Survey for filarial helminth infections of domestic dogs in Mahikeng, North West Province, South Africa

By

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There is a dearth of information pertaining to the occurrence and prevalence of filarial helminth infections of dogs and cats in several provinces in South Africa. In this context the dissertation is a pilot survey that was conducted to determine the occurrence and prevalence of filarial helminth infections of dogs in the greater Mahikeng Local Municipality of North West province. The incentive for the choice of this location in the North West province was a reported high overall prevalence of 18 % of canine filariosis in the town of Gaborone in neighbouring Botswana. EDTA blood samples were collected from 100 live dogs between the ages of 1-11 years (53 males and 47 females). Only dogs that had not received treatment with macrocyclic lactone actives during the previous 12 months were included in the survey. A total of 13 different dog breeds were involved in the survey and the crossbreed animals were the most abundant (59 %) followed by the Boerboel (9 %). Blood samples were screened for the presence of microfilariae by means of the membrane filtration technique. There was no evidence of filarial infections in the 100 dogs sampled.

Chapter 1
Introduction

Evidence suggests that the domestic dog (*Canis familiaris*) most likely diverged from wolves (*Canis lupis*) as long as 135 000 years ago (Vila, Savolainen, Maldonado, Amorim, Rice, Honeycutt, Crandall, Lundeberg & Wayne 1997). Humans and dogs share a long history as it is believed that dogs were most likely the first animals to be domesticated (Udell & Wynne 2008). Over time, a companionship has evolved which not only physically assists humans in herding, hunting, guarding and guiding but also makes important contributions socially and psychologically (Udell & Wynne 2008).

Several species of filarial parasites, such as *Dirofilaria immitis*, *Dirofilaria repens*, *Acanthocheilonema reconditum*, *Acanthocheilonema dracunculoides* and *Cercopithifilaria grassii*, have been described in the domestic dog (Genchi, Rinaldi, Cascone, Mortarino & Cringoli 2005). Of these species, it appears that *Dirofilaria immitis* is capable of spreading into non-endemic areas (Pantchev, Etzold, Daugschies & Dyachenko 2011). Important risk factors for the continuous spread of *D. immitis* include the increased movement of people with their pets and climatic changes which influence the distribution of the vector (Pantchev *et al*. 2011).

With the exception of *D. immitis,* filarial helminth infections are not as important as gastrointestinal helminth infections in domestic carnivores in terms of morbidity and mortality (Schwan 2009). However, with the introduction of macrocyclic lactone actives for deworming and ectoparasite control, particularly *D. repens* infections have become significant, since fatal side effects, although rare, can be observed following treatment of microfilaraemic dogs and cats (Schwan, Miller, de Kock & Van Heerden 2000).

Dirofilaria immitis and *D. repens* are two species of filarial nematodes of domestic carnivores which also have zoonotic implications (Muro, Genchi, Cordero & Simon 1999). Human dirofilariasis typically presents as subcutaneous or ocular masses (*D. repens*) or as pulmonary nodular lesions (*D. immitis*) (Muro *et al.* 1999). It is possible that the zoonosis

occurs more frequently than indicated in the literature, since it is suspected that cases are underdiagnosed, underreported or recovery occurs spontaneously without medical intervention. (Pampiglione, Canestri & Rivasi, 1995). Two cases of human dirofilariosis caused by *D. repens* have recently been reported in South Africa, one from the KwaZulu Natal province and the other from the Gauteng province. (Moodley, Govind, Peer, van der Westhuizen, Parbhoo, Sun, du Plessis & Frean 2015).

To determine the occurrence and prevalence of filarial nematode infections in dogs in South Africa a survey was conducted from 2001 to 2003 in Gauteng, Kwa-Zulu Natal and Mpumalanga provinces (Schwan 2009). Apart from isolated records very little or no information is available on the occurrence and prevalence of filarial infections in the remaining six provinces of South Africa. To complete the picture, the greater Mahikeng Local Municipality in North West Province was chosen as a locality for this survey. A further incentive for the geographical choice was a survey conducted in neighbouring Botswana, where 18 % of dogs in nearby Gaborone were found to be infected with filarial helminths (Ntesang 2016).

Several breeds of dogs are kept in the greater Mahikeng Local Municipality. The Cross breed animal are the most common, followed by Boerboel, Grey Hound, Great Dane and Pitbull Terrier. The results of this survey will contribute to the knowledge on the occurrence and prevalence of filarial helminths of dogs in South Africa.

Chapter 2
Literature review on the occurrence and prevalence of filarial helminth infections of dogs in South Africa and neighbouring countries

Canine filarial helminths are nematodes of the superfamily Filarioidea which are transmitted by haematophagous arthropods such as fleas, lice, ticks and mosquitoes (Rojas, Rojas, Montenegro & Baneth 2015; Bowman & Mannella 2011). The Filarioidea is a large superfamily within the order Spirurida, which include parasites of the tissue, tissue spaces, lymphatic vessels and body cavities of vertebrates, except fish (Bowman & Mannella 2011).

