twenty-six males and thirty-five females). For each helminth species differences in prevalence and intensity have been analysed in: *a*) between males and females of the same host population; *b*) between males and females of general sample; and *c*) between the helminthfaunas of both populations.

RESULTS

Fifteen helminth species were detected (one Digenetic Trematode, seven Cestodes and seven Nematodes); namely: *Dicrocoelium dendriticum* (Rudolphi, 1819) (Dicrocoeliidae); *Taenia pisiformis* (Bloch, 1780) *larvae* (Taeniidae); *Andrya cuniculi* (Blanchard, 1891), *Cittotaenia denticulata* (Rudolphi, 1804), *Neoctenotaenia ctenoides* (Railliet, 1890), *Leporidotaenia wimerosa* (Moniez, 1880), *L. pseudowimerosa* (Tenora, Murai, Valero & Cutillas, 1981-82) and *L. cf. wimerosa* (Moniez, 1880) (Anoplocephalidae); *Trichuris leporis* (Froelich, 1789) (Trichuridae); *Graphidium strigosum* (Dujardin, 1845) and *Trichostrongylus retortaeformis* (Zeder, 1800) (Trichostrongylidae);

Nematodiroides zembræ (Bernard, 1965) (Molineidae); *Passalurus ambiguus* (Rudolphi, 1819) (Oxyuridae); *Dermatoxys hispaniensis* (Simon Vicente, 1969) (Heteroxynematidae) and *Protostrongylus cuniculorum* (Joyeux & Gaud, 1946) (Protostrongylidae).

Distribution of the helminths in the two host populations, prevalences and parasitation intensities are shown in Table I.

Overall prevalences are high in both host populations and no significant differences can be observed between them (Table I). However, significant differences are observed in the prevalences by Cestodes ($\chi^2 = 7.44$). Such differences are mainly due to the presence of *Leporidotaenia* spp. in the southern population. Comparative analysis of prevalences of the parasite species has demonstrated the existence of significant differences in most of them (*T. pisiformis larvae*, $\chi^2 = 4.43$; *A. cuniculi*, $\chi^2 = 3.92$; *C. denticulata*, $\chi^2 = 11.74$; *L. wimerosa*, $\chi^2 = 27.60$; *L. pseudowimerosa*, $\chi^2 = 10.80$; *L. cf. wimerosa*, $\chi^2 = 12.80$; *G. strigosum*, $\chi^2 = 21.98$; *N. zembræ*, $\chi^2 = 57.17$; *P. ambiguus*, $\chi^2 = 16.27$; *D. hispaniensis*, $\chi^2 = 67.61$). The highest infestation prevalences of all species, with the exception of *T. pisiformis larvae*, were detected in the Southern Peninsula. When comparing parasitation intensities in both host populations, statistically significant differences have only been found in *T. retortaeformis* (significance level $p = 0.004$) (Table I).

Table II shows prevalences and mean parasitation intensities in all males and females of each population.

	General	North-Eastern n = 153 (<i>O. cuniculus cuniculus</i>)				South-Western n = 62 (<i>O. cuniculus algirus</i>)			
	P (%)	P (%)	Min. I.	Max. I.	Mean I.	P (%)	Min. I.	Max. I.	Mean I.
<i>Dicrocoelium dendriticum</i>	17.8	17.8	6	700	94.3				
Trematodes	17.8	17.8							
<i>Taenia pisiformis larvae</i>	28.4	33.3	1	29	4.7	16.1	1	31	4.7
<i>Andrya cuniculi</i>	15.4	12.4	1	45	10.0	22.6	1	92	17.0
<i>Cittotaenia denticulata</i>	5.1	1.3	2	2	2.0	14.5	1	3	1.8
<i>Neoctenotaenia ctenoides</i>	34.9	35.3	1	5	1.7	33.9	1	2	1.6
<i>Leporidotaenia wimerosa</i>	7.0	—	—	—	—	24.2			
<i>Leporidotaenia pseudowimerosa</i>	3.3	—	—	—	—	11.3	2	65	19.8
<i>Leporidotaenia cf. wimerosa</i>	3.7	—	—	—	—	12.9			
Cestodes	67.0	61.4				80.7			
<i>Trichuris leporis</i>	14.9	17.7	1	63	6.3	8.1	1	3	1.6
<i>Graphidium strigosum</i>	51.2	39.6	1	2,890	211.3	79.0	1	568	101.0
<i>Trichostrongylus retortaeformis</i>	77.7	77.8	1	2,906	186.7	77.4	1	162	43.2
<i>Nematodiroides zembræ</i>	47.0	32.6	1	217	37.7	82.3	1	771	71.2
<i>Protostrongylus cuniculorum</i>	1.5	1.5	110	172	14.0				
<i>Passalurus ambiguus</i>	20.5	11.8	2	18,715	2,581.7	41.9	1	9,110	2,117.0
<i>Dermatoxys hispaniensis</i>	17.2	2.0	1	7	3.0	54.8	1	133	25.5
Nematodes	92.1	92.2				91.9			
Helminths	94.0	94.8				91.9			

