Chapter 3
MATERIALS AND METHODS

3.1 Survey on the occurrence and prevalence of filarial helminths of domestic dogs in Gauteng, KwaZulu-Natal and Mpumalanga provinces, South Africa, and Maputo province, Mozambique

Samples were collected during the period of September 2001 to June 2003 and were subsequently analyzed at the Helminthology Section of the Faculty of Veterinary Science, Onderstepoort.

3.1.1 Description of survey areas

3.1.1.1 GAUTENG PROVINCE

In Gauteng province the survey involved private veterinary clinics and hospitals in Pretoria (25°42' S, 28°13' E). According to Mucina & Rutherford (2006) the Pretoria area is located partially in the Central Bushveld Bioregion, the Dry Highveld Grassland Bioregion and the Mesic Highveld Grassland Bioregion which are parts of the Savanna Biome and the Grassland Biome with wet summers and dry winters. The Central Bushveld Bioregion has the highest number of vegetation types within the Savanna Biome and covers most of the high-lying plateau west of the main escarpment from the Magaliesberg in the south to the Soutpansberg in the north (Rutherford, Mucina & Powrie 2006b). The macroclimatic traits characterising the Savanna Biome are: seasonality of precipitation and a subtropical thermal regime with no or usually low incidence of frost. (Rutherford, Mucina, Lötter, Bredenkamp, Smit, Scott-Shaw, Hoare, Goodman, Bezuidenhout, Scott, Ellis, Powrie, Siebert, Mostert, Henning, Venter, Camp, Siebert, Matthews, Burrows, Dobson, van Rooyen, Schmidt, Winter, du Preez, Ward,

Williamson & Hurter 2006a) The Dry Highveld Grassland Bioregion constitutes the western belt (Graaff-Reinet and Aliwal North to Mafikeng) of the Grassland Biome, mainly with a mean annual precipitation of below 600 mm (Rutherford *et al.* 2006a). The Mesic Highveld Grassland Bioregion has the highest number of vegetation types within the Grassland Biome. It is found mainly in the higher precipitation parts of the highveld and extends northwards along the eastern escarpment (Rutherford *et al.* 2006a). The temperate grasslands of southern Africa occur where there is strong summer rainfall, which may vary spatially from 400-2 500 mm per year, and winter drought (Mucina, Hoare, Lötter, du Preez, Rutherford, Scott-Shaw, Bredenkamp, Powrie, Scott, Camp, Cilliers, Bezuidenhout, Mostert, Siebert, Winter, Burrows, Dobson, Ward, Stalmans, Oliver, Siebert, Schmidt, Kobisi & Kose 2006a).

3.1.1.2 KWAZULU-NATAL PROVINCE

In KwaZulu-Natal province the survey involved private veterinary clinics and hospitals in Pongola (27°22' S, 31°37' E), Mtubatuba (28°25' S, 32°20' E), Eshowe (28°52' S, 31°28' E), Richards Bay (28°48' S, 32°6' E) Empangeni (28°45' S, 31°53' E) and Pietermaritzburg (29°37' S, 30°22' E). According to Mucina & Rutherford (2006), Pongola is located in the Lowveld Bioregion and Eshowe and Pietermaritzburg are located in the Sub-Escarpment Savanna Bioregion. Both, the Lowveld Bioregion and the Sub-Escarpment Savanna Bioregion are parts of the Savanna Biome with summer rainfall pattern and some rain in winter. The Lowveld Bioregion extends from the eastern foot of the Soutpansberg southwards along the base and lower slopes of the escarpment, through the lower parts of Swaziland to the low-lying parts of Zululand in KwaZulu-Natal (Rutherford *et al.* 2006a). The Sub-Escarpment Savanna Bioregion occurs mainly inland of the Indian Ocean Coastal Belt extending farther inland up major river valleys (Rutherford *et al.* 2006a). Mtubatuba, Richards Bay and Empangeni are all

located in the Indian Ocean Coastal Belt with marginal non-seasonal rainfall. The northern regions of the Indian Ocean Coastal Belt, close to the coast, have marginally, nonseasonal rainfall, with precipitation concentrated in summer (Mucina, Scott-Shaw, Rutherford, Camp, Matthews, Powrie & Hoare 2006b). In the KwaZulu-Natal part of the Indian Ocean Coastal Belt the mean annual rainfall ranges between 1 272 and 819 mm and the mean annual temperature ranges from about 22 ºC in the north, near the Mozambique border, to 20.4 ºC near Durban (Mucina *et al.* 2006b). Summers are hot to very hot, while winters are mild, with hardly any frost (Mucina *et al.* 2006b).

3.1.1.3 MPUMALANGA PROVINCE

In Mpumalanga province the survey involved a private veterinary practice in Nelspruit (25°28' S, 30°58' E) as well as the Malelane Research Unit of Intervet (SA) (Pty) Ltd in Malelane (25°28' S, 31°31' E). According to Mucina & Rutherford (2006), Nelspruit and Malelane are located in the Lowveld Bioregion which is part of the Savanna Biome with summer rainfalls and dry winters.

3.1.1.4 MAPUTO PROVINCE

In Maputo province the survey involved the National Directorate of Livestock and the Eduardo Mondlane University in Maputo covering the metropolitan area of Maputo (25º57' S, 32º35' E) and the villages of Namahacha (25º58' S, 32º1' E), Pessene (25º41' S, 32º21' E) and Régulo Mussumbuluco (26º9' S, 32º19' E). Maputo Province is located in the Tropical and Subtropical Moist Broadleaf Forests Biome (Burgess, D'Amico Hales, Underwood, Dinerstein, Olson, Itoua, Schipper, Ricketts & Newman 2004).

3.1.2 Survey animals, sample size and selection criteria

The animals involved in the survey were domestic dogs of either sex and any breed that were brought to the various practices or other facilities for routine procedures or health care examinations.

Since no data were available on the prevalence of filariosis in South Africa and Mozambique, a minimum sample size of 313 dogs per province was calculated, based on a prevalence estimate of 30 %, taking into account data obtained from a small-scale survey conducted in Mozambique (Schwan & Durand 2002). Only animals of 1 year of age and older were included in the survey. Dogs that were treated with macrocyclic lactones during the past 12 months prior to sample collection were excluded. A data capture form was completed for each animal.

3.1.3 Filarial diagnostic techniques

With the consent of their owners, approximately 2 ml of day blood were drawn from the cephalic vein into evacuated EDTA blood collection tubes. Immediately after collection the blood samples were vigorously shaken by hand to allow proper mixing of blood and anticoagulant. Blood samples were refrigerated until analyzed by one of the following techniques.

3.1.3.1 MEMBRANE FILTRATION

The membrane filtration technique as described by Dennis & Kean (1971) was applied to screen the blood samples for the presence of microfilariae. A Swinnex® (Millipore) 25 mm filter holder was assembled and fitted with a 3 µm Isopore® (Millipore) polycarbonate membrane filter. One ml EDTA blood and 4 ml of air were taken up in a 5 ml syringe and forced through the filter system with the syringe vertical and the filter

holder downmost which was held over a beaker to collect the filtrate. Subsequently, 10-20 ml of normal saline were washed through the filter holder, followed by a syringeful of air to clear any residual fluid. The filter was removed from the holder with a pair of forceps and placed on a slide where it was first air-dried, then fixed with methanol for 1 min and subsequently stained with Giemsa (Mehlhorn, Düwel & Raether 1993) for 20 min, air-dried and mounted in Entellan® (Merck). The slides were examined under a compound microscope at 40x magnification for the presence of microfilariae.

3.1.3.2 ACID PHOSPHATASE STAINING

To identify the species, the microfilariae in positive blood samples were first concentrated with the modified Knott's technique (Knott 1939). One ml of EDTA blood and 9 ml of 2 % formalin were mixed and centrifuged at 500 g for 5 min. The supernatant was discarded and three drops of the microfilariae-containing sediment were transferred to a slide for acid phosphatase staining using the technique of Yen & Mak (1978). Microfilariae were examined under a compound microscope at 100x and 200x magnification for species-specific differences in the somatic staining patterns, as described by Balbo & Abate (1972), Acevedo *et al.* (1981), Beugnet *et al*. (1993b), Ducos de Lahitte *et al*. (1993) and Valcárcel *et al.* (1990).

3.1.3.3 ANTIGEN CAPTURE ELISA

About 0.5 ml of EDTA blood was centrifuged at 1 000 g for 5 min at room temperature and the plasma collected was immediately screened for adult uterine *D. immitis* antigen with the DiroCHEK® (Synbiotics) ELISA test kit, following the instructions of the manufacturer.

3.1.4 Statistical analysis

Depending on the suitability of data, statistical analysis was performed using the SAS software system (SAS Institute Inc.). Differences in the microfilaria positive rates for sex, age groups and geographical origin among comparison groups were analyzed by multiple logistic regression or by the chi-squared test. In all tests, values of $p < 0.05$ were taken as significant.

3.2 Survey on the occurrence and prevalence of filarial helminths of cats in KwaZulu-Natal province

Samples were collected in the field during January to December 2005, and analyzed at the Helminthology Section of the Faculty of Veterinary Science, Onderstepoort.

3.2.1 Description of survey areas

The survey involved private veterinary practices and hospitals in cities and towns in the coastal areas, namely Mtubatuba (28°25' S, 32°10' E), Empangeni (28°45' S, 31°53' E), Umhlanga (29º43' S, 31º4' E), Durban (29°51' S, 31°1' E), Amanzimtoti (30º3' S, 30º52' E), Scottburgh (30°16' S, 30°45' E) and Port Shepstone (30°45' S, 30°26' E) which are all located in the Indian Ocean Coastal Belt with marginal non-seasonal rainfall.

3.2.2 Survey animals, sample size and selection criteria

The animals involved in the survey were domestic cats of either sex and any breed that were brought to the various practices for routine procedures or health care examinations. A total of 82 cats were sampled (Mtubatuba/8, Empangeni/2, Umhlanga/24, Durban/39, Amanzimtoti/2, Scottburgh/3, Port Shepstone/4). Only animals of 1 year of age and older were included in the survey. Cats that were treated

with macrocyclic lactones during the past 12 months prior to sample collection were excluded. A data capture form was completed for each animal.

3.2.3 Filarial diagnostic techniques

The same procedures and techniques were followed as described under *3.1.3*, with the exception that blood samples were not screened for *D. immitis* antigen.

3.3 Routine examinations for filarial infections of dogs and cats from South Africa between 1994 and 2008

During the period 1994 to 2008, samples were received from veterinary laboratories, private veterinarians and export kennels for the diagnosis of filarial infections of dogs and cats. Information concerning the origin and travel history was obtained from the owners of those animals that were diagnosed positive.

3.3.1 Filarial diagnostic techniques

The same procedures and techniques were followed as described under *3.1.3*, with the exception that blood samples were not screened for *D. immitis* antigen.

3.4 Routine examinations for filarial infections of dogs and cats imported from African countries between 1992 and 2008

During the period 1992 to 2008, samples were received from quarantine stations, veterinary laboratories and private veterinarians abroad for the diagnosis of filarial infection of dogs and cats. Information concerning the origin and travel history was obtained from the owners of those animals that were diagnosed positive.

3.4.1 Filarial diagnostic techniques

The same procedures and techniques were followed as described under *3.3.1*.

