Gastrointestinal and filarial helminth infections of domestic dogs in Gaborone, Botswana

By

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Submitted in partial fulfilment of the requirements for the degree of Masters of Science in the Department of Tropical Diseases in the Faculty of Veterinary Science, University of Pretoria.

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DECLARATION

I hereby certify that this research is the result of my own investigation. Where use was made of the work of others, it has been duly acknowledged in the text. The results in this dissertation have not been submitted, in whole or in part, for a degree at any other tertiary institution.

Kebonyemodisa Ntesang

I hereby release this dissertation for examination in my capacity as supervisor.

E. Volker Schwan

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SUMMARY

Gastrointestinal and filarial helminth infections of domestic dogs in Gaborone, Botswana

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There is a general lack on Information regarding gastrointestinal and filarial helminth infections of dogs in Botswana. Based on a first survey, the dissertation focuses on the occurrence and prevalence of gastrointestinal and filarial helminth infections of domestic dogs in the metropolitan area of the capital Gaborone. Faecal and blood samples were obtained from 150 live dogs aged 1-19 years (56 females and 84 males). Ten different dog breeds featured in the survey, with the indigenous Tswana as the most dominant breed (74.67 %) followed by the crossbreeds (14.67 %).

The overall prevalence of gastrointestinal helminth infections was 64 % (96/150) based on direct faecal flotation. The spectrum of gastrointestinal parasites detected included *Ancylostoma* spp. (64 %), *Dipylidium caninum* (4.66 %), *Toxocara canis* (1.33 %) and the coccidian *Isospora* spp (4 %). Dogs hosting a single gastrointestinal helminth species were more common (54.6 %) then those hosting 2 (6.67 %) or 3 species (0.67 %). *Ancylostoma spp., T. canis* and *D. caninum* have zoonotic implications.

The overall prevalence of filarial helminth infections was 18 % (27/150) based on demonstration of microfilariae in blood by membrane filtration. Based on acid phosphatase staining, microfilariae of 3 filarial helminths were identified, namely *Dirofilaria repens* (14.67 %) followed by *Acanthocheilonema reconditum* (2.67 %) and *Acanthocheilonema dracunculoides* (0.67 %). *Dirofilaria repens* has zoonotic implications.

Chapter 1
INTRODUCTION

Dog-like canids appear to have originated from multiple regional wolf populations at least 36 000 years ago with selective breeding by early humans probably occurring only at a later stage (Skoglund, Ersmark, Palkopoulou & Dalén 2015). Through the millennia a companionship has evolved which not only physically assists humans in herding, hunting and providing protection, but also makes important contributions on a social and emotional level (Dantas-Torres & Otranto 2014; Abere, Bogale & Melaku 2013; Bwalya 2012; Australian Council of Companion Animals n.d.; Robertson & Thompson 2002).

In Botswana, dogs are used privately for hunting game (*eg* antelopes), as a source of food, herding of farm animals, protection against predators and crime and as guide dogs. Furthermore, dogs are used by police, customs and security companies to uphold law and order as well as for specialized tasks such as detection of narcotics and explosives. There are several breeds of dogs kept in Botswana of which the most common ones are Tswana, Boerboel and German Shepherd. The Tswana is by far the most common and preferred dog breed because of its unique adaptation to the hard, semiarid conditions (Boitshepho 2013). According to the 2013 district livestock census (Statistics Botswana 2015) the size of the dog population was 133 932, with a number of 25 384 given for Gaborone.

Gastrointestinal helminth parasites of dogs are an important cause of morbidity and mortality in particularly puppies. Infections can negatively impact on the development of dogs, result in lower resistance to bacterial and viral diseases and affect performance in working and hunting dogs (Abere *et al.* 2013; Neves, Lobo, Simoes & Cardoso 2014; Bwalya 2012, Cook 1986). With the exception of *Dirofilaria immitis*, filarial helminths are of lesser importance as causes of morbidity and mortality. However, with the introduction of macrocyclic lactones for deworming and ectoparasite control, filarial infections have become significant as fatal side effects,

although rare, can be observed following treatment of microfilaraemic dogs and cats (Schwan, Miller, de Kock & van Heerden 2000; Schwan 2009). Several gastrointestinal (Macpherson & Craig 1991; Geerts, Kumar & Brandt 2012) and filarial (Pampiglione, Canestri Trotti & Rivasi, 1995; Muller 2002) helminths of domestic carnivores have zoonotic implications. Cases of human dirofilariosis caused by *D. repens* have been reported in South Africa (Moodley, Govind, Peer, van der Westhuizen, Parbhoo, Sun, du Plessis & Frean 2015).

In general, dogs are poorly managed in Botswana. This situation, in conjunction with very little public awareness of the zoonotic implications of canine and feline helminths, is reason for concern. Except for few records of helminths published in the Annual Reports of the Botswana National Veterinary Laboratory and isolated cases of filariosis (Schwan 2009), no systematic surveys have ever been conducted to determine the occurrence and prevalence of helminth parasites in domestic carnivores in Botswana.

In an attempt to overcome this situation, the aim of the study was to conduct a survey on helminths of dogs in the greater municipal area of Gaborone. The results of this survey will provide for the first time information on the occurrence and prevalence of gastrointestinal and filarial helminths of dogs in Gaborone. This information will assist in assessing the importance of helminth infections in dogs and to formulate recommendations for their control.

Chapter 2
LITERATURE REVIEW ON THE OCCURRENCE AND PREVALENCE OF HELMINTH PARASITES IN BOTSWANA AND NEIGHBOURING COUNTRIES

2.1 Botswana

There is limited published information on the occurrence of helminth parasites in domestic carnivores in Botswana. The only information available regarding helminth infections of dogs is found in the parasitology sections of the Annual Reports (1977-2008) and Monthly Reports (2009-2013) of the Botswana National Veterinary Laboratory (BNVL). The BNVL is the only veterinary laboratory in Botswana in which all veterinary samples are processed. According to the available BNVL reports from 1977-2013, there are frequent records of infections with *Ancylostoma* spp, *Ancylostoma caninum, Spirocerca lupi*, *Dipylidium caninum* and *Taenia* spp.