Table I. — Prevalences and intensities in wild rabbits.

Although prevalences of most species are higher in females, significant differences have only been found in overall prevalence of Cestodes ($\chi^2 = 5.02$) and *A. cuniculi* ($\chi^2 = 6.47$). No statistically significant differences have been found in the Northern Peninsula. In the Andalusian population, higher prevalences have been observed in females, but they were only significantly different in Cestodes ($\chi^2 = 6.58$), *A. cuniculi* ($\chi^2 = 7.56$), *T. retortaeformis* ($\chi^2 = 6.26$) and *D. hispaniensis* ($\chi^2 = 4.33$).

Mean parasitization intensities are also higher in females. However, statistically significant differences have only been found in the case of *T. retortaeformis* in the whole population ($p = 0.02$) and in each area (North $p = 0.04$; South $p = 0.05$).

DISCUSSION

The helminthfauna of *Oryctolagus cuniculus* in the Iberian Peninsula is mainly constituted by oligoxenous species. Therefore, the parasite fauna of the two studied populations shows the same

configuration. Similar results have been described in the remaining distribution area of the host (Tenora and Barus, 1957; Bernard, 1965; Hörning, 1974; Sugar *et al.*, 1978; Haupt and Hartung, 1984; Boag, 1985 and 1987; Butler, 1994; etc.). However, the highest number of species, among the cited helminthfaunas in the distribution area of the host, has been detected in the Iberian Peninsula. Moreover, species that are able to parasitize other Iberian mammals (*D. dendriticum* and *T. retortaeformis*) (Cordero del Campillo *et al.*, 1994) are also present in the peninsular rabbit and show high or very high prevalences. The percentage of infestation by *D. dendriticum* (17.8 %) is among the highest detected in Continental Europe (Casanova *et al.*, in press).

The comparative study of the Catalanian and Andalusian populations shows faunistic and ecological differences, which are often statistically significant. Qualitatively, the population from Southern Spain can be identified by the Cestodes of the genus *Leporidotaenia* (Table I). *D. dendriticum* has been occasionally found in the liver of some rabbits from Coto Doñana (Sevilla and Huelva provinces). This finding confirms the pre-