Chapter 4
RESULTS

4.1 Survey on the occurrence and prevalence of filarial helminths of domestic dogs in Gauteng, KwaZulu-Natal and Mpumalanga provinces, South Africa, and Maputo province, Mozambique

The survey was carried out on 1 379 blood samples collected from dogs in the 4 provinces, namely 316 from Gauteng, 417 from KwaZulu-Natal, 333 from Mpumalanga and 313 from Maputo. Out of the 1 379 samples analyzed, 196 (14.21 %) were found positive for microfilariae on membrane filtration (Fig. 4.1). By means of acid phosphatase staining 4 species were identified, namely *D. immitis*, *D. repens*, *A. reconditum* and *A. dracunculoides* (Figs. 4.2, 4.3, 4.4, 4.5).

4.1.1 Dirofilaria immitis

Three out of the 1 379 samples analyzed (0.22 %) were positive for microfilariae of *D. immitis.* Two of the cases were found in Maputo province, and 1 case in Nelspruit, Mpumalanga province. 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. The animal from Mpumalanga province was a female in the 1-5-year-old age group, born in Beira, Mozambique and brought by the owner to South Africa 4 months prior to sample collection. These 3 dogs were also seropositive for *D. immitis*.

A total of 25 animals tested positive for heartworm antigen, giving a seroprevalence of 1.8 %. The mean overall seropositive rates were 0.32 % for Gauteng province, 3.12 %

for KwaZulu-Natal province, 2.4 % for Mpumalanga province and 0.96 % for Maputo province.

4.1.2 Dirofilaria repens

Infection with *D. repens* was found in 70 of the 1 379 samples analyzed, giving a prevalence of 5.08%.

Dirofilaria repens had the highest prevalence in KwaZulu-Natal. The mean overall prevalence rates were 12.47 % for KwaZulu-Natal province, 1.5 % for Mpumalanga province and 3.83 % for Maputo province. A single microfilaraemic dog from Pretoria in Gauteng province was brought in by the owner from Durban in KwaZulu-Natal province 1 month prior to sample collection. When comparing the prevalence of infection in KwaZulu-Natal province to that in Gauteng province, Mpumalanga province or Maputo province there was a statistical difference (p = 0.0002, < 0.0001 and 0.0011 respectively). When comparing Mpumalanga province with Maputo province there was no statistical difference. The results obtained in each locality of sample collection are shown in Table 4.1.

The mean overall prevalence rate in male dogs was 5.28 % and 4.88 % in female dogs. There was no statistically significant difference in the prevalence of infection between male and female dogs (p = 0.64).

When analyzing the difference in prevalence of infection by age, the 6-10-year-old age group had the highest mean overall prevalence rate with 10.85 % compared to 3.45 % in the 1-5-year-old age group and 7.69 % in the ≥11-year-old age group. Statistically, a significant difference in prevalence was only observed between the 1-5-year-old age

group and the 6-10-year-old age group (p = 0.0003). The overall prevalence figures by age in each locality of sample collection are shown in Table 4.2.

Ten of the microfilaria-positive samples from KwaZulu-Natal province tested also positive for heartworm antigen.

4.1.3 Acanthocheilonema reconditum

Infection with *A. reconditum* was found in 122 of the 1 379 dog samples analyzed, giving a prevalence of 8.85 %.

Acanthocheilonema reconditum was the species with the highest overall prevalence in the survey and the highest prevalence rates in both Mpumalanga province and Maputo province. The p-value of the chi-squared test was < 0.0001, indicating a statistically significant relationship between province and prevalence of *A. reconditum*. Mpumalanga province and Maputo province had prevalence rates of 29.13 % and 6.39 % in comparison with Gauteng province and KwaZulu-Natal province with 0 % and 0.96 % respectively. The results obtained in each locality of sample collection are shown in Table 4.1.

The overall prevalence rate in male dogs was 12.9 % and 4.88 % in female dogs. There was a statistically significant relationship between the two gender groups and prevalence of *A. reconditum* (p < 0.0001).

There was also a statistical significant relationship between prevalence of infection and age (p < 0.0001). The 1-5-year-old age group had the highest overall prevalence rate with 11.24 % compared to 1.92 % in the 6-10-year-old age group and 0 % in the ≥11-

year-old age group. The overall prevalence figures by age in each locality of sample collection are shown in Table 4.3.

Two of the microfilaria-positive samples from Mpumalanga province and 1 sample from Maputo province tested also positive for heartworm antigen.

4.1.4 Acanthocheilonema dracunculoides

Infection with *A. dracunculoides* was found in only 1 of the 1 379 samples analyzed, giving a prevalence of 0.07 %. The animal was a female in the 6-10-year-old age group from Maputo province.

4.2 Survey on the occurrence and prevalence of filarial helminths of cats in KwaZulu-Natal province

The survey was carried out on 82 blood samples. Out of the 82 samples analyzed, 9 (10.98 %) were positive for microfilariae on membrane filtration. Acid phosphatase staining activity revealed *D. repens* as the only species involved.

The mean overall prevalence rates for the different localities in the province were 25 % for Mtubatuba, 4.17 % for Umhlanga, 10.26 % for Durban, 33.33 % for Scottburgh and 25 % for Port Shepstone. None of the samples from Empangeni and Amanzimtoti was found positive.

The mean overall prevalence in toms was 15.56 % and 5.41 % in queens.

Regarding the difference in prevalence of infection by age, the 1-5-year-old age group had the highest mean overall prevalence rate with 15.56 % compared to 3.85 % in the 6-10-year-old group and 9.09 % in the ≥11-year-old age group.

4.3 Routine examinations for filarial infections of dogs and cats from South Africa between 1994 and 2008

Microfilariae-positive samples from 39 dogs and 5 cats collected between 1994 and 2008 were received from the provinces of KwaZulu-Natal, Gauteng and Western Cape (Table 4.4). The microfilariae were identified as those of *D. repens* and *A. reconditum* only.

4.4 Routine examinations for filarial infections of dogs and cats imported from African countries between 1992 and 2008

Microfilariae-positive samples collected between 1992 and 2008 from 68 dogs and 2 cats originating from 20 different countries were received (Table 4.5). Microfilariae of *D. immitis*, *D. repens*, *A. reconditum*, *A. dracunculoides* and *B. patei* were identified.

Dogs infected with *D. immitis* were diagnosed in Northern Africa from Morocco, in Central Africa from the Democratic Republic of the Congo and Gabon, and in Eastern Africa from Madagascar, Mozambique, Réunion and Tanzania. *Dirofilaria immitis* has not been recorded before from the Democratic Republic of the Congo. The 2 infected dogs came from Kinshasa and had never left the country before. A single infected dog recorded from Zimbabwe originated from Beira in Mozambique.

Dogs infected with *D. repens* were diagnosed in Western Africa from Ghana, Ivory Coast, Mali, Niger and Nigeria, in Central Africa from Congo-Brazzaville and the

Democratic Republic of the Congo, in Eastern Africa from Kenya, Mozambique, Tanzania, Uganda and Zambia and in Southern Africa from Botswana and Namibia. All records except those from Kenya, Nigeria and Uganda are new and all animals had never left the respective countries before. Apart from several records in dogs in Namibia, *D. repens* was also diagnosed in 2 cats.

Dogs infected with *A. reconditum* were diagnosed in Central Africa from the Democratic Republic of the Congo, in Eastern Africa from Mozambique, Tanzania and Uganda and in Southern Africa from Botswana. All records except from Mozambique and Uganda are new and from animals that never had left the respective countries.

Dogs infected with *A. dracunculoides* were diagnosed in Eastern Africa from Kenya and in Southern Africa from Namibia. This filariid has already been recorded in both countries.

A single dog infected with *B. patei* was diagnosed from Tanzania, where the filariid had not been recorded ever before. The animal had never left the country. The sheathed microfilariae were identified on morphological grounds only (Buckley *et al.* 1958; Laurence & Simpson 1971) (Fig. 4.6). They had the two typically arranged tail nuclei, one terminal and one sub-terminal (Fig. 4.7). They varied in width from 5-6 µm at the widest part of the anterior end and varied in length from 252-265 µm. The cephalic space averaged 5 µm in length. Acid phosphatase staining which has never been reported before, showed enzyme activity at the cephalic vesicle, the excretory pore and the tail (Fig. 4.8).

4.5 Literature review on filariosis of dogs and cats in Africa

A critical review of published reports indicates the endemicity of *D. immitis*, *D. repens*, *A. reconditum*, *A. dracunculoides* and *B. patei.*

4.5.1 Dirofilaria immitis

According to Nelson (1966) it was common in veterinary practice to assume that dogs with microfilariae in their blood were infected with *D. immitis*, which has resulted in a great deal of confusion with other harmless species. The results of a critical review of the published information previously indicated endemicity of heartworm in 12 African countries and 4 islands. Infection has only been reported from dogs. In Northern Africa there is evidence of endemicity in Algeria (Rioche 1960), Egypt (Mahmoud & Ibrahim 1989), Morocco (Bouin 1921; Santucci *et al.* 1953, Pandey *et al.* 1987), Tunisia (Perrot 1985), and the offshore Canary Island of Tenerife (Valladares *et al.* 1987). The reports from Morocco have been confirmed in an imported dog from Rabat (Table 4.5). In Western Africa autochthonous infections have been diagnosed in Senegal (Pangui & Kaboret 1993). In Central Africa autochthonous infections are reported from Angola (Serrano 1962) and Gabon (Beugnet & Edderai 1998). Endemicity in Gabon was confirmed in 2 imported dogs from Libreville (Table 4.5). In Eastern Africa heartworm infection is documented from Ethiopia (Chiodi 1936; Graber 1975), Kenya (Heisch *et al.* 1959; Nelson *et al.* 1962; Murray 1968; Bwangamoi & Frank 1970; Bwangamoi *et al.* 1971), Malawi (Fitzsimmons 1964), Mozambique (Cruz e Silva 1971; Schwan & Durand 2002), Tanzania (Alley 1950) and the islands of Madagascar (Daynes 1964), Mauritius (Ware 1925; Webb & Nadeau 1958; Siebartie *et al.* 1983) and Réunion (Prunaux & Guignard 1991). The endemicity status has been confirmed in dogs imported from Kenya, Mozambique, Tanzania, Madagascar and Réunion (Table 4.5). Considering the

first record from the Democratic Republic of the Congo (Table 4.5), *D. immitis* is currently known to be endemic in 13 African countries and 4 islands (Fig. 4.9).

4.5.2 Dirofilaria repens

Published information previously indicated endemicity of *D. repens* in ten African countries. In animals, infection has been reported in various carnivores. In Northern Africa autochthonous infections were reported from Egypt (Myers *et al.* 1962), Sudan (Kellas & Webber 1955) and Tunisia (Chatton 1918; Bernard *et al.* 1967). In Western Africa there is evidence of endemicity in Nigeria (Schillhorn van Veen 1974; Schillhorn van Veen & Blotkamp 1975; Shonekan & Fabiyi 1975; Kamalu 1986,1991; Anyanwu *et al.* 1996) which was confirmed in 4 imported dogs from Lagos (Table 4.5). In Central Africa the only report came from the Central African Republic (Graber *et al.* 1972). In Eastern Africa autochthonous infections were reported from Kenya (Heisch *et al.* 1959; Nelson *et al.* 1962), Uganda (Bwangamoi 1973), Zambia (Le Roux 1958) and Zimbabwe (Jooste 1990). The endemicity status was confirmed for Kenya, Uganda and Zambia in imported dogs (Table 4.5). In Southern Africa autochthonous infections have been reported from South Africa (Schwan *et al.* 2000). Considering the first records from Ghana, Ivory Coast, Mali, Niger, Congo Brazzaville, Democratic Republic of the Congo, Mozambique, Tanzania, Botswana and Namibia (Table 4.5), *D. repens* is currently known to be endemic in 20 African countries (Fig. 4.10).