The BNVL Annual Report from 1987 (Department of Animal Health and Production 1987) mentions that microfilariae were found on several occasions in dogs and it was assumed that they belonged to *Dipetalonema* spp since no adult *Dirofilaria immitis* (canine heartworm) had ever been isolated from dogs during necropsies. The BNVL Annual Report from 1995 (Department of Animal Health and Production 1995) reports on a case of *Dirofilaria immitis* in a dog from Selebi Phikwe based on the mere demonstration of microfilariae in a Giemsa-stained blood film. *Dipetalonema reconditum* diagnosed in the blood of a dog from Selebi Phikwe was recorded in the BNVL Annual report from 2001 (Department of Animal Health and Production 2001). Based on acid phosphatase staining of microfilariae, infections with *Acanthocheilonema reconditum* and *Dirofilaria repens* have been diagnosed in dogs from Gaborone as part of the compulsory routine examination for filarial infections of dogs imported from foreign countries into South Africa (Schwan 2009).

2.2 Namibia

There are no reports on the occurrence of gastrointestinal helminths from dogs in Namibia. Filarial helminths reported from dogs based on acid phosphatase staining of microfilariae are *Acanthocheilonema dracunculoides* (Schwan & Schröter 2006) and *D. repens* (Schwan 2009).

2.3 Zambia

Several studies have been conducted in Zambia with respect to the occurrence and prevalence of helminth infections in dogs. Based on faecal examination and necropsy results, gastrointestinal helminths reported from dogs in Zambia comprise *D. caninum, Taenia hydatigena, Taenia multiceps, Echinococcus granulosus, Diphyllobothrium* spp, *Mesocestoides* sp, *Schistosoma mansoni, Toxocara canis, Toxascaris leonina, Ascaris* sp, *A. caninum, Ancylostoma braziliense, Spirocerca lupi, Gnathostoma spinigerum, Capillaria* sp and *Trichuris vulpis* (Islam & Chizyuka 1983; Nonaka, Nakamura, Inoue, Oku, Katakura, Matsumoto, Mathis, Chembesofu & Phiri 2011; Bwalya 2012; Chikweto, Bhaiyat, Mofya & Sharma 2015).

Based on acid phosphatase staining of microfilariae, *D. repens* infections have been diagnosed in several dogs from Lusaka and Chipata as part of the compulsory routine examination for filarial infections of dogs imported from foreign countries into South Africa (Schwan 2009). More recently, a survey conducted in Lusaka reports infections with *Acanthocheilonema reconditum* based on PCR (Siwila, Mwase, Nejsum & Simonsen 2015).

2.4 Zimbabwe

A checklist is available on the helminth parasites of mammals of Zimbabwe which also includes dogs (Jooste, 1990). Several studies have been conducted in Zimbabwe with respect to the occurrence and prevalence of helminth infections in dogs and the species recorded are *D.*

caninum, Joyeuxiella fuhrmanni, T. hydatigena, Taenia pisiformis, Taenia serialis, A. braziliense, A. caninum, Ancylostoma duodenale, Filaroides osleri, S. lupi, Strongyloides sp, *T. leonina, T. canis* (Mettrick 1962; Goldsmid 1965; Smith & Knottenbelt 1987; Rogers & Obwolo 1988; Obwolo, Ndikuwera, Musasira & Mushuku 1991; Mukaratirwa & Busayi 1995; Pfukenyi, Chipunga, Dinginya & Matenga 2010).

A checklist by Jooste (1990) indicates the endemicity of *Dirofilaria repens* and *Acanthocheilonema dracunculoides*.

2.5 Malawi

Although Malawi is not a neighbouring country of Botswana, it has been included in this literature review since in the colonial era it was, then as Nyasaland, part of the Federation with Rhodesia. The earliest record of helminths found in dogs comes from this time (Le Roux 1957). Apart from this, a checklist of helminth parasites form domestic animals which includes the dog (Fitzsimmons 1964) and results of two surveys on helminth infections of dogs in Malawi (Fitzsimmons, 1960; Fitzsimmons 1967) are available. Gastrointestinal and respiratory tract helminths recorded from dogs are *T. hydatigena, Mesocestoides lineatus, D. caninum, Dipylidium sexcoronatum, A. braziliense, A. caninum, T. canis, T. leonina, S. lupi, F. osleri* and *Chlamydonema* sp.

Regarding filarial helminths, *D. immitis* is recorded in Fitzsimmons' checklist (1964).

2.6 South Africa

Most information regarding gastrointestinal and filarial helminth infections of dogs is available from South Africa. The earliest information is provided in a check list by Mönnig (1928). Apart from a few case reports and reviews (Mönnig 1929; Smit 1960; Verster 1965; van Heerden &

Petrick 1980), several local and country-wide necropsy-based and coprological surveys have been conducted with respect to the prevalence of gastrointestinal helminth infections (Ortlepp 1934, 1937; Kaschula & Malherbe 1954, Neitz 1965; Verster 1979; Botha 1980; Minnaar, Krecek & Rajput 1999; Minnaar & Krecek 2001; Minnaar, Krecek & Fourie 2002; Lobetti 2000, 2014; Mukaratirwa & Singh 2010). Helminths of the gastrointestinal, respiratory tract, liver and nervous system recorded from dogs are *T. hydatigena, Taenia multiceps, Taenia serata, Taenia ovis, T. serialis, T. pisiformis, Taenia solium* (cysticercosis), *Echinococcus granulosus, Mesocestoides lineatus, D. caninum, D. sexcoronatum, Joyeuxiella sp, A. braziliense, A. caninum, T. canis, T. leonina, Trichuris vulpis, S. lupi, Physaloptera canis* and *Capillaria hepatica.*

Regarding filarial infections, case reports, diagnostic results and data collected from surveys on dogs are available from South Africa which indicate the endemicity of *A. reconditum, A. dracunculoides* and *D. repens* (Cobbold 1870; Verster 1991; Schwan *et al.* 2000; Schwan 2009).

Chapter 3
MATERIALS AND METHODS

3.1 Study area

The survey was conducted from mid of September 2014 to mid of November 2014 in the metropolitan area of Gaborone in the South East District of Botswana. As a landlocked country, Botswana is located in Southern Africa and covers a total area of about 582 000 km² (Burgess 2006) (Fig. 3.1). The climate is driven by the Zaire Air Boundary system and the South Atlantic Oscillatory climate system with mean rainfall ranging from 650 mm in the extreme northeast to 250 mm in the extreme southwest (Burgess 2006). Temperatures are extreme throughout the year and can also vary greatly within the daily cycle, depending on location, vegetation cover, wind reach and the presence of any large water bodies (Burgess 2006).

