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SUMMARY

FILARIOSIS OF DOMESTIC CARNIVORES IN GAUTENG, KWAZULU-NATAL AND MPUMALANGA PROVINCES, SOUTH AFRICA, AND MAPUTO PROVINCE, MOZAMBIQUE

By

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Degree: PhD

Based on two surveys, the thesis focuses on the prevalence of filarial parasites of domestic carnivores in Gauteng, KwaZulu-Natal and Mpumalanga provinces in South Africa and Maputo province of Mozambique. This is complemented by diagnostic results of routine examinations for filarial infections of dogs and cats from South Africa obtained between 1994 and 2008. Blood samples were collected and initially screened by membrane filtration for microfilariae. Other techniques employed were acid phosphatase staining for the identification of microfilariae and a commercial enzyme-linked immunosorbent assay for the detection of heartworm antigen. Combined with a critical literature review on filariosis of domestic carnivores in Africa, which is updated by diagnostic results obtained from animals in Africa between 1992 and 2008, the topic is addressed for the first time ever from a continental perspective.

In the South African provinces and Maputo province of Mozambique 196 of 1 379 dogs (14.21 %) were found positive for microfilariae. The species identified were *Dirofilaria immitis*, *Dirofilaria repens*, *Acanthocheilonema reconditum* and *Acanthocheilonema dracunculoides*. The endemic status of *D. immitis* was confirmed in 2 out of 313 dogs from Maputo province but not in the South African provinces. Infection with *D. repens* was found in 70 dogs (5.08 %). The highest prevalence rate was recorded in KwaZulu-Natal with 12.47 % (52/417), followed by Maputo Province with 3.83 % (12/313) and Mpumalanga with 1.5 % (5/333). Routine examinations have also confirmed autochthonous infections with *D. repens* in Gauteng and North West provinces. *Acanthocheilonema reconditum* was the species with the highest overall prevalence of 8.85 % (122/1 379). The highest prevalence rate was recorded in Mpumalanga with 29.13 % (97/333) followed by Maputo province with 6.39 % (20/313) and KwaZulu-Natal with 1.2 % (5/417). Routine examinations have also confirmed autochthonous infections in Gauteng, North West and Western Cape provinces. *Acanthocheilonema dracunculoides* was the species with the lowest overall prevalence of 0.07 % (1/1 379) and was only recorded in 1 dog from Maputo Province.

In KwaZulu-Natal 9 of 82 cats (10.98 %) were found positive for microfilariae, with *D. repens* as the only species involved.

Chapter 1
INTRODUCTION

Filariosis is the infection of vertebrate hosts with nematodes of the superfamily Filarioidea. Earlier known as filariasis, the term is derived from the generic name *Filaria* which according to Stiles (1907) has been used by zoologists and physicians 'as a generic catch-all for slender roundworms which could not be definitely determined'. The predilection sites of the preadult and adult stages of the filarial worms are the body cavities, blood or lymph vessels and connective tissue. The life cycles are indirect with mammals, amphibians, reptiles and birds acting as definitive hosts and haematophagous arthropods as intermediate hosts. Being viviparous nematodes, females produce incompletely differentiated first stage larvae, known as microfilariae, which are found in the blood and/or lymph. Several species are important pathogens of domestic animals and humans in mostly tropical and subtropical areas of the world. In the veterinary field, filariosis of domestic carnivores is of particular importance. Due to its severe pathogenic effects as well as profound financial implications for owners, *Dirofilaria immitis*, colloquially known as 'heartworm' or 'canine heartworm', constitutes the most important species for both dog and cat. Apart from the genus *Dirofilaria*, the spectrum of filarial helminths encountered in domestic carnivores belongs to the genera *Acanthocheilonema*, *Cercopithifilaria* and *Brugia*. Although *Acanthocheilonema*, *Brugia*, *Cercopithifilaria* and *Dirofilaria* species, other than *D. immitis*, were considered as largely non-pathogenic, there is growing evidence that infections are not so innocuous as generally assumed (Schwan, Miller, De Kock & Van Heerden 2000; Tarello 2003, 2004; Schwan & Schröter 2006). With the introduction of macrocyclic lactone-based dewormers for dogs and cats, filariosis of any aetiology has gained significance.

Similarly to the previously widely used diethylcarbamazine, macrocyclic lactones have microfilaricidal activity which can result in a potentially fatal shock-like syndrome and other adverse reactions as demonstrated in *D. immitis* and *Dirofilaria repens* infected dogs and cats (McGaughey 1952; Sasaki, Kitagawa, Ishihara & Shibata 1989; Euzéby 1990; Schrey 1996; Ware 2003; Plumb 2008; V. Schwan, unpublished data 2008).

Apart from the direct effects on the dog and cat population there are also zoonotic implications as humans can act as accidental hosts for some filarial helminths. The ever-increasing movement of people with their pets and climatic changes are regarded as important factors for the continuous spreading of filariosis (Russell 1985; Poglayen 1996; Rossi, Pollono, Meneguz, Gribaudo & Balbo 1996; Bucklar Scheu, Mossi & Deplazes 1998; Irwin 2002; Tarello 2003; Genchi, Rinaldi, Cascone, Mortarino & Cringoli 2005).

In contrast to most other continents, there is a lack of published information on the occurrence and distribution of filarial helminths of dogs and cats in Africa and its islands, which argues for a strong effort to conduct systematic field studies (Lok 1988). In a first attempt to remediate this situation, the objectives of this study are:

a) to determine the occurrence and prevalence of *Dirofilaria immitis* and other filarial helminths in dogs in Gauteng, KwaZulu-Natal and Mpumalanga provinces, South Africa and Maputo province, Mozambique,
b) to determine the occurrence and prevalence of filarial helminths of cats in KwaZulu-Natal province,

c) to evaluate the results of routine examinations for filarial infections of dogs and cats from South Africa conducted between 1994 and 2008,

d) to evaluate the results of routine examinations for filarial infections of dogs and cats imported from African countries conducted between 1992 and 2008, and

e) to conduct a literature review on filariosis of dogs and cats in Africa.

Chapter 2
LITERATURE REVIEW OF THE DIFFERENT AETIOLOGIES OF FILARIOSIS IN DOMESTIC CARNIVORES IN AFRICA

A total of ten confirmed filarial species has been reported worldwide in domestic carnivores (Table 2.1). As regards Africa and its islands, there are published reports of autochthonous cases of filarial infections in dogs and cats involving six of these species (Nelson, Heisch & Furlong 1962; Laub 1988).

Of these the species *D. immitis, Dirofilaria repens, Acanthocheilonema reconditum reconditum, Acanthocheilonema dracunculoides* and *Brugia patei*, that all belong to the family Onchocercidae of the superfamily Filarioidea, form the subjects of this study.

2.1 *Dirofilaria immitis*

2.1.1 Taxonomy

Dirofilaria immitis (Leidy, 1856), commonly known as heartworm or canine heartworm, belongs to the subfamily Dirofilariinae and was first described as *Filaria canis cordis* in 1850 by Leidy in Philadelphia. In 1856 the worm was renamed *Filaria immitis* by Leidy. Railliet & Henry (1911a) erected the genus *Dirofilaria* and designated *Filaria immitis* as its type species. As a result of subsequent descriptions by various authors the helminth figures in the literature under the following synonyms: *Filaria canis cordis* (Leidy, 1850), *Filaria papillosa haematica canis-domestica* (Gruly & Delafond, 1852), *Filaria immitis* (Leidy, 1856), *Filaria papillosa haematica* (Schneider, 1866), *Filaria spirocauda* (Leidy, 1858), *Filaria cordis phocae* (Joly, 1858), *Filaria haematica* (Leuckart, 1867), *Filaria sanguinis* (Cobbold, 1869), *Filaria hebetata* (Cobbold, 1873), *Filaria spirocauda* (Cobbold, 1879), *Filaria* sp. (Horst, 1889); *Microfilaria immitis* (Neumann & Mayer, 1914), *Dirofilaria nasuae* (Mazza, 1926), *Dirofilaria pongoi* (Vogel & Vogelsang, 1930), *Dirofilaria indica* (Chakravarty, 1936), *Filaria magalhaesi* (Blanchard, 1895), *Dirofilaria magalhaesi* (Blanchard, 1895), *Dirofilaria fausti* (Skrjabin & Schikhobalova, 1948) and *Dirofilaria louisianensis* (Faust, Thomas & Jones, 1941) (Anderson 1952; Sonin 1985).

