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Helminth parasites of the Eurasian otter *Lutra lutra* in southwest Europe

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Abstract

The helminth fauna in 109 Eurasian otters (*Lutra lutra* L.) from France, Portugal and Spain was analysed, together with 56 faecal samples collected in Portugal and 23 fresh stools from otters included in a reintroduction programme. Seven helminth species were found in *L. lutra* in southwest Europe: *Phagicola* sp. (Trematoda), *Aonchotheca putorii*, *Eucoleus schvalovoj*, *Strongyloides lutrae*, *Anisakis* (third stage larvae) and *Dirofilaria immitis* (Nematoda), and *Gigantorhynchus* sp. (Acanthocephala). *Eucoleus schvalovoj* was the dominant species throughout southwest Europe. *Strongyloides lutrae* was significantly more prevalent in the Iberian Peninsula than in France. Apart from these two dominant nematodes and *A. putorii*, the other helminth species were incidental parasites of *L. lutra* in southwest Europe. The helminth fauna of *L. lutra* in southwest Europe is, in general, poorer than that reported in eastern Europe and in all other aquatic mustelids in southwest Europe. *Phagicola* specimens are reported for the first time in a non-marine wild carnivore in Europe. The prevalences of *E. schvalovoj* and *S. lutrae* obtained by necropsy were higher than those observed by coprological analysis using a formalin-ether concentration method (Ritchie). Nevertheless, the culture of fresh faeces appears to be the best method to study infection of *L. lutra* by *Strongyloides*.

Introduction

The Eurasian otter (*Lutra lutra* L.) is a semi-aquatic mustelid widely distributed from the north of the Atlas to the Bering Strait (Mason & Macdonald, 1986). The Iberian Peninsula marks the southwest limit of its European range, where the distribution of *L. lutra* is well known (Delibes, 1990; Ruíz-Olmo & Delibes, 1998; Trindade *et al.*, 1998; Barbosa *et al.*, 2001, 2003). *Lutra lutra* feeds mainly fish and large aquatic invertebrates and its habitat is linked to relatively clean freshwater, shelter and abundant prey (Libois, 1995; Ruíz-Olmo & Palazón, 1997; Ruíz-Olmo & Delibes, 1998; Trindade *et al.*, 1998). Because of several factors (loss of habitat, water pollution, human disturbance, etc.), the worldwide distribution of the otter has declined sharply in recent decades (Mason & Macdonald, 1986; Macdonald & Mason, 1992), although in some areas a recovery has been noted (Ruíz-Olmo & Delibes, 1998). In this context, to restore the population of

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L. lutra in the basins of the rivers Muga and Fluvià (Catalonia, northeast Spain), a reintroduction programme was undertaken from 1995 to 2000 using specimens from other Iberian areas such as Asturias and Extremadura (Spain) and Portugal, where otters are abundant (Saavedra, 2003).

In western Europe, several studies in recent years have addressed the distribution, demography, biology, diet and causes of mortality of *L. lutra* (Libois & Rosoux, 1989; Nores *et al.*, 1991; Ruíz-Olmo, 1994, 1995, 2001; López-Marín & Ruíz-Olmo, 1996; Rosoux & Libois, 1996; Rosoux, 1998; Trindade *et al.*, 1998; Barbosa *et al.*, 2001, 2003; Ruíz-Olmo *et al.*, 2001a,b; Gutleb, 2002). The Eurasian otter has also been the subject of several parasitological studies, mainly in Britain (Jefferies *et al.*, 1990; Weber, 1991; McCarthy & Hassett, 1993), and more recently in eastern Europe (Barus *et al.*, 2000; Shimalov *et al.*, 2000; Anisimova, 2002). However, to date no study on the parasites of *L. lutra* has been undertaken in continental western Europe. The only data available on parasites of *L. lutra* in this area include those of Feliu *et al.* (1995), a redescription of *Eucoleus schvalovoj* by Torres *et al.* (1999) and a description of the protozoan *Isospora lutrae* by Torres *et al.* (2000).

Using a large temporal and spatial study, we provide the first comprehensive data on the helminth fauna of *L. lutra* in southwest Europe (France, Portugal and Spain). These data will be valuable in the characterization of the whole structure of the helminth fauna of aquatic mustelids (Torres *et al.*, 1996, 2003) at the limit of their western European ranges.