Gaborone (24°39' S, 25°54' E) is the capital and largest city of Botswana, 1012 m above sea level and has a population of 231 592, based on the 2011 census (Statistics Botswana 2014). Gaborone is divided into 14 suburbs called extensions, blocks and phases (Broadhurst, Block 5, Block 9, Block 6, Bontleng, Gaborone North, Gaborone West, Ginger, Ledumang, New Canada, Old Naledi, Phase 2, Phase 4, White City) (Fig. 3.2).

3.2 Study animals

Study animals were domestic dogs of either sex and of any breed belonging to private owners. Since no data were available on the prevalence of gastrointestinal and filarial infections in Botswana, a minimum size of the dog population to be sampled was determined at 138, based on a prevalence estimate of 10 % and using the formula after Daniel (1999):

$$n = \frac{Z^2 P(1-P)}{d^2}$$

where n = Size of sample
Z = Z statistic for a level of confidence = 1.96
P = Expected prevalence or proportion
d = precision = 0.05

Only dogs with a minimum age of 1 year or older were included in the survey because of the long prepatent periods of filarial helminths. Excluded for the survey were those animals that had received treatment with a macrocyclic lactone active during the past 12 months because of their microfilaricidal activity. For each dog sampled a data capture form was filled in.

3.3 Sample collection

Samples were collected during veterinary extension services provided to dog owners in various suburbs. With the consent of the owners, one faecal sample (1 heaped teaspoon) was collected from the rectum of each dog and transferred into a labelled 30 ml screw cap sample container. Additionally, one sample of about 4 ml blood was obtained from the cephalic vein into an EDTA Vacutainer® tube. Subsequent to collection, the blood samples were shaken for a few seconds by hand to ensure that anticoagulant and blood mixed properly. Both faecal samples and blood samples were stored in a refrigerator at approximately 4^{o} C until processed.

3.4 Diagnostic Procedures

3.4.1 Faecal flotation

Faecal samples were screened by direct faecal flotation using the commercial device Ovatector® and microscopically examined for the presence of helminth eggs, protozoan cysts, oocysts and trophozoites. Helminth eggs and protozoan developmental stages were identified

according to published morphological and morphometrical criteria (Levine 1980; Ash & Orihel 1987; Beugnet, Polack & Dang 2008).

3.4.2 Membrane filtration

EDTA blood samples were screened by membrane filtration for evidence of microfilariae (Dennis & Kean 1971). A 3 µm Isopore® (Millipore) polycarbonate membrane filter was inserted in a Swinnex® (Millipore) 25 mm filter holder. One ml EDTA blood followed by 4 ml of air were aspirated in a 5 ml nylon syringe and ejected through the assembled filter device. The filtrate was collected in a beaker. In a subsequent rinsing step, 10-20 ml of normal saline were forced through the filter holder. Residual fluid was removed by forcing 5 ml of air through the assembled filter device. With the aid of a pair of forceps, the membrane filter was removed from the filter holder and transferred on a slide where it was air-dried first and subsequently fixed with methanol for 1 min. This was followed by staining with Giemsa for 20 min (Mehlhorn, Düwel & Raether 1993). The filter was then allowed to dry and mounted in Entellan® (Merck). With the aid of a compound microscope, the stained membrane filters were examined at 40x magnification for the presence of microfilariae.

3.4.3 Acid phosphatase staining

To identify the filarial species involved, microfilariae in positive blood samples were first concentrated by means of the modified Knott's technique (Knott 1939). For this purpose, one ml of EDTA blood and 9 ml of 2 % formalin were mixed and centrifuged at 500 g for 5 min. After discarding the supernatant, three drops of the microfilariae-containing sediment were transferred on a slide, spread with the tip of a Pasteur pipette, air-dried and subsequently fixed with acetone. For acid phosphatase staining the technique described by Yen & Mak (1978) was employed. With the aid of a compound microscope, the stained microfilariae were examined at 100x and 200x magnification for species-specific differences in the somatic staining patterns (Acevedo, Theis, Kraus & Longhurst 1981; Beugnet, Costa & Lambert 1993; Ducos de Lahitte, Ducos de Lahitte & Davoust 1993; Valcárcel, Ferre, Gómez-Bautista & Rojo-Vázquez 1990).

3.5 Statistical Analysis

The data were entered into a Microsoft Excel spreadsheet and data quality was verified against raw data to minimize errors. Prevalence or infection rates were determined by dividing the total number of positive samples by the total number of samples tested (Thrusfield 1995; Hyera, Letshwenyo, Monyame, Thobokwe, Pilane, Mapitse & Baipoledi 2006) and was expressed as percentage. Relative frequency (RF) was also calculated according to Thrusfield (1995) and was also expressed as percentage. The standard error (SE) of percentages at the 95% confidence interval as well as the standard error difference (SE diff) was calculated according to the method by Swinscow (1980). The statistical table of Armitage (1971) was used to assess the statistical significance between infection rates. Probability (P) values < 0.05, < 0.01, and < 0.001 were considered respectively, as significant, very significant and highly significant.

