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Dynamics of the association between dung beetles (Coleoptera: Scarabaeidae) and the dog parasite Spirocerca lupi (Nematoda: Spiruromorpha: Spirocercidae)

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by

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This work is dedicated to my parents, Louw and Henriëtte du Toit

DECLARATION

I, Cornelius Andries du Toit declare that the thesis, which I hereby submit for the degree Philosophae Doctor in Entomology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution

SIGNATURE________________________

DATE_____________________

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Abstract

Spirocercosis is a canine disease caused by the nematode parasite *Spirocerca lupi* (Rudolphi, 1809) (Spirurida: Spirocercidae) and is a potentially fatal condition in domestic dogs (Canis familiaris). The larval life cycle of this parasite involves intermediate and paratenic (transport) hosts. Various species of coprophagous dung beetles (Scarabaeidae: Scarabaeinae) serve as the principle intermediate hosts. Despite extraordinary advances in biomedical research, it is unlikely that these alone will alleviate the burden of this parasitic disease in dogs.

Recently, there has been growing concern over the upsurge in incidence and reported cases of spirocercosis in domestic dogs in South Africa. There is a plethora of literature on the clinical, diagnostic and epidemiological aspects of this disease in dogs, yet no study has aimed at fully understanding the dynamic interactions between the various hosts and *S. lupi*, governed by the consequences of their behaviour under different and ever-changing environmental conditions. It is most likely that the impact of this disease is accentuated by constant changes in human demographics and behaviour.

Studies on spirocercosis in dogs have considered the consumption of the various paratenic hosts or the deliberate ingestion of dung beetles to be the main cause of

the transmission of *S*. *lupi* to dogs. However this study suggests that the coprohagous behaviour of dogs and the subsequent accidental ingestion of coprophagous dung beetles in or on faeces are mainly responsible for the transmission of this parasitic nematode to dogs. Changes in urban land use and subsequent changes along urban-rural gradients influence the nature of biological interactions partly due to changes in species assemblage structure and composition. Such alterations in assemblage structure of species pose a particular risk to altered rates of parasitism and disease transmission.

It is concluded that these changes in landscape use coupled to altered dung beetle species assemblage structure have influenced the pattern of events observed in this host – parasite relationship. Furthermore, the social organization of domestic dogs (pets versus feral animals) and the availability of exposed excrement as a direct or indirect consequence of human behaviour played a pivotal role in the rate these parasites are transmitted to dogs.

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CHAPTER ONE

"It may well be that it would take hundreds of generations for the progenitive instinct to develop in this way, but if it should do so, nature would have taken her revenge, and the variety *Homo contracipiens* would become extinct and would be replaced by the variety *Homo progenitivus*"

Charles Darwin

GENERAL INTRODUCTION

Parasitic diseases have shaped the course of human history and present a continual threat to the wellbeing of millions of people and domesticated animals across the world. They have for centuries ranked with war and disasters as the leading causes of death and disability and still present major challenges to human progress and survival (Morens *et al*. 2004). Parasitic infections can't be controlled or eradicated simply by the application of drugs or anti-vector measures after a parasite had been identified, its life cycle been demonstrated, and it been implicated as the causative agent of disease (Cox 1993). Careful consideration of recurrent lessons from history justifies the urgency of an understanding of the dynamic interactions between parasites, their hosts, and the environment. Emerging infectious diseases and the diversity and adaptability of pathogenic organisms are governed by evolutionary vigour, and associated with a range of underlying causal factors (Daszak *et al*. 2000; Morens *et al*. 2004). Most notably, these include a change in the ecology of the host, the parasite, or both, which is driven by human population expansion due to increasing population density and urbanisation, and expansion into previously natural habitats (Daszak *et al*. 2000). Thus, a variety of societal and cultural factors may contribute to increased host exposure or susceptibility to parasites. These factors induce changes that enhance the spread and transmission of parasitic diseases (Thompson 2001).

Background to spirocercosis

Spirocercosis is a canine disease caused by the nematode parasite *Spirocerca lupi* (Rudolphi, 1809) (Spirurida: Spirocercidae) (Van der Merwe *et al*. 2008) and is a potentially fatal condition in domestic dogs (*Canis familiaris*). This is a cosmopolitan parasite, though it is more commonly found in the warmer tropical and subtropical regions of the world (Bailey 1972). *S*. *lupi* parasitizes mainly domestic dogs but natural infections have been reported in other members of the family Canidae which serve as important reservoir hosts (Bailey 1972; Mazaki-Tovi *et al*. 2002). The larval life cycle of this parasite involves intermediate and paratenic (transport) hosts (Van der Merwe *et al*. 2008). Various species of coprophagous dung beetles (Scarabaeidae: Scarabaeinae) serve as the principle intermediate hosts after ingesting the embryonated eggs of *S*. *lupi* in the faeces of the definitive host (Bailey *et al*. 1963; Chhabra & Singh 1972; Chowdhury & Pande 1969). The nematode larvae encyst within the tissues of the dung beetle and reach communicability (Larval stage 3) within two months (Bailey 1972). Vertebrate paratenic hosts, including wild and domestic fowl, lizards, and certain mammals, may become infected only after ingestion of a suitable infective coprophagous dung beetle (Bailey 1972). Final hosts become infected after ingestion of either an infected scarabaeine or paratenic host (Bailey 1972; Van der Merwe *et al*. 2008).

Data on the prevalence of this disease in dogs is often cumbersome to interpret and can be misleading due a variety of factors. Limitations in diagnostic techniques employed by clinicians can lead to inaccuracies in the establishment of prevalence, such as false negative results given by faecal flotation tests, undetected variation in egg shedding by female worms, and challenges presented to clinical methods such as endoscopy and radiography (Dvir *et al*. 2010; Fisher *et al*. 2009; Van der Merwe *et al*. 2008). Furthermore, prevalence data on spirocercosis varies considerably between regions (countries), rural and urban areas, changes over time (seasonal), and must be interpreted in terms of the dog population sampled (feral dogs versus pets) (Van der Merwe *et al*. 2008). Differences in the prevalence of *S*. *lupi* have also been identified in pet dogs that show different behaviour in life and hunting styles (Mylonakis *et al*. 2001). Discovery of infection with *S*. *lupi* is often “coincidental”, during routine examinations for unrelated conditions or necropsies (Fisher et al. 2009; Van der Merwe et al. 2008). Moreover, a lack of awareness by the general public about spirocercosis, perhaps due to the large suite of clinical manifestations in dogs, and the scarcity of information in popular literature about the conditions that can lead to infection, may also contribute to underreporting of this parasitic infection in domestic dogs.

These factors have been clearly demonstrated in a study by Fisher *et al*. (2009) where they found a prevalence of 24% in apparently healthy dogs in St. Kitts, West Indies, brought to their clinic for neutering. This was found to be comparable with previous reports of infection with *S*. *lupi* in 22% of dogs in Kenya (Kagira & Kanyari 2000) and 28% of dogs in South Africa (based on surveys completed by privately practicing veterinarians) (Lobetti 2000). In Kenya, prevalence based on necropsy results was as high as 78% (85% in stray dogs and 38% in companion animals) (Brodey *et al*. 1977) while Minnaar & Krecek (2001) and Minnaar *et al*. (2002) reported infections of 14% and 13% in dogs belonging to people in two resource-limited communities in the provinces of Gauteng and the Free State, South Africa, respectively.

Treatment of spirocercosis

To date, the effectiveness of treatment with drugs or surgery and chemotherapy has been met with mixed success. A number of drugs have been used for the treatment and or prophylaxis of spirocercosis, but thus far none have been successful in killing both the adult and juvenile stages of this nematode without showing host side-effects (Van der Merwe et al. 2008). Doramectin, a cattle anthelmintic, and ivermectin were used as conventional treatment and have proved to be effective under clinical conditions over the last decade, although breed specific toxicity have been reported in in collies and other herding dog breeds following administration of both these treatments (Van der Merwe *et al*. 2008). Recently, Kok *et al*. (2011) tested the efficacy of Milbemycin oxime under clinical conditions against pre-adult *S. lupi*, which prevented the establishment and encapsulation of *S. lupi* in the oesophagus of experimentally infected dogs. Their results showed promising potential for the development of a prophylactic and therapeutic anthelmintic. Surgical removal of oesophageal sarcomas and surgery of the spinal cord to remove worms and the surrounding damaged tissue have been relatively unsuccessful up to date due to high post-surgical mortality rates (Dvir *et al*. 2010; Van der Merwe *et al*. 2008).