Faust (1937) proposed that the genus *Dirofilaria* be split into the subgenera *Dirofilaria* and *Nochtiella*. Species whose predilection site is the cardiovascular system were allocated to the subgenus *Dirofilaria*, whereas the subgenus *Nochtiella* contains those whose predilection site is the subcutaneous connective tissue.

2.1.2 Morphology

The morphological features that characterize the genus have been described by Railliet & Henry (1911a), Vogel (1927), Lent & Freitas (1937) and Sonin (1985). A detailed description of the adult stages of *D. immitis* is given by Fülleborn (1912) and Vogel (1927). According to these authors, females are 21-31 cm long and 1-1.3 mm wide whereas males are 12-20 cm long and 0.6-0.9 mm wide. The left spicule is 300-355 µm long, and the right spicule 175- 226 µm.

The microfilariae are unsheathed, and a detailed description was given by Fülleborn (1912) and Taylor (1960a). The cephalic end is conical and the posterior end is acute with the nuclear column (i.e. the cells that constitute the body of the microfilaria) not extending to the end of the body. The tail in unfixed and unstained microfilariae is straight (Marconcini, Magi, Macchioni & Sassetti 1996). Minimum and maximum measurements regarding length and width range from 180-340 µm and 5-7 µm respectively (Table 2.2).

The infective filarial larva in mosquitoes has been originally described by Nelson (1959) with additional information being provided by Taylor (1960b), Orihel (1961), Lichtenfels, Pilitt, Kotani & Powers (1985) and Bain & Chabaud (1986).

2.1.3 Life cycle

Dirofilaria immitis females produce microfilariae which are found in the blood of the definitive host. They are also capable of passing through the placenta and infect foetuses *in utero* (Mantovani & Jackson 1966; Atwell 1981; Todd & Howland 1983), and microfilariae have been found in urine (Kaewthamasorn, Assarasakorn & Niwetpathomwat 2008) and synovial fluid (Hodges & Rishniw 2008). The appearance of microfilariae in dogs in the peripheral blood is nocturnal subperiodic, with maximum levels of microfilaraemia being attained during late afternoon and at night with some geographical variation (Kosuge 1924; Schnelle & Young 1944; Euzéby & Lainé 1951; Webber & Hawking 1955; Newton & Wright 1956; Tongson & Romero 1962). Apart from the daily periodicity, there is also a seasonal periodicity with microfilariae being more abundant in the peripheral blood during spring and summer (Newton 1968; Kume 1975; Sawyer 1975). There exists a coincidence between the time microfilariae are most abundant in the peripheral blood and the time mosquito vectors obtain blood meals, a circumstance which is regarded as an evolutionary adaptation (Abraham 1988). Microfilariaemia in cats is only seen in less than 20 % of cases, and is inconsistent and transient when present (Cusick, Todd, Blake & Daly 1976). In cats the microfilaraemia is also nocturnal subperiodic (Nogami, Marasugi, Shimazaki, Maeda, Harasawa & Nakagaki 2000).

Mosquitoes act as intermediate hosts. Microfilariae develop into 3rd stage infective larvae in the mosquito and their development has been described by Taylor (1960b), Christensen (1977) and Bradley, Sauerman & Nayar (1984). The incubation period in mosquito vectors is largely temperature-dependent and may take as little as 14-17 days in *Aedes aegypti* (Taylor 1960b). While feeding, infective larvae emerge from the tips of the labella together with a drop of haemolymph onto the surface of the host's skin (Lavoipierre 1958). The haemolymph pool provides a medium in which the larvae can maintain their motility to search for and penetrate the puncture wound remaining after the withdrawal of the mosquito fascicle (Zielke 1973). The developing larvae are also pathogenic for the vector itself, which results in an increased mortality (Kartmann 1953; Galliard 1957; Christensen 1977; Hamilton & Bradley 1979).

In the definitive host the infective larvae undergo an extensive somatic migration to so-called intermediate locations, which are the submuscular membrane, subcutaneous tissue, adipose tissue, subserosa and muscles of the upper abdomen, thorax, head, neck and forelimb regions (Kume & Itagaki 1955). During this migration they moult to the L4-stage and then into young adults which finally enter veins to reach their predilection sites, the right ventricle, right auricle and pulmonary artery (Nelson 1966; Kotani & Powers 1982; Orihel 1961). Worms are found in the heart as early as day 67 after infection (Kume & Itagaki 1955) and the migration is always completed by day 90 (Orihel 1961). There are many reports of *D. immitis* found in aberrant sites (Otto 1975) which is more common in cats than in dogs (Dillon 1988). The prepatent period is 6-9 months in dogs (Bancroft 1904; Webber & Hawking 1955; Orihel 1961; Newton 1968; Kotani & Powers 1982) and 8 months in cats (Donahue 1975). The patent period is up to 7½ years in dogs (Newton 1968) and only about 2 years in cats (Donahue 1975;

Wong, Pedersen & Cullen 1983). The maximum life expectancy of microfilariae in the blood of dogs is 2½ years (Underwood & Harwood 1939).

2.1.4 Host range

Domestic dogs act as the preferential and principal definitive host (Abraham 1988). Cats are less prone to develop a patent infection and thus regarded as insignificant reservoirs of infection (Donahue 1975; Dillon 1988; Wong *et al.* 1983). Apart from these hosts, some wild canids, felids, other mammals, including man, and the Humboldt penguin (*Sphenicus humboldti*) have been found to be infected with adult *D. immitis* (Campbell & Blair 1978; Abraham 1988; Starr & Mulley 1988; Vellayan, Omar, Oothuman, Jefferey, Zahedi, Mathew & Krishnasamy 1989; Canestri Trotti, Pampiglione & Rivasi 1997; Sano, Aoki, Takahashi, Miura, Komatsu, Abe, Kakino & Itagaki 2005). In the majority of these hosts the adult worms are found in aberrant locations and do not produce microfilariae (Abraham 1988).

2.1.5 Vectors

About 70 anopheline and culicine mosquitoes throughout the world, that belong to the genera *Aedes, Anopheles, Coquillettidia, Culex, Culiseta, Mansonia* and *Psorophora* have been identified as potential intermediate hosts (Bemrick & Sandholm 1966; Ludlam, Jachowski & Otto 1970; Lok 1988). However, innate susceptibility is only a component of true vector competence, which is determined by the demonstration of infective larvae in field-captured mosquitoes (Lok 1988). As regards Africa there are very few references to natural infections of mosquitoes with animal filariae. Because infective larvae of *D. immitis* are practically indistinguishable on morphological criteria from those of *D. repens*, available records from Africa that specifically refer to *D. immitis* (Table 2.3) are therefore of only limited value (Nelson *et al.* 1962).

Geographical strains of mosquito species from Africa that have been found to be susceptible after experimental infections and might therefore act as natural vectors, are *Anopheles pembaensis* from Kenya (Nelson *et al.* 1962), *Aedes aegypti* from Kenya (Nelson *et al*. 1962) and Tanzania (Roubaud 1937) as well as *Culex pipiens fatigans* from Kenya (Heisch, Nelson & Furlong 1959; Nelson *et al*. 1962).