4.5.3 Acanthocheilonema reconditum

Published information previously indicated endemicity of *A. reconditum* in 6 African countries. Infection has been reported in various carnivores. In Western Africa autochthonous infections were reported from Liberia (Laub 1988) and Nigeria (Bobade *et al.* 1981). In Eastern Africa autochthonous infections were reported from Kenya

(Nelson 1962; Nelson *et al.* 1962) and from Mozambique (Schwan & Durand 2002). Previous reports from Uganda are inconclusive (Bwangamoi 1973; Bwangamoi & Isyagi 1973), however, the endemicity status was confirmed in an imported dog from Kampala (Table 4.5). In Southern Africa there is a doubtful report from South Africa (Van Heerden 1986), however, the endemicity status was confirmed in several dogs from various provinces (Table 4.4). Considering the first records from Democratic Republic of the Congo, Tanzania and Botswana (Table 4.5), *A. reconditum* is currently known to be endemic in nine African countries (Fig. 4.11).

4.5.4 Acanthocheilonema dracunculoides

Published information previously indicated endemicity of *A. dracunculoides* in 12 African countries. Infection has been reported from dog and spotted hyaena. In Northern Africa autochthonous infections were reported from Algeria (Rioche 1960; Montaron 1975), Morocco (Bouin 1921), Sudan (Baylis 1929) and Tunisia (Railliet *et al.* 1912; Bernard *et al.* 1967). In Western Africa *A. dracunculoides* is reported from Mali (Railliet *et al.* 1912) and Nigeria (Schillhorn van Veen *et al.* 1975). In Central Africa the only report comes from the Democratic Republic of the Congo (Gedoelst 1916). In Eastern Africa reports come from Kenya (Nelson *et al.* 1962), Tanzania (Sachs 1976) and Zimbabwe (Jooste 1990). The endemic status for Kenya was confirmed in an imported dog from Nairobi (Table 4.5). A report from Uganda (Carmichael & Bell 1943) which is regularly cited in the literature has not been considered as only a tentative diagnosis is made based on the unreliable parameters of length and width of microfilariae with no adult worms found during necropsy. In Southern Africa, autochthonous infections were reported from Namibia (Schwan & Schröter 2006) and South Africa (Cobbold 1870). The endemic status for Namibia was further confirmed in dogs from Windhoek and Otjiwarongo

(Table 4.5). Considering the first record from Mozambique (Table 4.5), *A. dracunculoides* is currently known to be endemic in 13 African countries (Fig. 4.12).

4.5.5 Brugia patei

Autochthonous infections were previously only reported from Kenya (Nelson & Heisch 1957; Nelson *et al.* 1962) in various carnivores and greater bushbaby. With the first record from a dog in Tanzania (Table 4.5), *B. patei* is currently known to be endemic in 2 African countries (Fig 4.13).

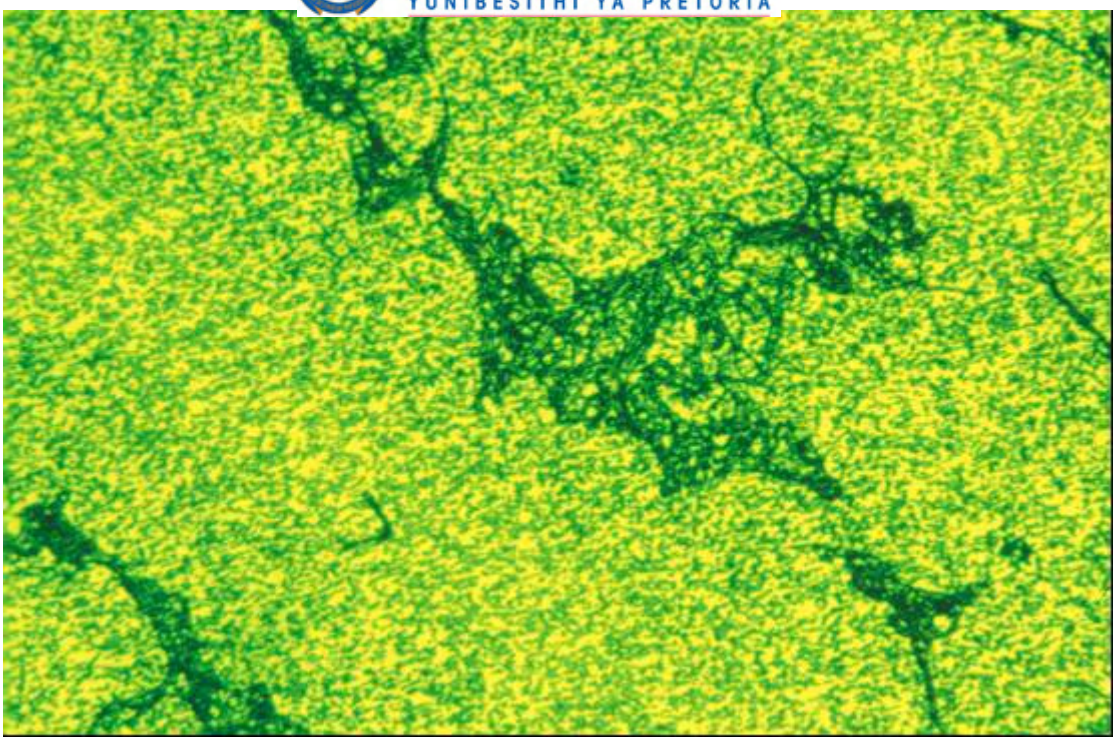

Figure 4.1: Microfilariae on a Giemsa-stained membrane filter

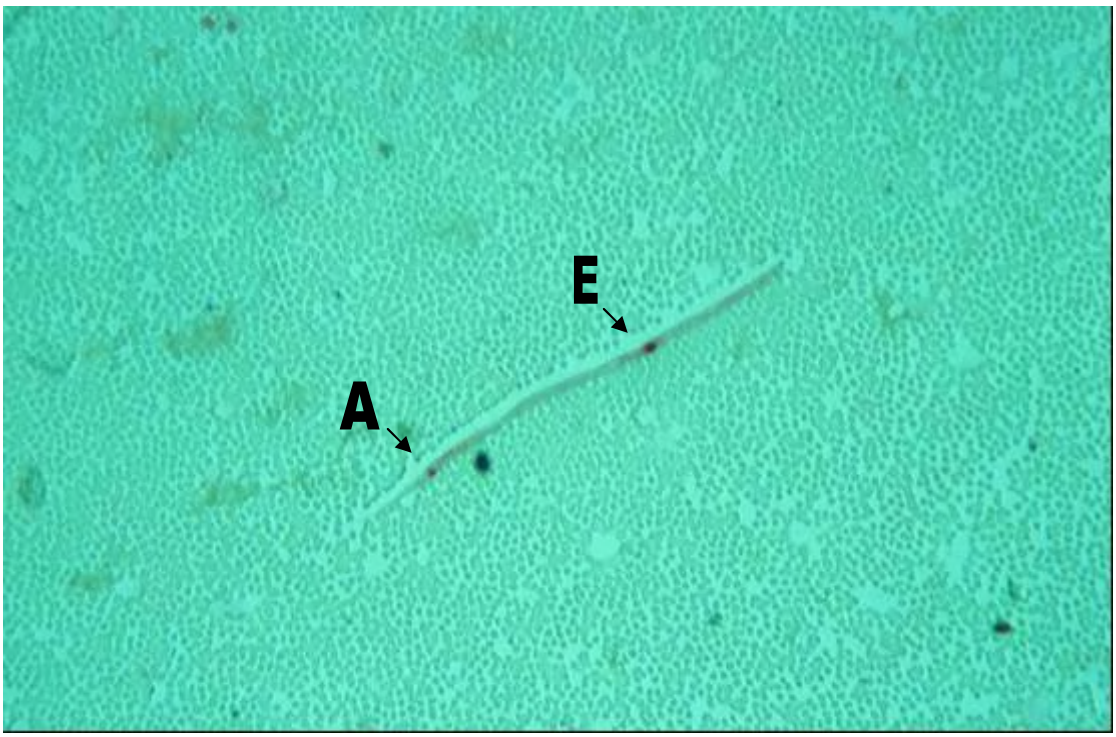

Figure 4.2: *Dirofilaria immitis* microfilaria showing acid phosphatase activity at the excretory pore (E) and anal pore (A)

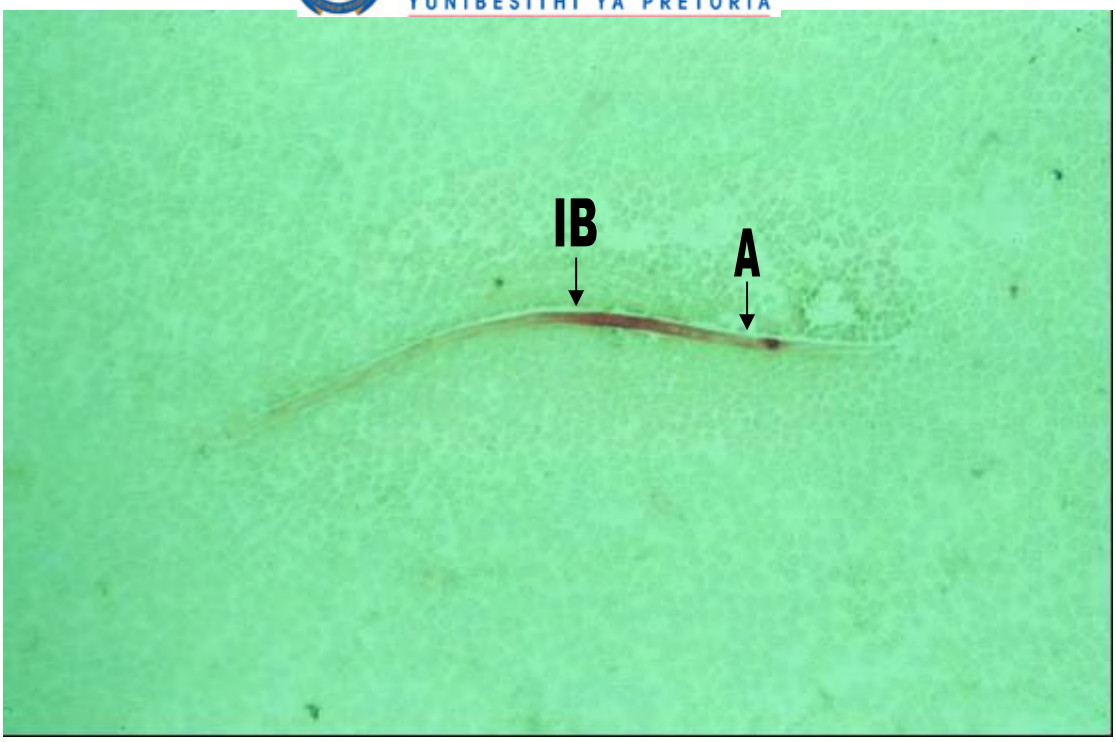

Figure 4.3: *Dirofilaria repens* microfilaria showing acid phosphatase activity at the inner body (IB) and anal pore (A)