Materials and methods

All the viscera of *L. lutra* specimens included in this study were provided by official institutions in France, Portugal and Spain over the last decade. Otters were usually found dead in the field or were killed on roads. One hundred and nine Eurasian otters were analysed from France (56 specimens), Portugal (5) and Spain (48). All French and Portuguese otters came from the Region Poitou-Charente and Alentejo, respectively. The Spanish otters came from: A Coruña (2 specimens), Asturias (4), Badajoz (7), Burgos (1), Cáceres (2), Cádiz (1), Cantabria (3), Ciudad Real (2), Cuenca (1), Girona (2), Guadalajara (1), Huelva (3), Huesca (3), Lleida (6), Lugo (1), Pontevedra (4), Sevilla (3) and Tarragona (2). To prevent the deterioration of helminths, the viscera of all specimens were quickly frozen before being sent to Barcelona (Spain) for analysis. After thawing at room temperature in the laboratory, routine techniques were used to systematically check each otter for helminths. Skulls were not examined for parasites. Helminths were removed and placed in vials containing fixing agents and were then processed, identified, and counted. Helminths were preserved in our laboratory collection.

To enlarge the sample from Portugal, during the 1999–2001 period 56 faecal samples of *L. lutra* were collected within 24 h of deposition at Serra de Grândola (southwest Portugal) and were then preserved in 10% formalin. A classic formalin-ether concentration technique (Ritchie) was used to analyse the faeces. In addition, we also studied the fresh stools of 23 otters from Asturias and Extremadura (Spain) and from Portugal, which were sent to the Barcelona Zoo for clinical evaluation prior to release in Catalonia (northeast Spain) as a part of a reintroduction project (Fernández-Morán *et al.*, 2002). These fresh faeces were studied by the Ritchie technique and were also cultured with charcoal in Petri dishes to identify the presence of *Strongyloides* before and after treating the otters with ivermectin.

Helminth identification was based on keys and descriptions given by Burton (1958), Little (1966), Petrochenko (1971), Butterworth & Beverley-Burton (1980), Goméz Bautista *et al.* (1999), Pereira Bueno (1999) and Torres *et al.* (1999). The use of descriptive ecological terms follows Bush *et al.* (1997). Prevalences were compared using the chi-square test with a Yates continuity correction.

Results

Seven helminth species were identified in *L. lutra*, including the stools of captive otters and faeces collected in the field. These helminths were *Phagicola* sp. (Trematoda), *Aonchotheca putorii* (Rudolphi, 1819), *Eucoleus schvalovoj* Kontrimavichus, 1963, *Strongyloides lutrae* Little, 1966, *Anisakis* (third stage larvae, L3), *Dirofilaria immitis* (Leidy, 1856) (Nematoda), and *Gigantorhynchus* sp. (Acanthocephala).

Post-mortem examination

More than half of the otters surveyed (56.9%) were free of helminths (69.6% from France, 40% from Portugal, and 43.7% from Spain). *Eucoleus schvalovoj* was the dominant species throughout the study area and no significant differences were observed between countries (table 1). In contrast, *S. lutrae* was significantly more prevalent in the Iberian Peninsula than in France ($P = 0.04$). The helminth fauna of *L. lutra* in Spain was richer than that in France and Portugal. Some incidental species such as *Phagicola* sp., *Anisakis* (L3) and *Gigantorhynchus* sp. were found only in samples from Spain and this may be due to the larger number of samples examined from this country, which included otters from several biogeographic areas.

Table 1. The prevalence (P%) and mean intensity (MI) of helminth in the Eurasian otter, *Lutra lutra*, in France, Portugal and Spain. Data for *E. schvalovoj* in France and Spain are derived from 30 and 38 otters respectively.

	France ($n = 56$)		Portugal ($n = 5$)		Spain ($n = 48$)	
Helminth	P (%)	MI	P (%)	MI	P (%)	MI
Phagicola sp.					2.1	27
Aonchotheca putorii	5.4	10.3	20.0	1	6.3	2.7
Eucoleus schvalovoj	46.7	8.9	20.0	4	50.0	12.2
Strongyloides lutrae	3.6	21.5			16.7	10.6
Anisakis (L3)					2.1	4
Dirofilaria immitis			40.0	6.5	2.1	2
Gigantorhynchus sp.					2.1	1

Coprological examination

In the faecal samples, only eggs of the two dominant species *(E. schvalovoj* and *S. lutrae*) were found. The prevalence of *E. schvalovoj* and *S. lutrae* observed (Ritchie method) from the faecal field samples were 32.1% and 12.5% respectively. The prevalence of *E. schvalovoj* determined by the same method but from fresh stool samples was 17.6%. The prevalence of *S. lutrae* (culture method) from fresh stool samples was 65.2%. There was a significant difference between the coprological and post-mortem results for *E. schvalovoj* in the Iberian Peninsula. Post-mortem studies showed a significantly higher prevalence of this nematode (46.5%) than that obtained using the Ritchie method ($P = 0.02$). However, following the culture of fresh stools, the prevalence of *S. lutrae* was statistically higher than the prevalence observed by post-mortem (13.1%; $P < 0.01$).