2.1.6 Laboratory diagnosis in live animals

The laboratory diagnosis in live animals can be attained by the demonstration and identification of microfilariae, by serology and by molecular techniques. Various methods have been described for the detection of microfilariae in the blood of animals and humans. The preparation of wet blood films, thin and thick blood films stained with Romanovsky-type stains as well as the capillary haematocrit tube method are appropriate if high levels of microfilaraemia prevail (Schalm & Lain 1966; Collins 1971; Kelly 1973; Bailey 1987). Standardized concentration techniques allow detection of low microfilaraemia levels and make it possible to quantify microfilaria densities. In the classical modified Knott's technique haemolysed blood is centrifuged and the sediment screened microscopically for microfilariae (Knott 1939; Newton & Wright 1956). Variations of this technique have been reviewed by Ho Thi Sang & Petithory (1963). In the membrane filtration technique 1 ml of blood treated with an anticoagulant is forced through a 3.0 µm polycarbonate membrane filter which is stained with Giemsa and examined microscopically (Bell 1967; Chularerk & Desowitz 1970; Dennis & Kean 1971; Chlebowsby & Zielke 1977). The membrane filtration technique is more sensitive than the Knott's technique in cases where the microfilaria density is low (100-50 microfilariae/ml blood) (Bell 1967; Watson, Testoni & Porges 1973; Southgate 1974; Feldmeier, Bienzle, Schuh, Geister & Guggenmoos-Holzmann 1986; Beugnet, Bima-Blum & Chardonnet 1993a; Martini, Capelli, Poglayen, Bertotti & Turilli 1996). Since there are several filarial species in both dog and cat that produce microfilariae which eventually appear in the blood, the mere demonstration of microfilariae remains meaningless unless they are identified (Valcárcel, Ferre, Gómez-Bautista & Rojo-Vázquez 1990).

2.1.6.1 MORPHOMETRICAL IDENTIFICATION OF MICROFILARIAE

Depending on the technique of processing, storage of blood samples and geographical origin, the morphometrical data given for heartworm microfilariae in the literature vary considerably, ranging from 180-340 µm and 5-7 µm respectively (Table 2.2) (Sawyer, Weinstein & Bloch 1963; Acevedo, Theis, Kraus & Longhurst 1981). As the size ranges of microfilariae of other species that concurrently occur on the African continent overlap with those of *D. immitis*, it is unreliable to establish a diagnosis on this criterion (Valcárcel *et al.* 1990). Described by Fülleborn (1924), the relative positions of somatic structures of the microfilarial body, which is constant in each species, is still one of the most accurate methods. The method is, however, sufficiently laborious to be impractical except for taxonomic purposes (Kelly 1973).

2.1.6.2 MORPHOLOGICAL IDENTIFICATION OF MICROFILARIAE

The morphology of the tail and the shape of the anterior extremity of the microfilariae have been described as features for identification (Sonin 1985; Marconcini *et al.* 1996). Tail morphology should only be considered in either unstained and unfixed microfilariae or in those isolated with the modified Knott's technique (Marconcini *et al.* 1996). In *D. immitis*, these morphological criteria are of limited value on the African continent, since *Dirofilaria repens* also has a straight tail and a conical anterior extremity (Valcárcel *et al.* 1990).

2.1.6.3 PHYSIOLOGICAL CHARACTERISTICS OF MICROFILARIAE

Motility in wet blood films is a criterion emphasized especially in the North American literature (Thrasher 1963). However, Valcárcel *et al.* (1990) could not confirm any difference in the motility of microfilariae of *D. immitis*, *D. repens*, *A. dracunculoides* and *A. reconditum*. Similarly, the claim that high microfilarial counts are indicative of *D. immitis* infection in contrast as opposed to low counts in *A. reconditum* infections (Wallenstein & Tibola 1960), has proved questionable, as high microfilarial counts have also been observed in dogs infected with *A. reconditum* (Herd 1978; Bobade, Ojebuoboh & Akinboade 1981).

2.1.6.4 HISTOCHEMICAL IDENTIFICATION OF MICROFILARIAE

In studies of filarial infections in monkeys, Chalifoux & Hunt (1971) observed differences in acid phosphatase activity of microfilariae in blood films using the method of Barka (1960). Subsequently, the method was applied to microfilariae of dogs and cats and has, with modifications, since been proven to be the most reliable, consistent and practical differential technique (Balbo & Abate 1972; Kelly 1973; Whitlock, Porter & Kelly 1978; Valcárcel *et al.* 1990; Beugnet, Costa & Lambert 1993b; Ducos de Lahitte, Ducos de Lahitte & Davoust 1993; Peribáñez, Lucientes, Arce, Morales, Castillo & Garcia 2001). In the microfilariae of *D. immitis*, acid phosphatase activity is uniquely restricted to the excretory pore and the anal pore (Chalifoux & Hunt 1971; Balbo & Abate 1972; Valcárcel *et al.* 1990). Histochemistry is ideally combined with the modified Knott's technique or the membrane filtration technique (Williams, Williams, Signs & Hokama 1977; Whitlock *et al.* 1978; Acevedo *et al.* 1981).

2.1.6.5 SEROLOGY

There are two groups of serological tests to detect circulating female heartworm antigen or circulating heartworm antibody. For the detection of antigen, enzyme-linked immunosorbent assay (ELISA) and immunochromatographic test systems are available. The main advantages of serological testing for antigen lie in the identification of 'occult' (amicrofilaremic) infections, the monitoring of adulticide treatment and the fast execution of the test by the veterinarian in the presence of the owner (Beugnet *et al.* 1993a). Disadvantages are the lack of sensitivity in only male worm infections, in prepatent infections and if low numbers of female worms are present (Beugnet *et al.* 1993b; Hoover, Campbell, Fox, Claypool & Mullins 1996). Lack of sensitivity is particularly a problem in low-endemic or newly colonized areas, where concentration tests provide more accurate results (Frank, Grieve, Mok, Smart & Salman 1992; Tarello 2001). False sero-positive results, due to cross-reactions with *D. repens* have to be considered (Valcárcel *et al.* 1990; Beugnet *et al.* 1993b; Schrey 1996; Schwan *et al*. 2000). The abundant North American literature currently regards antigen testing as the most sensitive diagnostic method (Datz 2003). However, data on the accuracy of the various commercial tests available cannot be extrapolated to other geographical areas since, with a single exception, all commercial test kits are manufactured in the USA where cross-reactions with *Dipetalonema reconditum* as the only other filarial species of dogs can be excluded (Schrey 1996). Heartworm antibody tests are used in cats, where infections are usually amicrofilaraemic and antigen is difficult to detect because of the low worm burdens (Datz 2003).

2.1.6.6 MOLECULAR DIAGNOSIS

The polymerase chain reaction (PCR) and DNA probes as tools in the molecular diagnosis have been used to differentiate *D. immitis* from other filarial helminths (WHO 1992; Favia, Lanfrancotti, Della Torre, Cancrini & Coluzzi 1996; Favia, Lanfrancotti, Della Torre, Cancrini & Coluzzi 1997; Bredal, Gjerde, Eberhard, Aleksandersen, Wilhelmsen & Mansfield 1998; Mar, Yan, Chang & Fei 2002; Rishniw, Barr, Simpson, Frongillo, Franz & Dominguez Alpizar 2006). However, due to technical inadequacies, a lack of practical trials to validate the techniques appropriately combined with exhaustive technical requirements, the routine application of molecular-biological techniques remains limited (WHO 1992; Pampiglione, Rivasi & Canestri Trotti 2000; Shaw & Day 2005; Olga & Éva 2006).

2.1.7 Veterinary and medical importance

Cardiovascular dirofilariosis is caused by the preadult and adult worms which exert a mechanical and phlogistic effect that, depending on the worm burden, duration of infection and host-parasite interaction, ultimately develops into a multisystemic disorder with the lungs, heart, liver and kidneys mainly affected (Pampiglione & Rivasi 2001; Ware 2003). The pulmonary arterial system is the prime site of pathology and the effects are reflected by alterations in the pulmonary vasculature and interstitial lung tissue (Sutton 1988). Due to the development of pulmonary hypertension, the heart and liver become affected which leads to right-sided circulatory failure with the kidneys also partially becoming involved in the cascade of events (Ducos de Lahitte 1990). The pathogenesis, pathology and clinical manifestations have been subject of many reviews (Knight 1977, 1987; Ducos de Lahitte 1990; Ducos de Lahitte *et al.* 1993; Ware 1998, 2003).

