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The prevalence and intensity of infection with helminth parasites in *Mus spretus* from the Setubal Peninsula of Portugal

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ABSTRACT

The results of a 5 year study of helminth parasites of *Mus spretus*, are reported. Six nematode and 5 cestode species were identified but no helminth showed 100% prevalence in *M. spretus*, the most commonly encountered nematode and cestode species being *Syphacia obvelata* (46·6%) and *Taenia taeniaeformis* (22·4%). Among the more unusual helminth species identified was *Eucoleus bacillatus*, a capillariid nematode inhabiting the stomach musculature. This species was identified in 3 of the 5 years of the study. The results are discussed in the broader context of previous studies and the epidemiology of rodent helminth infections in general.

KEY WORDS: *Mus spretus*, Portugal, Nematoda, Cestoda, prevalence

INTRODUCTION

Although intensive studies of parasitic infections in rodents have been systematically carried out in the UK over many years (SHARPE, 1964; LEWIS, 1968a,b; LEWIS & TWIGG, 1972; LANGLEY & FAIRLEY, 1982; MONTGOMERY & MONTGOMERY, 1988) and some parts of Europe, notably in Poland (FURMAGA, 1957; SOLTYS, 1957; KISIELEWSKA, 1970), Czechoslovakia (BAER & TENORA, 1970) and Hungary (TENORA & MURAI, 1972), reports on rodent parasites from Mediterranean countries usually represent no more than species lists. We are not aware of any detailed quantitative data on parasitic infections in *Mus* species from Mediterranean countries which consider such effects as host age, sex and seasonal fluctuations in parasite numbers and which contribute to a picture of the ecological relationships between the hosts and parasites concerned. Furthermore, almost nothing is known about the parasites of the little studied rodent *Mus spretus*, an aboriginal murine species found locally in western Mediterranean regions from France, through Spain, Portugal to Libya. In Portugal *M. spretus* occupies a niche in shrub-grassland habitats and seldom comes into contact with man in human habitations (SAGE, 1981). This species is thought to be closely related to *M. musculus domesticus* although it is now considered to merit separate specific status rather than the subspecific status reported earlier (SAGE, 1981).

In a series of two-week investigations carried out annually over 5 years, we accumulated quantitative information on parasitic helminth infections in *M. spretus*. This report describes our study sites and presents data on the identity, prevalence and intensity of infection with the major helminth taxa and species affecting *M. spretus*.

MATERIALS AND METHODS

Study site

The project was carried out during a two-week period in mid April from 1988 until 1992 and formed part of a terrestrial zoology field course. Most mice were caught at the Quinta de Sao Pedro in Sobreda near Lisboa but the Quinta Niza, about 3 km further south east also provided some animals for the study.

The Quinta de Sao Pedro (longitude 9°11′ west, latitude 38°39′ north) comprises 4 ha of land, mainly wooded with overgrown fields last used for arable purposes in 1965 and only occasionally grazed by sheep since then. It is located in the vicinity of Lisboa on the Setubal peninsula, 5 km from the resort of Costa da Caparica on the Atlantic coast. The entire plot is now walled off, although the boundary walls were not completed until 1990 and there are many potential entrance and exit points for small mammals. However, the site has become increasingly isolated from other reservoirs of small mammals in recent years. In 1988, dwellings surrounded about a third of the perimeter of the plot, the remainder being open grazing land, and a 50 m stretch adjoined a local road. Since 1991, however, there has been an extensive building programme in the region and in 1992, almost the entire perimeter, except for the road, had housing in various stages of completion.

Capture and treatment of mice

All mice were caught in standard Longworth traps baited with grain and peanut butter using conventional procedures. New mice, which had not previously been caught in that year, were identified to species, sexed, weighed to the nearest 0·5 g and the stretched body (tip of nose to anus) and tail length were measured. Mice destined to be released or to be kept in captivity for a day or more were marked by fur clippings. Those to be autopsied immediately were killed by cervical dislocation.

Within minutes of death, the body cavity was opened and inspected for overt cysts such as those of *Taenia taeniaeformis*. The bile duct was located and examined for evidence of the swelling characteristic of *Hymenolepis microstoma* infection. The entire small intestine was then removed and sectioned into oesophagus, stomach, anterior and posterior halves of the small intestine, caecum and colon. Each section was placed on a small piece of gauze, opened longitudinally and then suspended in a 50 ml plastic beaker containing Hanks's saline at 37°C. The only section to be treated slightly differently was the stomach, the contents of which were first removed by gentle immersion in saline. A different procedure was also adopted when *H. microstoma* infection was suspected on the basis of a hypertrophied bile duct. In such cases the intestine was removed with the entire liver. All portions of intestine from the posterior half of the small intestine and the stomach were treated as described above. The liver and anterior small intestine were then immersed in warm Hanks's saline in a Petri dish and the small intestine was opened from the posterior moving progressively anteriorly towards the bile duct. All beakers were incubated for a minimum of 2 h before examination. The contents of each beaker were transferred to a plastic Petri dish and examined under a binocular dissecting microscope. Parasites were then transferred with a Pasteur pipette, or using forceps for larger cestodes, to a separate dish for subsequent closer scrutiny. All gauzes were also examined and in no case were any parasites found to have been left attached to the gauze. All the tissues contained within the gauzes were also examined, first by shaking any remaining loose materials into saline and then after the entire mucosa had been scraped off with the edge of a glass slide.

Very few parasites were found after such examination of gut tissues. Only in exceptionally heavy infections with pinworms, were larvae occasionally detected and on rare occasions portions of cut worms were also identified. In general, all the parasites migrated out of the tissues and were easily located in the saline contained in the bottom of the beakers. The exception was *E. bacillatus* which did not detach from host tissues and required careful removal from the intestinal mucosa after the incubation period.

As far as possible, parasites were identified as they were encountered in Petri dishes and counted as they were removed to collecting dishes. Any unusual specimens were re-examined carefully. Tapeworms were measured and their scolices subjected to close

scrutiny. Hooks were counted, photographed and measured. All specimens were fixed: nematodes in 70% ethanol, cestodes in AFA.

Statistical procedures

Careful morphometric and parasitological records were kept of all data collected on each mouse. Where appropriate, data are presented as prevalence of infection (% of mice infected) or as Mean ± Standard Error of the Mean (S.E.M.) parasite taxa per host species.

RESULTS

Hosts

Table I shows the number of animals of each sex examined in each year. As can be seen, significantly more male mice than females ($\chi^2 = 5\cdot59$, $p < 0\cdot02$) were examined but, overall, the sample size was sufficient to enable quantitative analysis.

Parasites

Of the 58 mice examined, 75·8% were infected with at least one species of helminth parasite. In addition to the 10 helminth species recorded in Table II, one nematode and one cestode species were not identified. Each was found in only one mouse and sufficient material was recovered to establish that neither belonged to any of the species listed but insufficient to establish firmly the identity of the organisms involved. Data for these two unidentified species were excluded from the analysis. The mean number of

TABLE I. The number of *Mus spretus* examined during the study by gender and year.

Year	Male	Female
1988	6	4
1989	12	3
1990	5	5
1991	4	3
1992	11	5
Total	38	20

TABLE II. Prevalence (%), total and mean numbers of taxa, and mean worm burden of helminth parasites in *Mus spretus*.

Taxa	Prevalence	Total no. of taxa	Mean no. of taxa ± S.E.M.	Mean worm burden ± S.E.M.
ALL HELMINTHES	75·8	10	1·5 ± 0·2	15·6 ± 4·8
NEMATODA	60·3	5	0·9 ± 0·1	14·0 ± 4·8
Nippostrongylus brasiliensis	17·2	–	–	0·4 ± 0·2
Aspiculuris tetraptera	3·4	–	–	1·5 ± 1·5
Syphacia obvelata	46·6	–	–	11·5 ± 3·7
Heterakis spumosum	6·9	–	–	0·07 ± 0·03
Eucoleus bacillatus	8·6	–	–	0·4 ± 0·3
PLATYHELMINTHES	53·5	5	0·6 ± 0·1	1·6 ± 0·3
CESTODA	44·8	4	0·5 ± 0·1	1·0 ± 0·3
Taenia taeniaeformis	22·4	–	–	0·3 ± 0·1
Hymenolepis microstoma	13·8	–	–	0·5 ± 0·2
Hymenolepis nana	6·9	–	–	0·1 ± 0·1
Hymenolepis diminuta	1·7	–	–	0·02 ± 0·02
DIGENEA	15·5	1	0·2 ± 0·1	0·6 ± 0·3
Brachylaemus spp.	15·5	–	–	0·6 ± 0·3

taxa recorded per mouse was 1·5 (Table II) although, as can be seen from Fig. 1, this ranged from 0 to 4 with the largest category supporting 2 species (18/58, 31%). The Shannon Index of diversity was calculated as 0·267±0·045 and the effective number of abundant species (Hill's Index 1) as 1·38 ± 0·0768.

Table II lists the species of helminth parasites recovered and identified. Although identical numbers of nematode and platyhelminth taxa were identified (5 each), nematodes were the most common (60·3% prevalence) and accounted for the majority of worms recovered with a mean worm burden of 14 (Table II). Among the nematodes, the most common species was *S. obvelata* with a prevalence of (46·6%) and a mean worm burden of 11·5 which was largely dependent on a few heavily infected animals (Fig. 2). All other species were found more rarely. The dominant tapeworms were *T. taeniaeformis* (22·4%) and *H. microstoma* which varied from year to year but was found in a total of 8 *M. spretus* representing a prevalence of 13·8%. However, the mean worm burden for *H. microstoma* was slightly higher because in 1989 one female mouse was found to harbour 12 specimens (Fig. 3).
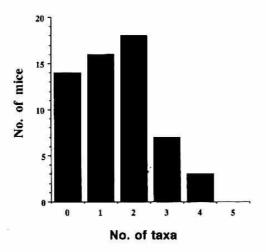

FIG. 1. Number of uninfected mice compared with mice from which parasites belonging to 1–4 helminth taxa were recovered.
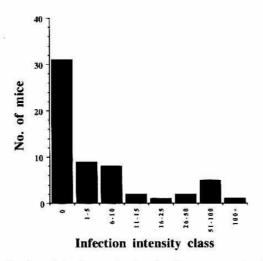

FIG. 2. The frequency distribution of *Syphacia obvelata* in *Mus spretus*. The index of dispersion *I* and the negative binomial constant *k* were estimated at 70·029 and 0·167 respectively.

DISCUSSION

This study, despite limited sample size, has provided for the first time detailed quantitative data on infections with parasitic helminths in *M. spretus*, a murine host which is relatively poorly studied and about which little is known. From available publications it appears that, unlike *M. m. domesticus*, this species shuns contact with man, preferring to live in a bush-grassland habitat in Mediterranean regions. Our experience of *M. spretus* suggests that it is relatively timid in comparison to *M. domesticus* and shows different patterns of behaviour (Hurst, personal observations).

Quite clearly, infections with *S. obvelata* accounted for the majority of helminth parasites in the mouse population and these were, in the main, attributable to relatively heavy infections in a few individuals (Fig. 2). Species of *Syphacia* are transmitted through eggs which embryonate within hours of deposition on the perianal skin surface of infected hosts (CHAN, 1952; LEWIS & D'SILVA, 1980; KERBOEUF & LEWIS, 1987). Transmission is therefore likely to be most efficient among hosts which congregate and occupy permanent territories and runs. In fact, transmission of *Syphacia* species is believed to be maximal when hosts congregate for rest and sleep, which in rodents coincides with midday. LEWIS & D'SILVA (1980) showed that transmission of *S. muris* in caged rats coincided with noon and the early afternoon hours with virtually no deposition of eggs in the early evening when rats were most active in feeding and when most faeces were produced. Similar observations have been made for *S. obvelata* (LEWIS & SHAVA, 1977). In contrast, *A. tetraptera*, whose eggs take about a week to embryonate (ANYA, 1966) and are released in host faeces, showed maximum output shortly before dawn when faecal output was at its highest (PHILLIPSON, 1974).

In our study, *M. spretus* exploited widely scattered resources in open grassland. As a consequence, individuals were widely distributed. *M. spretus* share semi-permanent tunnels through dense grass, but exhibit an interesting behavioural trait not shown by either *M. m. domesticus* nor *Apodemus sylvaticus* (Hurst, personal observations): these mice pick up or roll their own freshly-deposited faeces out of tunnels and away from
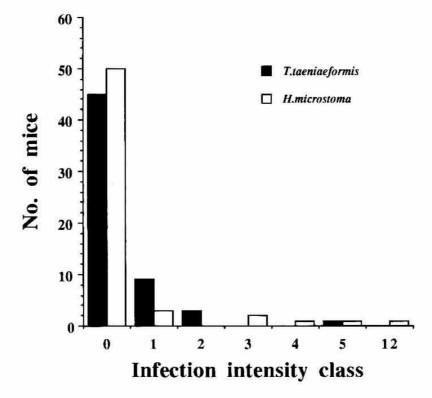
FIG. 3. The frequency distribution of *Taenia taeniaeformis* and *Hymenolepis microstoma* in *Mus spretus*. The index of dispersion *I* and the negative binomial constant *k* for *T. taeniaeformis* were estimated at 1·99 and 0·348 respectively and for *H. microstoma* at 6·461 and 0·095 respectively.

pathways. Transmission of parasites dependent on faecal eggs is thus likely to be less efficient in *M. spretus* than among *M. m. domesticus*: in this context it is interesting to note that nematode species transmitted through eggs voided in host faeces and requiring a period of days or even months to embryonate to the infective stage (e.g. *E. bacillatus, A. tetraptera*), were rare in *M. spretus* (Table I). The group-territorial social structure of commensal house mice (*M. musculus* and *M. m. domesticus*) allows them to live at very high densities where food resources are abundant (BRONSON, 1979; HURST, 1987a) and they share permanent well-made pathways to move between food and nest sites within buildings (HURST, 1987b). Under these conditions, parasite transmission through contact with conspecifics and their faeces is likely to be very high.

The patterns of infection with platyhelminth species also deserve comment. Five species of cestodes were recovered although the prevalence of none exceeded 25% among the sampled population. All the tapeworm species identified in this study are known to infect *M. m. domesticus*, which are found locally among farm buildings and human dwellings. Local cats are known to carry *T. taeniaeformis* with a relatively low prevalence of 5% (DA SILVA LEITAO & CRUZ E. SILVA, 1967) and are known to range throughout the study sites. The hymenolepiid infections are all transmitted via insect vectors (beetles and fleas) although *H. nana* has the potential for direct transmission.

All of the other parasites we identified were common helminths of house mice but some deserve special mention. Foremost among these is *Eucolleus bacillatus* (syn. *Trichosoma bacillatum*, Eberth, 1863; *Capillaria (Throminx) bacillata*, Hall 1916) a capillariid nematode species which has not been previously reported from *M. spretus* in Europe although it has been recorded from this rodent in Tunisia (BERNARD, 1987) and from other rodents in Spain (FELIU *et al.*, 1987a). OLDHAM (1931) listed *E. bacillatus* as a parasite of rats and SKRJABIN *et al.* (1957) gave Norwegian rats (*Mus [Epimys] norvegicus*) and house mice (*Mus musculus*) as the primary hosts but it has also been reported from *A. sylvaticus* (Spain, FELIU *et al.*, 19887b; Tunisia, BERNARD 1987; JUSTINE, 1989), *Arvicola terrestris* (Spain, FELIU *et al.*, 1987c; Belgium, BERNARD, 1969), *Arvicola terrestris, Clethrionomys glareolus* (Belgium, BERNARD,, 1969) and *Microtus majori* (in USSR, KURASHVILI, 1989). The adults of this species were located intertwined deep in the stomach walls with only a small section of the female worm protruding through the mucosa to allow eggs to be shed into the gut lumen. As far as we can ascertain, nothing more is known about the biology and life cycle of *E. bacillatus*, for example whether an intermediate host is required or whether transmission of embryonated eggs can take place directly. In this context we were able to embryonate eggs which were fed to laboratory house mice kept on immunosuppressive agents to maximize the chances of establishment and development, but without success.

Among the rarer species, which we believe to be incidental infections, was *Heterakis spumosum*, as parasite of rats (WINFIELD, 1933; SMITH, 1953) reported previously from local *Rattus norvegicus* (ARANDAS REGO, 1966; AFONSO-ROQUE *et al.*, 1984). This species was found at low prevalence and never more than 2 worms/host. Although primarily a parasite of rats, *H. spumosum* is known to cause transient, patent infections in house mice (ZINTZ & FRANK, 1982). Likewise, the few *N. brasiliensis* which we identified, also in low intensities, were likely to have been incidental infections from local rats which are known to carry this species with a high prevalence (>80%, see AFONSO-ROQUE *et al.*, 1984). *A. tetraptera* is primarily a parasite of *M. m. domesticus* (and laboratory house mice, *M. musculus*) and was also occasionally encountered in our *M. spretus* population.

Finally, although we were limited by the small sample sizes examined in some years, and our study was conducted only in a two-week period in April of each of the years, the present work has provided original detailed information on parasitic helminths in a little-studied rodent host species, *M. spretus*.

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