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

C. A. du Toit[1], C. H. Scholtz[1] & W. B. Hyman[2]

[1]*Scarab Research Group, Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002.*

[2]*VetPharm CC, P. O. Box 60, Bredell,1623*

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: χ^2 = 8.15, df = 1; $P < 0.05$; Fisher exact test: χ^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: χ^2 = 9.94, df = 1; $P < 0.05$; Fisher exact test: χ^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: χ^2 = 2.49, df = 1; $P < 0.05$; Fisher exact test: χ^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 semi-

natural 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.