A microfilaria-associated cutaneous syndrome characterized by erythematous, papulo-nodular and/or ulcerative pruritic lesions and a membranous glomerulonephritis have been described on very few occasions (Casey & Splitter 1975; Mozos, Ginel, López, Carrasco, Martín de las Mulas & Molleda 1992; Hargis, Lewis, Duclos, Loeffler & Rausch 1999). However, with the introduction of macrocyclic lactone-based dewormers for dogs and cats, microfilariae of any filarial species have gained significance. Similarly to the previously widely used diethylcarbamazine, macrocyclic lactones have microfilaricidal activity which can result in a potentially fatal shock-like syndrome and other adverse reactions as demonstrated in *D. immitis* and *Dirofilaria repens* infected dogs and cats (Sasaki *et al.* 1989; Euzéby 1990; Schrey 1996; Klotins, Martin, Bonnett & Peregrine 2000; Ware 2003; Plumb 2008; V. Schwan, unpublished data 2008). This is presumably due to immunological reactions against the substances released from dying microfilariae (Sasaki *et al.* 1989; Plumb 2008).

Clinically, cardiovascular dirofilariosis can present as a mild asymptomatic form, which is mostly detected incidentally, as a moderate form with exercise intolerance, chronic cough, dyspnoea and weight loss or as a severe form with right-sided congestive heart failure, syncope, acute or chronic *vena cava* syndrome and sudden death (Atwell 1988; Moraillon 1990).

About 230 human cases have been reported worldwide (Muller 2002). In almost all instances immature worms or unfertilized females have been isolated from the lungs (Pampiglione & Rivasi 2001). Most infections are asymptomatic, showing typical ‘coin lesions’ on chest radiography which are often mistakenly removed as neoplasms (Ciferri 1982).

The treatment of cardiovascular dirofilariosis consists of chemotherapy directed against preadult and adult worms by means of macrofilaricides (adulticides) and subsequently against microfilariae in the blood by means of 'microfilaricides' (McCall, Guerrero, Genchi & Kramer 2004). Possible systemic side effects such as pulmonary thromboembolism caused by the reaction of the body to the disintegrating adult worms and circulatory collapse following the rapid death of large numbers of microfilariae may require additional treatment with anti-inflammatories and parenteral fluids (Ware 2003).

Melarsomine (Immiticide®, Merial) is currently the drug of choice for the treatment of preadult and adult stages in dogs (Raynaud 1992). Macrofilaricidal therapy in cats should only be considered as a last resort, as severe complications are very likely to occur (Ware 2003). In dogs, mircofilaricidal therapy is recommended to be started 3-4 weeks after macrofilaricidal therapy in dogs (McCall *et al.* 2004). Ivermectin administered as a single dose of 50 µg/kg has to be regarded as the drug of choice for this purpose as it causes fewer side effects than other microfilaricides (Beugnet *et al.* 1993b; Ware 2003). At this dose, the drug is also safe in ivermectin-sensitive dog breeds (Pulliam, Seward, Henry & Steinberg 1985; Paul, Tranquilli, Seward, Todd & Di Pietro 1987).

For preventative use, specific formulations of the macrocyclic lactones ivermectin, milbemycin oxime, moxidectin and selamectin administered once a month and the piperazine diethylcarbamazine (DEC) given daily are available (Ware 2003; McCall *et al*. 2004; Plumb 2008).

2.1.8 Distribution on the African continent and its islands

A survey for parasitism in animals conducted by FAO, WHO and OIE (1984) indicates that filariosis of dogs is widespread in Africa. According to Nelson (1966) it was common in veterinary practice to assume that dogs with microfilariae in their blood were infected with *D. immitis* and that this has resulted in a great deal of confusion with other harmless species. This is supported by Levine (1980), who maintains that *D. immitis* is rather rare in Africa.

2.1.8.1 NORTHERN AFRICA

Dirofilaria immitis has been reported from Algeria, Egypt, Morocco, Tunisia and the offshore Canary Islands.

Algeria: The earliest reports come from Beni-Ounif de Figuig on the Algerian-Moroccan border were several small scale surveys were conducted between 1913 and 1923 (Foley 1921; Foley, Catanei & Vialatte 1926) with prevalence rates ranging from 10-22 % based on the demonstration and identification of microfilariae. According to the authors, the microfilariae were 175-253 µm long, 6-5 µm wide and had a cephalic hook. This description is contrary to published information for *D. immitis*, and only the microfilariae of *A. reconditum* possess a cephalic hook (Sawyer, Rubin & Jackson 1965). The misdiagnosis is also supported by the fact that the authors were unable to demonstrate adult worms in the heart of the microfilaraemic animals and that none of them showed any clinical signs attributable to heartworm infection. Choquette, Gayot & Poul (1952) report a case from a dog in the Alger region based on the finding of microfilariae. The authors have not provided any information on what criteria the microfilariae were identified. In a later survey involving 190 dogs from the Alger region, eight were found positive for microfilariae on blood examination with a description given that is in accordance with published information (Rioche 1960). In the most recent survey conducted in Alger, Montaron (1975) found one out of 215 dogs positive for *D. immitis* on examination of blood. The identification was based on microfilarial motility and the appearance of the tail.

Egypt: *Dirofilaria immitis* was incidentally found during postmortem examination in the pulmonary arteries in 5 out of 50 dogs in Assiut (Mahmoud & Ibrahim 1989). In a later report eight out of 19 police dogs in Assiut were found positive for microfilariae on blood examination (Abd El Rahim 1998). The microfilariae were identified morphologically by their tapered anterior end and their straight tail with no further information provided.

Morocco: Heartworm in Morocco was first reported by Bouin (1921), who conducted a necropsy survey in the south of the country with no exact locality given. The survey involved 109 dogs of which one was found positive with a single female specimen isolated from the right heart. Santucci, Haag & Sendral (1953) reported on a clinical case of dirofilariosis in a 2-year-old male Boxer. The diagnosis was based on the morphometrical identification of microfilariae in Giemsa-stained blood films using the data given by Neveu-Lemaire (1936) as a reference. In a more recent necropsy survey in the Rabat region 7 out of 57 stray dogs were reported to be infected (Pandey, Dakkak & Elmamoune 1987).

Tunisia: In a survey involving 207 dogs from Tunis, one dog was found positive for microfilariae which occurred in large numbers (Yakimoff & Kohl-Yakimoff 1911). The microfilariae are described as sheathless, 214-227 µm long and 4.2-5.6 µm wide with no further details provided. Juminer & Durand (1960) report on a dog with severe polyparasitism which was subsequently euthanased and necropsied. Although the authors failed to demonstrate adult parasites in the heart during necropsy, microfilariae found in stained bloodfilms were identified as those of *D. immitis* with no details provided on the criteria used for identification. In a survey conducted in Tunis, 25 out of 70 dogs were found to be microfilaraemic (Perrot 1985). The identification of the microfilariae as those of *D. immitis* was based on motility, morphometrical and morphological criteria and is in accordance with published information.

Canary Islands: In a prevalence survey on the Canary Island of Tenerife 130 out of 310 dogs were microfilaraemic (Valladares, Gijon & Lopez-Roman 1987). No details are given on what criteria the microfilariae were identified. However, *D. immitis* infection was confirmed by demonstration of adult parasites in the heart of 14 selected microfilaraemic animals during necropsy. In a more recent survey, heartworm seroprevalence in dogs from Tenerife Island was 21 % (172/823) (Montoya, Morales, Juste, Bañares, Simon & Genchi 2006). Seroprevalence surveys conducted during 1994 to 1996 involving 2034 dogs on Gran Canaria Island showed a mean prevalence of 58.89 % (Montoya, Morales, Ferrer, Molina & Corbera 1998).

2.1.8.2 WESTERN AFRICA

Dirofilaria immitis has been reported from Guinea-Bissau, Nigeria, Senegal and Sierra-Leone. However, most of the reports are based on the demonstration of microfilariae with negative necropsy results, a circumstance which is unlikely as is highlighted by Schillhorn van Veen & Blotkamp (1975), who also maintain that *D. immitis* is rare in Western Africa.

Guinea-Bissau: The existing reports are based on the demonstration of microfilariae only (Tendeiro 1948, 1949). Although Tendeiro (1949) claims that *D. immitis* is very common in dogs, in what was then Portuguese Guinea, the author also mentions that adult parasites have never been found and that no clinical signs were ever observed in infected animals. Tendeiro (1949) gives a detailed morphological and morphometrical description of the microfilariae and compares the obtained data with, amongst others, those of Fülleborn (1912) and Foley (1921). While Fülleborn's data are quoted incorrectly, Foley's description of the microfilariae having a cephalic hook and the circumstance that no clinical signs were observed in infected dogs suggests the aetiology of *A. reconditum*.