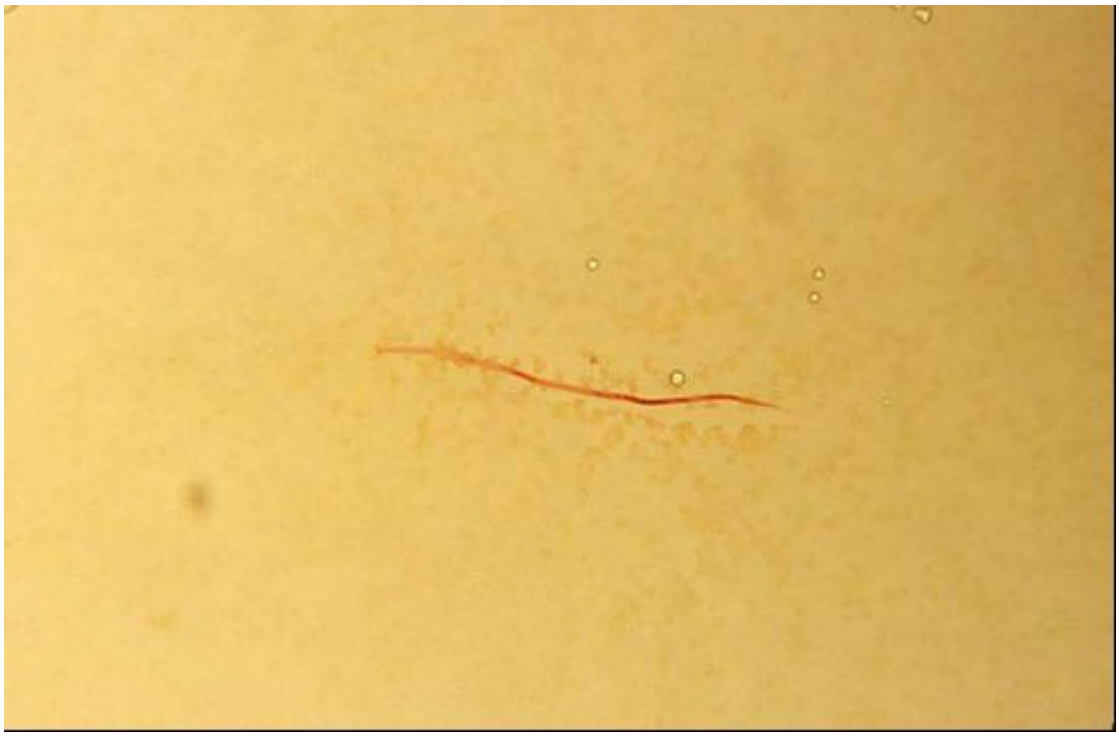

Figure 4.4: *Acanthocheilonema reconditum* microfilaria showing diffuse acid phosphatase activity in the area of the excretory pore, inner body and anal pore

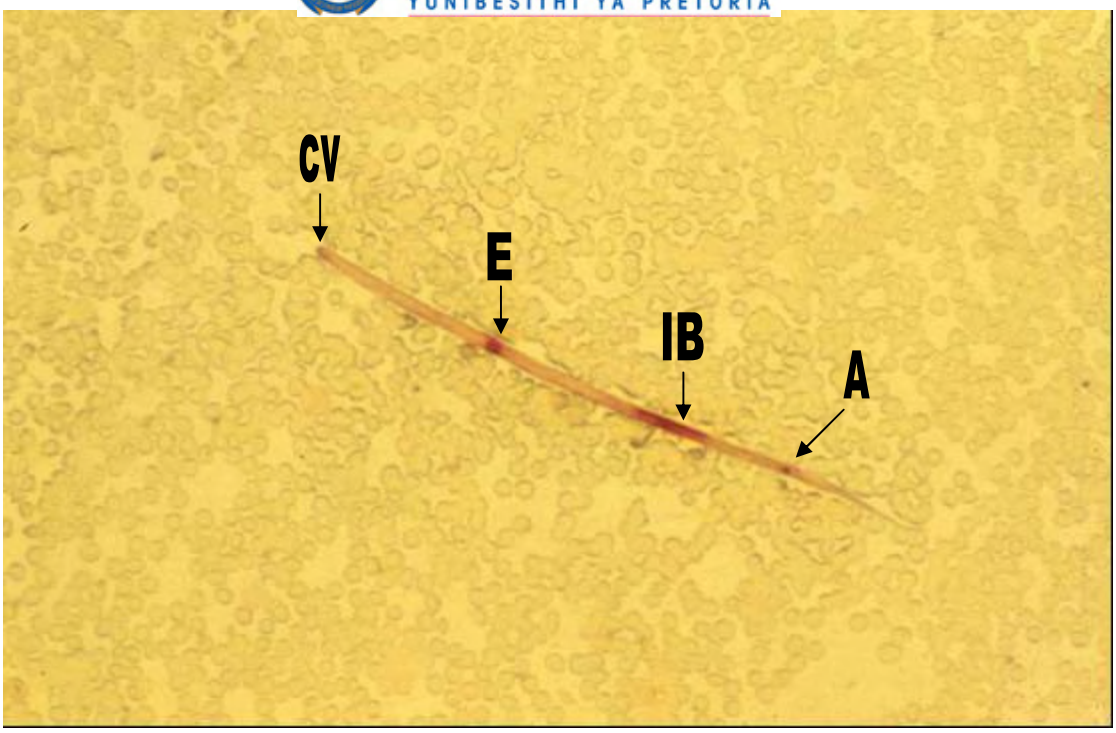

Figure 4.5: *Acanthocheilonema dracunculoides* microfilaria showing acid phosphatase activity at the cephalic vesicle (CV), excretory pore (E), inner body (IB) and anal pore (A)

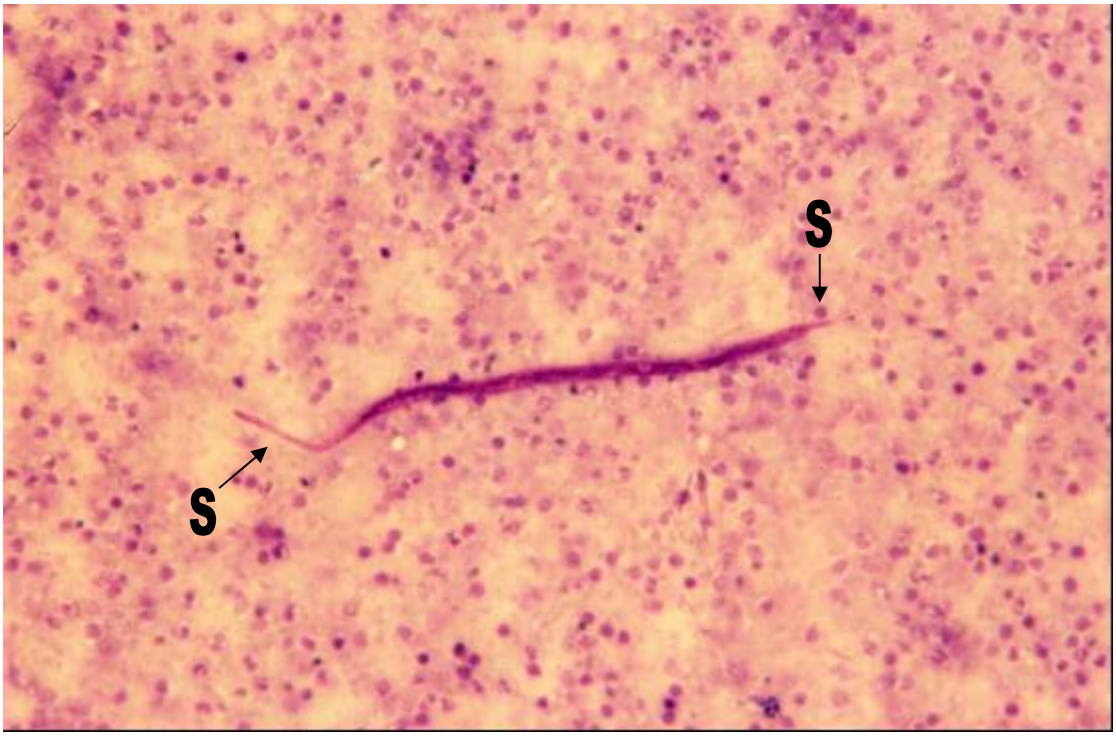

Figure 4.6: *Brugia patei* microfilaria with sheath (S) stained with Giemsa

Figure 4.7: Tail end of *Brugia patei* microfilaria with typical sub-terminal (ST) and terminal (T) tail nuclei

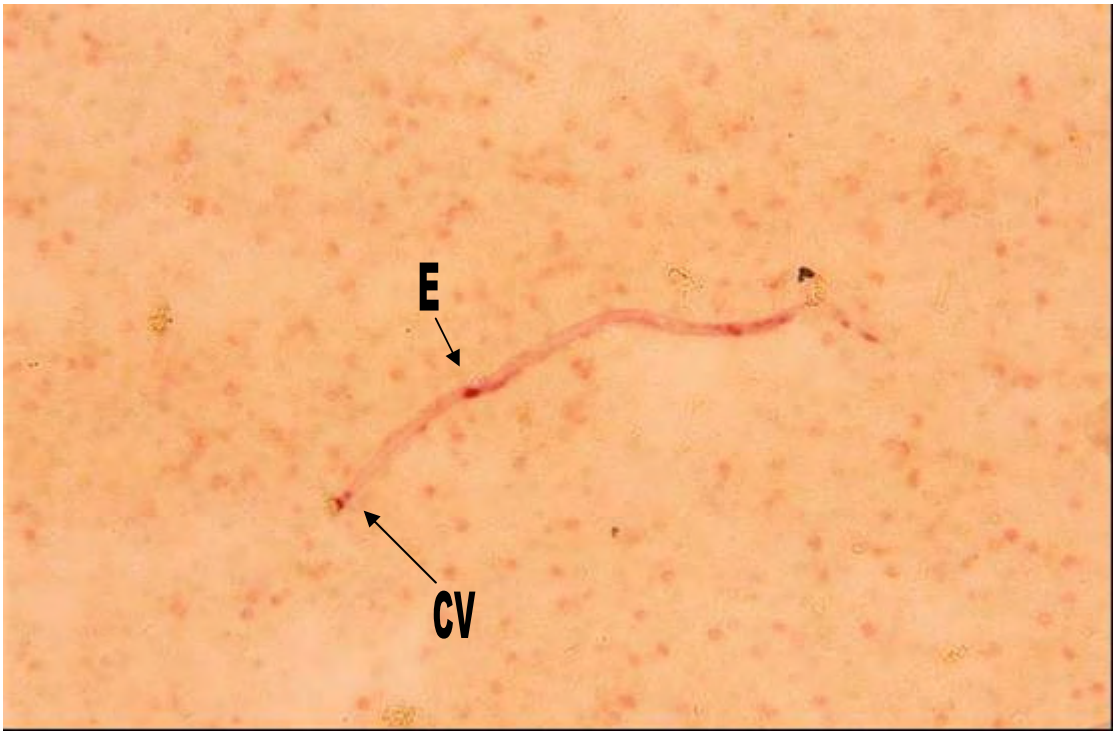

Figure 4.8: *Brugia patei* microfilaria showing acid phosphatase activity at the cephalic vesicle (CV), excretory pore (E) and the tail