A total of ten filarial helminth species have been reported worldwide in domestic carnivores (Schwan 2009), namely *D. immitis, D. repens*, *A. reconditum, A. dracunculoides, Brugia ceylonensis, Brugia malayi, Brugia pahangi, Brugia patei, Cercopithifilaria bainae* and *C. grassii* (Table 2.1).

Dirofilaria immitis, D. repens, A. reconditum and *A. dracunculoides* are the most common species found in domestic dogs (Rojas *et al*. 2015).

2.1 A check-list of filarial helminths of carnivores reported on the African continent and its islands

Published reports of filarial infections of domestic and sylvatic carnivores in Africa indicates the endemnicity of *D. immitis, D. repens, A. reconditum, A. dracunculoides, C. grassii* and *B. patei* (Nelson, Heisch & Furlong 1962; Schwan 2009). The objective of the checklist is to furnish background information to the original records of filarial helminths of domestic and sylvatic carnivores occurring in Africa and its islands. The format adopted in the compilation of the check-list is that of Round (1968) and Khalil & Polling (1997). In this checklist, the hosts are arranged alphabetically. The filarial helminths are listed under their scientific names, including their synonyms, and the domestic/sylvatic carnivore host and country/island from which they were recorded. The classification of the nematodes is done according to the system of Anderson, Chabaud & Willmott (1974).

PARASITE/HOST CHECK-LIST

Nematoda

Order Spirurida

Superfamily Filarioidea

Family FILARIIDAE/ ONCHOCERCIDAE (Anderson *et al*. 1974)

GENUS *DIROFILARIA* (Anderson *et al*. 1974)

1. *Dirofilaria immitis* (Leidy 1856)

Filaria canis cordis (Leidy 1856; Lok 1988); *Filaria immitis* (Leidy 1856; Lok 1988; Canestri, Pampiglione & Rivasi 1997); *Dirofilaria fausti* (Lok 1988); *Dirofilaria louisianensis* (Lewis, Williams & Tinsley 1969) and *Dirofilaria nasuae* (Lok 1988).

Canis familiaris

Tahir, Damene, Davoust & Parola (2017), **Algeria**
Abd El Rahim (1998), Egypt
Pandey, Dakkak & Elmamoune (1987), **Morocco**
Rjeibi, Rouatbi, Mabrouk, Tabib, Rekik & Gharbi (2017), **Tunisia**
Montoya, Morales, Juste, Banares, Simon & Genchi (2006), **Canary Islands**
Tendeiro (1949), **Guinnea Bassau**
Bobade, Ojebuoboh & Akinboade (1981), **Nigeria**
Pangui & Kaboret (1993), **Senegal**
Kamara (1977), **Sierre Leone**
Serrano (1962), **Angola**
Thys, Sawa & Guissart (1982), **Cameroon**
Beugnet & Edderai (1998), **Gabon**
Graber (1975), **Ethiopia**
Nelson *et al.* (1962), **Kenya**
Fitzsimmons (1966), **Malawi**
Schwan & Durand (2002), **Mozambique**
Alley (1950), **Tanzania**
Daynes (1964), **Madagascar**
Sibartie, Beeharry & Jaumally (1983), **Mauritius**
Prunaux & Guignard (1991), **Reunion**
Verster, Cilliers & Schroeder (1991) ; Schwan (2009) , **South Africa**
Schwan (2009) **Zimbabwe**

2. *Dirofilaria repens* (Railliet & Henry 1911)

Filaria palpebralis (Stuckey 1917) and *Filaria repens* (Buttiker 1948).

Canis aureus

Myers, Kuntz & Wells (1962), **Egypt**

Canis familiaris

Abdel-Rahman, Mahmoud, Galal, Gustinelli & Pampiglione (2008), **Egypt**
Rjeibi *et al*. (2017), **Tunisia**
Schillhorn van Veen & Blotkamp (1975), **Nigeria**
Nelson et al. (1962) **Kenya**
Bwangamoi & Isyagi (1973), **Uganda**
Siwila, Mwase, Nejsum & Simonsen (2015), **Zambia**
Schwan (2009), **Mozambique**
Jooste (1990), **Zimbabwe**
Schwan et al. (2000); Schwan (2009), **South Africa**
Ntesang (2016), **Botswana**
Schwan (2009), **Namibia**

Felis catus

Chatton (1918), **Tunisia**
Nelson et al. (1962) **Kenya**
Jooste (1990), **Zimbabwe**
Schwan (2000), **South Africa**

Genetta tigrina

Heisch, Nelson & Furlong (1959), **Kenya**

Panthera leo

Kellas & Webber (1955), **Sudan**
Graber, Euzeby, Gevrey, Troncy & Thal (1972), **Central African Republic**
Le Roux (1958), **Zambia**

GENUS *ACANTHOCHEILONEMA (*Napoli, Gaglio, Falsone, Giannetto, Dantas-Torres, Otranto & Brianti 2014)

1. *Acanthocheilonema reconditum (Napoli et al. 2014)*

Filaria recondita (Brianti, Gaglio, Napoli & Giannetto 2012); *Dipetalonema reconditum* (Sonin 1985)