Nigeria: Schillhorn van Veen (1974) maintains that *D. immitis* has not been demonstrated in dogs in Nigeria, although veterinary field officers often assume that dogs with microfilariae in their peripheral blood are infected (Idowu, Okon & Dipeolu 1977). This is the conclusion after routine necropsies of 400 dogs in Zaria failed to demonstrate heartworm.

Senegal: As the only reference available, Pangui & Kaboret (1993) report on a survey conducted in Dakar. Between 1984 and 1992, 72 stray dogs were caught and necropsied of which six were found to be infected with adult worms at the predilection site.

Sierra Leone: The two existing reports from Sierra Leone are based on the finding of microfilariae only (Kamara 1977; Hassan 1984) with no information provided on the criteria used for identification. Necropsies conducted on microfilaraemic dogs in the earlier study failed to demonstrate worms at the predilection site (Kamara 1977).

2.1.8.3 CENTRAL AFRICA

Dirofilaria immitis has been reported from Angola, Cameroon and Gabon.

Angola: Serrano (1962) reports that microfilariae of *D. immitis* have been found in the blood of dogs in Luanda and Nova Lisboa and that the adult stage was recovered in the heart of a dog in Luanda.

Cameroon: Thys, Sawa & Guissart (1982) published a case history of a 7-year-old male Boxer in Maroua who was brought to Cameroon from Yugoslavia 7 months before being presented for treatment. The animal was found to be infected with *D. immitis* based on demonstration and identification of microfilariae in stained bloodfilms. The diagnosis was confirmed at necropsy. However, the authors suggest that the dog was already infected before arriving in Maroua where *D. immitis* appears not to be endemic due to the unfavourable climatic conditions. Heartworm is endemic in the territory of the previously known Yugoslavia where it was first reported by Dzunkovski (1934) in a dog in Belgrade.

Gabon: In a survey involving 48 dogs from Libreville, 50 % tested positive for *D. immitis* based on the identification of microfilariae, positive necropsy results and heartworm antigen testing (Beugnet & Edderai 1998).

2.1.8.4 EASTERN AFRICA

Reports come from Ethiopia, Kenya, Malawi, Mozambique and Tanzania and the islands of Madagascar, Mauritius and Réunion.

Ethiopia: Chiodi (1936) reports that *D. immitis* is common in dogs in Abyssinia. In his collation of data on helminth infection of domestic and wild animals, Graber (1975) cites *D. immitis* which was recovered from the right ventricle and the pulmonary artery from a dog of unknown origin in Ethiopia by Chiodi in 1936.

Kenya: The earliest report comes from the Island of Pate (Heisch *et al*. 1959) where nine out of 12 dogs were infected with *D. immitis*. The diagnosis was based on the identification of microfilariae and demonstration of adults during necropsy. The identity of the microfilariae was confirmed by comparing them with those taken from the gravid uteri of adult worms found at autopsy. In a later survey on filarial infections in man, animals and mosquitoes on the Kenya coast from Somalia to Tanzania, 22 out of 252 dogs were found infected (Nelson *et al*. 1962). A necropsy survey involving 286 dogs from the Nairobi area yielded two cases (Murray 1968). There are two further reports were infection of dogs was demonstrated during necropsy from various unspecified localities in the country (Bwangamoi & Frank 1970; Bwangamoi, Frank, Moulton, Mugera & Wandera 1971).

Malawi: *Dirofilaria immitis* is mentioned in a check list of helminth parasites from domestic dogs (Fitzsimmons 1964).

Mozambique: In the earliest report, Dias (1954) states that *D. immitis* appears to be rare. The author reports on a single case from the Region of Maputo which was identified in a laboratory with, however, no details provided on what criteria the diagnosis was based. Cruz e Silva (1971) refers to adult specimens (several male and female worms) collected by Travassos Dias in 1969 in Quelimane as well as specimens from Beira collected in 1966 from dogs at necropsy. In a survey conducted in Maputo

Province between 1981 and 1984, Jurášek (1986) found five out of 86 dogs to be microfilaraemic. The author claims that the microfilariae were those of *D. immitis* with, however, no information provided on the criteria used for identification. In a small-scale survey in Quelimane 4 out of 13 indigenous dogs were found positive for microfilariae which were identified by acid phosphatase staining (Schwan & Durand 2002) as those of *D. immitis*.

Tanzania: Infection in a dog was first recorded by the Veterinary Department of Tanganyika (1934). Alley (1950) gives a clinical report on six cases of *D. immitis* infected dogs on the island of Zanzibar. In the annual report of the Veterinary Department, Roe (1958) lists *D. immitis* as having been diagnosed in dogs.

Madagascar: *Dirofilaria immitis* in dogs is mentioned in a host-parasite list of helminths in domestic animals (Daynes 1964). The author claims that *D. immitis* is known for a long time on the island.

Mauritius: Heartworm is mentioned for the first time for Mauritius as part of a helminth collection from domestic animals on the island (Ware 1925). The specimens were recovered from the dog's heart. In a necropsy survey, Webb & Nadeau (1958) found three out of 50 stray dogs to be infected. A more recent survey indicated that 30 out of 184 dogs were microfilaraemic (Sibartie, Beeharry & Jaumally 1983). The microfilariae were identified as those of *D. immitis* according to published criteria and infection was further confirmed by necropsy in four animals.

Réunion: Prunaux & Guignard (1991) report on the end result of a 4-year investigation of the Veterinary Departmental Laboratory of Réunion Island, in which 16 out 96 dogs were found positive on necropsy.

2.1.8.5 SOUTHERN AFRICA

Dirofilaria immitis has only been reported in imported animals (Van Heerden, Verster & Gouws 1980; Verster, Cilliers & Schroeder 1991; Schwan & Durand 2002).

2.2 *Dirofilaria repens*

2.2.1 Taxonomy

Dirofilaria repens (Railliet & Henry, 1911) has no vernacular name and has been known in the literature by the following names: *Filaria acutiuscula* (Molin, 1858); *Dirofilaria acutiuscula* (Molin, 1858); *Filaria palpebralis* (Pace 1867); *Filaria peritonaei hominis* (Babes, 1880); *Filaria conjunctivae* (Addario, 1885); *Dirofilaria conjunctivae* (Addario, 1885); *Filaria repens* (Braun, 1915) and *Loa extraocularia* (Skrjabin, 1917) (Anderson 1952; Sonin 1985; Chauve 1990).

2.2.2 Morphology

A detailed description of the adult stages of *D. repens* is given by Railliet & Henry (1911b), Vogel (1927) and Le-Van-Hoa & Le Thi-Ty (1971). According to these authors females are 8.4-17 cm long and 380-650 µm wide whereas males are 3.9-7 cm long and 270-450 µm wide. The left spicule is 338-590 µm long, and the right spicule 123-206 µm long.

The microfilariae are unsheathed and a detailed description is given by Gunewardene (1956) and Taylor (1960a). According to these authors the cephalic end is conical with 2-3 nuclei in the head space and the posterior end is acute with the nuclear column not extending to the end of the body. The tail in unfixed and unstained microfilariae is like the handle of an umbrella (Marconcini *et al.* 1996). Influenced by the technique of processing, geographical origin and host, the morphometrical data given in the literature vary considerably. Minimum and maximum measurements regarding length and width range from 207-385 µm and 5-9 µm (Table 2.4).

The infective filarial larva in mosquitoes has been described in detail by Nelson (1959, 1960) with additional information provided by Bain & Chabaud (1986).

2.2.3 Life cycle

Dirofilaria repens females produce microfilariae which are found in the blood of the definitive host. They are also capable of extravascular migration as is evidenced by passing through the placenta and infecting puppies (Mantovani 1966) and the demonstration of microfilariae in urine (Mantovani 1965). The appearance of microfilariae in the peripheral blood of dogs and cats is nocturnal subperiodic, with maximum levels of microfilaraemia between 20:00 and 03:00 (Webber & Hawking 1955; Mantovani & Restani 1965; Kamalu 1986).