Discussion

The present study shows that the helminth community of *L. lutra* in southwest Europe is qualitatively and quantitatively poorer than that reported in Spain for other semi-aquatic mustelids, such as *Mustela putorius* L.,*M. lutreola* (L.) and *M. vison* Schreber (Torres *et al.*, 1996, 2003). After examining 99*M. putorius*, 28 *M. lutreola* and 112 *M. vison* Torres *et al.* (1996, 2003) reported 18 helminth species (six digeneans, two cestodes, nine nematodes and one acanthocephalan). However, the helminth community in *L. lutra* in the present study shows that only one species *(A. putorii)* is shared with other semi-aquatic mustelids. Excluding incidental and non-specific species *(Phagicola* sp., *Anisakis* (L3), *D. immitis* and *Gigantorhynchus* sp.), the dominant helminths *(E. schvalovoj* and *S. lutrae)* were otter specialists. Surprisingly, we did not find any nematode that was a mustelid specialist, such as *Molineus patens* (Dujardin, 1845), *Crenosoma melesi* Jancev & Genov, 1988, *Filaroides martis* (Werner, 1783) or *Aelurostrongylus pridhami* Anderson, 1962. This finding is difficult to explain because, at least in Spain, large populations of both these nematodes and *L. lutra* are found (Motjé, 1995).

No cestodes were found, and digeneans were very rarely detected despite the large number of otters surveyed. This pattern strongly contrasts with data from eastern Europe, where several digeneans and cestodes have been recently reported in this species (Barus *et al.*, 2000; Shimalov *et al.*, 2000; Anisimova, 2002). However, we detected some digeneans that belong to the genus *Phagicola* in the intestine of one otter from Spain. This is the first finding of a representative of this genus in *L. lutra* in Europe. The specimens resembled *P. longa* Ransom, 1920, but their condition (especially the loss of some spines in the oral coronet) did not allow further identification. Species of the genus *Phagicola* are typical parasites of piscivorous birds and mammals since they require fresh-water fish as intermediate hosts (Yamaguti, 1975). Excluding Europe, species of *Phagicola* have been reported in some wild carnivores throughout the world (Font *et al.*, 1984; Dalimi & Mobedi, 1992). However, most reports refer to domestic carnivores (dogs and cats) infected under environmental or experimental conditions (Deiana, 1961; Jordan & Maples, 1966; Tinar, 1976; Costa *et al.*, 1984; Rajavelu & Raja, 1988; Nieddu & Lochi, 1990), as well as in experimental rodent models (Conroy, 1986) and even in humans (Chieffi *et al.*, 1992). To date, amongst wild carnivores in Europe, only some common seals have been reported harbouring *Phagicola* species (Strauss, 1990). Therefore, we report for the first time the presence of a representative of the genus *Phagicola* in a non-marine wild carnivore in Europe.

At least in Spain, *Aonchotheca putorii* is one of the most widespread nematodes that parasitizes mainly terrestrial mustelids, whose diet includes earthworms as intermediate hosts (Torres *et al.*, 1996, 2001). Aquatic mustelids such as *M. putorius*, *M. vison* and *M. lutreola* (Torres *et al.*, 1997, 2003) also appear to be infected by this nematode. *Aonchotheca putorii* was also detected in the present sample of *L. lutra*. The diet of the Eurasian otter has been extensively studied in the Iberian Peninsula (see summary in Ruíz-Olmo & Palazón, 1997) and *L. lutra* does not feed on earthworms. Eggs of *A. putorii* hatch in earthworms, in which the infective stage is reached one month later, but free fully embryonated eggs can also infect mustelids (Anderson, 2000). This pathway might constitute an efficient mechanism to increase its transmission among mustelids, but mainly in those such as *L. lutra*, which do not normally feed on earthworms.