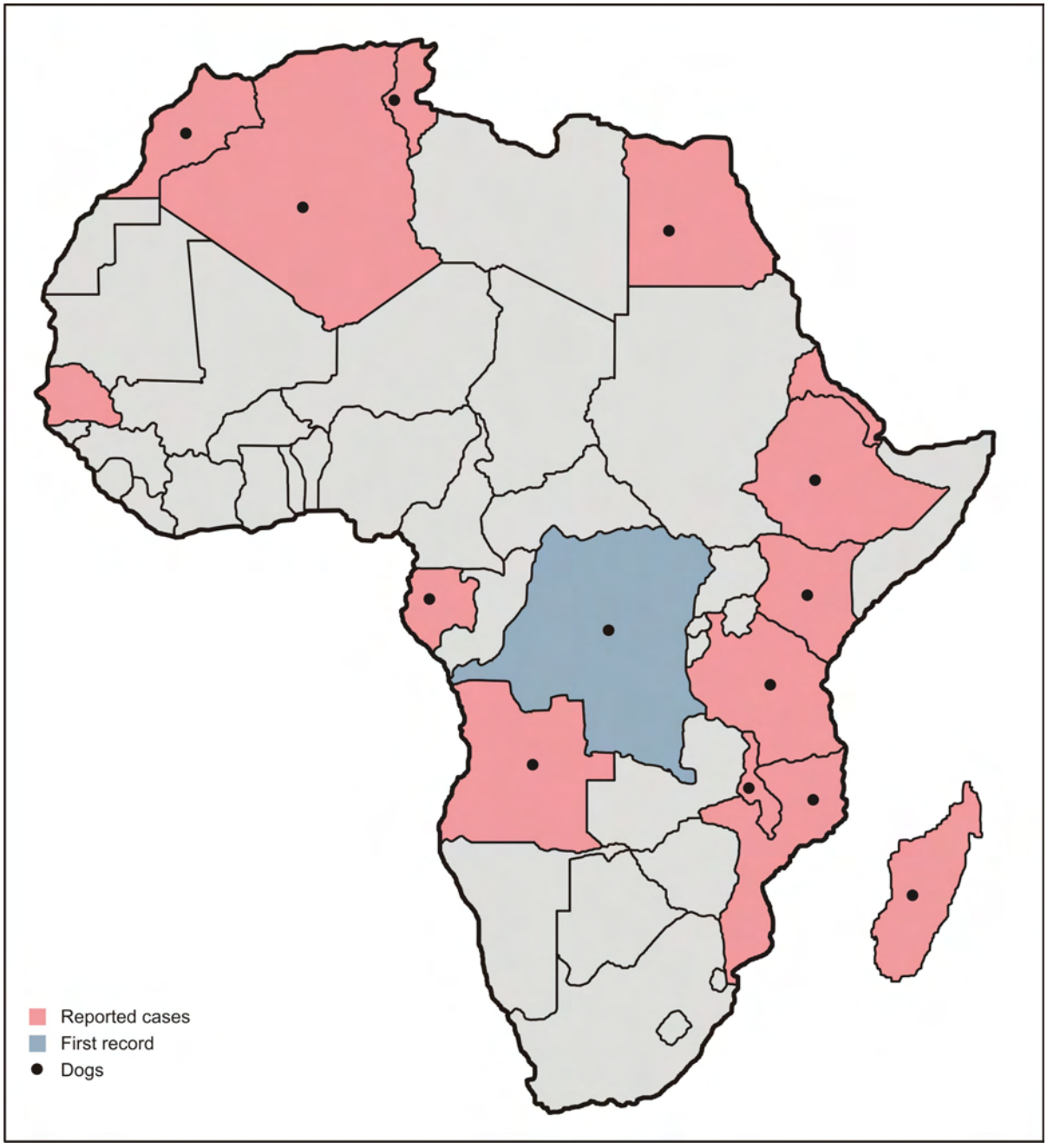

Figure 4.9: Geographical distribution of *Dirofilaria immitis* in dogs in Africa

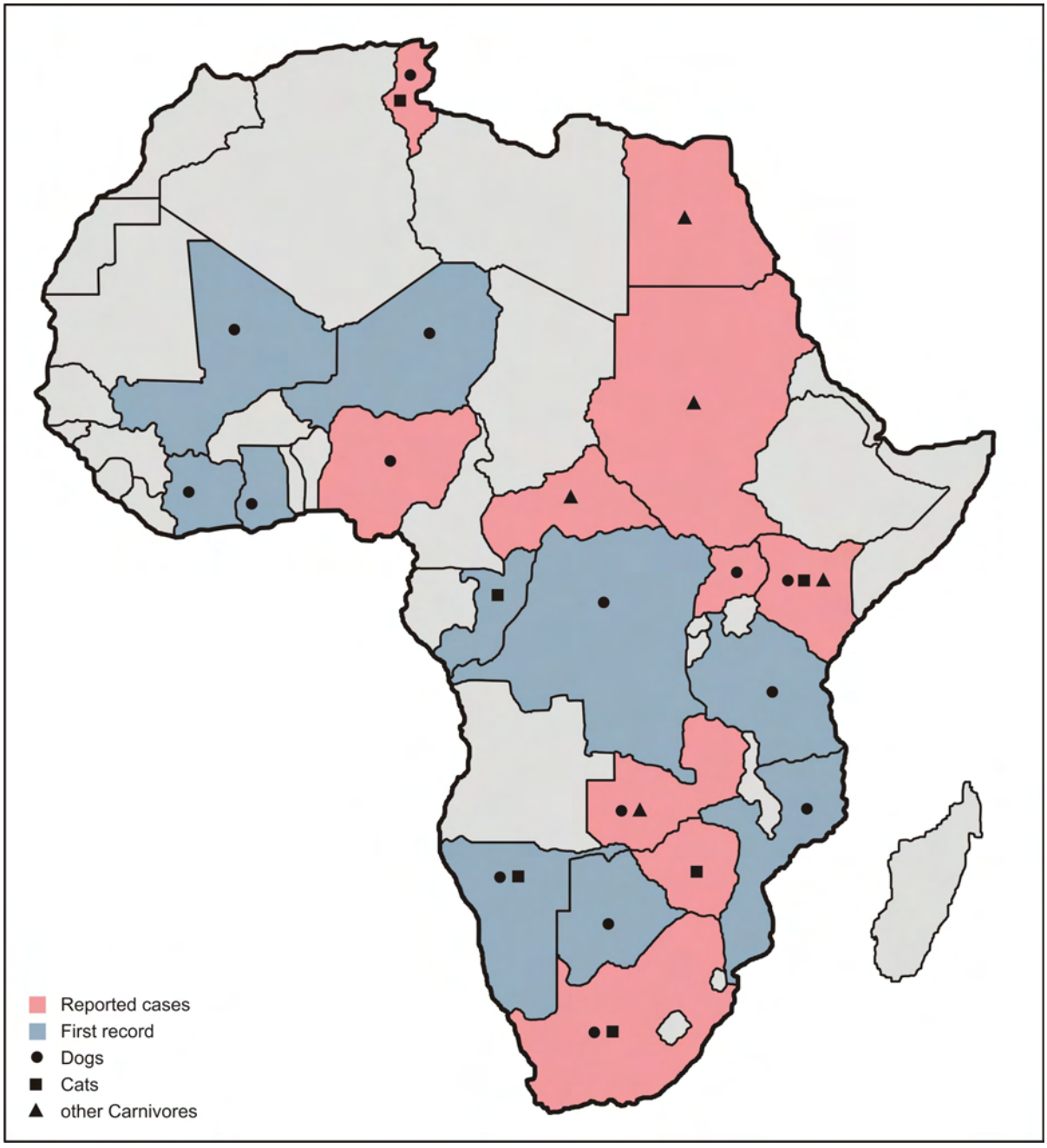

Figure 4.10: Geographical distribution of *Dirofilaria repens* in dogs, cats and other carnivores in Africa

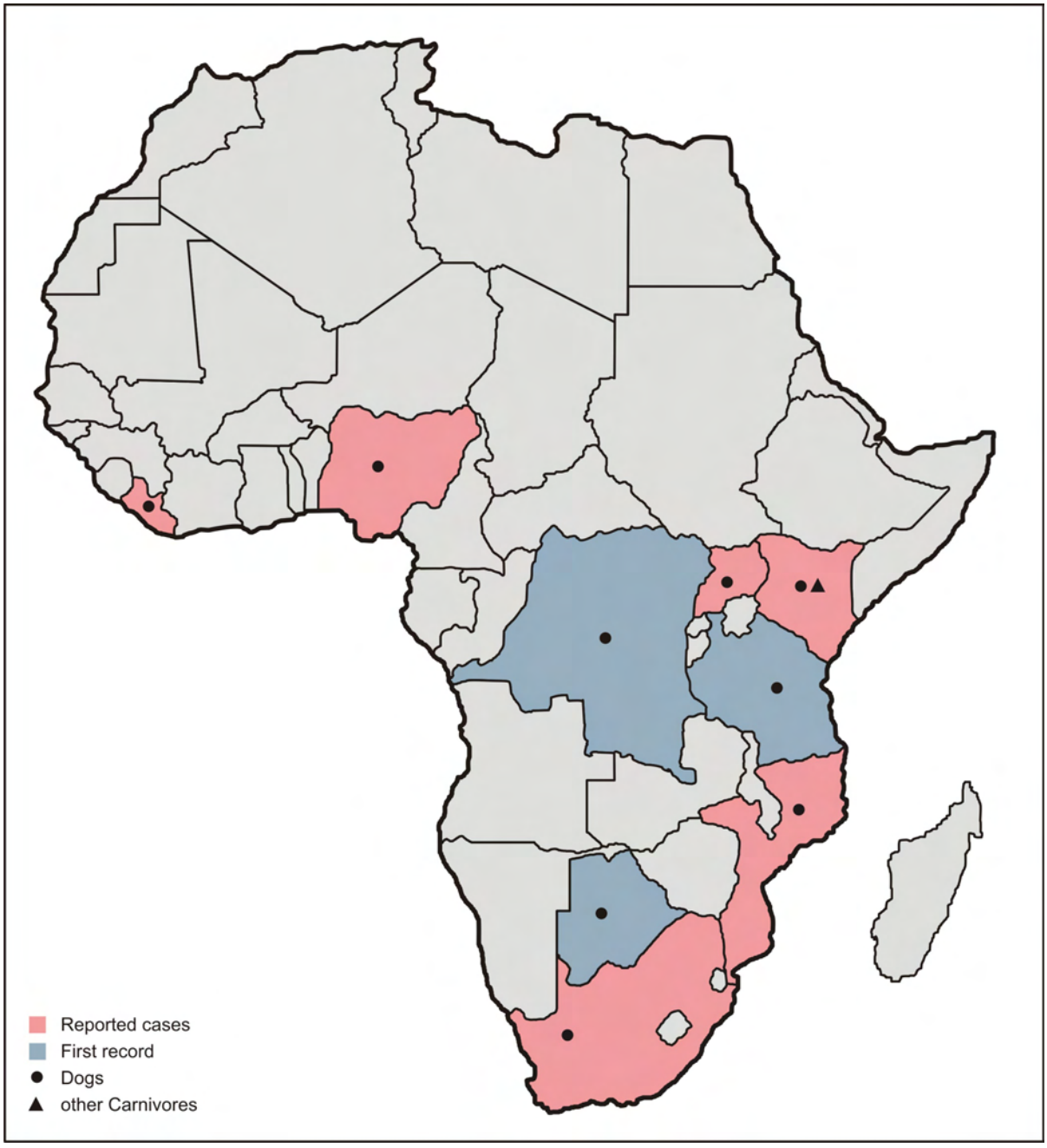

Figure 4.11: Geographical distribution of *Acanthocheilonema reconditum* in dogs and other carnivores in Africa