Despite extraordinary advances in biomedical research, it is unlikely that these alone will alleviate the burden of parasitic diseases. In fact, drug resistance in parasites will, in all likelihood, become more widespread due to an increase in the use thereof (Thompson 2001). Instead, an understanding of how human behaviour is influencing parasite transmission patterns, often sustaining such infection cycles, is essential if there is any hope in keeping emerging diseases in check. Spirocercosis is an emerging disease in several parts of the world (e.g. in Tel Aviv, Israel, where a sevenfold increase in incidence was reported in nine years (Van der Merwe et al. 2008)). Recently, there has also been growing concern over the upsurge in incidence and reported cases of spirocercosis in domestic dogs in South Africa. There is a plethora of literature on the clinical, diagnostic and epidemiological aspects of this disease in dogs, yet no study has aimed at fully understanding the dynamic interactions between the various hosts and *S. lupi*, governed by the consequences of their behaviour under different and ever-changing environmental conditions. It is most likely that the impact of this disease is accentuated by constant changes in human demographics and behaviour.

Interactions between the hosts, the parasite, and their environment

Humans and dogs share an ancient association that span nearly 14 000 years (Archer 1997; Daniels & Bekoff 1989). Viewed from an evolutionary perspective they resemble social parasites, manipulating human responses to ensure relationships for the procurement of resources. However, this statement would probably be refuted by most dog owners who would argue for the source of security and sense of wellbeing that they convey (Archer 1997). Domestic dogs are considered to be the most abundant extant terrestrial carnivore, and are found in every habitat that humans occupy (Daniels & Bekoff 1989). They show great behavioural and ecological plasticity: their social organisation being a response to the quantity and spatial distribution of food resources, and the strategies they have to employ to acquire it (Daniels & Bekoff 1989). This would account for the differences in social organisation and feeding behaviour encountered between dogs that are kept as companion animals and those that are free-ranging (feral).

Although dogs belong to the order Carnivora they are actually omnivores, because of their differences in food preferences and food selection behaviour to most members of the order, which they inherited from their ancestral association with humans (Bradshaw 2006). Furthermore, dogs are also known to be coprophagous, although the reasons for this practice have received little scientific attention (Soave & Brand 1991). The degree to which they are coprophagous should be similar in companion animals and feral dogs, although one would expect feral dogs to exhibit an increased frequency of coprophagy due to the factors that characterise their social organisation. Studies on spirocercosis in dogs have considered the consumption of the various paratenic hosts or the deliberate ingestion of dung beetles to be the main cause of the transmission of *S*. *lupi* to dogs. However this study suggests that the coprohagous behaviour of dogs and the subsequent accidental ingestion of coprophagous dung beetles in or on faeces are mainly responsible for the transmission of this parasitic nematode to dogs.

Urbanisation is increasing globally and more than half the world's population currently resides in cities and towns (Evans *et al*. 2009). In South Africa, about 17 million people (35% of the total population) live in informal settlements in urban and peri-urban areas. These are mostly very poor or low-income communities without access to any formal hygienic facilities such as sanitation. A further 20 million people are without on-site waterborne sanitation (Carden *et al*. 2008). Coupled to the enormous health risks posed by such absent, dysfunctional or inadequate sewage removal systems, is the accumulation of exposed human faeces. Moreover, dogs often become the most common large mammal in urban environments where a change in land use has led to the reduction or complete absence of grazing herbivores (Carpaneto *et al*. 2005) (e.g. where small holdings are converted into housing complexes). Pet owners are often reluctant to clean up after their dogs where they have defecated in their gardens or public areas, a habit that leads to the accumulation of dog faeces in public open spaces (e.g. parks) and on streets.

Dung beetles are mostly coprophagous and mediate several essential ecosystem services, such as the suppression of dung-dispersed nematodes and protozoa in the environment by removal of dung for feeding and breeding purposes (Nichols et al. 2008). They are known to colonise human faeces (Fincher et al. 1970) and dog dung (Wallace & Richardson 2005). Larger amounts of exposed faeces may lead to an increase in the abundance of certain species of coprophagous scarabaeines that are suitable intermediate hosts of *S*. *lupi*. Dogs do not seem to show any discrimination in the choice of dung they consume, and were observed on several occasions during this study to consume the dung of both humans and other dogs (Du Toit pers. obs.). The prevalence of infection with *S*. *lupi* in dung beetles and the subsequent infection of dogs with this nematode in any particular area depend in part on the abundance of susceptible beetles in that area and the contact rate between them and dogs (Bailey 1972). Changes in urban land use and subsequent changes along urban-rural gradients influence the nature of biological interactions partly due to changes in species assemblage structure and composition (Carpaneto *et al*. 2005; Evans *et al*. 2009). Such alterations in assemblage structure of species pose a particular risk to altered rates of parasitism and disease transmission (Evans *et al*. 2009). Higher rates of parasitism and disease are consistent in urban wildlife populations, in comparison to such populations in rural areas (Evans *et al*. 2009).

Rationale for the study

This study represents the first investigation into the dynamic interactions between *S. lupi* and its dung beetle intermediate hosts under natural conditions anywhere in the world. Although it was conducted under South African circumstances, it generated a novel understanding of the processes that govern the interactions between this parasitic nematode, the intermediate hosts, the definitive host, and humans, and should be representative for any region of the world where this disease is endemic. Furthermore, it is unique in that all previous studies on the intermediate dung beetle hosts were conducted in the laboratory or under experimental conditions.

There exists a real opportunity to prevent spirocercosis in dogs before it needs to be controlled or cured by veterinary intervention. But, who is responsible for preventing, or at least, managing an increase in the transmission rate of this disease to dogs?

The notion of treating spirocercosis in dogs with drugs or by means of surgery and chemotherapy is a member of the “no technical solution” problems (Hardin 1968). While progress in biomedical research is desirable and necessary, veterinary treatment of animals infected with this disease alone will not prevent this condition in uninfected dogs. People who are concerned about this emerging disease in dogs (with different perspectives on the problem ranging from personal to professional) are exploring new avenues of reducing infection in dogs without acknowledging (knowingly or unknowingly) the origin of the problem. This problem cannot be solved in a technical way.

In order to address this issue there must be a realisation of the actual problem, which is one of acknowledgement of personal responsibility. Such a responsibility would naturally extend to include a responsibility towards other humans in terms of their emotional well-being concerning the health of their dogs; their sense of pride and self-confidence with regards to having access to basic amenities of society; and their sense of community by cooperating with others in sustaining an agreeable way of life (living in an aesthetically appealing place). By adhering to this sense of self-responsibility, one is not necessarily conforming to altruism (for it is not truly attainable in our species), but rather ensuring self-preservation in terms of a preferred way of life.

All people want the maximum good, but as asked by Hardin (1968), what is this good? Perhaps it is survival. In order to attain an increased probability of survival (for dogs), there needs to be a common interest in managing the spread of this disease by decreasing the amount of exposed human and dog faeces. In this lies the “tragedy of the commons”. Rational man will find that his individual effort in fouling the commons (whether by him directly or indirectly, i. e. the dog) is less than the effort of cleaning the waste that goes into it. This is true for everyone and in a sense “we are fouling our own nest” as long as we act individually as rational free-riders (Hardin 1968). The problem of fouling is a consequence of population density:

as a population becomes denser, biological recycling of such waste becomes overburdened (Hardin 1968). As a function of population density, the problem of fouling discloses a principle of morality: “the morality of an act is a function of the state of the system at the time it is performed” (Hardin 1968). It calls for a redefinition of property rights and the betterment of basic human and societal needs.

Public open space, be it parks, streets or undeveloped urban land, is open to all. It is to be expected that dog owners would use this commons, to the mutual benefit of owner and dog, as often as possible. Crowding of the commons by dogs would not be an obvious problem, as long as the density of dogs is below a certain maximum. Such an arrangement will work reasonably well until that maximum capacity is reached. Being rational, every dog owner seeks to maximise his gain. This is the conclusion reached by every dog owner and therein is the tragedy. Of course, there are expected differences between a commons in an established neighbourhood and that in an informal settlement. All land is common in the “township” and there is no hindrance to dogs that frequent this commons. When the dogs are feral, there is no control over crowding. Privately owned land does not constitute a commons although the attitude of the owner with regards to crowding and fowling of his private property will contribute to the tragedy (dung beetles can fly some distance to a food resource and carry the parasite with them).

What will guide people’s behaviour in terms of cleaning-up after their dogs or themselves? Formal institution’s role in managing this problem is limited. It is not feasible to fence open spaces in informal settlements or impose fines on using this commons as latrines. A scenario more likely to succeed is to provide sanitation facilities to those who do not have such amenities (which would also contribute to uplifting basic human dignity) and to control the number of feral animals in these areas. In established public spaces a fine-system is more probable to aid in accomplishing this goal, although provision should be made for dog owners to have the opportunity to dispose of their pets' faeces (by e. g. providing plastic bags and bins). Here, formal institution can play a role by guiding land use (perhaps also by providing incentives for people to adhere to these principles). Unfortunately, formal institution will have little effect in privately owned property.

In the end it is the informal institution of moral values that will have the most effective solution to preventing the spread of this disease (and simultaneously contribute to community upliftment). Free-riders who refuse to conform to these values (either by not removing their dogs' faeces from public or private spaces, or by not disposing of their own (e.g. by burying it)) will ultimately be accountable for increased transmission and incidence of spirocercosis in dogs.