Figure 3.1: Map of Botswana with neighbouring countries

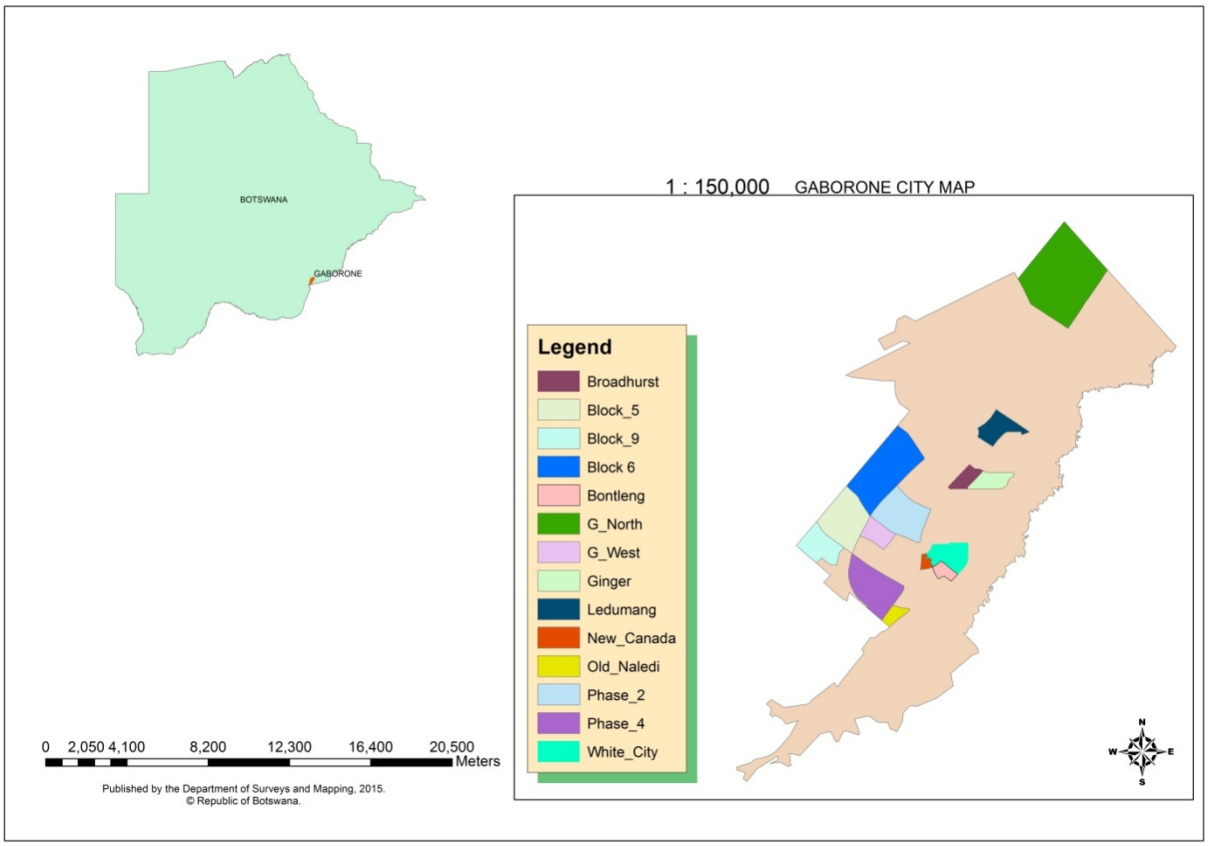

Figure 3.2: Map of Gaborone with suburbs

Chapter 4
RESULTS

4.1. Overview

A total of 150 dogs were sampled during the survey of which 66 (44 %) were female and 84 (56 %) were male. The ages of the dogs ranged from 1 to 19 years. Dog breeds which featured in the survey were the local breeds Tswana (Fig. 4.1), Sticky R (Fig 4.2), Grey Dorper, and Titie (Fig 4.3) as well as crossbreeds, Greyhound, Boerboel, German shepherd, Rottweiler, and Boxer. The indigenous Tswana (74.67 %) was the most dominant breed in the survey and this was followed by the crossbreeds (14.67 %). The dogs belonged to owners from 14 suburbs of Gaborone (Table 4.1, Table 4.2).

4.2 Gastrointestinal helminth infections

Based on faecal examination, 96 out of the 150 dogs (64.0 %) were found to be infected with gastrointestinal helminths and a coccidia (Table 4.3). The spectrum of gastrointestinal parasites included *Ancylostoma spp, D. caninum, T. canis* and *Isospora* spp. The prevalences of the helminth and *Isospora* sp infections in dogs of the various suburbs of Gaborone varied from zero to 100 % (Table 4.3).

Ancylostoma spp was found to be by far the most prevalent gastrointestinal helminth species. Of the 96 infected dogs, 82 (85.42 %) were diagnosed positive for *Ancylostoma* spp only and 14 (14.58 %) dogs had multiple infections (Table 4.3). Thirteen out of 14 multiple infected (13.54 %) dogs were found to be infected with one additional gastrointestinal parasite apart from *Ancylostoma* spp, namely *D. caninum* (6/14), *Isospora* spp (5/14) and *T. canis* (2/14) (Table 4.3).

One out of 14 multiple infected dogs (1.04 %; 1/96) with two additional gastrointestinal parasites apart from *Ancylostoma* spp, namely *D. caninum* and *Isospora* spp (Table 4.3).

In the 1–5 year-old age group the prevalence of *Ancylostoma* spp infection was 53.79 % (71/132), in the 6-10 year-old age group the prevalence was 61.54 % (8/13) and in the ≥ 11 year-old age group the prevalence was 60 % (3/5). There was no statistically significant difference in the prevalence of *Ancylostoma* spp infection between the 1-5-year-old age group and the 6-10-year-old age group ($p > 0.05$) (Table 4.4).

However, there was a statistical significant relationship between the overall prevalence of *Ancylostoma* spp infection and gender ($p < 0.001$). Male dogs had the highest overall prevalence rate with 76 % (56/85) compared to 40 % (26/65) (Table 4.5).

4.3 Filarial helminth infections

Out of the 150 dogs examined 27 (18.0 %) were found positive for microfilariae on membrane filtration. By means of acid phosphatase staining three species were identified, namely *D. repens, A. reconditum* and *A. dracunculoides,* with *D. repens* figuring as the predominant filarial species (Table 4.6).

Twenty-two out of the 150 dogs sampled (14.67 %) were positive for microfilariae of *D. repens.* Infection with this filarial helminth was only diagnosed in the Tswana breed and crossbreeds. Although the prevalence of *D. repens* was higher in crossbreeds (31.82 %) than in the Tswana breed (13.39 %), the difference was not statistically significant ($p > 0.05$) (Table 4.7).

Regarding the difference in prevalence of *D. repens* infection by age, the ≥11-year-old age group had the highest mean overall prevalence rate with 40 % compared to 30.77 % in the 6-10-year-old age group and 12.12 % in the 1-5-year-old age group. There was no statistically significant difference in the prevalence of infection between the age groups ($p > 0.05$) (Table 4.8).

The mean overall prevalence of *D. repens* infection in male dogs was 16.47 % and in female dogs 12.30 %. Statistically, there was no significant difference in the prevalence of *D. repens* infection between male and female dogs ($p > 0.05$) (Table 4.9).

Infection with *A. reconditum* was diagnosed in 4 of the 150 samples analyzed, giving a prevalence of 2.67 %. The 4 samples were collected from 3 male and 1 female dogs of the Tswana breed.

Infection with *A. dracunculoides* was diagnosed in only 1 of the 150 dogs sampled, giving a prevalence of 0.67 %. The dog which tested positive was a male of the Tswana breed.