Mosquitoes act as intermediate hosts. The incubation period in susceptible mosquito vectors is temperature- and species-dependent and may take as little as 10 days in *Anopheles stephensi* (Webber & Hawking 1955). Development within the vector and subsequent transmission is similar as for *D. immitis* and has been described by

Fülleborn (1908a), Bernard & Bauche (1913), Gunewardene (1956) and Mantovani (1965).

Whether infective larvae follow a complex migration in the definitive host is unknown (Webber & Hawking 1955). The predilection sites are the subcutaneous tissue in most parts of the body (Canestri Trotti *et al.* 1997) and the fascial sheaths overlying the muscles of the hind legs (Heisch *et al*. 1959) The prepatent period is 6-8 months in dogs (Webber & Hawking 1955) and 6 months in cats (Cancrini, Mantovani & Coluzzi 1979; Cancrini & Iori 1981). According to Webber & Hawking (1955) the patent period in dogs is at least 2-3 years. However, based on data obtained from experimentally infected dogs in Italy who remained microfilaraemic for 8-9 years, the patent period appears to be much longer (Cancrini & Iori 1981). The same authors state that experimentally infected cats remained microfilaraemic for about 2 years.

2.2.4 Host range

The domestic dog and cat act as the preferential and principal definitive hosts and both appear equally susceptible to infection in Africa (Heisch *et al.* 1959). Apart from these hosts some wild canids, felids and the large-spotted genet (*Genetta tigrina*) were found infected with adult *D. repens* (Canestri Trotti *et al*. 1997).

2.2.5 Vectors

Anopheline and culicine mosquito species belonging to the genera *Aedes*, *Anopheles*, *Culex*, *Mansonia* and *Taeniorhynchus* are considered intermediate hosts (Pampiglione, Canestri Trotti & Rivasi 1995).

There are no records on natural infections of mosquitoes with *D. repens* in Africa. Geographical strains of mosquito species from Africa that have been found to be susceptible following experimental infections are *Aedes pembaensis*, *Aedes aegypti*, *Mansonia uniformis* and *Mansonia africanus* from Kenya (Nelson *et al.* 1962) as well as *Aedes aegypti* from Nigeria (Anyanwu, Agbede, Ajanusi, Umoh & Ibrahim 2000).

2.2.6 Laboratory diagnosis in live animals

The laboratory diagnosis is based on the demonstration and identification of microfilariae in blood samples utilizing the same methodologies as outlined for *D. immitis*. Because of the unreliability of most characteristics, histochemical staining for acid phosphatase activity has proved to be the most reliable, consistent and practical differential technique to diagnose infection in dogs and cats (Kelly 1973; Valcárcel *et al.* 1990). In the microfilariae of *D. repens*, acid phosphatase activity is uniquely restricted to the anal pore or to the anal pore and innerbody (Balbo & Abate 1972; Yen & Mak 1978; Valcárcel *et al.* 1990). The utilization of the polymerase chain reaction (PCR) as a tool to differentiate *D. repens* microfilariae and immature adult stages removed from bioptic material from those of other filarial species of dogs and cats has been reported (Favia *et al*. 1996, 1997; Vakalis, Spanakos, Patsoula & Vamvakopoulos 1999; Rishniw *et al*. 2006).

2.2.7 Veterinary and medical importance

In the past *D. repens* has been regarded as apathogenic in natural and experimental infections (Webber & Hawking 1955; Heisch *et al.* 1959; Nelson 1966). However, since the 1960s it has been recognized that *D. repens* is not an innocuous parasite in at least a subgroup of infected dogs and cats. Pruritic dermatitis characterized by the presence of erythema, papules, focal or multifocal alopecia, crusting and subcutaneous nodules containing adult worms is the most commonly observed clinical manifestation of infection in both dogs and cats (Euzéby 1961; Kamalu 1986; Bredal *et al.* 1998; Tarello 2000a, 2002, 2003; Ananda & D'Souza 2006). The part of the body most affected are the lumbosacral and perineal areas, and the hind legs in dogs (Tarello 2002), and the flanks, back, neck, legs and paws in cats (Tarello 2000a). In dogs, the flanks, back and the hind limbs are commonly considered the preferential sites of dwelling for both the larvae and adults which may concentrate in large numbers in a single area (Mandelli & Mantovani 1966). The embolization of microfilariae, the movement of adults in the subcutaneous tissue and the immunological response to the L3, L4, adults and/or microfilariae are thought to cause these cutaneous lesions (Mozos *et al.* 1992; Chauve 1997; Pampiglione *et al.* 1995; Tarello 2002). Circular cutaneous ulcers, subcutaneous tumefactions, subcutaneous oedema and ascitis were reported from infected dogs which resolved following adulticidal and microfilaricidal treatment (Restani, Rossi & Semproni 1963). Cutaneous ulcers have also been reported from a cat (Tarello 2000b). Acute liver failure was reported from a microfilaraemic cat in South Africa which resolved after treatment with ivermectin (Schwan *et al.* 2000). The macropathological and histopathological changes of the spleen, liver, lungs, heart and kidneys, which have been described from some suspected clinical cases, are similar to those observed in cardiovascular dirofilariosis and comprise hyperplastic splenomegaly, plasmocytosis, erythrophagocytosis, haemosiderosis, chronic stasis of the liver with centrolobular steatosis and portal fibrosis, lung atelectasis and chronic bronchitis, glomerular fibrosis of kidneys, myocardosis, vascular tumours and other vascular alterations in the vessels of the nervous tissues (Mantovani 1965; Mandelli & Mantovani 1966; Kamalu 1991; Schwan *et al.* 2000; Martano, Veneziano, Santaniello, Carbone, Paciello, Cataldi, Russo & Maiolino 2004). There is increasing evidence that the pathogenicity may be influenced by concurrent infections, such as babesiosis, monocytic ehrlichiosis, leishmaniosis and haemobartonellosis (Tarello 2002).

Treatment of *D. repens* infection in dogs and cats is indicated if they are clinically affected and to decrease the risk of human infection in endemic areas (Baneth, Volansky, Anug, Favia, Bain, Goldstein & Harrus 2002). However, reports on treatment are scarce. The adulticide thiacetarsamide (no dosage provided) followed by the microfilaricide diethylcarbamazine (100 mg/kg *per os* daily for 30 days) were used in Italy (Restani *et al.* 1963) effectively. Diethylcarbamazine at 5.5 mg/kg *per os* daily for 1 month was used in Nigeria with no apparent clinical effect (Kamalu 1991). This is less than the recommended daily chemoprophylactic dosage of 6.6 mg/kg for heartworm (Roberson 1988). The adulticide melarsomine (2 x 2.5 mg/kg 24 hours apart intramuscular) subsequently followed by the microfilaricide ivermectin (50 µg/kg) 10 and 30 days later was used for the treatment of dogs and a cat in Italy with resolution of the cutaneous lesions (Tarello 1999, 2000a, b, 2002, 2003). Similarly, melarsomine followed by doramectin (0.4 mg/kg) has been reported from Israel to be effective in clearing infections (Baneth *et al.* 2002).

Three macrocyclic lactones are reported to be used successfully in monthly dosing regimens to prevent infection in dogs, two by oral administration, ivermectin (Marconcini, Magi & Hecht Contin 1993; Pollono, Pollmeier & Rossi 1998) and moxidectin (Rossi, Ferroglio & Agostini 2002), and selamectin by pour-on application (Genchi, Poglayen & Kramer 2002). An injectable, sustained-release formulation of moxidectin shown to confer six-month protection against the related *D. immitis*, has proved to be similarly effective as a prophylactic for *D. repens* (Lok, Knight, Wang, Doscher, Nolan, Hendrick, Steber & Heaney 2001; Rossi, Ferroglio & Agostini 2004).

Dirofilaria repens accidentally affects humans and has been reported about 400 times from 30 countries but mostly from Italy with a more common superficial manifestation and a visceral form which is often confused with neoplastic tumours (Pampiglione *et al.* 1995; Muro, Genchi, Cordero & Simón 1999). There is only a single report worldwide of a patent infection with a microfilariaemia in a human case from Corsica (Nozais, Bain & Gentilini 1994).

2.2.8 Distribution on the African continent

2.2.8.1 NORTHERN AFRICA

Dirofilaria repens has been reported from Egypt, Sudan and Tunisia.

