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BYTHINELLA
DIGENEAN
GALEMYS
NEOMYS
PSEUDOCEPHALOTREMA
SKRJABINOPHYETUS

ABSTRACT. – In the majority of digeneans infecting Insectivora, the larval stages are found in snails (cercaria) and terrestrial or aquatic invertebrates (metacercaria). The diets of both the Pyrenean desman *Galemys pyrenaicus* (Talpidae) and aquatic shrews (genus *Neomys*) include a wide variety of aquatic invertebrates that can act as the second intermediate host of several species of digeneans. In 2004, sampling was performed in the Py Natural Reserve (French Pyrenees). A total of 912 *Bythinella rufescens* (first intermediate host) and 167 non-mollusc aquatic invertebrates (second intermediate hosts) were studied for the detection of larval stages of digeneans. Two species of digenean trematodes were recovered as cercariae and metacercariae. These two species were *Pseudocephalotrema pyrenaica* (Lecithodendriidae) and *Skrjabinophyetus neomydis* (Nanophyetidae). In snails their general prevalence was 20.4 % and 1.97 % respectively. In the second intermediate hosts the corresponding prevalence rates were 22.15 % and 3 %, respectively. The study of larval digeneans of invertebrates can be used to detect the presence of small mammals.

INTRODUCTION

Faunistic studies of digenetic trematodes from small mammals in the Pyrenees have revealed the presence of several species (Ribas *et al*. 2005). In this study, conducted in the natural reserves of Py and Mantet (Eastern Pyrenees), ten digenean species were reported from six small mammal species (Rodentia and Insectivora). These helminths included members of Brachylaimidae and Dicrocoeliidae (four species) with terrestrial life cycles, while the remainder were six species from five different families (Lecithodendriidae, Nanophyetidae, Troglotrematidae, Collyriclidae and Prosthogonimidae) with aquatic life cycles. Other studies carried out in the Pyrenees have reported different digenean species, also with aquatic biology (Combes *et al*. 1974, Jourdane 1973a, 1974a, b, Jourdane & Vago 1973). The Py and Mantet reserves are inhabited by three protected species of semi-aquatic mammal insectivores: the water shrew *Neomys fodiens* (Pennant, 1771), Miller's water shrew *Neomys anomalus* (Cabrera, 1907) (Insectivora, Soricidae), and the Pyrenean desman *Galemys pyrenaicus* (Geoffroy-Saint-Hilaire, 1811) (Insectivora, Talpidae) (Fons unpubl data). While the two species of water shrews are widely distributed in Europe (*N. anomalus*) and in the Palaearctic region (*N. fodiens*), the Pyrenean desman inhabits the northern part of the Iberian Peninsula and can be found in isolated mountains in central Spain, as well as in Portugal and the south of France (Mitchell-Jones *et al*. 1999). All these species feed on similar prey, i.e. freshwater invertebrates (Castién 1995, Castién & Gosálbez 1995, Santamartina 1993). The rivers in which they are found are frequently inhabited by *Bythinella rufescens* (Dupy, 1851), a prosobranch mollusc of the family Hydrobiidae (Puig 1999, Tachet *et al*. 2003) that can act as the first intermediate host of digeneans (Faltinkova & Literak 2002).

The capture of the three above-mentioned species is complicated, as their semi-aquatic status means that alternatives must be found to the standard methods for capturing small mammals (Aymerich & Gosálbez 2004). Moreover, their protected status prohibits captures in the search for endohelminths. Given the above, the aim of the present study was to examine the diversity of the larval stages of digeneans present in freshwater invertebrates in a concrete locality, the Py Natural Reserve.

MATERIALS AND METHODS

The Py Natural Reserve is located in the most eastern part of the Pyrenees, between the Canigó and Carença Massifs, and ranges from 950 to 2463 m a.s.l. Samplings of freshwater invertebrates were carried out at the end of September 2004 in order to study larval trematode infections. Collection sites were selected according to the habitat preferences of the aquatic or semi-aquatic small mammals. Samples were collected from a single point in a small tributary of the river La Rotja. The substrate here consists mainly of stones and rocks, and is frequently covered by plant detritus. The stream flows through a small wooded valley with scattered meadows and the aquatic vegetation con-

Table I. – Quantitative results of parasite prevalence in species of invertebrates in general and in two periods of observation. N: number of hosts examined; NP: number of parasitized hosts; P (%): parasite prevalence in percentage in both periods; *S. n.*: *S. neomydis*. *P. p.*: *P. pyrenaica*.