Canis aureus

Nelson *et al*. (1962), **Kenya**

Canis familiaris

Diller (1947), **Liberia**
Bobade *et al.* (1981), **Nigeria**
Nelson *et al*. (1962), **Kenya**
Schwan & Durand (2002), **Mozambique**
Bwangamoi & Isyagi (1973), Schwan (2009), **Uganda**
Schwan (2009), **South Africa**
Ntesang (2016), **Botswana**

Crocuta crocuta

Nelson et al. (1962), **Kenya**

Hyaena brunnea

Nelson et al. (1962), **Kenya**

2. *Acanthocheilonema dracunculoides (Napoli et al. 2014)*

Acanthocheilonema dagestanica (Bain & Beaucournu 1974); Microfilaria sp. (Webber & Hawking 1955); Microfilaria lewisi (Wolfe, Aslamkhan, Sharif & Perves 1971) and Dipetalonema dracunculoides (Sonin 1985).

Canis familiaris

Schwan (2009), **Algeria**
Hawking (1957), **Morocco**
Kirk (1957), **Sudan**
Bernard et al. (1967), **Tunisia**
Schillhorn van Veen *et al.* (1975), **Nigeria**
Gedoelst (1916), **Democratic Republic of Congo**
Nelson *et al. (*1962) **Kenya**
Schwan (2009), **Mozambique**
Schwan & Schröter (2006), **Namibia**
Ntesang (2016), **Botswana**
Schwan (2009), **South Africa**

Crocuta crocuta

Railliet, Henry & Langeron (1912), **Mali**
Mukendi, Kimbita, Mbanzulu, Maindo & Misinzo (2016), **Tanzania**
Jooste (1990), **Zimbabwe**

Hyaena (species not specified)

Nelson *et al.* (1962) **Kenya**

*GENUS CERCOPITHIFILARIA (*Cortes, Cardoso, Giannelli, Latrofa, Dantas-Torres & Otranto, 2014)

1. *Cercopithifilaria grassii* (Cortes *et al*. 2014)

Filaria grassii (Magi, Guardone, Prati & Tozzini 2012); Acanthocheilonema grassii (Brianti, Gaglio, Napoli & Giannetto 2012) Dipetalonema grassii (Magi et al. 2011).

Canis familiaris

Heisch *et al.* (1959); Nelson *et al*. (1962), **Kenya**

2. *Cercopithifilaria baineae* (Cortes *et al*. 2014)

The occurrence has not been recorded in a domestic/sylvatic carnivore host on the African continent to date.

GENUS BRUGIA (Buckley, Nelson & Heisch, 1958)

1. *Brugia patei (Buckley et al. 1958)*

Wuchereria patei (Sonin 1985)

Canis familiaris
Nelson & Heisch (1957), **Kenya**
Schwan (2009), **Tanzania**

Felis catus
Nelson et al. (1962), **Kenya**

2. *Brugia ceylonensis (Jayewardene, 1963)*

The occurrence has not been recorded in a domestic/sylvatic carnivore host on the African continent to date.

3. *Brugia malayi (Buckley et al. 1958)*

The occurrence has not been recorded in a domestic/sylvatic carnivore host on the African continent to date.

4. *Brugia pahangi (Buckley et al. 1958)*

The occurrence has not been recorded in a domestic/sylvatic carnivore host on the African continent to date.

2.2 Studies on filarial infections of domestic dogs in South Africa and its neighbouring countries

2.2.1 South Africa

In a survey conducted between 2001 and 2003 to determine the occurrence and prevalence of filarial infections, blood samples from 1066 dogs were obtained in Gauteng (316), KwaZulu-Natal (417) and Mpumalanga (333) (Schwan 2009). Out of the 1066 samples, 160 were found to be positive for microfilariae based on membrane filtration (Schwan 2009). The species identified following acid phosphatase staining were *D. repens* and *A. reconditum,* which were found to have the highest overall prevalence of 9.57 % (Schwan 2009). The highest prevalence for *A. reconditum* was found in Mpumalanga with 29.13 %, followed by KwaZulu-Natal with 1.2 % (Schwan 2009). *Dirofilaria repens* infections were mostly found in the coastal areas of KwaZulu-Natal with a prevalence of 12.47 %, followed by Mpumalanga with 1.5 % (Schwan 2009).

Similarly, in another survey conducted in domestic cats in KwaZulu-Natal, 10.98 % of cats were found to be infected with *D. repens* (Schwan 2009). A report in 2000 documented a case of acute liver failure in a cat which was found positive for *D. repens* (Schwan, Miller, de Kock & Van Heerden, 2000). Only occasional diagnostic records are available from Western Cape province, Northern Cape province, Eastern Cape province, Free State and North West province. The species identified in these provinces were *D. repens* and *A. reconditum* (Schwan 2009).

These findings corroborate earlier findings in which 3 out of 132 samples were found to be positive for microfilariae based on membrane filtration in the Gauteng Province (Minaar & Krecek 2001). The species identified following acid phosphatase staining was *A. reconditum* which was found to have a prevalence of 2.3 % (Minaar & Krecek 2001). *Acanthocheilonema reconditum* was also identified in 2 out of 73 samples collected from the Gauteng and North West province by acid phosphatase staining (Minaar & Krecek 1999).