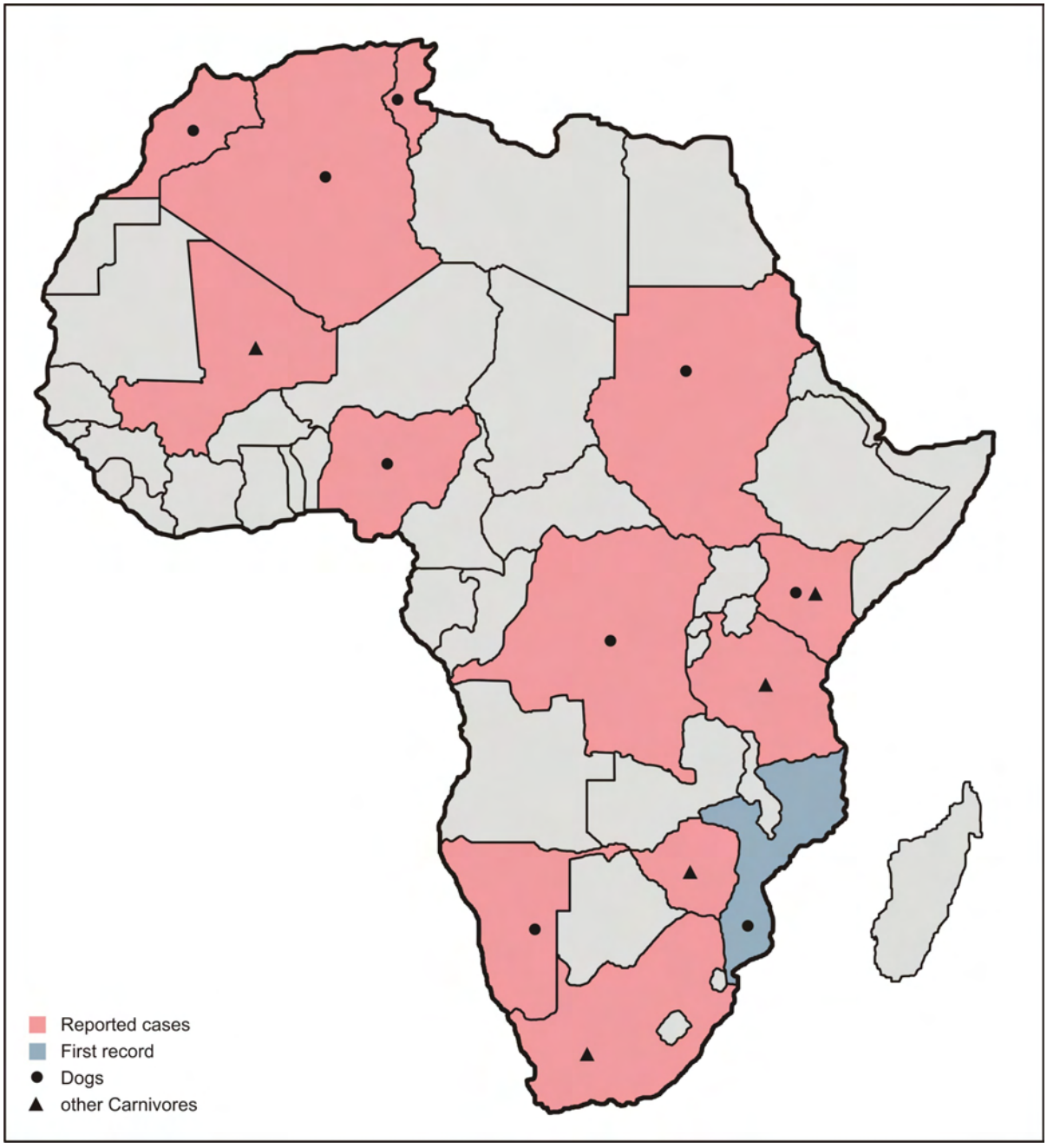

Figure 4.12: Geographical distribution of *Acanthocheilonema dracunculoides* in dogs and other carnivores in Africa

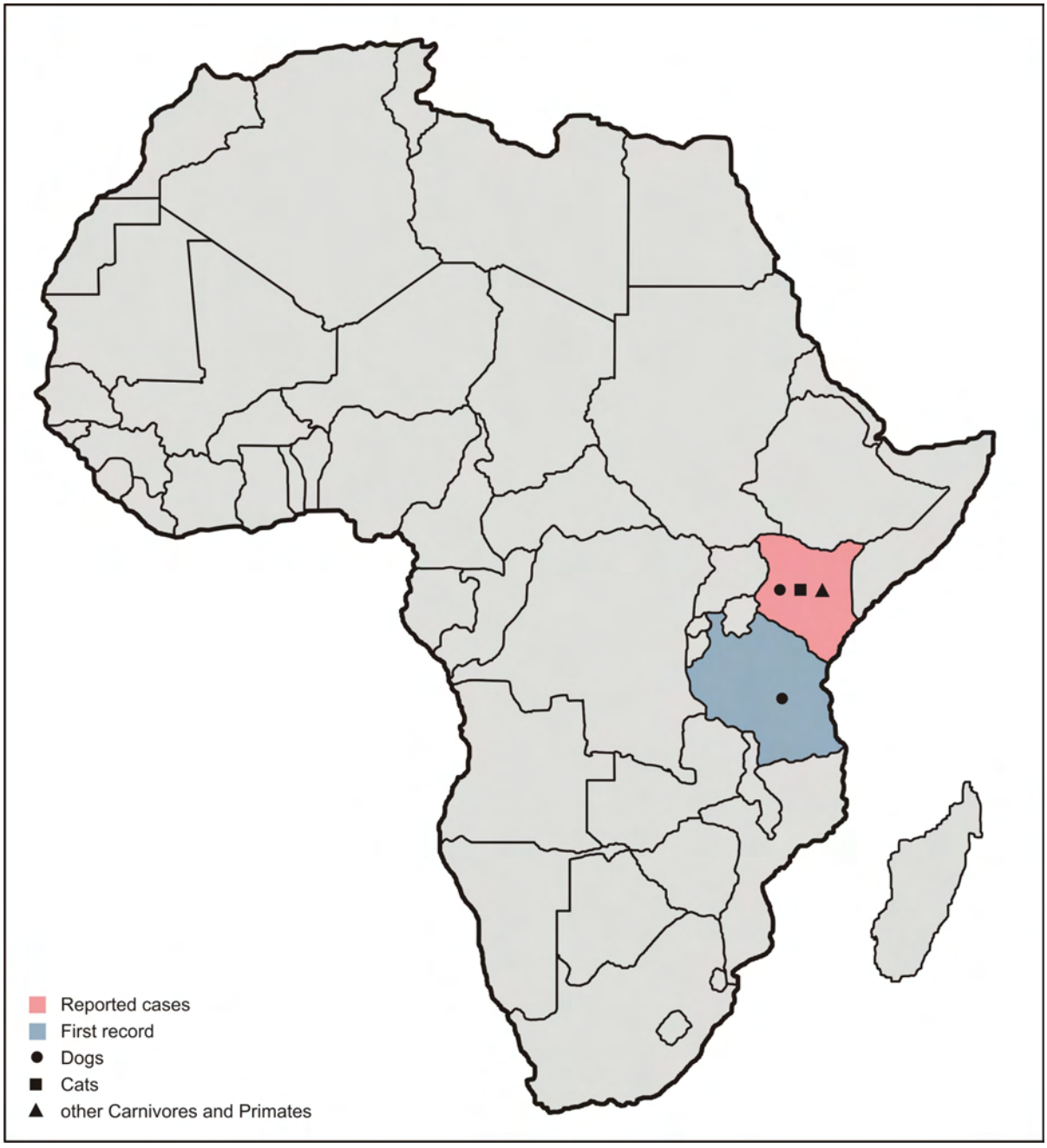

Figure 4.13: Geographical distribution of *Brugia patei* in dogs, cats and other carnivores and primates in Africa

Table 4.1: Overall filarial prevalence in dogs by locality in Gauteng, KwaZulu-Natal, Mpumalanga and Maputo provinces

Province/locality	No examined	*D. immitis* microfilariae	DiroCHEK® heartworm antigen	*D. repens* microfilariae	*A. dracunculoides* microfilariae	*A. reconditum* microfilariae
Gauteng						
Pretoria	316	0	1 (0.32 %)	1a	0	0
TOTAL	**316**	**0**	**1 (0.32 %)**	**1a**	**0**	**0**
KwaZulu-Natal						
Pongola	64	0	0	3 (4.69 %)	0	0
Mtubatuba	57	0	0	9 (15.79 %)	0	1 (1.75 %)
Empangeni	63	0	4 (6.35 %)	19 (30.16 %)	0	0
Richards Bay	63	0	6 (9.52 %)	10 (15.87 %)	0	0
Eshowe	62	0	2 (3.23 %)	6 (9.68 %)	0	0
Pietermaritzburg	108	0	1 (0.93 %)	5 (4.63 %)	0	4 (3.7 %)
TOTAL	**417**	**0**	**13 (3.12 %)**	**52 (12.47 %)**	**0**	**5 (1.2 %)**
Mpumalanga						
Nelspruit	96	1b	2 (2.08 %)	4 (4.17 %)	0	0
Malelane	237	0	6 (2.53 %)	1 (0.42 %)	0	97 (40.93 %)
TOTAL	**333**	**1b**	**8 (2.4 %)**	**5 (1.5 %)**	**0**	**97 (29.13 %)**
Maputo						
Maputo	266	2 (0.75 %)	2 (0.75 %)	9 (3.38 %)	1 (0.38 %)	10 (3.76 %)
Namahacha	17	0	0	2 (11.76 %)	0	0
Pessene	22	0	1 (4.55 %)	0	0	10 (45.45 %)
Régulo Mussumbuluco	8	0	0	1 (12.5 %)	0	0
TOTAL	**313**	**2 (0.64 %)**	**3 (0.96 %)**	**12 (3.83 %)**	**1 (0.32 %)**	**20 (6.89 %)**
Total	**1379**	**3 (0.22 %)**	**25 (1.81 %)**	**70 (5.08 %)**	**1 (0.07 %)**	**122 (8.85 %)**

[a] Animal brought in from Durban one month prior to sample collection.
[b] Animal imported from Mozambique 4 months prior to sample collection.

Table 4.2: Overall prevalence of *Dirofilaria repens* in dogs by age

Province	Age groups (years)	No examined	Microfilaria positive	% Positive
Gauteng	1-5	226	0	0
	6-10	67	1a	1.49a
	≥ 11	23	0	0
KwaZulu-Natal	1-5	269	23	8.55
	6-10	109	23	21.1
	≥ 11	39	6	15.38
Mpumalanga	1-5	280	3	1.07
	6-10	39	1	2.56
	≥ 11	14	0	0
Maputo	1-5	268	10	3.73
	6-10	43	2	4.65
	≥ 11	2	0	0

[a] Animal brought in from Durban one month prior to sample collection.

