CHAPTER THREE

ROLE OF DUNG BEETLE FEEDING MECHANISMS IN LIMITING THEIR SUITABILITY AS HOSTS FOR THE NEMATODE *Spirocerca lupi*

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Introduction

The feeding mechanisms of coprophagous dung beetles (Scarabaeidae: Scarabaeinae) may make them efficient vectors for the transmission of *Spirocerca lupi* (Nematoda: Spiruromorpha: Spirocercidae) to dogs (Canidae). This is somewhat paradoxical, since adult and larval scarabaeine dung beetles mediate several essential ecological processes, including parasite suppression in the environment, through their relocation and consumption of both animal and human faeces (Nichols *et al*. 2008). Furthermore, the feeding biology of adult dung beetles is not yet fully understood, despite extensive research on their behaviour, and practical applications such as the biological control of dung and dung-breeding parasites (Holter 2000). Scarabaeid dung beetles have highly specialised mouthparts (Hata & Edmonds 1983; Holter 2000) which are adapted to restrict food ingestion to minute particles, ranging from 2 – 150 µm (Holter & Scholtz 2007), suspended in the dung. Based mainly on mouthpart morphology, Miller (1961) and Hata & Edmonds (1983) assumed that dung beetles grind their food prior to ingestion. However, subsequent studies by Holter (2000) and Holter *et al*. (2002) have shown that dung beetles *do not* masticate their food, but rather that larger indigestible fragments are avoided by filtration, thus resulting in only very small particles being ingested. This filtration may limit the ability of coprophagous Scarabaeines to ingest parasite eggs and hence determine their suitability as hosts/ vectors for *S*. *lupi*.

The aim of the present study was to determine the size of ingested food particles in various species of dung beetles, sampled from study sites in two geographical regions of South Africa. Specific objectives of this paper are: (1) to exclude certain dung beetle species as possible intermediate hosts of *S*. *lupi* based on the size of ingested food particles; and (2) to identify future research objectives that will lead to a better understanding of the dynamics of the intermediate host-parasite associations between dung beetles and *S*. *lupi* in dynamic urban landscapes.

Material and methods

Sampling methods and experimental animals

Dung beetles were collected at two sites in different geographical regions of South Africa: Pretoria Metropole (Gauteng) (25°43' S, 28°11' E) and Grahamstown (Eastern Cape) (33°18’ S, 26°32’ E). These sites were found to be focal areas of high incidence of spirocercosis in domestic dogs by the Department of Veterinary Tropical Diseases, University of Pretoria and ClinVet International Research Organisation. Trapping was conducted during November 2008, and February and April 2009, in 10 localities in each of the two collecting sites.

As a surrogate for dog dung, pig dung-baited pitfall traps were used for sampling dung beetles. In each locality, five pitfall traps were set at 10 m intervals along a single transect line. The plastic buckets used as traps had a 1000 m*l* capacity (11 cm in

diameter and 12 cm deep) and were sunk into the ground so that the rims of the buckets were level with the soil surface. They were filled to about one-third their volumes with damp soil, which served as a refuge for the live dung beetles used in this study. A plastic funnel was placed inside each bucket for channelling dung beetles into the pitfall trap and preventing their escape. Chiffon-wrapped dung baits were suspended on u-shaped metal wire supports, which were placed over the buckets. Traps were covered with lids supported on wire legs to avert flooding of the buckets by rain, and shield live specimens from direct sunlight. Trap contents were collected 48 h after the traps had been set and only dung beetles were collected from the buckets. Dung beetles were positively identified to species level in the laboratory. All conspecific specimens from each geographical region were housed together in plastic buckets filled with damp soil, and kept at a constant ambient temperature (30°C) and relative humidity (80%).

Measuring the ratios of ingested particles

The experimental design of this study was identical to the procedure described by Holter (2000), Holter *et al*. (2002) and Holter & Scholtz (2005). Latex beads (Coulter®, Miami, Florida) of two known diameters were mixed into fresh cattle dung and presented to dung beetles that had been starved for three days. Combinations of beads with diameters 5/ 10, 5/ 14, and 10/ 20 µm were used in these experiments. Relative numbers of the two bead sizes in the feeding mixture, which was offered to dung beetles, were determined by microscopical counts of three sub-samples of each feeding mixture. Amalgamation of the beads with the feeding mixture was considered sufficient

when the three small sub-samples were homogenous ($P > 0.05$; 2 x 3 χ^2) in terms of the relative abundance of the two particle sizes.

Small portions (about 5g) of each of the three available combinations of feeding mixture were transferred into vials and a single dung beetle placed in each. Beetles were allowed to feed for 45 minutes in darkness, whereafter they were instantaneously killed by dropping them into boiling water. A sample of the midgut content was removed by dissection, mixed with glycerol and water (1:1) on a microscope slide and covered with a coverslip. Beads in these samples were counted using a microscope and samples with less than 50 particles of the most abundant size category were omitted from the results. Counts from as many beetles as were available from the collection sites were determined for each combination of species and bead sizes. All assays were conducted in the Department of Zoology and Entomology, University of Pretoria.

Bead numbers in the gut samples are often highly variable (even between similar-sized conspecifics) and to make counts comparable they had to be standardised. For any combination of bead sizes, the probability, β (%), that the larger beads in the feeding mixture would pass through the mouthpart filter and be ingested, was calculated. This was based on the assumption that the smaller beads in the same combination in the feeding mixture would have a 100% probability of ingestion. β = 100% suggests uninhibited ingestion of both particle sizes, while β < 100% implies that the mouthpart filter discriminated against the larger beads compared with the smaller ones. Thus, β is

the percentage chance of a larger bead passing the mouthpart filter and being ingested, assuming free passage for the smaller beads. Holter (2000) and Holter & Scholtz (2005) described these calculations for the standardisation of counts.

The maximum diameter of ingested particles, for a specific species, is defined as the diameter of a particle that has a 5% chance of passing the mouthpart filter and being ingested (Holter 2000; Holter *et al*. 2002). A decision whether a particular species can or cannot serve as a vector of *S*. *lupi*, was based on the mean β-values of feeding mixtures. This can be illustrated using the following example. In *Onthophagus asperilus* (Table 2), the mean β-value of 4.8% (three replicates) for the 5/ 10 µm combination indicated that 10 µm is near the maximum size of particles ingested by this species. This is supported by the fact that neither 14 µm nor 20 µm beads were ingested in any replicate for the 5/ 14 µm (six replicates) and 10/ 20 µm (six replicates) combinations.

Results

Table 1 summarises information on the collection sites and biology of the dung beetle species used in this study. Sizes of ingested particles' mean β-values are presented in Table 2 for all species tested. Information on the ranges of β-values and number of replicates for all species and combinations of bead diameters is also included in this Table. A mean β-value of 0 indicates that the large bead size in the feeding mixture was completely discriminated against by the mouthparts and was absent from the midgut

sample. An absence of data for mean β-values and β-value ranges indicates that the beetles used in those specific feeding trials did not feed during the experiment.

Only one species, *Onthophagus fritschi* can be excluded as a possible intermediate host of *S*. *lupi*, while one other species, *O*. *asperilus*, is a poorly suitable intermediate host for this nematode under natural conditions, based on the mean β-values for the 5/ 14 µm and 10/ 20 µm feeding mixtures (Table 2). *O*. *fritschi* restricts its ingestion of food particles to those below 5 µm, while *O*. *asperilus* rarely ingest particles larger than 10 µm. No conclusion can be reached about the potential intermediate host-status of *Onthophagus deterrens* for this nematode, due to a lack of data from these feeding experiments (Table 2). All the other species could serve as potential intermediate hosts of *S. lupi* under natural conditions (Table 2).

Table 1. Tribe and species names, and collection sites, of the dung beetle species used in the feeding experiments.

Tribe	Species	Collection site
Canthonini	*Epirinus* sp.	Grahamstown
Coprini	*Catharsius vitulus*	Grahamstown
Dichotomiini	*Sarophorus striatus*	Grahamstown
Onitini	*Onitis pecaurius*	Grahamstown
Onthophagini	*Euonthophagus carbonarius*	Pretoria
	Onthophagus asperilus	Grahamstown
	O. binodis	Grahamstown
	O. deterrens	Grahamstown
	O. fritschi	Grahamstown
	O. lugubris	Grahamstown
	O. pilosus	Grahamstown
	O. pugionatus	Pretoria
	O. vinctus	Pretoria
Sisyphini	nr. *Sisyphus rubrus*	Grahamstown, Pretoria

Table 2. Mean β-values, β-value ranges, and number of replicates for tunnelers and rollers used in the feeding experiments for three feeding mixtures with different bead size combinations. A mean β-value of 0 indicates that one, or both of the two bead sizes in any of the three feeding mixtures were completely discriminated against by the mouthparts and were absent from the midgut sample. An absence of data for mean β-values and β-value ranges indicates that the beetles used in those specific feeding trials did not feed during the experiment.

Species	**Measurements**								
	Diameter (µm) small: large latex beads in food: **5/10**			Diameter (µm) small: large latex beads in food: **5/14**			Diameter (µm) small: large latex beads in food: **10/20**		
	$\bar{x}$ β-value	Range β-values	Number replicates	$\bar{x}$ β-value	Range β-values	Number replicates	$\bar{x}$ β-value	Range β-values	Number replicates
Sarophorus striatus	-	-	4	6.4	0 – 7.7	7	0	-	4
Catharsius vitulus	79.9	59.9 – 100	5	-	-	4	-	-	5
Onitis pecuarius	-	-	5	-	-	3	40.3	20.9 – 69.4	3
Onthophagus asperilus	4.8	3.8 – 6.0	3	0	-	6	0	-	6
O. binodus	95.3	92.0 – 98.0	6	2.0	0 – 2.0	2	-	-	4
O. deterrens	0	-	5	-	-	3	-	-	4

O. fritschi	0	-	5	0	-	3	0	-	5
O. lugubris	39.0	15.8 – 59.4	11	20.3	0 – 54.6	9	0.4	0 – 2.4	7
O. vinctus	-	-	5	9.8	1.5 – 14.5	6	1.4	0.5 – 2.5	6
Euonthophagus carbonarius	0.7	0.4 – 0.9	6	9.1	5.0 – 13.0	6	-	-	8
Epirinus sp.	-	-	5	100	-	5	-	-	5
nr. *Sisyphus rubrus*	0	-	3	13.3	0 – 53.0	4	0	-	4

3.4 Discussion

This study has shown that the majority (11/ 14) of dung beetle species that were used in these feeding experiments could serve as intermediate hosts of *Spirocerca lupi* because their mouthparts allow the passage of food particles larger than the minimum size range of the eggs of this parasite. *S. lupi* eggs measure 11 – 15 x 30 – 37 µm (Mönnig 1938), thus dung beetles that only ingest food particles smaller than the eggs, cannot serve as intermediate hosts of the nematode. This, rather than the masticating action of the mandibles of dung beetles (Miller 1961; Hata & Edmonds 1983), might explain the absence of parasites in certain species, such as *Onthophagus asperilus* and *O. fritschi*. It is assumed that the mouthpart filters of these dung beetles would discriminate against *S. lupi* eggs, suspended in a dung pat, because both species ingest particles smaller than the lower size limit (11 µm) of the eggs. *S. lupi* third stage larvae (L3) were recorded in five dung beetle species in a previous study on the prevalence of this nematode in populations of its intermediate dung beetle host in the Pretoria Metropole (du Toit *et al*. 2008). Two of these species, *Onthophagus pugionatus* and *Gymnopleurus virens*, were included in the feeding trials of this study. *G. virens* did not feed during the experiments and those data were omitted from Table 2. However, the estimated maximum size of ingested particles in this species is between 10 and 16 µm (Holter & Scholtz 2005). The others were excluded because they were absent from the pitfall traps when the collection of specimens took place. Although the maximum diameter of particles ingested by *O. pugionatus* is close to the lower limit of *S. lupi* egg size, this beetle had shown, during an earlier study, a prevalence of infection of 12.5 % in certain of its urban populations (du Toit *et al*. 2008). *G. virens* has a similar upper limit for the

size of its ingested food particles. However, this species showed a prevalence of infection with the L3 of *S*. *lupi* of 29 % in its urban populations during the same study (du Toit *et al*. 2008). The fresh body weight of adult dung beetles does not seem to be a good criterion for the exclusion of scarabaeine species as potential intermediate hosts for *S*. *lupi*. Presently, there is no evidence of significant, intraspecific correlations between dung beetle body weight and maximum ingested particle size (Holter *et al*. 2002).

The availability of excrement as a food source influences the abundance of dung beetles in a specific area (Bailey 1972). Some dung beetles show preferences for certain dung types (Lumaret *et al*. 1992) and with the exception of a few generalist species, usually avoid carnivore faeces (Carpaneto *et al*. 2005), although there is little data on the exploitation of dog faeces as a resource. This holds important implications for the prevalence of *S. lupi* 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). However, a reduction of green areas and parks within city boundaries and an increase in the density of dogs, lead to higher numbers of these animals (feral, vagrant or pets) frequenting such open spaces. The faeces of these dogs become an abundant resource in these areas and could provide temporary refuge to species that would otherwise encounter local extinction in the urban environment (Carpaneto *et al*. 2005).

The prevalence of canine spirocercosis is influenced by the proximity of the final host to the intermediate hosts in the environment where they feed (Mazaki-Tovi *et al*. 2002). Urbanisation may indirectly lead to increased transmission rates of *S*. *lupi* to dogs in urban environments, due to the higher contact rates between them and dung beetles, and dogs (which is mediated through the coprophagous behaviour observed in the domestic dog).

Future research should be directed at determining specific dung preferences for the species of dung beetles that are encountered in urban areas. This would help create a better understanding of the factors that influence the prevalence of *S*. *lupi* in populations of its intermediate dung beetle host, and ultimately a better approach at implementing management objectives for spirocercosis among dogs. There is an urgent need for better control of dog faeces by humans in urban environments, such as the provision of disposable bags in recreational parks, a culture that is lacking in South Africa, but that will surely contribute to a decreased probability for dogs to contract this fatal parasitic infection.