Observation period in lab	Invertebrates	N	NP	P (%)	NP *S. n.*	P (%) *S. n.*	NP *P. p.*	P (%) *P. p.*
From 13 Oct to 22 Nov 2004	Hydracnidae gen. sp.	4	-	-	-	-	-	-
	Psephenidae gen. sp.	5	-	-	-	-	-	-
	Chironomidae gen. sp.	4	-	-	-	-	-	-
	Culicidae gen. sp. (Larvae)	13	-	-	-	-	-	-
	Baetidae gen. sp. (Larvae)	6	5	83.3	-	-	5	83.3
	Paraleptophlebia sp. (Larvae)	14	10	71.4	-	-	10	71.4
	Leptoceridae gen. sp. (Larvae)	69	14	20.3	-	-	14	20.3
	Polycentropodidae gen. sp. (Larvae)	8	5	62.5	-	-	5	62.5
	Subtotal 1	123	34	27.6	-	-	34	27.6
From 23 Nov to 10 Dec 2004	*Atractides* sp. (Adult)	2	-	-	-	-	-	-
	Elmidae gen. sp. (Larvae)	1	-	-	-	-	-	-
	Psephenidae gen. sp. (Adult)	3	-	-	-	-	-	-
	Baetis sp. (Larvae)	6	-	-	-	-	-	-
	Ecdyonurus sp. (Larvae)	1	-	-	-	-	-	-
	Xanthoperla sp. (Larvae)	4	-	-	-	-	-	-
	Leptoceridae gen. sp. (Larvae)	1	-	-	-	-	-	-
	Glyphotaelius pellucidis (Larvae)	23	8	34.8	5	21.7	3	13.0
	Hyporhyacophila sp. (Larvae)	1	-	-	-	-	-	-
	Erpobdella octoculata (Adult)	2	-	-	-	-	-	-
	Subtotal 2	44	8	34.8	5	21.7	3	13.0
	Total	**167**	**42**	**25.1**	**5**	**3**	**37**	**22.1**

sists mainly of mosses. In the field, invertebrates were collected and placed separately.

A total of 912 prosobranchid snails *Bythinella rufescens* (Hydrobiidae) were collected and transported to the Parasitology Laboratory of the University of Barcelona, where they were placed on plates with an air pump at 6 degrees. To study cercariae emission, snails were placed individually into each well of a microplate (Elisa Plate) with water from sampled river; water was renewed daily and the snails were fed with crushed dry lettuce. Three observation periods were used (4-18 October 2004; 19 October to 12 November 2004, and 15 November to 10 December 2004). Individuals were checked with a binocular stereomicroscope (3x) between 11 a.m. and 3 p.m., except for at weekends, and we observed the presence/absence of cercariae and identified the species present. Cercariae were collected using a Pasteur pipette with a modified end (narrowed with the flame) and transferred to a micro slide. The measurements taken were length and width of cercaria, diameter of both suckers, length of stylet, length of tail, and the morphology allowed for identification at the species level. From each species of cercariae detected a total of 30 individuals were studied.

Invertebrates (excluding molluscs) were also brought alive to the laboratory. They were separated onto plates according to their taxonomic group in order to minimise the risk of predation, and maintained at 6 degrees with air pumps. To detect metacercariae, arthropods were dissected and metacercariae isolated in Petri dishes containing Ringer's frog solution (Taylor & Baker 1978). Metacercarial cysts were observed and measured under a light microscope. For excystment, metacercariae were placed in Ringer solution at 39ºC. Fresh excysted metacercariae were fixed in Bouin solution, stained with Semichon acetocarmine and mounted in Canada balsam. Larval stages of digeneans and invertebrates were identified according to the criteria of Jourdane (1973 a, b), Brendow (1970) and Tachet *et al*. (2003). Terms of prevalence and mean intensity are those of Bush *et al*. (1997).