Table 4.3: Overall prevalence of *Acanthocheilonema reconditum* in dogs by age

Province	Age groups (years)	No examined	Microfilaria positive	% Positive
Gauteng	1-5	226	0	0
	6-10	67	0	0
	≥ 11	23	0	0
KwaZulu-Natal	1-5	269	4	1.49
	6-10	109	1	0.92
	≥ 11	39	0	0
Mpumalanga	1-5	280	93	33.21
	6-10	39	4	10.26
	≥ 11	14	0	0
Maputo	1-5	268	20	7.46
	6-10	43	0	0
	≥ 11	2	0	0

Table 4.4: Results of routine examinations for filarial infections of dogs and cats from South Africa between 1994 and 2008 based on the identification of microfilariae by acid phosphatase staining

Province	Locality	Year	Breed	Age group 1 1-5 years 2 6-10 years 3 ≥ 11 years	Sex 1 male 2 female	Filarial species
KwaZulu-Natal	Doonside	1994	Labrador	1	1	*D. repens*
	Pietermaritzburg	1994	Staffordshire Bull Terrier	2	1	*D. repens*
	Empangeni	1998	Toy Pom	1	1	*D. repens*
	PMB	1998	Labrador	2	2	*D. repens*
	Durban	1998	Corgi	2	1	*D. repens*
	Durban	1998	Staffordshire Bull Terrier	2	1	*D. repens*
	Mtubatuba	1999	Jack Russel Terrier	2	1	*D. repens*
	Scottburgh	2000	Crossbreed	1	1	*A. reconditum*
	Durban	2001	Labrador	2	2	*D. repens*
	Durban	2001	Border Collie	3	1	*D. repens*
	Scottburgh	2001	Border Collie	2	2	*D. repens*
	Umhlanga	2002	Crossbreed	2	2	*D. repens*
	Umhlanga	2002	Staffordshire Bull Terrier	3	2	*D. repens*
	Durban	2002	Crossbreed	2	1	*D. repens*
	Durban	2003	Border Collie	2	2	*D. repens*
	Durban	2003	Domestic Shorthair Cat	1	2	*D. repens*
	Pietermaritzburg	2003	Crossbreed	1	2	*D. repens*
	Richards Bay	2003	Crossbreed	3	1	*D. repens*
	Ballito	2004	Dalmation	1	1	*D. repens*
	Durban	2004	German Shepherd Dog	1	2	*D. repens*
	Durban	2004	Labrador	3	2	*D. repens*
	Durban	2004	Maltese Poodle	3	2	*D. repens*
	Durban	2004	Labrador	3	2	*D. repens*
	Durban	2004	Scottish Terrier	1	1	*A. reconditum*
	Durban	2005	Staffordshire Bull Terrier	2	1	*D. repens*
	Durban	2005	Crossbreed	3	2	*D. repens*
	Durban	2005	Staffordshire Bull Terrier	3	1	*D. repens*
	Durban	2006	German Shepherd Dog	1	1	*D. repens*
	Durban	2006	Domestic Shorthair Cat	2	2	*D. repens*
	Durban	2006	Domestic Shorthair Cat	2	1	*D. repens*
	Durban	2006	Dachshund	2	2	*D. repens*
	Meerensee	2007	Fox Terrier	2	1	*D. repens*
	Durban	2008	Miniature Pinscher	2	2	*A. reconditum*

Table 4.4 (*cont.*)

Province	Locality	Year	Breed	Age 1 1-5 years 2 6-10 years 3 ≥ 11 years)	Sex 1 male 2 female)	Filarial species
Gauteng	GaRankuwa	1994	Crossbreed	1	1	*A. reconditum*
	Onderstepoort (ex Pongola)	1998	Rottweiler	2	1	*D. repens*
	Pretoria	1999	Domestic Shorthair Cat	3	1	*D. repens*
	Johannesburg	1999	Domestic Shorthair Cat	3	1	*D. repens*
	Randburg	2003	Greyhound	2	1	*D. repens*
	Johannesburg	2007	Beagle	2	1	*D. repens*
North West	Rustenburg	2001	Maltese Poodle	2	2	*D. repens + A. reconditum*
Western Cape	Cape Town	1994	Crossbreed	2	1	*A. reconditum*
	Wellington	1999	Crossbreed	1	1	*A. reconditum*
	Cape Town	2003	Cocker Spaniel	2	1	*A. reconditum*
	Cape Town (ex Durban)	2003	Jack Russel Terrier	2	1	*D. repens*
	Cape Town	2007	Greyhound	2	1	*A. reconditum*

Table 4.5: Results of routine examinations for filarial infections of dogs and cats imported from countries in Africa and its islands into South Africa between 1992 and 2008 based on the identification of microfilariae by acid phosphatase staining

Country	Locality	Year	Breed	Age 1 1-5 years 2 6-10 years 3 ≥ 11 years	Sex 1 male 2 female	Filarial species	First record
Botswana	Gaborone	1999	Crossbreed	1	2	*D. repens*	X
	Gaborone	2000	Bouvier des Flandres	1	2	*A. reconditum*	X
	Gaborone	2004	Rhodesian Ridgeback	3	1	*D. repens*	
Congo-Brazzaville	Pointe Noire	2006	Airedale	1	1	*D. repens*	X
Democratic Republic of the Congo (Zaire)	Kinshasa	1998	Crossbreed	1	1	*A. reconditum*	X
	Kinshasa	2003	Crossbreed	2	1	*D. immitis*	X
	Kinshasa	2003	Crossbreed	3	1	*D. immitis*	X
	Kinshasa	2007	Rottweiler	1	1	*D. repens*	X
Gabon	Libreville	2004	Cocker Spaniel	1	1	*D. immitis*	
	Libreville	2007	German Shepherd	1	2	*D. immitis*	
Ghana	Accra	1999	Crossbreed	1	2	*D. repens*	X
	Accra	2002	German Shepherd	1	2	*D. repens*	
Ivory Coast	Abidjan	2002	Crossbreed	1	2	*D. repens*	X
Kenya	Nairobi	2005	Rhodesian Ridgeback	2	1	*D. repens*	
	Nairobi	2005	Labrador	1	1	*D. repens*	
	Nairobi	2005	German Shepherd	2	1	*A. dracunculoides*	
	Watamu	2008	Jack Russel Terrier	1	1	*D. immitis*	
Madagascar	Antananarivo	2008	Labrador	2	1	*D. immitis*	
Mali	Kayes Region	2004	Labrador	1	2	*D. repens*	X
Morocco	Rabat	2007	Chinese Sharpei	1	2	*D. immitis*	
Mozambique	Maputo	1992	German Shepherd	1	1	*D. immitis*	
	Quelimane	1996	Crossbreed	1	2	*D. immitis*	
	Quelimane	1996	Crossbreed	1	1	*D. immitis*	
	Quelimane	1996	Crossbreed	1	1	*D. immitis*	
	Quelimane	1996	Crossbreed	1	2	*D. immitis + A. reconditum*	X (*A. recond.*)
	Pemba	2003	Crossbreed	1	2	*D. repens*	X
	Beira	2004	Fox Terrier	1	1	*D. repens*	
	Maputo	2008	Border Collie	1	2	*A. reconditum*	

Table 4.5 (*cont.*)

Country	Locality	Year	Breed	Age 1 1-5 years 2 6-10 years 3 ≥ 11 years	Sex 1 male 2 female	*Filarial species*	First record
Namibia	Windhoek	1992	Cocker Spaniel	1	2	*A. dracunculoides*	X
	Windhoek	1992	St. Bernard	2	2	*A. dracunculoides*	
	Windhoek	1993	Crossbreed	1	1	*A. dracunculoides*	
	Otjiwarongo	1994	Fox Terrier	3	2	*D. repens*	X
	Omaruru	1998	Staffordshire Bullterrier	2	2	*D. repens*	
	Windhoek	2002	Crossbreed	1	1	*D. repens*	
	Windhoek	2002	Crossbreed	1	2	*D. repens*	
	Windhoek	2002	Crossbreed	1	1	*D. repens*	
	Windhoek	2002	Crossbreed	1	1	*D. repens*	
	Windhoek	2002	Crossbreed	1	1	*D. repens*	
	Otjiwarongo	2003	German Shepherd	2	2	*A. dracunculoides*	
	Windhoek	2007	Domestic Shorthair Cat	2	1	*D. repens*	
	Windhoek	2007	Domestic Shorthair Cat	2	1	*D. repens*	
	Windhoek	2008	Dobermann	2	1	*D. repens*	
Niger	Niamey	2008	Crossbreed	2	1	*D. repens*	X
Nigeria	Lagos	2004	Boerboel	1	2	*D. repens*	
	Lagos	2004	Labrador	2	2	*D. repens*	
	Lagos	2006	Bull Terrier	1	2	*D. repens*	
Réunion	-	2001	Boxer	2	1	*D. immitis*	
Tanzania	Dar-es-Salaam	1998	Staffordshire Bull Terrier	1	1	*D. repens*	
	Dar-es-Salaam	1998	Labrador	2	2	*D. repens* + *A. reconditum*	X (*A. recond.*)
	Dar-es-Salaam	1998	Rhodesian Ridgeback	1	2	*D. repens*	
	Dar-es-Salaam	1999	German Shepherd	1	1	*D. repens*	
	Dar-es-Salaam	2000	Staffordshire Bullterrier	1	1	*D. immitis*	
	Unknown	2001	Crossbreed	1	1	*D. repens*	
	Dar-es-Salaam	2001	Labrador	2	2	*D. immitis*	
	Dar-es-Salaam	2002	Jack Russel Terrier	2	2	*D. repens*	
	Dar-es-Salaam	2002	Fox Terrier	1	1	*D. immitis*	
	Dar-es-Salaam	2003	German Shepherd	1	2	*D. repens*	
	Kilombero Valley	2005	Rhodesian Ridgeback	1	2	*B. patei*	X
	Dar-es-Salaam	2007	Crossbreed	1	1	*D. repens*	
	Dar-es-Salaam	2007	Dalmation	1	2	*D. repens*	
	Dar-es-Salaam	2007	Cocker Spaniel	2	2	*D. repens*	
	Dar-es-Salaam	2008	Dalmatian	2	2	*D. repens*	

Table 4.5 (*cont.*)

Uganda	Kampala	1999	German Shepherd Dog	2	2	*A. reconditum*	X
	Kampala	2006	Labrador	2	2	*D. repens* + *A. reconditum*	X (*D. repens*)
Zambia	Lusaka	2001	Maltese Poodle	2	2	*D. repens*	X
	Lusaka	2002	Crossbreed	1	1	*D. repens*	
	Lusaka	2004	Boerboel	1	1	*D. repens*	
	Chipata	2005	Jack Russel Terrier	1	2	*D. repens*	
	Lusaka	2007	Labrador	3	2	*D. repens*	
Zimbabwe	Harare	2002	Crossbreed	1	1	*D. immitis*	X

[a] Animal originally from Beira in Mozambique; moved to Zimbabwe 1 year prior to sample collection.

Chapter 5
DISCUSSION

Although a survey for parasitism in animals conducted by FAO, WHO and OIE (1984) indicates that filariosis of dogs is widespread in Africa, there is a dearth of published information on the occurrence and prevalence of filarial helminths in both dogs and cats. In two independent surveys a first attempt was made to map canine and feline filariosis with focus on Gauteng, KwaZulu-Natal and Mpumalanga provinces in South Africa and Maputo province in Mozambique. This attempt was complemented by diagnostic results of routine examinations for filarial infections of dogs and cats from South Africa obtained between 1994 and 2008. Combined with a critical literature review on filariosis of domestic carnivores in Africa which was updated by diagnostic results of routine examinations for filarial infections obtained from animals originating from other African countries between 1994 and 2008, the topic is comprehensively addressed for the first time ever from a continental perspective. The results indicate the endemic status of 5 filarial species in dogs and cats on the African continent, namely *D. immitis*, *D. repens*, *A. reconditum*, *A. dracunculoides* and *B. patei*. The supposed diagnosis of *C. grassii* by Heisch *et al.* (1959) and Nelson *et al.* (1962) in 2 dogs from Kenya and Tanzania is unlikely as the sparse description of the microfilariae, which were encountered in the skin, very much deviates from the original descriptions given by Noè (1907, 1908). The validity of *Microfilaria auquieri*, first reported by Foley in 1921 in dogs on the Algerian-Moroccan border and subsequently by Rioche (1960) in a dog in Algeria, requires further investigation. Although, with the exception of *D. immitis*, filarial species of dogs and cats were regarded as largely non-pathogenic, there is growing evidence that infections are not so innocuous as assumed (Piercy 1951; Restani *et al.* 1963;

Mantovani 1965; Mandelli & Mantovani 1966; Bobade *et al.* 1981; Hubert 1985; Kamalu 1986, 1991; Ortega-Mora & Rojo-Vázquez 1988; Bredal *et al.* 1998; Schwan *et al.* 2000; Tarello 2000a, 2003, 2004; Bolio *et al.* 2002; Martano *et al.* 2004; Schwan & Schröter 2006). Particularly, with the introduction of macrocyclic lactone-based dewormers for dogs and cats, filariosis of any aetiology has gained significance due to the microfilaricidal activity of the anthelmintic group which can result in a potentially fatal shock-like syndrome and other adverse reactions (Sasaki *et al.* 1989; Schrey 1996; Ware 2003; Plumb 2008; V. Schwan, unpublished data 2008).