Dirofilaria immitis has to date only been reported in cases of dogs imported from heartworm endemic countries (Verster, Cilliers & Schroeder 1991; Schwan 2009). Out of the 1066 samples screened, only one sample was found positive for *D. immitis* by acid

phosphatase staining. The animal from Mpumalanga province was a female, that was born in Beira, Mozambique and brought to South Africa by the owner 4 months prior to sample collection (Schwan 2009). An earlier case of *D. immitis* involved a female dog imported from Brisbane, Australia (Verster *et al.* 1991).

2.2.2 Mozambique

Filarial species reported from dogs in Mozambique are *D. immitis, D. repens, A. reconditum* and *A. dracunculoides*.

Dirofilaria immitis was first reported by Dias (1954) and later by Cruz e Silva (1971). Claims of a *D. immitis* prevalence of 5.81 % in dogs in Maputo province are controversial since the criteria used for identification of the microfilariae detected were not published (Schwan 2009). The endemic status was confirmed in a pilot survey conducted in Zambezia province, in which 4 out of 13 dogs (31 %) tested positive for *D. immitis* by acid phosphatase staining (Schwan & Durand 2002). In a latest survey conducted in Maputo province, 2 out of 313 dogs (0.64 %) tested positive (Schwan 2009). Both animals from Maputo were females, one in the 1-5 year-old age group and the other in the 6-10 year-old age group (Schwan 2009).

Dirofilaria repens was first recorded in Maputo province by Schwan in 2009, in which 12 out of 313 dogs (3.83 %) were positively identified via acid phosphatase staining.

Acanthocheilonema reconditum was detected in a small survey conducted in Zambezia Province, in which 1 out of 13 dogs (11.3 %) tested positive by acid phosphatase staining (Schwan & Durand 2002). Out of 313 dogs sampled in the Maputo province, 20 tested positive (6.39 %) for *A. reconditum* using acid phosphatase staining (Schwan 2009).

Acanthocheilonema dracunculoides was first recorded in the Maputo Province by Schwan in 2009, in which 1 out of 313 dogs was positively identified using acid phosphatase staining.

2.2.3 Botswana

Microfilariae of *D. repens* were first detected by acid phosphatase staining in a crossbreed in 1999 and then 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 first report of *A. reconditum* was made in 2000, from a Bouvier des Flandres in Gaborone (Schwan 2009).

The endemic status of *D. repens* and *A. reconditum* was confirmed in a survey conducted during 2014 and 2015 to determine the occurrence and prevalence of filarial infections, in blood samples from 150 dogs obtained in the Gaborone Province (Ntesang 2016). Out of the 150 samples, 27 were found to be positive (18 %) for microfilariae based on membrane filtration (Ntesang 2016). The species identified following acid phosphatase staining were *A. reconditum, A. dracunculoides* and *D. repens*, which were found to have the highest overall prevalence of 14.67 % (Ntesang 2016). Infection with *A. reconditum* was diagnosed in 4 of the 150 samples analysed, giving a prevalence of 2.67 % (Ntesang 2016). Infection with *A. dracunculoides* was diagnosed in 1 of the 150 dogs sampled, giving a prevalence of 0.67 % (Ntesang 2016).

2.2.4 Namibia

The first record of *D. repens* in dogs was reported from a Cocker Spaniel in Otjiwarongo in 1994 (Schwan 2009).

The first record of *A. dracunculoides* was reported in 2006 from two dogs in Windhoek, based on acid phosphatase staining of microfilariae (Schwan & Schröter 2006).

2.2.5 Zimbabwe

The first record of *D. immitis* in a dog was reported in 2002 in Harare, involving a crossbreed that originated from Beira in Mozambique one year prior to the sample being collected (Schwan 2009).

A checklist by Jooste (1990) indicates the occurrence of *D. repens* in a cat and *A. dracunculoides* from a spotted hyena.

2.2.6 Lesotho

There are no records of filarial infections in domestic carnivores from Lesotho.

2.2.7 Swaziland

There are no records of filarial infections from Swaziland.

Table 2.1: Filarial helminths described from dogs and their geographical distribution (Schwan 2009)

Species	Geographical distribution
Acanthocheilonema dracunculoides	Africa , Asia, Europe
Acanthocheilonema reconditum	Africa, America, Asia, Europe, Australia
Brugia ceylonensis	Asia
Brugia malayi	Asia
Brugia pahangi	Asia
Brugia patei	Africa
Cercopithifilaria baineae	South America
Cercopithifilaria grassii	Europe
Dirofilaria immitis	Africa, America, Asia, Europe, Australia
Dirofilaria repens	Africa, Asia, Europe

Chapter 3
Materials and methods

3.1 Study area

This survey was conducted from August 2016 to August 2017 in the greater Mahikeng Local Municipality in the North West Province of South Africa. South Africa occupies the southernmost tip of Africa (Fig. 3.1). Although located in a subtropical region, it is a relatively dry country with a mean annual rainfall of 464 mm (Tibane & Lentsoane 2016). The Western Cape province of South Africa receives winter rainfall, but most other provinces, including North West Province, receive predominantly summer rainfall.