RESULTS

In the sample examined, a total of 167 non-mollusc invertebrates were studied. These invertebrates were identified as: *Atractides* sp. (n = 6) (Acarina Hydracnidae); Psephenidae gen. sp. (n = 8) and Elmidae gen. sp. larvae (n = 1) (Coleoptera); Chironomidae gen. sp. larvae (n = 4) and Culicidae gen. sp. larvae (n = 13) (Diptera); *Baetis*

Table II. – Quantitative results of general prevalences in the three observational times. N: number of examined hosts; P(%): prevalence.

Period of observation	N	P (%)	P (%) *S. neomydis*	P (%) *P. pyrenaica*
4 - 18 Oct	432	27.70	-	27.70
19 Oct - 12 Nov	240	18.70	4.10	14.60
15 Nov -10 Dec	240	16.20	3.30	13
Total	912	22.36	1.97	20.40

sp. larvae (n = 12), *Paraleptophlebia* sp. larvae (n = 14) and *Ecdyonurus* sp. larvae (n = 1) (Ephemeroptera); Leptoceridae gen. sp. larvae (n = 69), Policentropodidae gen. sp. larvae (n = 8), *Glyphotaelius pellucidis* larvae (n = 23) and *Hyporhyacophila* sp. larvae (n = 1) (Trichoptera); and *Erpobdella octoculata* (n = 2) (Turbellaria).

Two species of larval digeneans were found: *Skrjabinophyetus neomydis* (Dimitrova et Genov, 1967) (Nanophyetidae) and *Pseudocephalotrema pyrenaica* (Combes et Jourdane, 1970) (Lecithodendriidae). Metacercariae of these two species were isolated from: *Baetis* sp. larvae, *Paraleptophlebia* sp. larvae, Leptoceridae gen. sp. larvae, Policentropodidae gen. sp. larvae, *Glyphotaelius pellucidis* larvae, and *Hyporhyacophila* sp. larvae. A total of 42 non-mollusc invertebrates (25.15 %) were infected: 5 (3 %) by *S. neomydis* and 37 (22.15 %) by *P. pyrenaica*. Prevalence rates of metacercariae in each arthropod species are given in Table I. *Atractides* sp., Psephenidae gen. sp., Elmidae gen. sp., Chironomidae gen. sp., Culicidae gen. sp., *Ecdyonurus* sp., *Hyporhyacophila* sp., and *Erpobdella octoculata* were not infected. Infection intensities ranged from 1 to 5 metacercariae for *S. neomydis* and 1 to 10 metacercariae for *P. pyrenaica*. A double infection was only detected in one specimen of *G. pellucidis*. The same digenean species of cercaria were found in *B. rufescens*, *S. neomydis* (20.4 %) and *P. pyrenaica* (1.97 %). Quantitative results are summarized in Table II.

DISCUSSION

The life cycles of *S. neomydis* and *P. pyrenaica* were first described by Brendow (1970) and Jourdane (1973a), respectively. Brendow (1970) studied the biology of *Skrjabinophyetus repens* Bregenzer, 1916 and cited the snail *Bythinella compressa* as the first intermediate host and stone flies and caddis flies (Plecoptera) as second intermediate hosts. This author adopted the specific name of *S. repens* Bregenzer, 1916 due to the similarity of their larval stages with *Cercaria repens*. Later authors synonymized both species as *S. neomydis*. The study of Brendow (1970) is not as exhaustive as that of Jourdane (1976), in which *Bythinella reyniesii* is cited as the first intermediate host and Trichoptera Limnephilidae (*Potamophylax cingulatus*, *Chaetopterygopsis maclachlami*, *Chaetopterygopsis* sp.) and Plecoptera Perlodidae (*Arcynopteryx compacta*) as second intermediate hosts in the Pyrenees. Comparing our results with those of Jourdane (1976), we have identified for the first time the limnephilid *G. pellucidis* as a second intermediate host of *S. neomydis* in different biotopes in the Pyrenees. The life cycle of *P. pyrenaica* is well documented, with *B. reyniesii* as the first intermediate host and Plecoptera of the Setipalpia group in the families Perlidae (*Perla marginata marginata*, *Dinocras cephalotes*) and Perlodidae (*Arcynopteryx compacta*, *Perlodes intricata*, *Isoperla* spp.) as second intermediate hosts (Jourdane 1973a). In this study *B. rufescens* and different species of Ephemeroptera (*Baetis* spp.) and Trichoptera (*Leptocerus* sp., *G. pellucidis* and Policentropodidae gen spp.) are reported as new host species for *P. pyrenaica*. *P. pyrenaica* was not found in the plecopters examined here (*Xanthoperla* sp.), perhaps due to the low number of animals dissected.