Figure 4.1: Local dog breed 'Tswana'

Figure 4.2: Local dog breed 'Sticky R'

Figure 4.3: Local dog breed 'Tities'

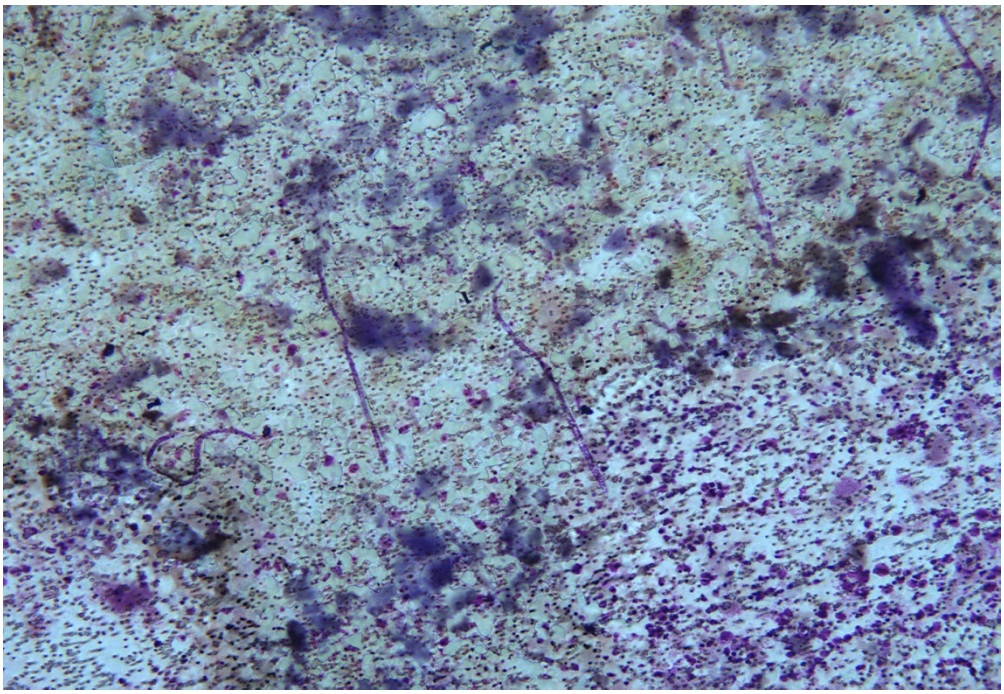

Fig. 4.3: Giemsa-stained polycarbonate membrane filter with microfilariae

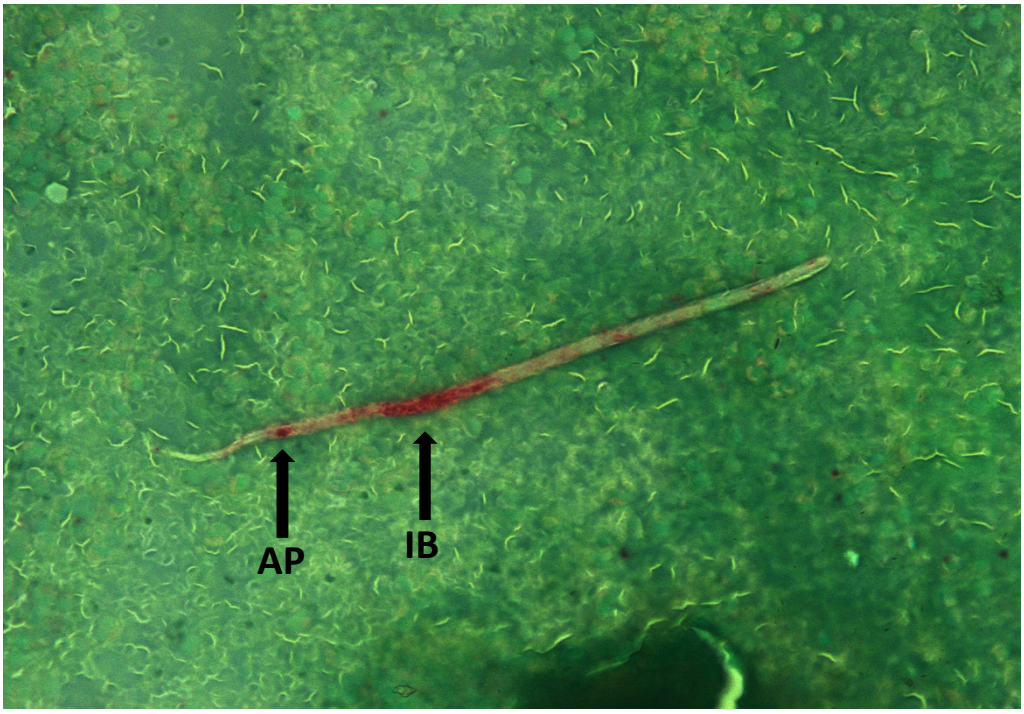

Fig. 4.4: *Dirofilaria repens* microfilaria: Acid phosphatase activity at the anal pore (**AP**) and innerbody (**IB**)

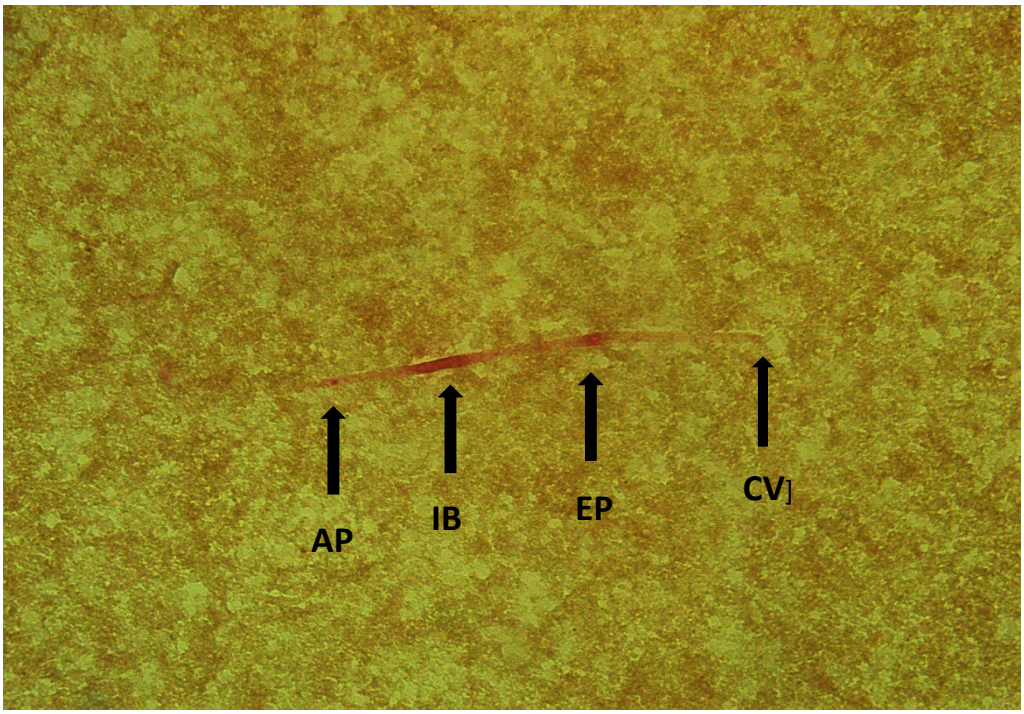

Fig. 4.5 *Acanthocheilonema dracunculoides* microfilaria: Acid phosphatase activity at the anal pore **(AP)**, innerbody **(IB)**, excretory pore **(EP)** and cephalic vesicle **(CV)**

Table 4.1: Overview of dogs sampled according to Gaborone suburbs

Suburb	Male	Female	Total [%]
Broadhurst	0	1	1 (0.67 %)
Block 5	3	4	7 (4.67 %)
Block 9	2	2	4 (2.67 %)
Block 6	15	19	34(22.67 %)
Bontleng	8	10	18 (12.00 %)
G/North	3	0	3 (2.00 %)
G/West	10	5	15 (10.00 %)
Ginger	1	2	3 (2.00 %)
Ledumang	14	8	22 (14.67 %)
New Canada	2	5	7 (4.67 %)
Old Naledi	17	7	24 (16.00 %)
Phase 2	2	0	2 (1.33 %)
Phase 4	4	0	4 (2.67 %)
White City	4	2	6 (4.00 %)
Total	**85**	**65**	**150**

Table 4.2: Overview of dogs sampled according to breed and sex

Dog Breed	Male	Female	Total [%]
Tswana	58	54	112 (74.67 %)
Cross	15	7	22 (14.67 %)
Greyhound	4	1	5 (3.33 %)
Boerboel	3	1	4 (2.67 %)
German Shepherd	2	-	2 (1.33 %)
Rottweiler	1	-	1 (0.67 %)
Boxer	-	1	1 (0.67 %)
Sticky R	-	1	1 (0.67 %)
Grey Dorper	1	-	1 (0.67 %)
Tities	1	-	1 (0.67 %)
Total No	**85**	**65**	**150 (56.67 % ♂, 43.33 % ♀)**

Table 4.3: Overall faecal examination results of dogs from Gaborone by suburb

Suburb	No examined	*Ancylostoma* spp	*Ancylostoma* spp & *D. caninum*	*Ancylostoma* spp & *Isospora* spp	*Ancylostoma* spp & *T. canis*	*Ancylostoma* spp & *Isospora* spp & *D. caninum*
Broadhurst	1	0	0	0	0	0
Block 5	7	1 (14.29 %)	0	0	0	0
Block 9	4	2 (50 %)	0	0	0	0
Block 6	34	20 (58.82 %)	2 (5.88 %)	1 (2.94 %)	0	0
Bontleng	18	9 (50 %)	0	0	1 (5.56 %)	0
G/North	3	3 (100 %)	0	0	0	0
G/West	15	11 (73.33 %)	0	0	1 (6.67 %)	0
Ginger	3	2 (66.67 %)	0	0	0	0
Ledumang	22	13 (59.09 %)	2 (9.09 %)	2 (9.09 %)	0	0
New Canada	7	1 (14.29 %)	0	0	0	0
Old Naledi	24	14 (58.3 %)	2 (8.33 %)	1 (4.17 %)	0	1 (4.17 %)
Phase 2	2	0	0	0	0	0
Phase 4	4	2 (50 %)	0	1 (25 %)	0	0
White city	6	4 (66.67 %)	0	0	0	0
Total	150	**82 (54.6 %)**	**6 (4 %)**	**5 (3.33 %)**	**2 (1.33 %)**	**1 (0.67 %)**

Table 4.4: Overall prevalence of *Ancylostoma* spp infection in dogs from Gaborone by age group

Age groups (years)	No of dogs examined	Faecal exam positive	Prevalence (%)
1-5	132 (74 ♂, 58 ♀)	71 (47 ♂, 24 ♀)	53.79% (35.6 % ♂, 18.18 % ♀)
6-10	13 (7 ♂, 6 ♀)	8 (6 ♂, 2 ♀)	61.54% (46.15 % ♂, 15.38 % ♀)
≥ 11	5 (4 ♂, 1 ♀)	3 (3 ♂)	60 %
Total	**150 (85 ♂, 65 ♀)**	**82**	**54.67 %**

Table 4.5: Overall prevalence of *Ancylostoma* spp infection in dogs by gender

Gender	Number of dogs infected with *Ancylostoma* spp	% Positive
Male	56/85	68.29
Female	26/65	31.71
Total	**82/150**	**54.67 %**

Table 4.6: Overall filarial prevalence in dogs from Gaborone by suburb

Suburb	No of dogs examined and positive	*D. repens* microfilariae	*A. reconditum* microfilariae	*A. dracunculoides* microfilariae
Broadhurst	1/0	0	0	0
Block 5	7/1	1 (14.29 %)	0	0
Block 9	4/2	2 (50 %)	0	0
Block 6	34/8	7 (20.59 %)	1 (2.94 %)	0
Bontleng	18/3	2 (11.11 %)	0	1 (5.56 %)
G/North	3/1	1 (33.33 %)	0	0
G/West	15/4	4 (26.67 %)	0	0
Ginger	3/0	0	0	0
Ledumang	22/4	4 (18.18 %)	0	0
New Canada	7/1	1 (14.29 %)	0	0
Old Naledi	24/3	0	3 (12.5 %)	0
Phase 2	2/0	0	0	0
Phase 4	4/0	0	0	0
White city	6/0	0	0	0
Total	**150/27 (18 %)**	**22 (14.67 %)**	**4 (2.67 %)**	**1 (0.67 %)**

Table 4.7: Overall prevalence of *D. repens* in dogs from Gaborone by breed

Breeds	No examined	Microfilaria positive	% Positive
Tswana	112 (58 ♂, 54 ♀)	15 (9 ♂, 6 ♀)	13.39 (8.04 ♂, 5.36 ♀)
Crossbreed	22 (15 ♂, 7 ♀)	7 (5 ♂, 2 ♀)	31.82 (22.73 ♂, 9.09 ♀)

Table 4.8: Overall prevalence of *D. repens* in dogs from Gaborone by age

Age groups (years)	No examined	Microfilaria positive	% Positive
1-5	132 (74 ♂, 58 ♀)	16 (10 ♂, 6 ♀)	12.12 % (7.56 ♂, 4.55 ♀)
6-10	13 (7 ♂, 6 ♀)	4 (2 ♂, 2 ♀)	30.77 % (15.38 ♂, 15.38 ♀)
≥ 11	5 (4 ♂, 1 ♀)	2 (2 ♂)	40 %
Total	**150**	**22**	**14.67 %**

Table 4.9: Overall prevalence of *D. repens* infection in dogs by gender

Gender	Number of dogs infected with *D. repens*	% Positive
Male	14/85	16.47 %
Female	8/65	12.30 %
Total	**22/150**	**14.67 %**

Chapter 5
DISCUSSION

Most information regarding gastrointestinal and filarial helminth infections of domestic carnivores in the Southern African region is available from South Africa. In Botswana the occurrence and prevalence of helminths in domestic carnivores is largely unknown with only scant information available from the Annual/Monthly Reports of the Botswana National Veterinary Laboratory. In a first attempt to remediate this situation a survey was conducted on domestic dogs in the metropolitan area of Gaborone in Botswana, focussing on the occurrence and prevalence of gastrointestinal and filarial helminth infections of domestic dogs. In this survey helminth infections were found to be more prevalent than gastrointestinal protozoal infections, which is in accordance with results obtained in a survey conducted in neighbouring South Africa (Mukaratirwa & Singh 2010).

5.1 Gastrointestinal helminth infections

Dog owners in the metropolitan area of Gaborone are well informed about rabies vaccination but have very little awareness concerning endoparasites and their zoonotic implications (Sehularo 1995). There was not a single record of deworming concerning the dogs which were sampled for the survey and the only document that was readily available was a rabies vaccination certificate. Dogs in Botswana are generally poorly managed and roam around in the streets scavenging for food. Similar observations have been reported from other African countries (Yacob, Ayele, Fikru & Basu 2007).

The direct faecal flotation results indicate the endemic status of *Ancylostoma* spp, *T. canis* and *D. caninum* as well as the protozoal coccidian *Isospora* sp. The overall prevalence of gastrointestinal helminth infections with 1 or more species found in this survey was 64 % and

this finding is in agreement with results obtained from surveys conducted in other African countries (Islam & Chizyuka 1983; Mukaratirwa & Busayi 1995; Yacob, Ayele, Fikru & Basu 2007; Swai, Kaaya, Mshanga & Mbise 2010; Zwedu, Semahegn, Y. & Mekibib, B. 2010; Minnaar, Krecek & Fourie 2002). The gastrointestinal helminth infection prevalences found in this survey are lower when compared to results obtained from surveys conducted in Hawassa town, Ethiopia (Paulos, Addis, Fromsa & Mekibib 2012; Zwedu *et al.* 2010), with prevalences of, respectively 86.9 % and 86.54 %, Zambia with 78.4 % (Bwalya, 2012) and Durban in South Africa with 82.5 % (Mukaratirwa & Singh 2010).

With an overall prevalence rate of 64 %, *Ancylostoma* sp *was* the most prevalent gastrointestinal helminth species diagnosed in this survey and this is in accordance with the findings of surveys conducted in South Africa (Ortlepp 1934; Verster 1979; Minnaar *et al.* 1999; Minnaar & Krecek 2001; Minnaar *et al.* 2002; Mukaratirwa & Singh 2010), Zambia (Islam & Chizyuka 1983), Zimbabwe (Obwolo *et al.* 1991; Mukaratirwa & Busayi 1995); Minnaar *et al.* 1999) Tanzania (Swai *et al.* 2010) and Zaire (Schandevyl, Mbundu & Sumbu 1987). A prevalence rate of 100 % is reported from Chinamora communal land dogs in Zimbabwe with no clinical signs associated with infection (Obwolo *et al.* 1991). *Ancylostoma* species described from domestic dogs in Africa are *Ancylostoma caninum* and *Ancylostoma braziliense* (Soulsby 1982). As other animal hookworm species, both have zoonotic implications as causative agents of cutaneous larva migrans, with *A. braziliense* regarded as more significant (Bowman, Montgomery, Zajac, Eberhard & Kazacos 2010). Since the survey was based on faecal examination, the exact *Ancylostoma* species involved could not be determined since the morphometrical data of the eggs of *A. caninum* and *A. braziliense* are overlapping (Levine 1980; Soulsby 1982). Eosinophilic enteritis in humans as the result of intestinal infection with *A. caninum* has first been described from northeastern Australia (Prociv & Croese 1990). A higher prevalence was observed in male dogs (68.29 %) than female dogs and the difference was statistically highly significant. The finding is consistent with results obtained in surveys conducted in Brazil and Argentina (Oliveira-Sequeira, Amarante, Ferrari & Nunes 2002; Fontanarrosa, Vezzani, Basabe & Eiras 2006). Considering the high prevalence of *Ancylostoma* spp infection in dogs of Gaborone, reports of pyrantel-resistant strains of *A. caninum* (Jackson,

Lance, Townsend & Stewart 1987; Kopp, Coleman, McCarthy & Kotze 2008) are of a concern since the drug is low cost compared to other actives and readily available in various supermarket chains in Botswana. Unfortunately, no surveys have ever been conducted in Africa concerning emerging anthelmintic resistance in the gastrointestinal helminths of dogs and cats.

Toxocara canis prevalence in this survey was 1.33 % and very much lower than reported from South Africa (Ortlepp 1934), Zambia (Islam & Chizyuka 1983) and Zimbabwe (Obwolo *et al.* 1991; Mukaratirwa & Busayi 1995; Mukaratirwa & Singh 2010). The lower prevalence rate observed amongst the dogs in Gaborone could be explained by the exclusion of dogs younger than 1 year of age. Toxocarosis is mainly acquired by puppies through the transplacental and lactogenic route and infection stimulates immunity (Oberem, Krieck & Lange 2015).

Egg capsules of *D. caninum* were only recovered from a single faecal sample, giving a prevalence of 0.67 %. This is in sharp contrast to findings from South Africa were in a survey conducted in Free State Province *D. caninum* was the most common helminth and was recovered from 44 % of dogs (Minnaar *et al.* 2002). Minnaar and Krecek (2001) suggest that a reliable method for diagnosing patent infections of *D. caninum* is currently lacking.