Mahikeng (25° 51' S 25° 38' E) is the capital city of the North West Province of South Africa (Materachera 2016). Samples were primarily collected at the Dale Beigle Animal Health Centre, the Mahikeng Society for the Prevention of Cruelty to Animals (SPCA) as well as the Angel's Refuge Animal Shelter. The incentive for the choice of this location was a reported high overall prevalence of 18 % of canine filariosis in Gaborone in neighbouring Botswana, which lies in close proximity to Mahikeng (Fig. 3.2) (Ntesang 2016).

3.2 Study animals

Domestic dogs were selected as the study animals in this survey. Both male and female dogs, older than 1 year of age and of any breed, belonging to private owners or housed at animal shelters in the Mahikeng area, were selected for this study. It has been shown that dogs younger than 12 months are rarely found to be infected due to the long prepatent period of 6-9 months of filarial helminths (Vezzani, Carbajo, Fontanarros, Scodellaro, Basabe, Cangiano & Eiras 2011). Dogs that were treated with macrocyclic lactones in the previous 12 months

were also excluded from the survey because of their microfilaricidal activity (Vezzani *et al.* 2011).

Since no data was available on the prevalence of filarial infections in Mahikeng, the minimum size of the dog population to be sampled was determined at 97 dogs, given an estimated prevalence of 10 % in the population. The formula of Naing (2006) was used to calculate the sample size:

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

n = Size of sample
Z = Z statistic for a level of confidence = 1.64
P = Expected prevalence or proportion
d = precision = 0.05

Samples were not repeated. A data capture form was completed for each animal.

3.3 Sample collection

Prior to sample collection, consent was obtained from the dog owner or management at the animal shelter/ SPCA. Thereafter, approximately 3 ml of blood was collected from each dog and immediately expressed into an evacuated EDTA tube. Blood was collected from the cephalic vein. The blood sample was shaken by hand immediately following collection in order to allow for adequate mixing of the blood with the anticoagulant present in the EDTA tube and thus avoid clotting of the blood. Blood samples were stored refrigerated at approximately 4 ^{o}C until analysed by the technique described below. Blood samples were stored for a maximum of two weeks prior to analysis.

3.4 Diagnostic procedures

3.4.1 Membrane filtration

The blood samples were screened for the presence of microfilariae by using the membrane filtration technique as described by Dennis & Kean (1971). A Swinnex® (Millipore) 25 mm diameter filter holder was assembled. Thereafter, a 3 µm Isopore® (Millipore) polycarbonate membrane filter was fitted into the filter holder. One ml EDTA blood was mixed with 1 ml of saline and drawn into a 5 ml syringe, followed by 3 ml of air. The mixture was then ejected through the filter system with the syringe held vertically and the filter holder pointing downwards. The filter holder was held over a beaker, into which the filtrate was collected. Thereafter, 10 ml of saline was pushed through the filter holder using the same syringe with even pressure applied throughout. This was followed by pushing a syringe full of air through the assembled filter device in order to clear it of any remaining fluid.

The filter was then removed from the filter holder with a pair of forceps and placed onto a microscope slide with a frosted edge. The filter was initially left to air dry, then fixed with methanol for 1 min and subsequently stained with a 10% Giemsa solution for 20 min (Mehlhorn, Düwel & Raether 1993). The filter was again air dried prior to being mounted under a coverslip with Entellan® mounting medium (Merck). Finally, the mounted and stained membrane filters were examined with a compound microscope for the presence of microfilariae using a x40 objective and 10X ocular lenses providing effectively 400X magnification.

3.4.2 Acid phosphatase staining of microfilariae

Blood samples that were identified as positive on membrane filtration for the presence of microfilariae were further processed to identify the filarial species involved. In the first step, the microfilariae had to be concentrated by using the modified Knott's technique (Knott 1939). In this technique, 9 ml of 2 % formalin was mixed with 1 ml of blood collected in EDTA and centrifuged at 500 g for 5 min. The microfilariae would be concentrated in the sediment, of which three drops were transferred onto a glass microscope slide. The supernatant was discarded. The sediment was spread out on the slide by using the tip of a Pasteur pipette. This

was followed by drying and fixing with acetone. For the acid phosphatase staining procedure the technique of Yen & Mak (1978) was followed.

Subsequent to the acid phosphatase staining, microfilariae were examined with the compound microscope at x10 and x20 objective and 10X ocular lenses providing effectively x100 and x200 magnification respectively for species-specific differences in the somatic staining patterns. Species identification of microfilariae was conducted with the aid of published keys illustrating the species-specific staining patterns (Acevedo, Theis, Kraus & Longhurst 1981; Beugnet, Costa & Lambert 1993).

As no microfilariae-positive blood samples were identified by means of membrane filtration in this survey, acid phosphatase staining was not performed.

3.5 Statistical analysis

The data captured from the owner consent forms were entered into a Microsoft Excel spreadsheet.