Eucoleus schvalovoj was the most prevalent helminth species (46.5%) in the present study. The higher prevalence (50.0%) and mean intensity (12.2) of this species were observed in Spain but *E. schvalovoj* was also found frequently and with quite elevated intensities in Portugal and France. This nematode, recorded for first time in *L. lutra* from the Khabarovsk region, was recently redescribed by Torres *et al.* (1999) from otters in Spain. Torres *et al.* (1999) pointed out that *E. schvalovoj* is an oesophageal parasite specific to *L. lutra*, whose distribution is restricted to the two extremes of the Palaearctic region. They also proposed that the lack of reports of this parasite in other European studies on *L. lutra* might be due to the difficulty in detecting this small nematode, as it is threaded into the stratified squamous epithelial lining of the oesophagus. Torres *et al.* (1999) suggested that the distribution of *E. schvalovoj* is wider than it seems and the present results confirm this. Furthermore, eggs of *E. schvalovoj* were also easily recognized in faecal samples. These eggs, measuring $59.5 \times 27.7\ \mu$m, pass unembryonated through the faeces and present a network of ridges on the surface with protruding polar plugs (Torres *et al.*, 1999). However, eggs in the uterus of *E. schvalovoj* were not very numerous, which may explain why low intensities of *E. schvalovoj* go unnoticed in coprological examinations.

With reference to *Strongyloides*, female worms degenerate rapidly after death of the host (Speare, 1989) and hence the majority of females isolated from the intestine could not be identified. However, using cultures of fresh stools from otters, the growth and development of larval stages and free-living males and females were observed and the specimens were identified as *S. lutrae*. This species was described in *Lutra canadensis* (Schreber) by Little (1966) and has been reported several times in this host in the Nearctic region, sometimes with considerable prevalences (44% and 33%) in the Pacific northwest (Hoberg *et al.*, 1997). To our knowledge, the present study constitutes the first report of *S. lutrae* from Eurasian otters in the Palaearctic region. The higher prevalence of *Strongyloides* in otters from the Iberian Peninsula with respect to France and eastern Europe is probably due to the high population density of *L. lutra* in several Iberian regions (Ruíz-Olmo, 2001; Ruíz-Olmo *et al.*, 2001b). The otter population in Portugal is considered one of the most viable in Europe (Trindade *et al.*, 1998) and otter populations in some regions of Spain (Extremadura and Asturias) are also large (Ruíz-Olmo & Delibes, 1998).

The adult female of *S. lutrae* is a minute, very slender nematode that lives in tunnels within the intestinal mucosa, therefore making its detection difficult. There are only sporadic reports of *Strongyloides* specimens in otters in Europe. Shimalov *et al.* (2000) reported only one otter (4%) infected with *Strongyloides martis* (Petrow, 1940) in Belorussia. On the other hand, female *S. lutrae* are ovoviviparous and present few eggs in both uteri, which makes it difficult to find in a small sample of faeces. Therefore, the culture of fresh faeces is the best method to study the infection of otters by *Strongyloides* specimens.

One otter from the coastal Esba river (Asturias), 5–10 km from the Cantabrian sea, was infected with four nematodes identified as the third stage larvae of *Anisakis* (probably *A. simplex* Rudolphi, 1809). Adult *Anisakis* are typical nematodes of pinnipeds and cetaceans, whereas infective larvae have been reported in a variety of marine and anadromous teleosts (Anderson, 2000), which are common food for these otters (Kruuk, 1995). *Anisakis* larvae have never been reported in otters from riparian systems. However, larvae of species of anisakids (genus *Anisakis* and *Pseudoterranova*) have previously been observed in otters (*L. canadensis* and *L. lutra*) from coastal sites in the Pacific (Hoberg *et al.*, 1997) and in Britain (Jefferies *et al.*, 1990; Weber, 1991).

We detected some specimens of *Dirofilaria immitis* from the right ventricle of three Eurasian otters. This appears to be the first report of this nematode in *L. lutra* in Europe. *Dirofilaria immitis* has been reported in the South American otter, *Pteronura brasiliensis* (Gmelin), in Venezuela (Vogelsang, 1940) and in *L. canadensis* from Louisiana (Snyder *et al.*, 1989). In the Iberian Peninsula, *D. immitis* has also been found in dogs, foxes and wolves (Pérez-Sánchez *et al.*, 1989; Gortázar *et al.*, 1998; Segovia *et al.*, 2001). The finding of *D. immitis* in several carnivores indicates that this pathogenic nematode, which has a life cycle that involves diverse species of culicids (Anderson, 2000), is more frequent in irrigated lands close to rivers, probably because of the increased presence of vectors.