Aims of this research

This study aimed to investigate the dynamic associations between the parasitic dog nematode *Spirocerca lupi* and its intermediate dung beetle hosts. From these data generalities are explored by drawing conclusions about the interactions between this parasite and its intermediate hosts under natural conditions.

Chapter 2 investigates the prevalence of *S*. *lupi* in populations of its intermediate dung beetle hosts under natural conditions in two geographical regions of South Africa (Grahamstown in the Eastern Cape Province, and Pretoria in Gauteng). Specific objectives of this chapter are (1) to determine which species of dung beetles are hosts under natural conditions; and (2) to establish the proportion of infection with *S*. *lupi* in the samples of species collected from their populations in these geographical regions. The role of dung beetle feeding mechanisms in limiting their suitability as hosts for *S*. *lupi* is discussed in Chapter 3. A specific objective of this chapter is to exclude certain dung beetles as possible intermediate hosts of this nematode based on the size of ingested food particles. Chapter 4 investigates the effects of trophic preference and urbanization on dung beetle assemblage structure and transmission of *S*. *lupi* to dogs. Objectives of this chapter are to demonstrate the effect of location and food preference on dung beetle assemblage structure, and the effect those may have on infestation rates; and to indicate consequences of the effect of a larger suite of hosts on possible increased transmission rates of *S*. *lupi* to dogs. Chapter 5 serves as a conclusion to the study.

CHAPTER TWO

PREVALENCE OF *Spirocerca lupi* IN POPULATIONS OF ITS INTERMEDIATE DUNG BEETLE HOSTS IN TWO GEOGRAPHICAL REGIONS OF SOUTH AFRICA

2.1 General introduction

Defining the host and parasite population is important for studying host-parasite interactions and disease epidemiology (Cox 1993). A population is an assemblage of organisms belonging to the same species that occupy the same place at a specifically defined point in space and time (Cox 1993). Parasites are aggregated across their host populations with the majority of them occurring in the minority of their hosts (Wilson 2002). Host populations should be viewed as dynamic variables, which will lead to a more comprehensive understanding of the biology of infectious diseases (Anderson & May 1979).

Prevalence is defined as the proportion of host individuals (from a specific population) in a sample that are infected by a particular parasite, although the actual prevalence of infection is usually not known because the number of hosts sampled is generally lower than the total population size of that host (Jovani & Tella 2006). Often, prevalence of infection with a parasite is negatively correlated with sample size: the larger the sample of hosts investigated, the smaller the number of individuals in such a host population found to harbour a particular parasite (Gregory & Blackburn 1991). For every sample size, there are clearly defined upper and lower boundaries of prevalence (Gregory & Blackburn 1991). Reasons for the negative association between prevalence and host sample size are open to debate (it could have a biological basis or be artificial). However, these interspecific negative correlations are usually attributed to a couple of biases in the data set: the exclusion of zero prevalence from comparative data and prevalence having a lower boundary (by excluding zeros) that is not independent of sample size (Gregory & Blackburn 1991).

Cyclic prevalence is driven by environmental factors or result from processes that are fundamental to a specific host-parasite system (Lass & Ebert 2006). Environmental factors include climatic conditions, food availability, and host behaviour in response to these, while intrinsic factors may arise from dynamic feedback between host and parasite populations and include host immunity and methods of parasite transmission (Lass & Ebert 2006). Prevalence regularly varies on a seasonal basis, and is often caused by the effect of temperature and precipitation. Host size, population density, and nutritional status underlie seasonal variation in prevalence and could be responsible for driving prevalence dynamics (Cox 1993; Lass & Ebert 2006).

Sampling efficiency is vital for several reasons. Although there is no single method that could be employed to sample all taxa, the use of pitfall traps for surveying surface-active invertebrates is usually a widely used method (Ward *et al*. 2001). However, there are a number of factors that produce biases in pitfall catches that could affect the number of taxa caught and their abundance (Ward *et al*. 2001). There is a potential to introduce confounding effects between treatments in a study that rely exclusively on this method (Ward *et al*. 2001).

Two separate studies were conducted to determine the prevalence of infection with the larvae of *S*. *lupi* in populations of its intermediate dung beetle hosts, in two geographical regions of South Africa. The first was conducted in the Pretoria Metropole (Gauteng) as a pilot study to investigate the prevalence of this nematode in dung beetle populations. The second study was carried out in Grahamstown in the Eastern Cape Province. These studies were executed in different ways to find the most effective manner to establish the prevalence of infection in dung beetle intermediate host populations.

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

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The Pretoria study was published in the Onderstepoort Journal of Veterinary Research **75:** 315-321 (2008). The format of the journal article was adapted to suit the style of this thesis.

2.2.1 Methods and Materials

Description of the study area

A study was conducted in 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-urban areas. The prevalence of infection with this parasite was also compared between dung specific and non-specific dung beetle species from the same communities. The study was conducted north of the Magaliesberg range (25° 40'S 28° 16'E). This mountain range separates the Metropolitan into two large vegetation types: cooler Bankenveld (Bredenkamp & van

Rooyen 1998) to the south and Sour Bushveld and warmer Clay Thorn Bushveld (Van Rooyen & Bredenkamp 1998) 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.

Sampling 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 *Spirocerca lupi* in dogs**.** The Department of Veterinary Tropical Diseases at the University of Pretoria provided information about the infection rates in dogs from various areas, which they compiled from clinical reports of necropsies performed at the Onderstepoort campus. Dung beetles were sampled in three localities per area.

Pig, dog and cow dung baited pitfall traps were used for sampling 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 traps had a 1000 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. The pitfall traps were filled to about one-fifth their volumes with a solution of liquid soap and water to immobilise trapped dung beetles. Dung baits were suspended on u-shaped metal wire, placed over the traps. Trap contents were collected 48 hours 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 *Spirocerca 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 *et al*. 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 *et al*. 2000) and reported as a percentage.

2.2.2 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 *Spirocerca lupi* larvae in dung beetles varied considerably among 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 *Spirocerca lupi* larvae (Table 3).

Table 1. Results of the dissection of various dung beetle species from an urban area in the Tshwane Metropolitan 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 Metropolitan 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 Metropolitan 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	_	_

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

Table 4. Observed frequencies of uninfected and infected dung beetles from three areas in the Tshwane Metropolitan.

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

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.

Table 5. Observed frequencies of uninfected and infected dung beetles from a rural and an urban area in the Tshwane Metropolitan.

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 Metropolitan.

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 Metropolitan.

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

2.2.3 Discussion

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) Metropolitan.Conditions for maximum dung beetle activity were sub-optimal 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, however, 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 the 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.

In this study only omnivore dung specific dung beetles were found to be parasitized by Spirocerca 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.

Prevalence of *Spirocerca lupi* in populations of its intermediate dung beetle hosts in Grahamstown, Eastern Cape Province, South Africa

2.3.1 Materials and Methods

Description of the study area

This study was conducted in Grahamstown, in the Eastern Cape Province of South Africa (33°18'S, 26°32'E) on the basis of being a focal area of high infection with *S. lupi* in dogs. Information on incidence of infection in dogs was obtained from ClinVet International Research Organisation, South Africa. The study area was classified into two main regions: a high human density region and a low human density region, based on characteristics of their respective land use, the number of people that resided in each of the two regions, and the potential free-range limits of dogs. The high human density region comprised of informal settlements and the general landscape was severely transformed by human activity. Flora consisted of mostly non-woody exotics; large areas were devoid of any vegetation with signs of advanced erosion damage. These areas were heavily polluted with household refuse and a noticeable feature of the landscape was the large amount of exposed faeces (predominantly human, dog, cattle and donkey). Dogs that frequented in this region were mostly feral. The low human density region was situated within the suburban zone of Grahamstown. This region consisted of well watered gardens, public open spaces in the form of parks and sports fields, and natural or seminatural green spaces. Open green spaces comprised principally of natural indigenous vegetation of the Grahamstown Grassland Thicket vegetation type (McConnachie et al. 2008).

Sampling design

Dung beetles were sampled over one breeding season on three separate occasions: December 2007, February 2008, and April 2008, which coincides with high dung beetle activity (Davis 2002) in summer rainfall areas of South Africa. Sampling was conducted in four sites in the high human density region and in five sites in the low human density region. Collecting sites in the high human density region were selected on the basis of being frequented by high densities of feral dogs. The selection of specific sites for trapping in the low density region was based on information obtained from a local veterinarian on patient records pertaining to dogs that were infected by *S*. *lupi* and consultation with dog owners on where dogs had been taken for daily exercise. Exactly the same locations and pitfall trap positions were used for all three sampling occasions.

Pig dung-baited pitfall traps were used for sampling dung beetles. In this study the domestic dog was treated as an omnivore (see Chapter 1). Pig dung served as a surrogate for dog dung, because it is also an omnivore and strong smelling, and due to difficulties in procuring enough dog dung for baiting purposes. Pig dung used for bait was collected from a piggery to the east of Pretoria. Five pitfall traps were placed at 10 m intervals along a single transect line in sunny situations. Plastic buckets were used as pitfall traps and had a 1 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 of their volume with a water and soap solution to immobilise trapped beetles. On each trapping occasion the 0.5 L dung baits were suspended on u-shaped metal wire supports, which were placed over the buckets at ground level. Baits were wrapped in chiffon to allow for the diffusion of volatile compounds but at the same time exclude beetles from the dung baits.