Studies carried out in Spanish regions indicate that Crustacea, Diptera, Ephemeroptera, Odonata, Oligochaeta, Plecoptera and Trichoptera are habitual components of the desman diet (Santamarina 1993). In the Pyrenees, Castién & Gosálbez (1995) analyzed the digestive tracts of 46 *G. pyrenaicus* and identified 2629 aquatic prey. In this study, Trichoptera larvae accounted for 23.1 % of the total prey and provided 56.8 % of the biomass. Most of these invertebrate species are intermediate hosts of the potential desman helminths. The most common digenean in *G. pyrenaicus* is *Omphalometra flexuosa* (Rudolphi, 1819) (Omphalometridae), a parasite of the desman and *Talpa europaea* L., 1758 (Talpidae) in France and Spain, which is found (metacercariae) in crustaceans (*Gammarus pulex* L., 1758) (Vaucher 1975, Yamaguti 1971). *Mathovius galemydis* Mas Coma, Roset et Montoliu, 1985 is a Lecithodendriidae that is now only found in the Pyrenean desman that inhabits the Iberian Peninsula. In lecithodendrid worms with a known life cycle, metacercariae are found in larvae and adult arthropods with an aquatic life cycle (Yamaguti 1971). The microphallid *Maritrema pyrenaica* Deblock et Combes, 1965 is a parasite of *G. pyrenaicus* and *N. fodiens* in Spain (Casanova *et al*. 1998) and France (Jourdane 1979). However, we did not find it in our study because even though *B. reyniesii* is the first intermediate host, metacercariae encyst in crustaceans (*Echinogammarus berilloni*, which were misidenti-

fied by Casanova *et al*. (1998) as *Gamarus pulex*). In the Pyrenees the latter authors found gammarids infested by *M. pyrenaica* in the typical habitats of the desman. The biological pattern is the same as that of *Microphallus gracilis* Baer, 1943 (Microphallidae), a Pyrenean parasite of water shrews (Jourdane 1977). We did not find *Nephrotrema truncatum* (Leuckart, 1842), a digenean with aquatic invertebrates as intermediate hosts (Jourdane 1974a), which has not been recovered in its larval form despite the fact that it has been recorded in its adult stage (Ribas *et al*. 2005).

Prevalence of parasitism in snails differed between the two digeneans recovered (20.4 % vs. 1.97 %). This variability in infestation rates (between localities) has been previously observed (Faltinkova & Literak 2002), but as our study only includes a single sample point such comparisons cannot be made here. Despite the high number of snails in our study (close to one thousand) the species richness is poor when compared with the data of Faltinkova & Literak (2002), who found 12 species from 549 snails. The high number of localities sampled by these authors probably contributed to a higher number of digenean species. As regards the detection of aquatic small mammals, our results suggest that the study of aquatic snails is an easier method than focusing on the second intermediate host; if a pool (e.g. 100) of *Bythinella* can be placed in the same recipient and examined the next day in order to check for the presence/absence of cercariae this will be easier than dissecting one of the invertebrates. This proposed methodology enables different points of a river to be examined at the same time by grouping snails by points and examining them the next day. The transport and survival of snails is also easier. In Mediterranean rivers where only *Neomys anomalus* is present, this should be an excellent methodology to detect its presence. Nevertheless, future studies are needed to improve this methodology.

In conclusion, our study illustrates the utility of non-aggressive methods to detect parasitic species without host damage and provides new data on the intermediate hosts of digenean parasites of semiaquatic mammals (*Galemys pyrenaicus* and *Neomys* spp.).

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