There was no evidence of infection with other cestodes by direct faecal flotation and it is suggested that in further surveys adhesive tape swabs from the perianal area are taken which is regarded more sensitive to diagnose carriers of particularly taeniids (Deplazes & Eckert 1988).

5.2 Filarial helminth infections

Despite a survey for parasitism in animals carried out by FAO, WHO and OIE (1984) which points out that filarial infections of domestic dogs are widespread on the African continent, there is a lack of published reports regarding the occurrence and prevalence of filarial helminths in domestic carnivores. Published surveys, case reports and diagnostic results of routine examinations for filarial infections obtained from dogs and cats originating from foreign

countries present evidence that 5 filarial helminth species, namely *D. immitis, D. repens, A. reconditum, A. dracunculoides* and *Brugia patei* are endemic in Africa(Schwan 2009).

In Gaborone the survey was conducted on 150 EDTA blood samples. The overall prevalence of filarial helminth infection in dogs was 18 % (27/150) based on membrane filtration for microfilariae. By means of acid phosphatase staining, the microfilariae were identified as those of *D. repens, A. reconditum* and *A. dracunculoides.* With 14.67 %, *D. repens* was the most prevalent species. Earlier records of filarial infections of dogs in Botswana are found in the BNVL Annual Reports from 1987, 1995 and 2001 based on the mere demonstration of microfilariae in stained bloodfilms. According to these reports microfilariae were those of *Dipetalonema* (now *Acanthocheilonema*), *D. immitis* and *Dipetalonema reconditum* (now *A. reconditum*). Since not even morphometrical data are furnished, the records are inconclusive.

Microfilariae of *D. repens* were first diagnosed by acid phosphatase staining in a crossbreed in 1999 and again in 2004 from a Rhodesian Ridgeback, both from Gaborone, as part of the compulsory routine examination for filarial infections of dogs imported from foreign countries into South Africa (Schwan 2009). The prevalence of *D. repens* in dogs in the current survey was 14.67 % which should be regarded as high. *Dirofilaria repens* is also endemic in Namibia, Zambia, Zimbabwe, Mozambique and South Africa (Schwan 2009). In South Africa the species is particularly prevalent in dogs (12.47 %) and cats (10-98 %) in the coastal areas of KwaZulu-Natal and the Eastern Cape (Schwan 2009). Microfilariae of *D. repens* have only on 1 occasion been demonstrated in a dog from North-West Province which is bordering on Botswana (Schwan 2009). Based on the current information, *D. repens* is widespread on the continent and is endemic in 20 African countries (Schwan 2009)). Anopheline and culicine mosquitoes of the genera *Anopheles, Aedes, Culex, Mansonia* and *Taeniorhynchus* have been identified as vectors (Pampiglione *et al.* 1995). Awareness of the high prevalence of *D. repens* should be created amongst veterinarians and animal health technicians to check the microfilarial status of dogs and cats before choosing dewormers containing macrocyclic lactone actives. Although rare, there is evidence from South Africa and Namibia where *D. repens* infected cats and dogs developed a shock-like syndrome which can be fatal (Oberem *et al.* 2015). Apart from studies

conducted in Kenya, eastern Africa, (Nelson, Heisch & Furlong 1962), the mosquito vectors involved in the transmission of *D. repens* in southern Africa are unknown. Human dirofilariasis caused by *D. repens* is well documented and a case was recently reported in South Africa (Moodley *et al.* 2015).

The endemic status of *A. reconditum* in dogs was first confirmed in a dog from Gaborone in 2000, as part of the compulsory routine examinations for filarial infections of dogs imported from foreign countries into South Africa (Schwan 2009). The prevalence of *A. reconditum* in dogs in the current survey was 2.67 %. In the southern African region, *A. reconditum* is also endemic in South Africa (Schwan 2009). It was recently also reported from dogs in Lusaka (Siwila *et al.* 2015). In Mpumalanga Province of South Africa the prevalence was particularly high (29.13 %), followed by KwaZulu-Natal with 5 % (Schwan 2009). In North-West Province, which is bordering on Botswana, the species has only been isolated on a single occasion from a dog in 2001 (Schwan 2009). Vectors identified are *Ctenocephalides felis* and *Ctenocephalides canis* as well as the lice species *Heterodoxus spiniger* and *Linognathus setosus* (Levine 1980). *Acanthocheilonema reconditum* has only very minor veterinary importance, as the species is regarded as largely nonpathogenic. Based on the current information, *A. reconditum* is known to be endemic in 9 African countries (Schwan 2009).

Acanthocheilonema dracunculoides was diagnosed in only 1 of the 150 dogs sampled, giving a prevalence 0.67 %. The endemicity of *A. dracunculoides* in Botswana confirms claims by Nelson (1963) that the species is widespread in the more arid areas of Africa. *Acanthocheilonema dracunculoides* is also endemic in South Africa, Namibia and Zimbabwe. Apart from Botswana, *A. dracunculoides* has been recorded in 13 African countries. The louse fly *Hippobosca longipennis* as well as the kennel tick *Rhipicephalus sanguineus* have been identified as vectors (Nelson 1963; Olmeda-García, Rodríguez-Rodríguez & Rojo-Vázquez 1993). *Rhipicephalus sanguineus* is widespread throughout Africa and also serves as a vector of canine monocytic ehrlichiosis in southern Africa. *Acanthocheilonema dracunculoides* is regarded as only minor pathogenic (Montaron 1975), however there are some reports were dermal clinical signs and

lesions as well as nervous clinical signs were associated with infection (Rodríguez 1990; Bolio, Montes, Gutierrez, Alonso, Bernal, Sauri & Rodríguez-Vivas 2002; Schwan & Schröter 2006).

Chapter 6
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APPENDIX 1 ANIMAL ETHICS APPROVAL

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Gastrointestinal and filarial helminth infections of domestic dogs in Gaborone, Botswana**
PROJECT NUMBER	**V037-14**
RESEARCHER/PRINCIPAL INVESTIGATOR	**K Ntesang**

STUDENT NUMBER (where applicable)	**10 646 303**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Canine**	
NUMBER OF ANIMALS	**150**	
Approval period to use animals for research/testing purposes		**June 2014 - July 2015**
SUPERVISOR	**Dr. EV Schwan**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	30 June 2014
CHAIRMAN: UP Animal Ethics Committee	Signature	