Figure 3.1: Map of South Africa with its neighbouring countries (maps.google.com 2005)

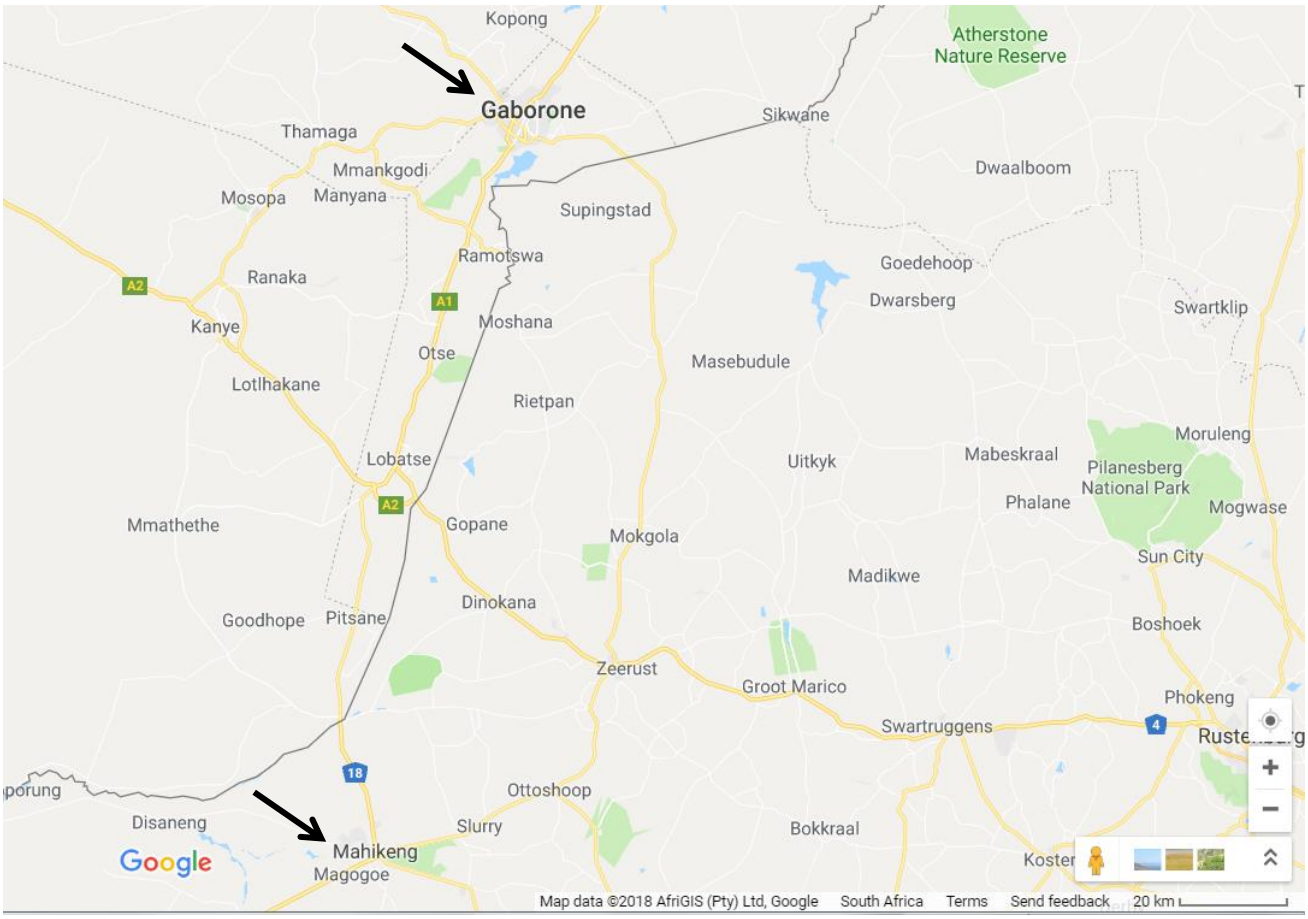

Figure 3.2: Map showing proximity of Mahikeng to Gabarone, Botswana (maps.google.com 2005)

Chapter 4
Results

A total of 100 dogs were sampled during the survey of which 47 (47 %) were female and 53 (53 %) were male. The ages of the dogs ranged from 1 to 11 years. Dog breeds which were sampled included the Boerboel, Greyhound, Great Dane, Pitbull terrier, Africanis, Siberian Husky, Miniature Pinscher, Jack Russel terrier, Pekingese, Boxer, German short haired pointer, Standard poodle, Dachshund as well as cross bred animals. The cross bred animal (59 %) was the most common dog identifiedin the survey. This was followed by the Boerboel (9 %), the Greyhound (6 %), the Great Dane (4 %) and the Pitbull terrier (4 %). The dogs were sampled in three primary locations, namely the Dale Beigle Animal Health Centre, the Mahikeng Society for the Prevention of Cruelty to Animals (SPCA) and the Angel's Refuge Animal Shelter.

4.2 Blood samples examined by the membrane filtration technique

The 100 blood samples were screened for the presence of microfilariae by means of the membrane filtration technique. There was no evidence of filarial infections in the 100 dogs sampled.