Egypt: In a check list of nematodes collected during 1948-1955, *D. repens* is listed for the golden jackal (*Canis aureus*) (Myers, Kuntz & Wells 1962).

Sudan: Adult *D. repens* adult specimens were recovered from the *Gluteus superficialis* and *Biceps femoris* muscles of 2 lions from Bahr-el-Ghazal Province (Kellas & Webber 1955).

Tunisia: Chatton (1918) reports on a survey conducted in Médine and Gabès in the south of the country, where 2 out of 26 cats were found microfilaraemic. The microfilariae were described as 240-350 µm long and 7-9 µm wide. In a later necropsy survey involving 348 dogs in Tunis, 1 dog was found infected (Bernard, Ben Osman & Juminer 1967). Adult worms were isolated from the supracostal connective tissue. The identification was based on a detailed morphometrical study with results being in accordance with published information.

2.2.8.2 WESTERN AFRICA

Dirofilaria repens has only been reported from Nigeria. Schillhorn van Veen (1974) maintains that *D. repens* is the most common filarial worm in Nigerian dogs and cats. According to this author it is found mainly in the subcutaneous connective tissue without causing any marked pathological changes. The author reports on a 9.4 % prevalence in dogs in the Zaria area based on the demonstration of microfilariae in blood. The size range given is 300-369 µm. There are several other reports of *D. repens* in dogs from Nigeria with information provided on the criteria used for identification of microfilariae and adults which confirm its endemic status (Schillhorn van Veen & Blotkamp 1975; Schillhorn van Veen, Shonekan & Fabiyi 1975; Kamalu 1986, 1991; Anyanwu, Umoh, Ogbogu, Essien, Galadima, Adawa & Hassan 1996).

2.2.8.3 CENTRAL AFRICA

The only report comes from the Kapa River in the northeast of the Central African Republic, where the adult specimens of *D. repens* were recovered from the subcutaneous connective tissue of a lion (Graber, Euzéby, Gevrey, Troncy & Thal 1972). The authors provide morphometrical data which are in accordance with published information.

2.2.8.4 EASTERN AFRICA

Dirofilaria repens has been reported from Kenya, Uganda, Zambia and Zimbabwe.

Kenya: In a survey on the Island of Pate, 2 out of 12 dogs, 27 out of 29 cats and 8 out of 9 large-spotted genet cats were found to be infected with *D. repens* (Heisch *et al.* 1959). Adult worms were found under the skin and were particularly common in the fascial sheaths overlying the muscles of the hind legs with no obvious pathological lesions. The identity of microfilariae in the blood of animals was cross-checked by comparing them with those taken from the gravid uteri of known adult worms found at necropsy. The authors report that the parasite is not confined to the Island of Pate but that it is fairly common in cats and dogs on the Kenya coast. This was confirmed in a subsequent survey involving the entire Kenya coast from Somalia to Tanzania in which 6 out of 252 dogs and 43 out of 240 cats were found to be infected on day blood films (Nelson *et al*. 1962).

Uganda: Bwangamoi (1973) recovered two female specimens of *D. repens* from the subcutis of a dog from Kampala during necropsy. Except of their length (12.7 and 16.2 cm) no further criteria are presented for their identification. In a microfilarial survey involving 836 dogs from various parts of the country, 8.6 % were found positive (Bwangamoi & Isyagi 1973). The authors present a confusing morphometrical study with no references provided for identification and record, amongst other filarial species, the presence of *D. repens.*

Zambia: Le Roux (1958) reports that a lion on Mbesuma Ranch in the Chinsali District was found heavily infected with amongst other helminths *D. repens*. No details are given on what criteria the identification was based.

Zimbabwe: *Dirofilaria repens* is listed for cats in a checklist of helminth parasites of domestic and wild mammals of Zimbabwe (Jooste 1990).

2.2.8.5 SOUTHERN AFRICA

Dirofilaria repens has only been reported from South Africa in a cat from Pretoria (Schwan *et al.* 2000). The diagnosis was based on the demonstration of microfilariae and their identification by acid phosphatase staining.

2.3 *Acanthocheilonema reconditum*

2.3.1 Taxonomy

Acanthocheilonema reconditum (Grassi, 1889) has no vernacular name and has been known in the literature by the name *Filaria recondita* Grassi, 1889 and the widely used previous name, *Dipetalonema reconditum* (Grassi, 1889) (Sonin 1985). This latest allocation is based on an attempt to rearrange the complex genus *Dipetalonema* on evolutionary lines (Chabaud & Bain 1976; Bain, Baker & Chabaud 1982a). As a result of this the revived genus *Acanthocheilonema* includes those dipetalonematids which were similar to *Dipetalonema s.s.* of New World primates (Muller 1987).

2.3.2 Morphology

The genus *Acanthocheilonema* accommodates those dipetalonematids which have a well-chitinised buccal capsule, a sturdy, divided oesophagus and a right spicule that is provided with a well-developed sheath (Bain *et al.* 1982a).

A detailed description of the adult stages is given by Noè (1907), Nelson (1962), Korkejian & Edeson (1978) and Laub (1988). According to these authors, females are 21-36 mm long and 70-205 µm wide whereas males are 9-17 mm long and 70-133 µm wide. The left spicule is 220-300 µm long, and the right spicule 92-104 µm long.

The microfilariae are unsheathed. A detailed description is given by Laub (1988). They have a long clear head space (Nelson 1962) and a cephalic hook which was first described by Fülleborn (1913) and redescribed by Sawyer *et al.* (1965). The tail is attenuated and free of nuclei (Nelson 1962). The tail in unfixed and unstained microfilariae assumes the shape of a hook (Marconcini *et al.* 1996). Influenced by the technique of processing and geographical origin, the morphometrical data in the literature vary considerably. Minimum and maximum measurements regarding length and width range from 168-292 µm and 4-6.7 µm respectively (Table 2.5).

The infective filarial larva in *Heterodoxus spiniger* and *Ctenocephalides felis* has been described in great detail by Nelson (1962), Bain & Beaucournu (1974) and Laub (1988).

2.3.3 Life cycle

Information in the literature concerning the periodicity of microfilariae in the peripheral blood is controversial. Newton & Wright (1956) in the United States reported a diurnal subperiodicity. Gubler (1966) in Hawaii illustrated a periodic cycle with a diurnal and nocturnal peak in naturally infected dogs. Bobade *et al.* (1981) report on a marked nocturnal subperiodic appearance of microfilariae in an infected dog in Nigeria. However, the aetiology of the filarial infection in the latter report remains questionable since the identification was only based on the length and width of microfilariae, which according to Laub (1988) is inappropriate for species identification. Korkejian & Edeson (1978) report a nocturnal subperiodicity in naturally infected dogs in Lebanon. The results obtained from studies on naturally infected dogs in Brazil and Okinawa are inconclusive (Pennington & Phelps 1969; Lima & Costa 1972). A study conducted on naturally infected dogs in Liberia showed no periodicity of microfilariae (Laub 1988).

The fleas *Ctenocephalides canis*, *Ctenocephalides felis* and *Pulex irritans*, as well as the chewing lice *Heterodoxus spiniger* and *Linognathus setosus* have been identified as intermediate hosts (Grassi & Calandruccio 1890; Newton & Wright 1956; Nelson 1962; Pennington & Phelps 1969). The incubation period in *C. canis* is 20-23 days at 28 ºC and 80 % humidity (Laub 1988) and 7 days in *C. felis* (Farnell & Faulkner 1978).

According to Nelson (1962) the predilection sites of the adult worms are the subcutaneous fascial spaces of the limbs and back. Korkejian & Edeson (1978) recovered adult worms from the trunk and hindlegs only. Grassi & Calandruccio (1890) found adults near the kidney.

The prepatent period is 61-101 days in experimentally infected dogs (Farnell & Faulkner 1978; Lindemann & McCall 1984). No information is available on the patent period of the species.

2.3.4 Host range

Apart from the domestic dog, which is the preferred and principal definitive host, *A. reconditum* has also been isolated from some wild canids as well as the spotted hyaena (*Crocuta crocuta*) and the brown hyaena (*Hyaena brunnea*) (Sonin 1985).