There are few reports of acanthocephalans in otters. Only *Corynosoma strumosum* (Rudolphi, 1802) has been reported frequently in otters, having been found in the sea otter, *Enhydra lutris* (L.) (Rausch & Locker, 1951), in *L. canadensis* (Hoberg *et al.*, 1997) and in *L. lutra* from marine coastal areas in Britain (Jefferies *et al.*, 1990; Weber, 1991) and in Ireland (McCarthy & Hassett, 1993). Unidentified specimens of the genus *Acanthocephalus* were reported in *L. canadensis* by Fleming *et al.* (1977) in Alabama and by Kollars *et al.* (1997) in Tennessee. In Europe other species such as *Gigantorhynchus moniliformis* (Bremser, 1811), *Heterosentis plotosi* Yamaguti, 1935 and *Pomphorhynchus laevis* Van Cleave, 1924 have been found in *L. lutra* (Petrochenko, 1971; Butzeck, 1984). The current Spanish report of one immature specimen of the genus *Gigantorhynchus*, which could not be identified, corroborates the previous finding of a representative of this genus (*G. moniliformis*) in the Eurasian otter in the Caucasus region (Butzeck, 1984). With respect to aquatic mustelids in West Europe, *Centrorhynchus ninnii* (Stossich, 1891) is another acanthocephalan that sporadically parasitizes the American mink (Torres *et al.*, 2003) in Spain. The life cycle of the *Gigantorhynchus* species reported here probably involves a crustacean, as the case of other species of the genus (Crompton & Nickol, 1985).

Studies on the parasites of *L. lutra* in Britain include those of Jefferies *et al.* (1990) who examined 56 otters from coastal sites in Britain and reported only *Pseudoterranova decipiens* (Krabbe, 1878) and *C. strumosum*, and Weber (1991) who examined 38 otters from Shetland, mostly living in or very close to marine habitats, and reported the helminths *Diphyllobothrium medium* (Fahmy, 1954), *P. decipiens*, *A. simplex* and *C. strumosum*. It is difficult to compare the helminth fauna of freshwater otters with those reported in coastal zones because the helminth fauna may differ considerably according to the habitat. For example the intermediate host of *C. strumosum* is an amphipod and the absence of this acanthocephalan in freshwater otters in western and eastern Europe could be explained by the absence of this intermediate host in the location of the otters examined. However, in one otter examined from a Spanish locality relatively close to the coast, we found an anisakid nematode, which is found in hosts from coastal zones in Britain.

Nevertheless, the most interesting data to compare with the present results were those reported by Shimalov *et al.* (2000) and Anisimova (2002) in Belarus. From 25 *L. lutra* carcasses and 117 faecal samples examined by Shimalov *et al.* (2000) from the Brest and Gomel regions in the southern part of Belarus, 15 helminth species (seven digeneans, two cestodes and six nematodes) were reported and this constitutes a considerably larger helminth community than that reported in the present study. *Isthmiophora melis* (Schrank, 1788) was the dominant digenean (24%) and, in contrast to our study, *L. lutra* shared several digeneans with *M. lutreola* and *M. vison* in these Belorussian regions. The nematode *E. schvalovoj* was not found and only one otter was reported to be infected with a species of *Strongyloides* which, after post-mortem and coprological analysis, was identified as *S. martis* Petrow, 1940. On the other hand, Anisimova (2002) reported five helminth species (*Euparyphium melis, Rossicotrema donicum, Spirometra erinacei, Capillaria mucronata* and *Skrjabingylus nasicola)* from 60 Eurasian otters from Belarus from 1989 to 1997. Quantitatively, these results are similar to the present data and reflect that the helminth fauna of *L. lutra* in Belarus is also poor. However, qualitatively these results differ greatly because otters from Belarus and southwest Europe do not share the same helminth species.

Populations of the Eurasian otter have declined in recent decades in western Europe (Mason & Macdonald, 1986). Consequently, there has been a negative impact on the diversity of the helminth fauna harboured by *L. lutra*. Only a few oioxenous species (*E. schvalovoj* and *S. lutrae*) have not been affected by this decline which, together with the fact that *L. lutra* is piscivorous, explains the nature of the helminth composition of *L. lutra* in southwest Europe.

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