Table 4.1: Overview of dogs sampled according to the three primary sample locations

Suburb	Male	Female	Total [%]
Dale Beigle Animal Health Centre	21	14	35 %
Angel's Refuge	14	12	26 %
Mahikeng SPCA	18	21	39 %
Total	**53**	**47**	**100**

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

Dog Breed	Male	Female	Total [%]
Cross	30	29	59 %
Boerboel	6	3	9 %
Greyhound	3	3	6 %
Great Dane	2	2	4 %
Pitbull	1	3	4 %
Africanis	3	1	4 %
Siberian Husky	1	2	3 %
Miniature Pinscher	1	2	3 %
Jack Russel Terrier	2	1	3 %
Pekingese	1	0	1 %
Boxer		1	1%
German Shorthaired Pointer	1		1 %
Poodle	1		1 %
Dachshund	1		1 %
Total	**53**	**47**	**100 (53 % Male, 47 % Female)**

Chapter 5
Discussion

In an attempt to address the general lack of published information available on the prevalence of filarial infections in domestic dogs in South Africa, a survey was conducted between 2001 and 2003 (Schwan 2009). Blood samples from 1066 dogs were obtained in Gauteng Province (316), KwaZulu-Natal (417) and Mpumalanga Province (333) (Schwan 2009). Out of the 1066 samples, 160 were found to be positive for microfilariae based on the membrane filtration (Schwan 2009). The species identified following acid phosphatase staining were *D. repens* and *A. reconditum,* which was found to have the highest overall prevalence of 9.57 % (Schwan 2009). The highest prevalence for *Acanthocheilonema reconditum* was found in Mpumalanga with 29.13 %, followed by KwaZulu-Natal with 1.2 % (Schwan 2009). *Dirofilaria repens* infections were mostly found in the coastal areas of KwaZulu-Natal with a prevalence of 12.47 %, followed by Mpumalanga with 1.5 % (Schwan 2009).

The present study aimed to address the dearth of information available pertaining to the occurrence of filarial helminth infections of domestic dogs in the North West Province of South Africa by conducting a survey in the greater Mahikeng Local Municipality. The justification for the choice of this location was a reported high overall prevalence of 18 % of canine filariosis in Gaborone in neighbouring Botswana, which lies within a distance of 150 km of Mahikeng (Ntesang 2016). The filarial species identified in Gabarone following acid phosphatase staining were *A. reconditum, A. dracunculoides* and *D. repens*, which was found to have the highest overall prevalence of 14.67 % (Ntesang 2016). It was hypothesized that, due to the close proximity to Gaborone, the occurrence and prevalence of filarial helminth infections in domestic dogs in Mahikeng would be similar.

Blood samples were screened for the presence of microfilariae by means of the membrane filtration technique. There was no evidence of filarial infections in the 100 dogs sampled. These findings are reported with a confidence of 90 % for the estimated initial prevalence of 10 % in the population. These findings are dissimilar to earlier findings by Schwan (2009) and Ntesang (2016) on the occurrence and prevalence of filarial helminths in the provinces of Gauteng, Limpopo and Mpumalanga of South Africa and Gaborone in Botswana respectively.

The difference noted in the occurrence of filarial infections in the Mahikeng Local Municipality compared to that of neighbouring Gaborone, Botswana, could be attributed to several factors. The sample size was calculated at an estimated prevalence of 10% in the population, based on the prevalence of 18% obtained in Gabarone, Botswana (Ntesang 2016). Should the true prevalence of filariosis in the canine population be lower than 10 % in Mahikeng, a larger sample size would have been required.

The average age of dogs sampled in this survey was 2.7 years, with a maximum age of 11 years and a minimum age of 1 year. Most dogs sampled were cross breeds. Most pure bred dogs were either used as guard dogs, such as the Boerboel or were used for recreational activities such as hunting and racing (Greyhounds and Great Danes). Considering the purposes for which these dogs were kept, it can be assumed that they would have had exposure to the vectors of canine filariosis.

The distribution of canine filarial helminths is dependent on a number of other factors such as the distribution of the vector, the climatic conditions (such as temperature, humidity and precipitation) present in the study area, the density of the canine population which could serve as a reservoir for infection and the density of the human population (Rojas, Rojas, Montenegro & Baneth 2015; Harizanov, Jordanova & Bikov 2014). Possible causes for the unexpected result obtained in this study could be related to a number of factors including the low prevalence or absence of appropriate vectors, incorrect information furnished by owners regarding treatment

with macrocyclic lactones and regular control of fleas and ticks that are the vectors of *A. reconditum* and *A. dracunculoides*.

As the microfilariae of *D. repens* and *A. reconditum* are nocturnal subperiodic (Ionica, Matei, D'Amico, Bel, Dumitrache, Modry & Mihalca 2017; Webber & Hawking 1955) and those of *A. dracunculoides* appear to be controversial, however diurnal subperiodity was reported in one dog from Pakistan (Wolfe, Aslamkhan, Sharif & Perves 1971) they should have been detected by the membrane filtration technique which is regarded as the most sensitive one for this purpose (Feldmeier, Bienzle, Schuh, Geister & Guggenmoos-Holzmann 1986).