2.3.5 Laboratory diagnosis in live animals

The laboratory diagnosis is based on the demonstration and identification of microfilariae in blood samples by means of acid phosphatase staining (Kelly 1973; Valcárcel *et al*. 1990). In the microfilariae of *A. reconditum* acid phosphatase activity is either uniform with slighter lighter area from the cephalic end to the excretory vesicle or in some instances with diffuse denser staining in the area of the excretory pore, innerbody and anal pore (Chalifoux *et al.* 1971; Acevedo *et al.* 1981). The presence of a cephalic hook as a differentiating feature is only visible in dehaemoglobinized, undried blood films that are stained with brilliant cresol blue (Sawyer *et al.* 1965). Nelson *et al.* (1962) discovered that by using *Ctenocephalides felis* for xenodiagnosis, infections in dogs were detected with microfilarial densities of less than 10/ml. The utilization of the polymerase chain reaction (PCR) as a tool to differentiate *A. reconditum* microfilariae from other filarial species of dogs has been reported (Mar *et al.* 2002; Rishniw *et al.* 2006).

2.3.6 Veterinary and medical importance

Acanthocheilonema reconditum is widely regarded as apathogenic (Grassi & Calandruccio 1890; Newton *et al.* 1956; Nelson 1962). However, cases of pruritic dermatosis and focal alopecia have been attributed to the action of microfilariae if present in large numbers (Bobade *et al.* 1981; Hubert 1985; Chauve 1990).

2.3.7 Distribution on the African continent

2.3.7.1 WESTERN AFRICA

Acanthocheilonema reconditum has been reported from Liberia and Nigeria.

Liberia: In a survey conducted in Bong County, Montserrado County, Cape Mount County and Grand Bassa County, 56 out of 137 dogs were found positive for microfilariae of *A. reconditum* (Laub 1988). The identification is based on a detailed morphological and morphometrical analysis.

Nigeria: Schillhorn van Veen & Blotkamp (1975) report on 'short-type microfilariae' found in 9.2 % of 369 dogs in the Zaria area which based on morphometrical analysis were similar to those of *A. reconditum* and *A. dracunculoides*. Idowu *et al.* (1977) report on a survey in Ibadan in which two out of 488 dogs were found positive for microfilariae of *A. reconditum.* The authors do not provide any information on the microfilariae and the criteria used for identification. Bobade *et al*. 1981 report on a dog from Ibadan with microfilariae identified as *A. reconditum* by measuring length and width and tail shape.

2.3.7.2 EASTERN AFRICA

Acanthocheilonema reconditum has been reported from Kenya, Mozambique and Uganda.

Kenya: Nelson (1962) gives the first description of the parasite from Africa from material collected from dogs in Mombasa and Nairobi. The author reports that *A. reconditum* is the most widely distributed and common filarial species of dogs in Kenya. It is particularly common in dogs on the hot coastal strip and in the cooler highlands. It also occurs in jackals (*Canis adustus, Canis aureus*, *Canis mesomelas*) and hyaenas (*C. crocuta, Hyaena hyaena*). In a survey on filarial infections in man, animals and mosquitoes on the Kenya coast from Somalia to Tanganyika 40 out of 252 dogs were found positive for *A. reconditum* microfilariae on day blood films (Nelson *et al.* 1962).

Mozambique: In a small scale survey conducted in the Quelimane area 1 out of 13 dogs was found positive for *A. reconditum*, as identified by acid phosphatase staining of microfilariae (Schwan & Durand 2002).

Uganda: Bwangamoi (1973) reports on a dog from Kampala with microfilariae which were not identified. At necropsy 16 adult and one immature filarial worms were recovered and identified as *A. reconditum.* Although the overall body measurements fall in the range of *A. reconditum*, the measurements given for the spicules (left spicule 161 µm, right spicule 48 µm) are not in accordance with published information. In a microfilarial survey involving 836 dogs from various parts of the country, 8.6 % were found positive (Bwangamoi & Isyagi 1973). The authors present a confusing morphometrical study with no references for identification, and conclude, amongst other filarial species, the occurrence of *A. reconditum*.

2.3.7.3 SOUTHERN AFRICA

The only report comes from South Africa. Van Heerden (1986) reports on microfilariae of *A. reconditum* in 6 out of 13 blood samples obtained from wild dogs (*Lycaon pictus*) with no data and details provided on what criteria the diagnosis was based.

2.4 *Acanthocheilonema dracunculoides*

2.4.1 Taxonomy

Acanthocheilonema dracunculoides (Cobbold, 1870) has no vernacular name and has been known in the literature by the names *Acanthocheilonema dagestanica* (Yarulin, 1962), *Microfilaria sp.* (Leger, 1911); *Microfilaria lewisi* (Korke, 1924); *Haematozoon lewisi* (Rao, 1923) and the widely used previous name *Dipetalonema dracunculoides* (Cobbold, 1870) (Sonin 1985).

2.4.2 Morphology

A detailed description of the adult stages is given by Leger (1911), Railliet, Henry & Langeron (1912), Rao (1938), Fraga de Azevedo (1943) and Nelson (1963) with additional information provided by Rioche (1960) and Chauve (1990). According to these authors females are 30-60 mm long and 200-370 µm wide whereas males are 15-

31 mm long and 100-310 µm wide. The left spicule is 320-402 µm long, and the right spicule 120-186 µm long.

Microfilariae are unsheathed and a detailed description was given by Rioche (1960) and Ortega-Mora, Gomez-Bautista & Rojo-Vázquez (1989). They have a clear head space and a short attenuated tail free of nuclei that ends bluntly. There is no cephalic hook (Leger 1911). The tail in unfixed and unstained microfilariae is straight (Marconcini *et al.* 1996). Minimum and maximum measurements regarding length and width range from 121-277 µm and 3.1-7.4 µm respectively (Table 2.6).

The infective larva in *Hippobosca longipennis* has been described by Nelson (1963) and Bain (1971).

2.4.3 Life cycle

Acanthocheilonema dracunculoides females produce microfilariae which are found in the blood of the definitive host. The information in the literature concerning periodicity of microfilariae is controversial. While Bouin (1921) and Montaron (1975) could not observe any periodicity in naturally infected dogs in Algeria and Morocco, Wolfe, Aslamkhan, Sharif & Pervez (1971) reported a diurnal subperiodicity in an infected dog in Pakistan.

So far, the louse fly *Hippobosca longipennis* and the hard tick *Rhipicephalus sanguineus* have been identified as intermediate hosts (Nelson 1963; Olmeda-García, Rodríguez-Rodríguez & Rojo-Vázquez 1993; Olmeda-García & Rodríguez-Rodríguez 1994).

The predilection sites of the adult worms are the abdominal and thoracic cavities (Chauve 1990).

2.4.4 Host range

Apart from the domestic dog, *A. dracunculoides* has been isolated from the aardwolf (*Proteles cristatus*), the spotted hyaena and the red fox (*Vulpes vulpes*) (Sonin 1985).

2.4.5 Laboratory diagnosis in live animals

Similar to other filarial infections of dogs and cats, histochemical staining for acid phosphatase activity of microfilariae has proved to be the most reliable, consistent and practical differential technique to diagnose infection (Valcárcel *et al*. 1990). Acid phosphatase activity is restricted to the cephalic space, the excretory pore, inner body and the anal pore (Ortega-Mora *et al.* 1989; Chauve 1990; Peribáñez *et al*. 2001). The utilization of the polymerase chain reaction (PCR) as a tool to differentiate *A. dracunculoides* microfilariae from other filarial species of dogs has been reported (Rishniw *et al.* 2006).

2.4.6 Veterinary and medical importance

Although *A. dracunculoides* is regarded as non-pathogenic in the dog (Nelson 1966; Montaron 1975), there is some evidence reported from Spain, Kenya, Uganda and Namibia that suggests that the parasite may not be as innocuous as generally assumed. Infection occasionally presents with dermal clinical signs and lesions ranging from pruritus, alopecia, erythema to skin ulcers as well as other clinical signs such as ataxy, incoordination, cachexia, cyanosis, ascitis and pleural effusion (Piercy 1951; Ortega-Mora & Rojo Vázquez 1988; Chauve 1990; Bolio, Montes, Gutierrez, Alonso, