Prevalence of the dog nematode *Spirocerca lupi* in populations of its intermediate dung beetle host in the Tshwane (Pretoria) Metropole, South Africa

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ABSTRACT

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Spirocerca lupi (Spirurida: Spirocercidae) is a cosmopolitan parasite, principally of domestic dogs and dung beetles are its main intermediate hosts. In South Africa there has recently been growing concern over the upsurge of reported cases of clinical spirocercosis in dogs, while little is known or understood about the dynamics of the host-parasite associations between dung beetles and this nematode. We determined and compared the prevalence of infection in dung beetles between rural, urban and peri-urban areas of Tshwane (Pretoria) Metropole. Dung beetles were sampled during April and October 2006, at various localities in each of these areas. Localities were selected on the basis of being focal areas of high infection with *S. lupi* in dogs. Pig, dog and cow dung-baited pitfall traps were used for sampling the beetles. Trap contents were collected 48 h after the traps had been set and only dung beetles were collected from the traps. In total, 453 specimens belonging to 18 species were collected from 63 pitfall traps in all three areas. The numbers of species that were collected varied among the three areas. Dung beetles, irrespective of species (18) and numbers (447), predominantly preferred pig dung. The prevalence of dung beetles infected with the larvae of *S. lupi* varied considerably in the three areas. In the urban area 13.5 % of the dung beetles dissected were infected, while the prevalence of *S. lupi* in dung beetles in the rural area was 2.3 %. All the dung beetles that were infected with this nematode showed a preference for omnivore (pig and dog) dung.

Keywords: Dung beetle, intermediate host, Metropole, prevalence, *Spirocerca lupi*, spirocercosis, Tshwane (Pretoria)

INTRODUCTION

Spirocercosis is a canine disease caused by the nematode *Spirocerca lupi* (Rudolphi, 1809) (Spirurida: Spirocercidae) (Van der Merwe, Kirberger, Clift, Williams, Keller & Naidoo, in press). This is a cosmopolitan parasite, but is found more commonly in the warmer tropical and subtropical regions of the world (Bailey 1972). *Spirocerca lupi* is a parasite mainly of domestic dogs, although it also infects other members of the family Canidae (Mazaki-Tovi, Baneth, Aroch, Harrus, Kass, Ben-Ari, Zur, Aizenberg, Bark & Lavy 2002). Natural infections have been reported in coyotes, wolves, foxes and jackals, which serve as important reservoir hosts (Bailey 1972). Dung beetles are the main intermediate hosts of this parasitic nematode (Bailey 1972).

There is a plethora of literature on the clinical, diagnostic and epidemiologic aspects of spirocercosis in dogs, while very few studies have focused on the host-parasite associations between dung beetles and *S. lupi* (Bailey 1972; Chhabra & Singh 1973,

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1977). In a study to determine the prevalence of helminth parasites in dogs from a resource-limited urban community in Gauteng Province, Minnaar & Krecek (2001) found that 14.5 % of dogs were infected with this parasite. A similar study by Minnaar, Krecek & Fourie (2002) in a resource-limited peri-urban community in the Free State Province showed a prevalence of infection of 13 % in dogs from that region. Recently, there has been growing concern over the upsurge of reported cases of spirocercosis in dogs in South Africa, while little is known or understood about the dynamics of the host-parasite associations between dung beetles and this nematode. In this paper we discuss the prevalence of infection in populations of dung beetles with the larvae of *S. lupi* in rural, urban and peri-urban areas in the Tshwane (Pretoria) Metropole of South Africa.

SPIROCERCA LUPI LARVAL LIFE CYCLE

Intermediate as well as paratenic (transport) hosts are involved in the larval life cycle of this parasite. However, in light of the scope of this paper the discussion is limited to larval development in the coprophagous beetle intermediate host. Embryonated *S. lupi* eggs (L1) are passed in the faeces of the definitive host, whereafter it is ingested by a number of suitable species of coprophagous beetle intermediate hosts (Van der Merwe *et al*., in press). The eggs hatch after ingestion and within 2 months the larvae develop into the infective stage (L3). In the intermediate beetle host the infective stage larvae become encysted mainly on the tracheal tubules (Mazaki-Tovi *et al*. 2002). The coprophagous beetle, containing L3 larvae is ingested either by a paratenic or definitive host. The final host (dog) may become infected after ingestion of such a coprophagous beetle (Van der Merwe *et al*., in press).

MATERIALS AND METHODS

We conducted a pilot study during 2006 in the Tshwane (Pretoria) Metropole to determine and compare the prevalence of infection in dung beetles with the larvae of *S. lupi* between rural, urban and peri-unban areas. The prevalence of infection with this parasite was also compared between dung-specific and non-specific dung beetle species from the same communities.

Description of the study area

The study was conducted in the Tshwane (Pretoria) Metropole north of the Magaliesberg range. This mountain range separates the Metropole into two large vegetation types: cooler Bankenveld (Bredenkamp & Van Rooyen 1998) to the south and warmer Sour Bushveld and Clay Thorn Bushveld (Van Rooyen & Bredenkamp 1998a, b) to the north. The study area was classified into rural, urban and peri-urban areas, based on characteristics of their individual land use and the potential free-range limits of the dogs within each area. This distinction between areas translated into agricultural smallholdings being classified as rural areas, suburban gardens as being urban areas and resource-limited townships and informal settlements as being peri-urban areas.

Experimental design

Dung beetles were sampled during April and October 2006 at various localities in each of these areas. Localities were selected on the basis of being focal areas of high infection with *S. lupi* in dogs. The Department of Veterinary Tropical Diseases, University of Pretoria provided information concerning the infection rates in dogs from various areas, which they had compiled from reports of necropsies performed at the Onderstepoort campus. Dung beetles were sampled in three localities per area.

In this study the domestic dog was treated as an omnivore. Pig, dog and cow dung-baited pitfall traps were used for capturing dung beetles. Nine pitfall traps were placed in three transects in each locality. Transects were separated by 15-m intervals and each of the three traps per transect were placed 10 m apart. Each transect was baited with one of the three different dung types. The plastic buckets used for the traps had a 1 000 mℓ capacity and were 11 cm in diameter and 12 cm deep. Traps were sunk into the ground so that the rims of the buckets were level with the soil surface and were filled to about one fifth their volumes with a solution of liquid soap and water to immobilize trapped dung beetles. Dung baits were suspended on u-shaped metal wire, placed over the traps. Trap contents were collected 48 h after the traps had been set and only dung beetles were collected from the traps. Morphospecies were identified and conspecific beetles, collected from the same dung type and area (rural, urban or peri urban), were pooled and stored together in absolute ethanol in labelled jars. The beetles were then positively identified in the laboratory.

Data collection and analysis

A maximum of 20 specimens per species per dung type and locality were dissected. The dung beetles were dissected in distilled water and examined under a stereoscopic microscope for the presence or absence of *S. lupi* larvae (Mönnig 1938). Individual beetles were recorded as being either positive or negative for infection. The data for all the localities in an area were combined for statistical analysis.

The significance in difference of prevalence of infection between areas was tested using the Chi-square test (Fowler, Cohen & Jarvis 1998). The 2x3 contingency table was subdivided (Zar 1984) into three 2x2 contingency tables in a series of multiple comparisons between areas. Yates' corrected Chi-square tests (Fowler *et al*. 1998) were used to test which areas' prevalence of infected beetles occurred at relative frequencies significantly different from those of the others. Furthermore, Fisher exact tests (Zar 1984) were performed for all the 2x2 tables that had more than 20 % of their expected frequencies below five. A sequential Bonferroni correction (Rice 1989) was applied for the multiple comparisons. The prevalence of infected dung beetles in each area was calculated (Rózsa, Reiczigel & Majoros 2000) and reported as a percentage.

RESULTS

The results of the sampling effort that took place during April 2006 were omitted from this study, due to the data being insufficient for statistical analysis. However, a sampling protocol was established for the subsequent sampling that was done during October 2006. In total, 453 specimens belonging to 18 species were collected from the 63 pitfall traps in the three areas during October 2006. The numbers of species that were collected varied among the three areas. Dung beetles, irrespective of species (18) and numbers (447), predominantly preferred pig dung. Only six individuals of three species were collected from pitfall traps baited with dog dung, and no dung beetles were attracted to cattle dung. The rural area, where 11 species were collected, showed the highest species richness, followed by the peri-urban area, where nine species were collected. The urban area, with only six species collected, had the lowest richness.

The prevalence of infection with *S. lupi* larvae in dung beetles varied considerably in the three areas. In the urban area 13.5 % (7/52) of the dung beetles dissected were infected with the nematode and the number of parasite larvae per beetle varied between 1 and 119 (Table 1). Prevalence of infection in the rural area was 2.3 % (3/129) (Table 2), with the number of larvae per beetle ranging from 1 to 10. No dung beetles collected from the peri-urban area were found to be infected with *S. lupi* larvae (Table 3).

The three areas differed significantly from one another with regard to the prevalence of dung beetles infected with *S. lupi* (Chi-square test: $\chi^2 = 16.19$, df = 2; $P < 0.05$) (Table 4).

The prevalence of infected dung beetles differed significantly between the rural and urban areas (Yates' corrected Chi-square test: $\chi^2 = 8.15$, df = 1; $P < 0.05$; Fisher exact test: $\chi^2 = 7.61$, df = 1; $P < 0.05$) (Table 5), as well as between the urban and peri-urban areas (Yates' corrected Chi-square test: $\chi^2 = 9.94$, df = 1; $P < 0.05$; Fisher exact test: $\chi^2 = 9.64$, df = 1; $P < 0.05$) (Table 6). However, there was no significant difference in the prevalence of infected dung beetles between the rural and peri-urban areas (Yates' corrected Chi-square test: $\chi^2 = 2.49$, df = 1; $P < 0.05$; Fisher exact test: $\chi^2 = 1.24$, df = 1; $P < 0.05$) (Table 7). The results remained unchanged after a sequential Bonferroni correction was applied to the multiple comparisons.

All the dung beetles that were infected with this nematode showed a preference for omnivore (pig and dog) dung.

TABLE 1 Results of the dissection of various dung beetle species from an urban area in the Tshwane (Pretoria) Metropole to investigate the incidence of infection with *Spirocerca lupi* under natural conditions

Dung beetle species	Number dissected	Number positive for *S. lupi*	Number of parasite larvae per beetle	
			Range	Average
Gymnopleurus virens	1	0	–	–
Onthophagus ebenus	6	1	9	9.0
Onthophagus pugionatus	40	5	1– 119	37.8
Onthophagus spp. B	3	0	–	–
Onthophagus sugillatus	1	1	105	105.0
Onthophagus vinctus	1	0	–	–

TABLE 2 Results of the dissection of various dung beetle species from a rural area in the Tshwane (Pretoria) Metropole to Investigate the incidence of infection with *Spirocerca lupi* under natural conditions

Dung beetle species	**Number dissected**	**Number positive for *S. lupi***	**Number of parasite larvae per beetle**	
			Range	**Average**
Euonthophagus carbonarius	2	0	–	–
Gymnopleurus virens	6	2	1– 10	6.5
Onthophagus aeruginosis	20	0	–	–
Onthophagus obtusicornis	20	1	9	9.0
Onthophagus pugionatus	21	0	–	–
Onthophagus spp. B	9	0	–	–
Onthophagus spp. nr. *pullus*	1	0	–	–
Onthophagus sugillatus	22	0	–	–
Onthophagus vinctus	2	0	–	–
Sisyphus goryi	20	0	–	–
Tiniocellus spinipes	6	0	–	–

TABLE 3 Results of the dissection of various dung beetle species from a peri-urban area in the Tshwane (Pretoria) Metropole to investigate the incidence of infection with *Spirocerca lupi* under natural conditions

Dung beetle species	**Number dissected**	**Number positive for *S. lupi***	**Number of parasite larvae per beetle**	
			Range	**Average**
Euoniticellus intermedius	3	0	–	–
Liatongus militaris	2	0	–	–
nr. *Sisyphus ruber*	7	0	–	–
Onitis alexis	1	0	–	–
Onthophagus aeruginosis	11	0	–	–
Onthophagus lamelliger	3	0	–	–
Onthophagus spp. B	1	0	–	–
Onthophagus stellio	21	0	–	–
Onthophagus sugillatus	22	0	–	–

TABLE 4 Observed frequencies of uninfected and infected dung beetles from three areas in the Tshwane (Pretoria) Metropole

Beetles	**Area**			**Total**
	Rural	**Urban**	**Peri-urban**	
Uninfected dung beetles	126	45	71	242
Infected dung beetles	3	7	0	10
Total	**129**	**52**	**71**	**252**

Dung beetle—*Spirocerca lupi* associations

The prevalence of canine spirocercosis varies within its geographical range (Mazaki-Tovi *et al*. 2002) and the dung beetle intermediate hosts are widely distributed throughout the distribution area of *S. lupi* (Bailey 1972). This study showed that the prevalence of this parasite in its intermediate dung beetle hosts differs significantly among rural (2.3 %), urban (13.5 %) and peri-urban (0 %) areas in the Tshwane (Pretoria) Metropole.

Parasites are aggregated across their host populations with the majority of them occurring in the minority of their hosts. Moreover, parasite prevalence changes temporally and spatially (Wilson, Bjornstad, Dobson, Merler, Poglayen, Randolph, Read & Skorping 2002). These heterogeneities arise from the variation between individuals in their exposure to parasite infective stages and by differences in their susceptibility once an infective agent has been encountered (Wilson *et al*. 2002). It seems that the

TABLE 5 Observed frequencies of uninfected and infected dung beetles from a rural and an urban area in the Tshwane (Pretoria) Metropole

Beetles	Area		Total
	Rural	Urban	
Uninfected dung beetles	126	45	171
Infected dung beetles	3	7	10
Total	**129**	**52**	**181**

TABLE 6 Observed frequencies of uninfected and infected dung beetles from an urban and a peri-urban area in the Tshwane (Pretoria) Metropole

Beetles	Area		Total
	Urban	Peri-urban	
Uninfected dung beetles	45	71	116
Infected dung beetles	7	0	7
Total	**52**	**71**	**123**

TABLE 7 Observed frequencies of uninfected and infected dung beetles from a rural and a peri-urban area in the Tshwane (Pretoria) Metropole

Beetles	Area		Total
	Rural	Peri-urban	
Uninfected dung beetles	126	71	197
Infected dung beetles	3	0	3
Total	**129**	**71**	**200**

prevalence of spirocercosis is influenced by the proximity of the final host to the intermediate hosts, as well as the density of such infected hosts in the environment where they are preyed upon by the definitive host (Mazaki-Tovi *et al*. 2002). There are also several selective factors that control beetle associations in dung beetle communities (Lumaret, Kadiri & Bertrand 1992). These factors include the nature of the soil substrate (Lumaret *et al*. 1992), fauna and flora of the specific region, rainfall and temperature (Bailey 1972). The widespread use of pesticides in an area might lead to a decrease in the population size of dung beetles, which will lead to a decrease in the prevalence of this parasite in that area (Bailey 1972). Maximum dung beetle activity is correlated with the onset of the rainy season in many parts of the world. During this season there would be optimal opportunity for a final host to ingest infected dung beetles (Brodey, Thompson, Sayer & Eugster 1977).

Conditions for maximum dung beetle activity were suboptimal during October 2006 when sampling took place. Although temperatures were constantly above 25 °C, no rain had yet been recorded for any of the localities in the rural, urban or peri-urban areas. The rural area was devoted to mainly small scale livestock and crop production, but sampling sites were always located in patches of natural vegetation, which might explain why the highest number of species (11 species) was collected in that area. Although the peri-urban area had the second highest number of recorded species (nine species), sites in this area were heavily polluted by rubbish such as plastic bags, broken glass, paper and biological waste material. Furthermore, these sites were mostly ecologically degraded and the vegetation predominantly alien. The fact that the peri-urban sites had the second highest number of species might be attributable to the ever present and seemingly abundant goats and cattle which roam the area. The urban area had the lowest species number (six) of all three areas. Although the majority of gardens in this area are watered throughout the year, they represent a modified environment of which the vegetation is almost exclusively alien. A small patch of natural vegetation was found in only one of the urban sites, where a few ostriches were kept. Pesticides are also often applied to maintain the integrity and aesthetic value of gardens.

The availability of excrement as a food source influences the abundance of dung beetles in a specific area (Bailey 1972), although it seems that food is not an important determinant of local species distributions (Lumaret *et al*. 1992). Dung beetles show preferences for certain dung types (Lumaret *et al*. 1992). This holds important implications for the prevalence of this parasite in dung beetle populations. Dung beetles that are not attracted to the faeces of any of the various definitive hosts might not be good intermediate hosts under natural conditions (Bailey 1972). In this study only omnivore dung-specific dung beetles were found to be parasitized by *S. lupi* larvae. This might be related to the fact that the definitive hosts are mainly domestic dogs and a few other members of the family Canidae. There was a high concentration of domestic dogs in the urban area and the sampling sites in the rural area were all close to pig farms. Furthermore, owners of properties in the rural area often kept more than three dogs. A sufficient explanation cannot be offered for the absence of herbivore dung-specific or generalist dung beetles from the peri-urban area.

The feeding biology of adult dung beetles is not fully understood (Holter, Scholtz & Wardhaugh 2002). Miller (1961) attributed the absence of helminth eggs in the digestive tracts of dung beetles to the masticating action of their mandibles. From his experiments he deduced that the mandibles of dung beetles serve the dual purpose of gathering and masticating faecal material and that helminth eggs are destroyed or damaged in this process. However, this does not sufficiently explain the consistent presence of *S. lupi* parasites in members of certain taxa. In subsequent research Holter *et al*. (2002) and Holter & Scholtz (2005) have shown that dung beetles do not masticate their food prior to ingestion and determined the size of ingested food particles in different species of dung beetles. In these studies it was shown that dung beetles from various taxa and across different body size classes and ecotypes (tunnellers, rollers and endocoprids) ingest food particles that varied from less than 5 µm to more than 83 µm in diameter. Food particles are filtered through filtration channels connected to the molar surface through narrow fissures prior to ingestion (Holter *et al*. 2002). The eggs of *S. lupi* measure 11–15 x 30–37 µm (Mönnig 1938) and this, rather than the masticating action of the mandibles of dung beetles, might explain the absence of parasites in members of certain taxa. Thus, dung beetles that can only ingest food particles that are larger than the eggs, will be able to serve as intermediate hosts of this parasite.

The prevalence of spirocercosis also varies over relatively short periods of time (Bailey 1972). In a study by Chhabra & Singh (1973) it was shown that the prevalence of infection in beetles increased towards the middle of the breeding season of dung beetles infected in the laboratory. In Israel the rate of detection of spirocercosis is significantly higher during the colder months. This might be explained by the seasonality of the main dung beetle intermediate host, *Onthophagus sellatus*, in that country (Mazaki-Tovi *et al*. 2002). Moreover, if any of the beetles dissected during this study contained parasite eggs or first instar larvae they would have been recorded as being negative for infection with this parasite since they are too small to detect under the light microscope that was used in this study. The developmental time from egg to the infective third instar larva is about 2 months (Mazaki-Tovi *et al*. 2002).

There seems to be an increased incidence of clinical spirocercosis among dogs in South Africa in both urban and rural areas, perhaps due to improved techniques for early diagnosis as well as an increased global multidisciplinary interest in this disease and its agents. This study indicated that the area with the least species diversity had the highest prevalence of infection. Thus, the simple classification of areas as rural, urban or peri-urban might not be sufficient and we want to investigate whether there may be a correlation between human population density and the prevalence of infection among dung beetle populations from corresponding areas. Therefore, we will determine and compare the prevalence of infection in dung beetles in a specific number of areas of high and low human population densities in two geographical regions [Tshwane (Pretoria) Metropole and Grahamstown]. These regions have been selected on the basis of being focal areas of high infection with *S. lupi* in dogs.

It is not known exactly which or how many species of dung beetles transmit this parasite or what the effect of dung preference on susceptible and non-susceptible dung beetle species is. Thus, we want to determine which species of dung beetles in each geographical range are susceptible to infection under natural conditions. Moreover, we want to determine whether dung beetle species that are not attracted to the faeces of the definitive host and that may not be good intermediate hosts under natural conditions, are capable of being good intermediate hosts under experimental conditions.

CONCLUSIONS

There is an urgent need for better control and preventative measures to be investigated for this disease in dogs. A better understanding of the dynamics of the intermediate host-parasite associations between dung beetles and *S. lupi* under South African conditions may contribute to establishing preventative measures for the spread of this disease as well as finding more effective treatment for spirocercosis in domestic dogs. Furthermore, it might aid in identifying objectives and priorities for management for those with a technical interest in the problem, as well as those who might be affected emotionally and economically.

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