Trap contents were collected 48 h after traps had been set and only scarabaeine dung beetles were collected from the traps. Species-level identification of dung beetles were carried out in the laboratory and conspecifics collected from the same locality in each of the two regions were pooled and stored together in absolute ethanol in labelled jars. Voucher specimens were deposited at the University of Pretoria Insect Collection.

Data analysis

All beetles (total catch) collected from each transect in both regions were dissected. Dung beetles were dissected in distilled water and examined under a light microscope to observe the presence or absence of *S. lupi* larvae (Mönnig 1938).

Individual beetles were recorded as being either positive or negative for infection with this nematode.

2.3.2 Results

December 2007 sampling effort

In total, 182 dung beetles belonging to eight species were collected from 45 pitfall traps in two regions (high human density and low human density) during 48 h in December 2007. Only 49 beetles (26.9% of the total from both regions) from five species (Table 8) were collected from the high human density region, while the remaining 133 beetles (73.1% of the total from both regions) belonging to eight species (Table 9) were collected in the low human density region.

The prevalence of infection with the larvae of *S*. *lupi* was found to be low in both regions for the total number of beetles from all species collected. However, all beetles that were found to be harbouring S. *lupi* larvae belonged to the genus *Onthophagus*. In the high human density region, larvae were recovered from two *O*. *sugillatus* (sp. 3) females (11 and two parasites, respectively), representing a prevalence of 6.6% for the population sampled (Table 8). Four beetles from three species were positive for infection in the low human density region (Table 9). One male *O*. *asperulus* was infected with a single *S*. *lupi* larva indicating representing a prevalence of 1.8% of the population sampled for this species. *O*. *cyaneoniger* yielded two infected individuals, one male (2 parasites) and one female (one parasite). The prevalence of infection as a total for the population sampled was the highest in this species at 27.3%. A single male *O*. *lugubris* was infected with 31 *S*. *lupi* larvae, representing a prevalence of 5.9% of beetles from the population in this region sampled.

The sample size of beetles found to be positive for infection with the larvae of *S*. *lupi* was too small for any meaningful statistical analyses to be performed on the data set.

February 2008 sampling effort

During this collecting exercise a total of 155 dung beetles from 11 species were collected in both sampling regions during the 48 h sampling event. Five species and 46 individual beetles (29.7% of total number of beetles collected in both regions) were trapped in the high human density region (Table 10). In the low human density region 109 beetles belonging to 10 species (70.3% of total number of beetles collected from both regions) were sampled (Table 11). QAlthough the December 2007 collecting effort yielded more individual dung beetles (Tables 8 and 9), a greater number of species were collected during this specific sampling exercise. A 0% prevalence of infection of dung beetles with *S*. *lupi* larvae was observed. Thus, no statistical analyses were performed on these data.

April 2008 sampling effort

This sampling effort yielded the lowest number of individuals and the fewest species of dung beetles of all three trapping occasions. In total, 83 specimens from five species were collected in both regions combined during 48 h that sampling was conducted. Three species and 41dung beetles (49.4% of total number of beetles collected for both regions combined) were trapped in the high human density region (Table 12). However, 35 individuals belonged to only one of the three species, *Onthophagus sugillatus* (sp. 3). In total, 42 beetles belonging to five species (50.6% of total number of dung beetles collected from both regions) were sampled from the low human density region on this trapping occasion (Table 13). During this sampling occasion a 0% of infection with the larvae of *S*. *lupi* was observed. No statistical analyses were performed on these data.

Table 8. Number of infected and uninfected dung beetles for both sexes from the high human density region in Grahamstown during December 2007. Numbers in brackets indicate the number of *S. lupi* larvae recovered per individual infected dung beetle.

Grahamstown: High human density region (December 2007)

Species	**Male infected**	**Male uninfected**	**Female infected**	**Female uninfected**
Epirinus spp.	0	2	0	6
Onthophagus asperulus	0	1	0	2
O. lugubris	0	2	0	0
O. sugillatus (sp. 3)	0	10	2 (11; 2)	23
Sisyphus alveatus	0	1	0	0

Table 9. Number of infected and uninfected dung beetles for both sexes from the low human density region in Grahamstown during December 2007.

Grahamstown: Low human density region (December 2007)

Species	**Male infected**	**Male uninfected**	**Female infected**	**Female uninfected**
Catharsius tricornutus	0	1	0	0
Epirinus spp.	0	1	0	0
Euoniticellus triangulatus	0	2	0	0
Onthophagus asperulus	1 (1)	26	0	29
O. cyaneoniger	1 (2)	2	1 (1)	7
O. lugubris	1 (31)	10	0	6
O. sugillatus (sp. 3)	0	9	0	23
Sisyphus alveatus	0	7	0	6

Table 10. Number of infected and uninfected dung beetles for both sexes from the high human density region in Grahamstown during February 2008.

Grahamstown: High human density region (February 2008)				
Species	**Male infected**	**Male uninfected**	**Female infected**	**Female uninfected**
Epirinus aquilus	0	1	0	2
Onthophagus asperulus	0	5	0	12
O. binodis	0	0	0	3
O. cyaneoniger	0	4	0	10
O. sugillatus (sp. 3)	0	4	0	5

Table 11. Number of infected and uninfected dung beetles for both sexes from the low human density region in Grahamstown during February 2008.

Grahamstown: Low human density region (February 2008)

Species	**Male infected**	**Male uninfected**	**Female infected**	**Female uninfected**
Drepanocerus kirbyi	0	1	0	0
Epirinus aquilus	0	4	0	4
Onthophagus asperulus	0	2	0	8
O. binodis	0	0	0	1
O. cyaneoniger	0	24	0	23
O. lugubris	0	8	0	4
O. naso	0	3	0	2
O. pilosus	0	3	0	2
O. sugillatus (sp. 3)	0	10	0	9
Scarabaeus convexus	0	1	0	0

Table 12. Number of infected and uninfected dung beetles for both sexes from the high human density region in Grahamstown during April 2008.

Grahamstown: High human density region (April 2008)				
Species	**Male infected**	**Male uninfected**	**Female infected**	**Female uninfected**
Onthophagus asperulus	0	1	0	3
O. sugillatus (sp. 3)	0	15	0	20
Sisyphus spinipes	0	0	0	2

Table 13. Number of infected and uninfected dung beetles for both sexes from the low human density region in Grahamstown during April 2008.

Grahamstown: Low human density region (April 2008)				
Species	**Male infected**	**Male uninfected**	**Female infected**	**Female uninfected**
Epirinus aquilus	0	0	0	1
Copris antares	0	1	0	1
Onthophagus asperulus	0	3	0	0
O. sugillatus (sp. 3)	0	14	0	17
Sisyphus spinipes	0	0	0	5

2.3.3 Discussion

Dung beetles (four species and five individuals) were only found to be positive for infection with *S. lupi* larvae during the December 2007 sampling occasion. The low accuracy of prevalence estimates associated with small sample size has a mathematical basis (Jovani & Tella 2006) and statistical analysis of the results was rejected on the basis of the small sample size. Although large amounts of exposed faeces were present in the high human density region, it had the lowest abundance of dung beetles. A possible explanation for this phenomenon is the extent to which this region has been transformed by human activity. It was heavily polluted, large areas were devoid of vegetation cover and the soil was compacted from being trampled by high volumes of humans and cattle. The low human density region, on the other hand, consisted of well watered gardens and parks, which offered better conditions for dung beetles to breed in.

Conditions during April 2008 were suboptimal for dung beetle activity, which was characterised by long dry spell accompanied by high temperatures. Furthermore, traps were often disturbed by human activity and baits were found to be absent on inspection of sites, possibly due to being consumed by coprophagous mammals that frequented the area.

2.4 General discussion

Both the studies conducted in Pretoria and Grahamstown were characterised by small sample sizes of the dung beetle intermediate host populations and low prevalence of infection was indicated in both cases. High statistical uncertainties of prevalence can be overcome by rejecting data from such small sample sizes (Jovani & Tella 2006). However, establishing a minimum sample size is usually a subjective decision on the part of the researcher. Larger sample sizes deliver more reliable results, and uncertainty decreases with increasing sample size up to 10-20, but not more with further increases in sample size (Jovani & Tella 2006). 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 *Spirocerca lupi* (Bailey 1972). It seems that the prevalence of this disease in dogs 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).

Several factors could cause a decline in prevalence of *S*. *lupi* larvae in dung beetle populations. There are a number of selective factors that control beetle associations in dung beetle assemblages (Lumaret *et al*. 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 and abundance of dung beetles, which will lead to a decrease in the prevalence of this parasite in that area (Bailey 1972). Winter and summer diapause can cause a decrease in prevalence and the magnitude of the decline depends on varying climatic conditions during these seasons (Lass & Ebert 2006). 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 suitable dung beetle intermediate hosts to become infected and for the final host to ingest infected dung beetles (Brodey *et al*. 1977).