In the following, separate accounts on the filarial species confirmed in the study are given.

5.1 *Dirofilaria immitis*

With the exception of South Africa and Namibia, there is only very little attention given to companion animals in African countries. Hence there is little awareness of heartworm and the severe disease it can elicit. Chemoprophylactic and adulticidal drugs are not only unavailable in many African countries but also unaffordable for most owners. The situation in Africa is compounded further by the fact that filarial diagnostic services are only available in South Africa.

Dirofilaria immitis infections have been reported very occasionally in South Africa in imported dogs only (Van Heerden *et al.* 1980; Verster *et al.* 1991). The survey conducted on dogs in the South African provinces did not provide convincing evidence for autochthonous heartworm infections. Microfilariae of *D. immitis* were detected in a single dog in Nelspruit, Mpumalanga province. However, the clinical history revealed that the dog originated from Beira in Mozambique and was brought by the owner to

South Africa 4 months prior to sample collection. Considering the long prepatent period of 6-9 months, the obvious conclusion is that the infection was acquired in Mozambique. In the South African provinces, 2.06 % of samples (21/1 066) tested positive for *D. immitis* antigen in the absence of microfilariae. The result has to be interpreted with reserve as the blood samples, for logistical reasons, were generally analyzed only after the maximum recommended storage period of 7 days at 2-7 °C specified by the manufacturer of the DiroCHEK® test kit. Also to consider is the simultaneous occurrence of *D. repens* in dogs, since there is evidence that infections can be antigenically crossreactive with *D. immitis* antigen tests (Valcárcel *et al.* 1990; Beugnet *et al.* 1993b; Schrey 1996; Schwan *et al.* 2000). This is supported by the finding that 19.23 % of the *D. repens*-positive samples from Kwa-Zulu-Natal tested positive in the *D. immitis* antigen test. According to Frank *et al.* (1992) and Tarello (2001), concentration tests provide more accurate results in low-endemic and newly colonized areas than serological tests. However, the sample size of 313 dogs per province is insufficient to make a representative statement on the absence of autochthonous infections in South Africa. Since dogs imported into South Africa are subject to heartworm screening, the percentage of potentially infected animals in the overall population of the country, if existing at all, must be very small. Nevertheless, there are several reports where a newly introduced infected host has established autochthonous cycles in previously free regions (Zimmerman, Knapp, Foreyt, Erekson & Mackenzie 1992).

Prerequisites for the transmission of *D. immitis* are a high density of genetically suitable polycyclic mosquito vectors with high transmission potential as well as suitable climatic conditions to allow the development of metacyclic larvae (Abraham 1988). Several mosquito species with high transmission potential such as *Aedes aegypti*, *Aedes vexans*, *Aedes cinereus*, *Anopheles pharoensis*, *Anopheles tenebrosus*, *Culex pipiens*,

Culex quinquefasciatus, *Mansonia africana* and *Mansonia uniformis* are endemic in South Africa (Gillies & De Meillon 1968; Jupp 1996). Whereas heartworm transmission is all year round in tropical latitudes, it is seasonal in subtropical and particularly temperate regions. A threshold of approximately 14 ºC has been determined, below which development will not proceed in the mosquito vector and transmission ceases (Fortin & Slocombe 1981). The total environmental heat required for development can be expressed in terms of degree-days in excess of this threshold, known as heartworm development units (HDUs) (Fortin & Slocombe 1981; Slocombe, Surgeoner & Srivastava 1989). By additionally incorporating other variables, models have been developed for Canada, the United States and Europe that permit to determine the seasonal transmission period which is useful for timing of annual blood testing and preventive medication programmes (Slocombe *et al.* 1989; Lok & Knight 1998; Genchi *et al.* 2005). Such models are not available for the African continent. In the South African context, favourable climatic conditions for heartworm transmission are prevailing particularly in the Indian Ocean Coastal Belt and to a lesser extent in the Lowveld Bioregion, the Mopane Bioregion and the Central Bushveld Bioregion (Mucina & Rutherford 2006). The high prevalence of *D. repens* discovered in dogs and cats in the Indian Coastal Belt might hold an explanation why heartworm has not become established in this bioregion. In Italy, Genchi *et al.* (2005) discovered an immunological-based interaction between *D. immitis* and *D. repens* which plays an important role in the establishment of the parasite in the host, thus influencing different patterns of prevalence. The studies in Italy suggest that establishment of *D. immitis* infection by superimposition of this parasite on an existing *D. repens* infection is more difficult than establishment of *D. repens* infection in dogs with existing *D. immitis* infection.

The widespread use of tetracyclines and macrocyclic lactones in South Africa might be another reason why heartworm has never become established. Since high tick infestations and subsequent *Ehrlichia canis* infections are common in Southern Africa, tetracyclines and derivatives are used indiscriminately and extensively to treat suspected ehrlichiosis cases and other infectious diseases. Particularly oxytetracycline which is readily available in a broad range of low-cost, over-the-counter injectable formulations is widely used by laymen (Jan G. Myburgh, personal communication 2009). However, intracellular bacteria of the genus *Wolbachia*, a filarial endosymbiont upon which filarial helminths appear to be dependent for embryogenesis, larval development and survival as adult worms, are highly susceptible to tetracyclines (Bandi, McCall, Genchi, Corona, Venco & Sacchi 1999; McCall, Jun & Bandi 1999; Smith & Ranjan 2000). Macrocyclic lactones on the other side are known to have microfilaricidal properties, and hence can reduce the number of microfilaraemic dogs and the source of infection for the mosquito population. When used in combination, tetracyclines and macrocyclic lactones appear to have a synergistic effect on filarial helminths which is supported by field trials on human onchocercosis in Western Africa (Hoerauf, Adjei & Büttner 2002).

With Mozambique as the only neighbouring country of South Africa where *D. immitis* is known to be endemic, there are valid concerns of transborder infections. The study was able to confirm endemicity of *D. immitis* for Maputo province in Mozambique, with a prevalence of 0.64 %. Previous reports by Dias (1954) and Jurášek (1986) for Maputo Province are controversial as no details were provided on what criteria the diagnoses were based. The very low prevalence for Maputo province is in sharp contrast with findings of a small-scale survey conducted in the Province of Zambézia where 4 out of 13 dogs (30.8 %) were found positive (Schwan & Durand 2002). The widespread off-

label use of the over-the-counter injectable bovine ivermectin formulation, which according to Luis Neves (personal communication 2003) is widespread among dog owners and veterinarians in Maputo province, might explain the discrepancy in prevalence.

Based on the literature review and diagnostic results, *D. immitis* is currently known to be endemic in 13 African countries and 4 islands and has only been reported from dogs. With a general lack of recent surveys the importance of *D. immitis* is difficult to assess.

5.2 *Dirofilaria repens*

Since 1994 autochthonous *D. repens* infections have been diagnosed regularly in blood samples received from dogs and cats in the South African provinces of KwaZulu-Natal and occasionally from Gauteng and North West provinces. The apparent predominance of *D. repens* in KwaZulu-Natal was confirmed in both surveys with 12.47 % (52/417) of dogs and 10.98 % (9/82) of cats infected. The rather close prevalence rates for dogs and cats are in contrast with results reported from Kenya, where *D. repens* was more prevalent in cats (18-93 %) than in dogs (2.4-16.7%) (Heisch *et al.* 1959; Nelson *et al.* 1962). The prevalence in dogs from Mpumalanga province was 1.5 % (5/333) only. A single dog found to be infected in Pretoria, Gauteng, was brought in by the owner from Durban in KwaZulu-Natal 1 month prior to sample collection. Considering a 6-months prepatent period, infection must have been contracted in KwaZulu-Natal. Several mosquito species, such as *Aedes aegypti*, *Mansonia africana* and *Mansonia uniformis*, were identified by Nelson *et al.* (1962) to have a high transmission potential for *D. repens* in Africa. All of them are widely distributed in South Africa (Jupp 1996). Veterinarians should be aware of the high prevalence of *D. repens* in KwaZulu-Natal and check the microfilarial status of their patients before opting for a macrocyclic

lactone-based dewormer. There is strong evidence coming from the province that suggests the development of a shock-like syndrome, with in some instances fatal outcome, following the administration of macrocyclic lactones in *D. repens* infected cats and dogs (V. Schwan, unpublished data 2008). The comparatively low prevalence of 3.83 % (5/333) in dogs of Maputo province came as a surprise as the prevailing climatic conditions are similar to those encountered in the Indian Coastal Belt of KwaZulu-Natal and *Aedes pembaensis*, as an additional vector with high transmission potential, is endemic (Nelson *et al.* 1962; Jupp 1996). An explanation can be the previously mentioned extensive off-label use of the injectable bovine ivermectin formulation by dog owners and veterinarians in the province (Luis Neves, personal communication 2003).

Dirofilaria repens is currently known to be endemic in 20 African countries which illustrates that the filariid is far more widespread on the continent than has been previously claimed by Pampiglione *et al.* (1995).

5.3 *Acanthocheilonema reconditum*

The study identified *A. reconditum* as the species with the highest overall prevalence (Table 4.1). The data for Mpumalanga with the highest prevalence rate of 29.13 % (97/333) merit discussion since none of the dogs from Nelspruit (0/96) were found to be infected, but 40.93 % (97/333) of dogs from Malelane were positive. This discrepancy can most likely be explained by the poor socioeconomic background of pet owners in Malelane where sampling was conducted in an exclusively rural catchment area with basic animal care, such as regular ectoparasite control, being unaffordable. Since 1994 autochthonous infections have been diagnosed in dogs from KwaZulu-Natal, Gauteng, North West and Western Cape provinces (Table 4.4) which indicates that the filariid is

probably the most widely distributed in the country. Similar findings are reported from Kenya (Nelson 1962).

Acanthocheilonema reconditum is currently known in 9 African countries with no reports yet available from Northern Africa.

5.4 *Acanthocheilonema dracunculoides*

Although Nelson (1963) claims that *A. dracunculoides* is widespread in the drier areas of Africa, extending from the Mediterranean to South Africa, where it was also first discovered and described by Cobbold (1870), the filariid has not been diagnosed again in the country. Future surveys should focus on the Northern Cape province where climatically more appropriate conditions prevail.

5.5 *Brugia patei*

Autochthonous infections with *B. patei* were previously only reported by Nelson *et al.* (1962) from Kenya in various carnivores and greater bushbaby. There is now a first record for Tanzania, where a dog from Kilombero Valley was diagnosed with typically sheathed microfilariae sharing the morphological features described by Buckley *et al.* (1958) (Table 4.5). A sheathed microfilaria was already described briefly by Fülleborn (1908b) in a dog from Dar-es-Salaam and named *Filaria ochmanni*. Buckley *et al.* (1958) already suggested a link with *B. patei*, but re-examination of Fülleborn's material is impossible as it has been lost. Considering the wide distribution of *A. pembaensis, M. africanus* and *M. uniformis* as the currently known vectors, one can expect *B. patei* to be more widespread in Eastern Africa.