The distribution of canine filarial helminths also depends on the occurrence of suitable vectors. *Dirofilaria repens* was found to have the highest overall prevalence in 2014 and 2015 in Gaborone, which is situated within a proximity of 150 km from Mahikeng (Ntesang 2016). *Dirofilaria repens* is widespread in Africa and has been reported from South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Zambia, Tanzania, Democratic Republic of Congo, Central African Republic, Uganda, Kenya, Sudan, Egypt, Niger, Nigeria, Mali, Cote d'Iore, Ghana and Tunisia (Schwan 2009).

Anopheline and culicine mosquitoes of the genera *Aedes, Anopheles, Culex, Mansonia* and *Taeniorhynchus* have been identified as vectors of *D. repens* (Pampiglione & Rivasi 2000; Pampiglione *et al.* 1995). Mosquito species endemic to the African continent that have been found to have a high transmission potential as vectors, following experimental infection, are *Aedes pembaensis, Aedes aegypti, Mansonia uniformis* and *Mansonia africana* (Nelson *et al.* 1962; Anyanwu, Agbede, Ajanusi, Umoh & Ibrahim 2000).

There is a general lack of knowledge concerning the composition and distribution of mosquito species in southern Africa (Cornel, Lee, Almeida, Johnson, Mouatcho, Venter, de Jager & Braack 2018). *Aedes aegypti, Mansonia uniformis* and *Mansonia africana* are known to be endemic in South Africa and were recorded from the formerly known province of Transvaal of which the current NorthWest province was a part (Gillies & Coetzee 1987; Jupp 1996). It should be further

investigated whether the spectrum of suitable vectors for *D. repens* in Mahikeng is comparable to that in Gaborone, Botswana.

A recent study investigating the mosquito composition in South Africa and some neighbouring countries found that the lowest abundances and diversities of mosquito species were found in the semi-desert Kalahari, close to the South African border to Botswana (Cornel *et al*. 2018). However, the species found in this area included *Mansonia uniformis*, which has been identified as a potential vector for *D. repens* (Cornel *et al*. 2018; Nelson *et al*. 1962). Unfortunately, the mosquito sampling areas chosen did not include Mahikeng in South Africa or Gaborone in Botswana.

In addition to lice, *Ctenocephalides felis* and *Ctenocephalides canis*, flea species commonly found on dogs have been, apart from lice, identified as the principle vectors of *A. reconditum* (Ionica *et al*. 2017; Newton & Wright 1956; Nelson 1962, Pennington & Phelps 1969). Apart from the louse fly *Hippobosca longipennis,* the kennel tick, *Rhipicephalus sanguineus,* is recognized as the vector for *A. dracunculoides* (Nelson 1963; Olmeda-Garcia, Rodriguez-Roderiguez & Rojo-Vazquez 1993; Olmeda-Garcia & Rodriguez-Roderiguez 1994). *Rhipicephalus sanguineus* is commonly found on dogs in South Africa and it is also the vector of canine monocytic ehrlichiosis which has a high prevalence (Mtshali, Nakao, Sugimoto & Thekisoe 2017). Is this also in Mahikeng?

A further consideration for the results obtained could be that the dogs were harbouring adult infections in which there were circulating microfilariae in the bloodstream. This could occur in cases with unisexual infection (for example when only the female worm is present), if immune mediated clearance of microfilariae had occurred or if the dogs had been previously treated with macrocyclic lactone therapy and this was not made known to the study participants (Siwila, Mwase, Nejsum & Simonsen 2015). The use of tetracyclines to control diseases such as ehrlichiosis could also result in a decreased observation of the number of circulating microfilariae (Labarthe & Guerrero 2005).

Further work should be aimed at investigating the composition of the mosquito population in Mahikeng and Gaborone, Botswana to identify if the possible vectors of *D. repens,* which was found in with the highest prevalence in Gaborone, overlap. The study could also be expanded to include a larger sample size of domestic dogs, thereby assuming that the prevalence of infection is lower.

Chapter 6
Conclusion

From the results of this study, the null hypothesis, stating that the prevalence of filarial infections in domestic dogs in Mahikeng is 10%, is rejected. It can thus be concluded, with a confidence of 90 %, that the prevalence of filarial species in domestic dogs in Mahikeng, North West Province is less than 10 %. It is recommended that additional surveys be conducted in the Mahikeng municipality area, screening larger sample sizes of domestic dogs and also domestic cats, to obtain more accurate data on the prevalence of filarial infections. Furthermore, comparative surveys should be conducted on the occurrence of mosquito species in the larger Mahikeng municipality area and Gaborone municipality, in an attempt to clarify the reasons for the high prevalence of *Dirofilaria repens*-infected dogs in Gaborone. Questionnaires involving dog and cat owners could also provide additional information on the use and awareness of health-related aspects of the companion animals in their care.

Chapter 6
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Animal Ethics Committee

PROJECT TITLE	**Survey on the occurrence and prevalence of filarial helminth infections of domestic dogs in Mahikeng, North West province**
PROJECT NUMBER	**V101-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. B Voigts**

STUDENT NUMBER (where applicable)	**UP_292 881 78**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Canine**	
NUMBER OF ANIMALS	**138**	
Approval period to use animals for research/testing purposes		**August 2016 – August 2017**
SUPERVISOR	**Prof. 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 18 January 2017
CHAIRMAN: UP Animal Ethics Committee	Signature