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) (See Chapter 4). 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).

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).

Several factors affect pitfall trap efficiency, such as trap diameter, layout of traps within transects, bait type used, disturbance of traps, and depletion of baits (Ward *et al*. 2001). Baits were regularly found to be absent on inspection of traps, possibly due to being scavenged by coprophagous mammals, since high densities of feral dogs were present in some of the study sites. Furthermore, the plastic buckets that were used as pitfall traps were often removed by people in the course of an experiment due to the value associated with its usefulness to such persons. Large amounts of exposed faeces were characteristic of some of the study areas, which might have influenced the effectiveness of the baits used for sampling dung beetles.

Moreover, pig dung was used as a surrogate for dog faeces and although a pig is also an omnivore, direct sampling from dog scats may provide a clearer indication of the prevalence of infection in dung beetles.

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. $\beta = 100\%$ suggests uninhibited ingestion of both particle sizes, while $\beta < 100\%$ 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.

CHAPTER FOUR

EFFECTS OF TROPHIC PREFERENCE AND URBANIZATION ON DUNG BEETLE ASSEMBLAGE STRUCTURE AND TRANSMISSION OF *Spirocerca lupi* TO DOGS

Introduction

Mammalian faeces represent very patchy and ephemeral habitats. They are patchy due to the distribution of the producer from which it is excreted and ephemeral as a result of the activities of a variety of dung colonisers (Dormont *et al*. 2007; Scholtz *et al*. 2009; Tshikae *et al*. 2008). However, dung is a highly sought-after and nutritious resource that, under favourable conditions, is quickly colonised by coprophagous beetles belonging to the subfamily Scarabaeinae, for the purposes of feeding and breeding (Scholtz *et al*. 2009). It constitutes a combination of characters such as age, size, water content, physico-chemical attributes, seasonality, and temporal and spatial distribution, which can be regarded as important niche dimensions for dung beetles (Scholtz *et al*. 2009; Sowig & Wassmer 1994; Tshikae *et al*. 2008). As these factors influence its species-specific attractiveness, selection of a particular dropping that is to be colonised results in differences between species assemblages in different dung types (Scholtz *et al*. 2009; Sowig & Wassmer 1994).

The physical and chemical composition of dung varies considerably between that of herbivores, omnivores and carnivores (Dormont 2007; Martin-Piera & Lobo 1996). There can also be substantial variation in the dung produced by different mammalian herbivores, since grazers and browsers produce quite different dung types (Scholtz *et al*. 2009). An additional complexity is whether the herbivore is a ruminant, producing fine-textured faeces, or a non-ruminant producing coarse dung, as well as variation in dung quality arising from such factors as disparities in pasture quality or the season when the dung is produced (Gittings & Giller 1998; Scholtz *et al*. 2009).

The assumption is often made that most dung beetles are polyphagous and colonise the faeces of several vertebrates without any discrimination between dung types (Dormont 2007). Although most species of dung beetles are indeed opportunistic without discriminating between various types of dung, specialist coprophages with clear trophic preferences have been documented (Davis 1994; Dormont *et al*. 2007; Fincher *et al*. 1970; Hanski & Cambefort 1991; Martin-Piera & Lobo 1996; Tshikae *et a*l. 2008). Moreover, some studies have empirically shown that dung beetles do display differences in colonisation activity among the dung of various herbivorous mammals (Dormont *et al*. 2007).

Urbanisation is increasing worldwide, and it is expected that more than 66% of the global human population will reside in cities within the next three decades (Bradley & Altizer 2006). Changes in urban land use influence shifts in the geographical ranges and densities of host species, interspecific interactions (Bradley & Altizer 2006), and more specifically, the structure of dung beetle species assemblages (Carpaneto *et al*. 2005). These changes in urban environments may lead to a reduction or complete absence of grazing herbivores. Dogs, both pets and feral animals, often then become the most common large mammal in these urban environments (Carpaneto *et al*. 2005). Dog dung may provide a temporary refuge for species of coprophagous dung beetles that do not prefer omnivore dung (the dog was treated as an omnivore in this study), but would otherwise encounter local extinction in these urban environments (Carpaneto *et al*. 2005).

The aims of the present study were to assess abundance, diversity, and trophic preference of dung beetles across three dung types along an urban-peri-urban-rural gradient in Grahamstown (Eastern Cape, South Africa). This area was found to be a focal area of high incidence of spirocercosis in domestic dogs by the ClinVet International Research Organisation, South Africa. The selection of specific sites for trapping was based on information obtained from a local veterinarian on patient records pertaining to dogs that were infected by *S*. *lupi* and consultation with dog owners on where dogs had been taken for daily exercise. Furthermore, this study served to identify omnivore dung beetle specialists which could potentially act as vectors for *S*. *lupi* under natural conditions in these environments. A specific objective of this chapter is to understand whether changes in dung beetle species assemblages and trophic choice due to changes in landscape use, can lead to altered transmission rates of *S*. *lupi* to dogs.

Materials and Methods

Sampling localities

Dung beetles were collected at three localities along an urbanisation gradient in Grahamstown, a medium-sized town with 57 030 inhabitants (McConnachie *et al*. 2008), in the Eastern Cape Province of South Africa (33°18'S, 26°32'E). Locality one was situated within an urban environment in an open field adjacent to a military base. This urban site was severely degraded in terms of having reduced woody vegetation cover, and most of the flora comprised of alien invasive and non-invasive species.

Locality two was situated on the periphery of the town in a peri-urban greenspace area and was less transformed by human activity than the urban site. The current landscape of this study site consists of grassland, dotted by a mosaic of evergreen shrubs and low woody plants. The area is used by urban dwellers for a variety of activities, such as hiking, horse riding, bird watching, and harvesting of fuel-wood (Du Toit pers. comm.). During the sampling period, dogs were regularly encountered in both the urban and peri-urban sites, as these areas were used extensively by dog owners for exercising their pets (either restricted on a leash or by letting the animals run freely) (Du Toit pers. obs.).

Locality three was situated on a sheep farm, approximately 5 kilometres outside Grahamstown. This rural study site was characterised by indigenous vegetation that forms part of the Grahamstown Grassland Thicket vegetation type (McConnachie *et* *al*. 2008). Dogs were conspicuously absent from the site and the area was grazed by mainly sheep, although a few indigenous antelope were observed during the study.

By comparing the abundance, trophic associations, and assemblage structure of dung beetles between these three localities, this study may identify potential effects of changes in landscape use and composition on the transmission rate of *S*. *lupi* to dogs.

Sampling design

Dung beetle assemblage structure and trophic associations with bait type were studied using three different types of mammalian dung. The three dung types consisted of (1) relatively smooth and rancid-smelling pig dung as a surrogate for dog dung (2) fine-fibred dung of a ruminant herbivore (cattle); and course-fibred dung of a hay-fed, non-ruminant herbivore (horse). Pig dung served as a surrogate for dog dung because it is also an omnivore and strong smelling, and due to difficulties in obtaining sufficient quantities of dog dung for baiting purposes. Dung for baits were collected from a commercial pig farm, from pasture-grazing cattle on a small holding West of Pretoria (Gauteng), and from stabled horses on a small holding in Grahamstown (Eastern Cape).

Trapping was conducted during November 2009, which coincides with high dung beetle activity (Davis 2002) in summer rainfall areas of South Africa. As dung beetle activity is strongly influenced by insolation (Tshikae *et al*. 2008), pitfall traps were placed in predominantly sunny situations to standardise sampling design according to microhabitat. In each locality, 30 pitfall traps were set 10 m apart along three transect lines. Transects were separated by 50 m intervals. All traps in a specific transect were baited with one of the three dung types. The plastic buckets used as traps had a 1 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 of their volume with a water and soap solution to immobilise trapped beetles. On each trapping occasion the 0.5 L dung baits were suspended on u-shaped metal wire supports, which were placed over the buckets at ground level. Baits were wrapped in chiffon to allow for the diffusion of volatile compounds but at the same time exclude beetles from the dung baits. Traps were covered with lids supported on wire legs to prevent flooding of the buckets by rain.

Trapping was carried out in all sites simultaneously for a continuous 48 h period. Traps were baited between 06h00 and 08h00 and re-baited between 16h00 and 18h00 to ensure that diurnal as well as crepuscular/nocturnal species were presented with fresh baits. The trap contents were collected on each baiting occasion and samples were preserved in absolute ethanol for species-level identification and counting in the laboratory. Voucher specimens were deposited at the University of Pretoria Insect Collection.

Data analysis

The data were analysed using methods similar to Davis (1994) and Tshikae *et al*. (2008). Rank abundance curves were generated and used to compare abundance patterns and species evenness (Krebs 1999) across the three different dung types along an urban-peri-urban-rural gradient. Furthermore, species were classified along a gradient that ranges from specialist to generalist with regard to trophic niche width. A value for niche width across the three dung types was calculated for each species using the Shannon-Wiener diversity index (Krebs 1999) and niche width indices were standardised by dividing all values by -1.029, which was the most generalist value generated by the data set (Davis 1994). This provided an index scale for trophic niche width (W) where zero represented the most specialist species and one the most generalist.

Patterns of trophic associations (omnivore, ruminant-herbivore, and non-ruminant-herbivore) were classified by arranging the trap data as a matrix of eight species by total numbers attracted to each of the three dung types. The data matrix only included the eight most abundant species, which comprised 90% of all individuals collected. A cluster analysis with Bray-Curtis Similarity Index (**PRIMER** v5.0) was used to investigate differences in dung beetle assemblage structure between the three localities along the urban-peri-urban-rural gradient. The results were summarised and presented as a dendogram (Figure 3) from which groups of species with similar trophic associations were defined. For the eight most common dung beetle species Kruskal-Wallis tests (STATISTICA 10) were conducted to evaluate differences in abundance per trap between the three dung types (horse, cattle, and pig) and the three study sites (urban, peri-urban, and rural).

Results

In total, 2396 dung beetles were collected in the study representing 29 species in 16 genera and eight tribes (Appendix 1). Of the 29 species, 26 (90%) were sampled from pig dung, 24 (83%) from cattle dung, and 12 (41%) from horse dung (Figure 1a; Appendix 1). Omnivore dung attracted more beetles than the two types of herbivore dung combined. Pig dung baits attracted 1539 (64.2%) individuals, followed by cattle dung with 740 (30.9%). Only 115 (4.8%) dung beetles were collected from horse dung baits (Figure 1b, Appendix 1). Three species were collected exclusively from only one dung type (Appendix 1), and 15 species of dung beetles were attracted to two of the three dung types, while 11 species were attracted to all three dung types (Appendix 1).

Table 1 summarises trophic preference and abundance for the eight most abundant species, which comprised 90% of the total number of dung beetles sampled from three different dung types along the Grahamstown urbanisation gradient. *Onthophagus* spp. showed a strong trophic preference for pig dung (Table 1) and were most abundant in the urban and peri-urban sites. A similar pattern was observed for *Sarophorus striatus*. The most abundant species on cattle dung was *Drepanocerus kirbyi*, which reached peak numbers in the rural site furthest from the town (Table 1).

Rank abundance curves (Figure 2) of dung beetles trapped along the urban-peri-urban-rural gradient in Grahamstown show clear patterns of species diversity in the dung baits. The curves for species sampled from pig and cattle dung show a similar assemblage structure and indicate higher species diversity than that for species assemblages on horse dung. Species diversity was highest in the cattle dung assemblage, even though more dung beetle species were attracted to pig dung baited traps (a few species were much more abundant in pig dung baited traps than they were in cattle). Greatest evenness is observed in the curves for pig and cattle dung among species with intermediate and low abundance.

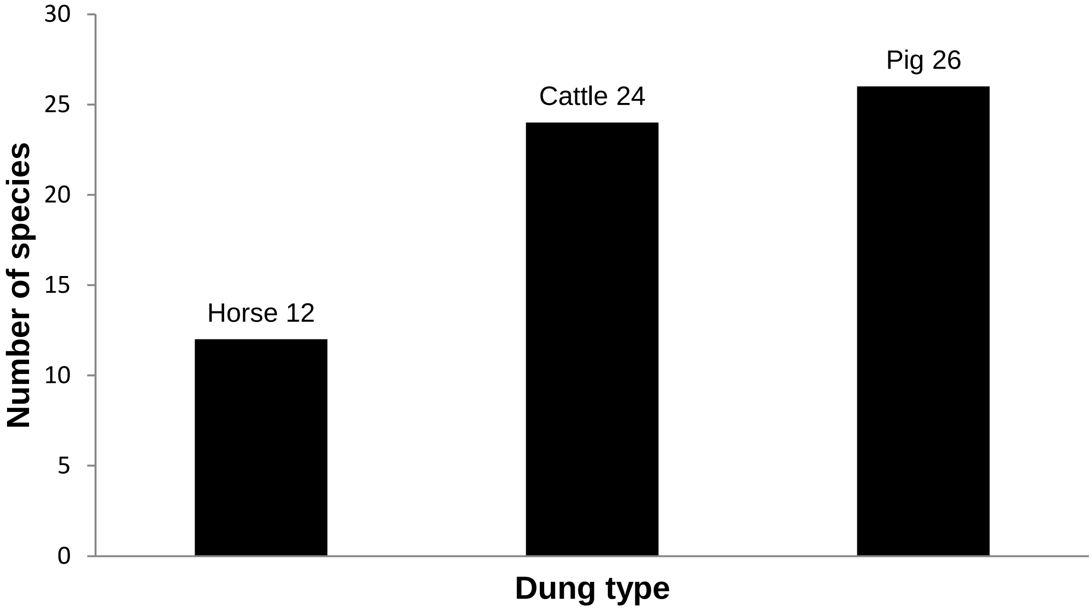

Figure 1a. Number of species trapped on different dung types.

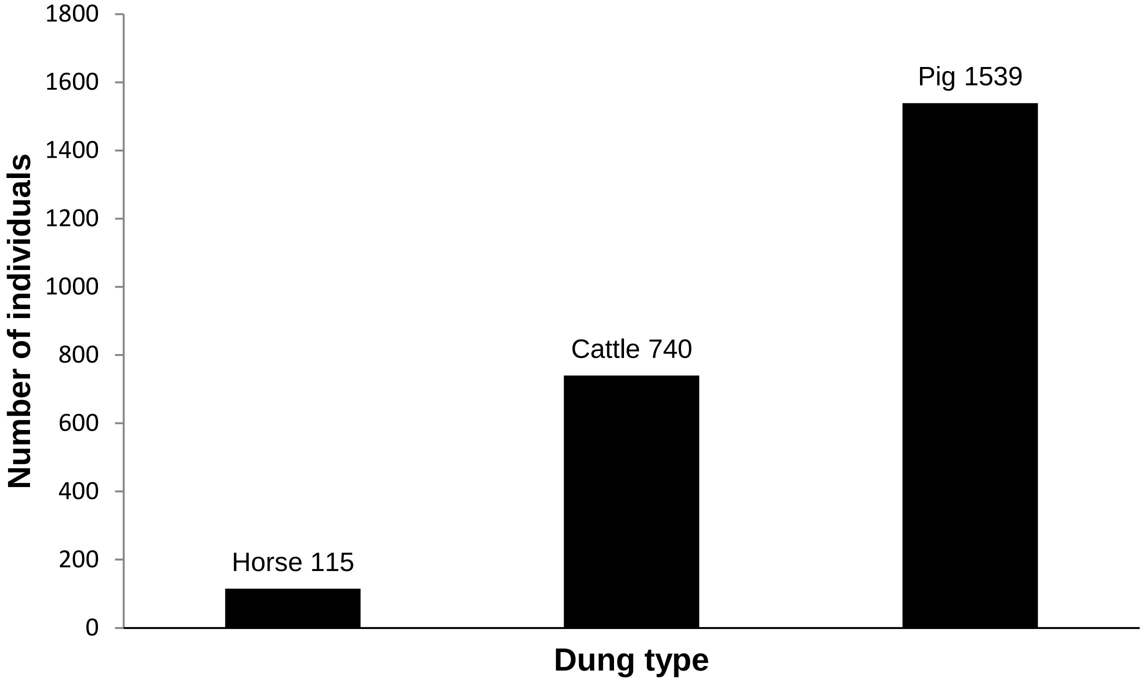

Figure 1b. Number of individual dung beetles trapped on different dung types.

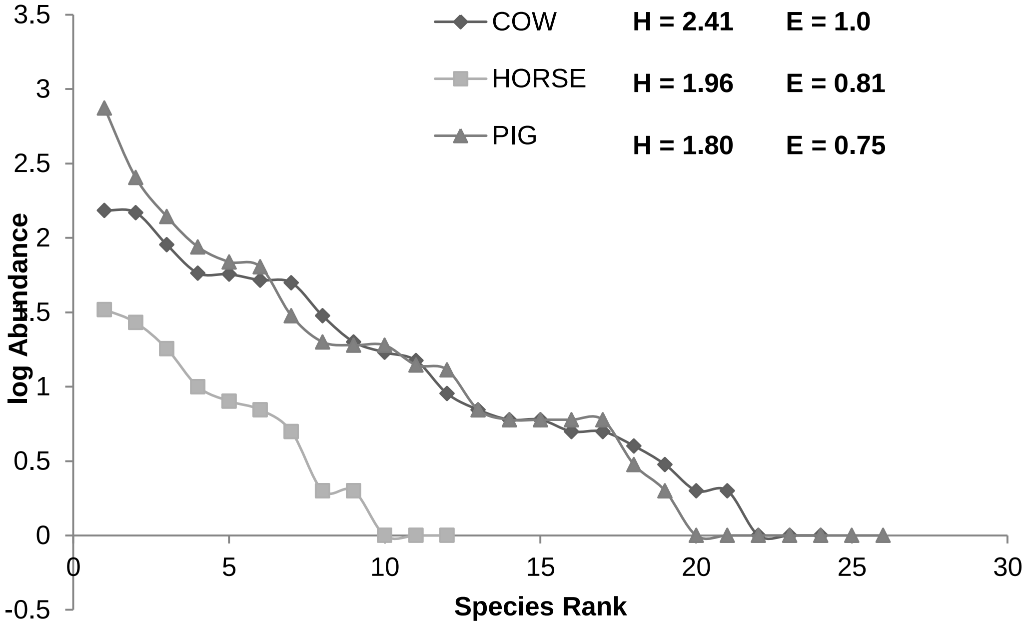

Figure 2. Rank-abundance curves for dung beetle species on three dung types (H, Shannon-Weiner; E, evenness).

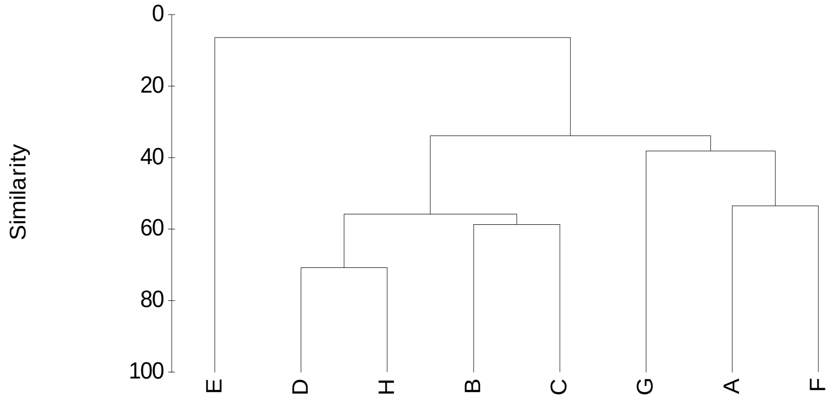

Figure 3. Dung beetle trophic associations of the eight most abundant species between the three localities along an urbanisation gradient. A= *Sarophorus striatus*; B= *Drepanocerus kirbyi*; C= *Euoniticellus africanus*; D= *Onthophagus asperulus*; E= *Onthophagus fritschi*; F= *Onthophagus lugubris*; G= *Onthophagus sugillatus*; H= *Sisyphus alveatus*

Table 1. Numbers showing trophic association of the eight most abundant dung beetle species collected along an urban-peri-urban-rural gradient in Grahamstown, Eastern Cape.

		Numbers on bait type										H
Species	Code	Horse 1	Horse2	Horse 3	Cattle 1	Cattle 2	Cattle 3	Pig 1	Pig 2	Pig 3	Total	(2, N=90)
Sarophorus striatus	A	0	6	2	18	24	15	78	112	65	320	33.21*
Drepanocerus kirbyi	B	0	0	1	9	27	117	1	18	20	193	26.61*
Euoniticellus africanus	C	0	0	0	7	13	28	5	19	41	113	22.31*
Onthophagus asperulus	D	0	6	1	5	14	71	0	11	76	184	8.17**
Onthophagus fritschi	E	0	0	0	57	1	0	1	0	0	59	11.96**
Onthophagus lugubris	F	1	16	1	0	21	9	1	68	15	132	6.32***
Onthophagus sugillatus (sp. 3)	G	2	18	13	32	75	41	185	365	196	927	26.97*
Sisyphus alveatus	H	0	3	26	0	0	52	1	11	127	220	5.44
Total		3	49	44	128	175	333	272	604	540	2148	

*P < 0.001, **P < 0.03, ***P < 0.05

4.4 Discussion

This study investigated trophic preferences in dung beetles along an urbanisation gradient to ascertain whether changes in species assemblages and trophic choice could lead to altered transmission rates of *S*. *lupi* to dogs. The prevalence of infection in dung beetles and the epidemiology of spirocercosis in any particular area depend in part on the abundance of these beetles and the degree of contact between them and domestic dogs (Bailey 1972). Higher contact rates with the faeces of infected dogs by coprophagous beetles lead to an increased probability of infection in dung beetles, and higher abundance and population density of susceptible dung beetle species on dog scats may lead to an increased transmission rate of *S*. *lupi* to dogs. Several factors influence the population density of scarabaeines in any specific region: vegetation cover; soil type and pH; dung type diversity (carnivore/ omnivore, and herbivore); temporal patterns such as successional processes associated with dung (age, size, water content), diel activity and seasonality; and physico-chemical attributes of the dung itself (Bailey 1972; Hanski & Cambefort 1991).

Eight out of a total of 29 species collected during the sampling effort, constituted 90% of the individual beetles trapped in the Grahamstown area (Appendix 1; Table 1). The most abundant species in terms of individuals trapped, belonged to the genus *Onthophagus*. Three of these, *O*. *sugillatus*, *O*. *lugubrus*, and *O*. *asperulus*, have been found positive for infection with *S*. *lupi* in a separate study. (Chapter 2). Although *Onthophagus cyaneoniger* was also found to harbour this nematode

(Chapter 2) it is excluded from analyses in this study because only one individual was collected. In a recent study on the prevalence of this nematode in populations of its intermediate dung beetle hosts in the Pretoria Metropole (Chapter 2), Du Toit *et al*. (2008) have shown *O*. *sugillatus* to be a vector of *S*. *lupi* in that region too, along with four other species, three of which also belonged to the genus *Onthophagus*. Gottlieb *et al*. (2011) identified *O*. *sellatus* as the main intermediate host of this parasite in an endemic urban area in central Israel. Therefore, it seems that *Onthophagus* spp. could be regarded as a major vector of *S*. *lupi* and the preferred host to support larval development and transmission to paratenic and definitive hosts (Gottlieb *et al*. 2011) under natural conditions, at least in urban environments where this disease in dogs is considered to be endemic. However, since *Onthophagus* is the largest dung beetle genus, the preference of a few species for dog dung may simply be a factor of large numbers of species of which some have niches wide enough to encompass dog dung as food source.

Species showing a preference for omnivore dung and a higher abundance in urban environments, can be expected to be more active in spreading *S*. *lupi* to dogs. Within urban and peri-urban areas in Grahamstown, the replacement of grazing herbivores by a single omnivorous species (domestic dog) may account for the high numbers of *Onthophagus* spp. and *Sarophorus striatus*. The dominance of domestic (sheep and cattle) and indigenous herbivores (kudu (*Tragelaphus strepsiceros*) and grey duiker (*Sylvicapra grimmia*)) in in the rural agro-ecosystem may explain both lower abundances in dung beetle species associated primarily with pig dung and much higher numbers in cattle dung frequenting species, such as *Drepanocerus kirbyi*.

Differences in beetle numbers between sites one (urban) and two (peri-urban) (Table1) could be a result of the differences in disturbance between those sites. Site one was situated on the edge of a military base golf course, which was more transformed in terms of the proportion of natural vegetation still intact, while site two served as an urban greenbelt area.

Landscapes modified by humans lead to altered local species assemblage structures (Radtke *et al*. 2008; Carpaneto *et al*. 2005). Of particular concern to this study, is the conversion of land previously used as pastures into urban parks, built-up residential areas, or informal, high density human settlements (“townships”). Where this takes place, grazing herbivores are often replaced by a single large omnivore, the domestic dog, which may be kept either as pets or roam freely as feral animals (Carpaneto *et al*. 2005). This leads to an increase in the numbers of dogs and the density of dog faeces. In turn, this may lead to a higher abundance of dung beetles that show a preference for carnivore/ omnivore dung. Another factor to consider is the socio-economic attributes of a particular area. Lower income level communities are significantly negatively correlated with the quality of public green spaces in towns in the Eastern Cape (McConnachie *et al*. 2008). This situation may arise because of a lack of proper sanitation, which is a consequence of the low income level of such a community. This would result in decreased hygienic conditions and an abundance of exposed human faeces often associated with socio-economic inequalities encountered in poorer communities (Du Toit pers. obs.).

Under such conditions, human faeces may serve as an additional food resource to dung beetles, which may play a pivotal role in their ability to persist under unfavourable conditions (in terms of trophic preference) in urban areas. Moreover, dog dung (and human faeces (Du Toit pers. comm.)) may provide a temporary refuge to dung beetles that do not primarily prefer this resource, but will otherwise encounter local extinction in urban environments (Carpaneto *et al*. 2005). In fact, species assemblages occurring in dog and human dung in India, were found to be distinct from those associated with herbivore dung (Carpaneto *et al*. 2005). This holds important implications for the suite of dung beetle species that can be considered as suitable intermediate hosts for *S. lupi* under natural conditions. See Chapter 3. Few data exist on the colonisation of dog faeces by coprophagous dung beetles in any world region (Carpaneto *et al*. 2005), although Wallace and Richardson (2005) have compiled an inventory of scarabaeines observed to utilise the dung of domestic dogs in Austin, Texas. Changes in traditional grazing regimes have been shown to lead to declines in several dung beetle species in that particular region (Nichols *et al*. 2009). A myriad of other examples exist on the dramatic effects that a reduction in large mammal diversity (and thus, a reduction in the diversity of dung types available to Scarabaeine dung beetles) has had on the structure of dung beetle assemblages (Nichols *et al*. 2009; Scholtz *et al*. 2006).

Dung quality in terms of water content varies widely between different dung types and is an important factor affecting patch choice. Larger droppings, such as those produced by cattle, differ in their water retention qualities from smaller droppings, such as those produced by sheep, which are able to rehydrate by dew or during rainfall (Sowig & Wassmer 1994). Almost all adult dung beetles feed exclusively on the minute particles in the micro-organism-rich, liquid fraction of dung (Holter 2000; Holter *et al*. 2002). Thus, since there is considerable variation in the size of dung produced by different mammals, it might play an important role in niche separation (Sowig & Wassmer 1994). Canine dung undergoes a more rapid change of microclimate conditions because of its coarse structure (Carpaneto *et al*. 2005). Furthermore, changes in the quality of available dung resources (when one dung type is substituted with another) cause shifts in dung beetle communities with regards to competition within and between ecological guilds (Lumaret *et al*. 1992). In warmer climates competition is exacerbated by factors such as dryness and temperature (Lumaret *et al*. 1992).

4.5 Conclusion

It is imperative to have a comprehensive understanding of the incidence of species persisting in dynamic equilibriums between local extinction and colonisation events (Roslin & Koivunen 2001). At the landscape scale such events are expected to be higher in a dense network of patches than in a sparser one. Differences in the densities of patch networks cause differences in population densities. Thus, higher densities of suitable habitat patches in the landscape translate into higher local population densities (Roslin & Koivunen 2001). This necessitates the study of urban dung beetle assemblage structures because they indicate ecological changes in the local environment (Radtke *et al*. 2008). Dog dung (Carpaneto *et al*. 2005) and human faeces (Du Toit pers. comm.) are the most abundant resources for dung beetles in urban environments and pose major hygiene problems if not removed. Furthermore, dog and human faeces may favour certain rare species of dung beetles, or provide temporary refuge to species that do not usually show a preference for omnivore dung, which could allow for the persistence of their metapopulations in urban areas (Carpaneto *et al*. 2005). However, coprophagous dung beetles provide essential ecological services through their feeding and nesting activities, which not only allow for the recycling of faeces in urban environments (Wallace & Richardson 2005), but also serve to control the abundance of dung-dispersed nematodes and protozoa (Spector et al. 2008). These ecological services hold enormous implications for the health and wellbeing of humans and their companion animals.

CHAPTER FIVE

5.1 Concluding remarks

Finally, having reached a point where the hosts, the parasite, humans and their dynamic relationship with one another and their environment have been investigated, a clearer understanding of the underlying causal factors associated with the transmission success of *Spirocerca lupi* to dogs has been achieved. The rate of urbanisation is accelerating with the effect that land previously used for grazing purposes is converted to urban and suburban environments (Pickett *et al*. 2001). These changes in landscape use coupled to altered dung beetle species assemblage structure have influenced the pattern of events observed in this host – parasite relationship. Furthermore, the social organization of domestic dogs (pets versus feral animals) and the availability of exposed excrement as a direct or indirect consequence of human behaviour played a pivotal role in the rate these parasites are transmitted to dogs.

Transmission rate is paramount to a parasite’s fitness (Agnew & Koella 1999). On the one hand dung beetles may act as vectors for nematode parasites. On the other hand however, coprophagous dung beetles mediate several important ecosystem functions, such as nutrient re-cycling and parasite suppression, by removing dung from the environment for feeding and breeding purposes (Nichols *et al*. 2008). In sodoing they provide valuable ecosystem services, such as the control of pest and parasite numbers (“biological pest control”), removal of the breeding medium for flies, and soil fertilisation (Nichols *et al*. 2008). A decline or local extinction of dung beetle populations would have dramatic short and long-term effects on ecosystem integrity, which make it all the more important to protect these processes since dung beetles are highly sensitive to human disturbances (Nichols *et al*. 2008). Assuming that dung beetles persist in the modified urban environment, eradicating them would not be a useful solution to controlling infection of dogs by *S*. *lupi*. There are now clear indications that dung beetles belonging to the genus *Onthophagus* are important vectors for this parasitic nematode of dogs. One could monitor one or more *Onthophagus* species populations to determine both the incidence of parasite eggs and the relative danger of dogs contracting spirocercosis from such populations.

Imposing proper sanitation and hygienic habits through health education in poor, resource-limited communities would contribute to the reduction in transmission of *S. lupi* to dogs. Provision of simple pit latrines and education about disposal of faeces where latrines are not available (by e.g. burying it) should go a long way in improving the current situation among dogs, especially in informal settlements. However, dog owners in wealthier neighbourhoods must take responsibility to remove their dogs’ faeces from the environment, not only from public spaces, but especially on private property as this is where the animals most likely spend most of their time. Formal institutions can aid in this process by providing disposable bags and bins in public open spaces, and impose fines on those who do not comply with such regulations. This would surely help foster a moral culture of cleaning up after one's own dog.

Information should be made available to the lay public through popular literature about the underlying causes of spirocercosis and its transmission to domestic dogs, and about current advances being made in scientists' understanding of the dynamics of spirocercosis. People should be made aware of the fact that dogs *are* coprophagous despite the diet they are fed. This might foster a better understanding among dog owners about the transmission of this parasite to dogs and how it is sustained in the environment. The role of the intermediate dung beetle hosts in the transmission of this disease should be better communicated to veterinarians and veterinary students. There seems to be a lack of awareness of their exact role (at least in South Africa) in transmitting *S. lupi* to dogs. A possible solution would be to include veterinary entomology as part of the undergraduate curriculum.

Future research objectives

Refinement of sampling methods is required for studies on the associations between dung beetles and *S. lupi*. Although the method of pitfall trapping applied in this study is currently the standard method used in all studies on various aspects of dung beetle ecology and biodiversity, a new approach may be needed for studies on parasite prevalence in dung beetle populations. The degree to which dung beetles in urban areas are influenced by dung in the environment immediately surrounding a trapping site, may provide a false indication of the real prevalence of parasites in such populations. Comparative studies between pitfall trap results on prevalence of infection and those obtained from collecting dung beetles directly from a host's faeces should be explored. Dung beetle sampling should occur more regularly throughout the year to obtain a clearer understanding of the parasite's fate during months of lower dung beetle activity. Sampling in urban areas should take place over longer periods at a time to maximise the catch, since dung beetles occur in much lower densities in urban environments than in natural areas. Similar studies should be conducted in rural environments, and over a wider geographical range with different climatic conditions, to improve our understanding of certain trends identified in this study and extend the list of suitable dung beetle intermediate hosts. The development of mathematical models based on data obtained from studies such as the current one would aid in making meaningful predictions about future trends in parasite burdens in scarabaeine host populations and transmission rates of *S. lupi* to dogs.

Appendix 1. Abundance of 29 species of coprophagous Scarabaeine dung beetles trapped over 48 h along an urbanisation gradient in Grahamstown, Eastern Cape Province, South Africa.

		Dung-baited pitfall traps		
Tribe	Species	Horse dung	Cattle dung	Pig dung
Canthonini	*Epirinus aquilus*	0	5	0
	Epirinus obtusus	5	2	14
Coprini	*Catharsius tricornutus*	1	6	6
	Copris antares	10	17	6
Dichotomiini	*Sarophorus striatus*	8	57	255
Oniticellini	*Drepanocerus kirbyi*	1	153	19
	Euoniticellus africanus	0	50	64
	Euoniticellus intermedius	0	4	1
	Euoniticellus triangulatus	0	0	1
Onitini	*Cheironitis scabrosus*	0	3	7
	Onitis alexis	2	6	0
Onthophagini	*Caccobius obtusus*	0	0	1
	Digitonthophagus gazella	0	1	1
	Onthophagus asperulus	7	90	87
	Onthophagus binodis	0	20	13
	Onthophagus cribripennis	0	0	2
	Onthophagus cyaneoniger	0	0	1
	Onthophagus fimetarius	0	1	0
	Onthophagus fritschi	0	58	1
	Onthophagus lugubris	18	30	69

	Onthophagus sugillatus (sp. 3)	33	148	746
	Onthophagus suturalis	0	1	3
	Proagoderus lanista	0	0	1
Scarabaeini	*Scarabaeus ambiguus*	2	9	20
	Scarabaeus convexus	1	5	30
Sisyphini	*Neosisyphus barbarossa*	0	15	19
	Neosisyphus rubrus	0	7	6
	Sisyphus alveatus	27	52	139
	Neosisyphus spinipes	0	2	6
Total		**115**	**742**	**1518**

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