	General n = 175				North-Eastern n = 114 (<i>O. cuniculus cuniculus</i>)				South-Western n = 61 (<i>O. cuniculus algirus</i>)			
	Males n = 77		Females n = 98		Males n = 51		Females n = 63		Males n = 26		Females n = 35	
	P (%)	Mean I.	P (%)	Mean I.	P (%)	Mean I.	P (%)	Mean I.	P (%)	Mean I.	P (%)	Mean I.
<i>Dicrocoelium dendriticum</i>	19.6	96.8	19.1	107.7	19.6	96.8	19.1	107.7				
Trematodes	19.6		19.1		19.6		19.1					
<i>Taenia pisiformis</i> larvae	20.8	4.6	31.6	5.5	25.5	5.3	38.1	5.3	11.5	1.3	20.0	6.1
<i>Andrya cuniculi</i>	6.5	11.0	21.4	13.6	7.8	16.3	12.7	6.0	3.9	1.0	37.1	18.2
<i>Cittotaenia denticulata</i>	5.2	1.5	6.1	2.0	2.0	2.0	—	—	11.5	1.3	17.1	2.0
<i>Neoctenotaenia ctenoides</i>	31.2	1.5	37.8	1.6	31.4	1.5	38.1	1.6	30.8	1.5	37.1	1.6
<i>Leporidotaenia wimerosa</i>	9.1		8.2		—	—	—	—	26.9		22.9	
<i>Leporidotaenia pseudowimerosa</i>	2.6	14.6	5.1	22.7	—	—	—	—	7.7	14.6	14.3	22.7
<i>Leporidotaenia cf. wimerosa</i>	1.3		7.1		—	—	—	—	3.9		20.0	
Cestodes	58.4		75.5		54.9		65.1		65.4		94.3	
<i>Trichostrongylus axei</i>	11.7	6.6	12.2	7.6	13.7	8.0	14.3	9.6	7.7	1.5	8.6	1.7
<i>Graphidium strigosum</i>	48.1	168.2	61.2	179.3	37.3	235.8	46.0	260.2	69.2	96.8	88.6	103.5
<i>Trichostrongylus retortaeformis</i>	70.1	88.6	81.6	189.4	74.5	113.8	76.2	282.0	61.5	28.8	91.4	50.4
<i>Nematodiroides zembrae</i>	36.4	37.1	50.0	73.1	17.7	44.1	27.0	34.9	73.1	33.8	91.4	93.4
<i>Protostrongylus cuniculorum</i>	2.0	110.0	1.6	172.0	2.0	110.0	1.6	172.0				
<i>Passalurus ambiguus</i>	19.5	2,483.0	27.6	1,752.9	9.8	3,905.0	17.5	909.6	38.5	1,772.0	45.7	2,332.7
<i>Dermatophyes hispaniensis</i>	14.3	24.6	25.5	24.2	2.0	7.0	1.6	1.0	38.5	26.4	68.6	25.1
Nematodes	89.6		92.9		90.2		90.5		88.5		97.1	
Helminths	90.9		94.9		94.1		93.7		88.5		97.1	

Table II. — Prevalences and mean intensities according to sexes.

sence of the Trematode in Andalusia (Casanova, unpublished data).

Our results show once again that origin and evolution of Cestodes belonging to the genus *Leporidotaenia* are difficult to explain (Genov *et al.*, 1990). *L. wimerosa* and *L. cf. wimerosa* infest species of *Oryctolagus* and *Lepus* in Western Europe (Genov *et al.*, 1990). However, in the Iberian Peninsula, they have only been found in Southern Spain. Recent studies performed in rabbits from Navarra (Northern Peninsula) have not shown either the presence of these species. On the other hand, *L. pseudowimerosa*, detected originally in Southern Spain (Tenora *et al.*, 1981-82), seems to have a restricted chorology to this area of the Iberian Peninsula. The peninsular parasite fauna of *Oryctolagus cuniculus* shows the relic-tual trait of the Iberian helminthfaunas (Hugot and Feliu, 1990). Such condition is observed in the evolution of a genus of Cestodes (*Leporidotaenia*), which has been probably influenced by the peninsular origin of *Oryctolagus*. The absence of these Anoplocephalid Cestodes in Australian rabbits (Bull, 1960 and 1964; Dunsmore, 1978) substantiates such hypothesis. The present study demonstrates for the first time the presence of *L. cf. wimerosa* in Iberian rabbits. Thus, evolution, distribution and specificity of *L. wimerosa* and *L. cf. wimerosa* could be similar (Western Europe/Leporidae). However, *L. pseudowimerosa* seems to follow a different process (Southern Spain/*Oryctolagus*).

The genetically different Iberian rabbit populations, *O. c. cuniculus* (north-eastern) and *O. c. algirus* (south-western), can also be differentiated by their *Leporidotaenia* species. However, species of this genus have not been found in *Lepus* spp. (Cordero del Campillo *et al.*, 1994) of Iberian Peninsula. This surprising fact does not correlate with the range hosts that *L. wimerosa* and *L. cf. wimerosa* show in other European countries (Genov *et al.*, 1990). Moreover, other endemic Nematode species (*D. hispaniensis*) infest also Iberian rabbits and hares (Simon Vicente, 1969). *T. pisiformis* (33.3 % in Catalonia/16.1 % in Andalusia; $\chi^2 = 4.43$) evolves in the Iberian Peninsula using foxes as definitive hosts. No data exist about infestation of this Canid in Southern Spain. In Catalonia, the Carnivore appears to be infested at low rates by adult tapeworms (6.1 %; Miquel *et al.*, 1994). Due to the segregation of the functional space between foxes and lynxes in the studied Andalusian area (Frediani, 1993), the cycle of *T. pisiformis* could have greater limitations to be closed and, therefore, lower rates of infestation by larvae in rabbits are detected.

Quantitative results are difficult to explain for the remaining species, except for the afore-mentioned *Leporidotaenia* spp. The data of some monoxenous

pseudogeohelminth Nematodes (*P. ambiguus* and *D. hispaniensis*) and geohelminths (*G. strigosum* and *N. zembrae*) are specially controversial. Evidently, humidity and temperature conditions are more adverse for these Nematodes in the Southern regions than in the Northern Peninsula. Thus, higher prevalences should be expected in the Northern biotopes. Such phenomena was described by Dunsmore (1966 *b*) in a study about *G. strigosum* prevalences in four Australian areas with different climatic conditions. Lower prevalences were detected in areas with extreme climatic conditions. Lack of precise information about the biology of these Nematodes leads us to the hypothesis that higher density of wild rabbits in the Southern Peninsula could be the main conditioning factor influencing our results. Moreover, maximum and mean intensities are, for most Nematode species, higher in the Catalanian population. This would indicate that climatic conditions in this area are more advantageous for the majority of parasite cycles.

The effect of sex, *per se*, on the helminthfauna of *O. cuniculus* in both populations seems to be low. In this sense, no significant differences have been found in Catalanian hosts. Respecting to Andalusian hosts three species significantly different (*A. cuniculi*, *T. retortaeformis*, *D. hispaniensis*) display completely different life cycles, which makes difficult to speculate about possible causes of their higher prevalences in females.

The relationship between helminthfauna and host sex has always been controversial. Thus, Dudzinski and Mykityowycz (1963) showed that parasitization intensity does not depend on host sex for *T. retortaeformis*, but it does for *G. strigosum*. This Nematode seems to show higher parasitization in females. Dunsmore (1972) cited higher intensities for both species (*T. retortaeformis* and *G. strigosum*) in females. However, Boag and Kolb (1989) described opposite results. Mead-Briggs and Vaughan (1973) stated that *C. denticulata* is more frequent in males. Boag (1985) and Butler (1994) agreed in that no significant differences exist between males and females in prevalences by Cestodes and Nematodes. Most authors believe that differences in parasitization intensities of *T. retortaeformis* (Bull, 1959; Dunsmore, 1966 *a*; Dunsmore and Dudzinski, 1968), *G. strigosum* (Dunsmore, 1966 *b*) and *P. ambiguus* (Dunsmore, 1966 *c*; Dunsmore and Dudzinski, 1968) between males and females are related to the season. Thus, females show higher rates than males during reproductive periods and an opposite tendency is observed during the rest of the year. The influence of hormones in the parasite-host relationship was already described in a study about the influence of the host reproductive state on the number of *T. retortaeformis* and *G. strigosum* (Dunsmore, 1966 *d*).

ACKNOWLEDGEMENTS

The authors want to thank D. Antoni Arrizabala (Granollers, Spain) and Dr. Roger Fons (Banyuls-sur-Mer, France) for conceding some of the studied hosts. The study has been partially supported by the Spanish DGICYT Project PB 92-0517-CO2-02 and the « Comissionat per Universitats i Recerca de la Generalitat de Catalunya » (GRQ 94-1057).

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Reçu le 14 mars 1996
 Accepté le 10 août 1996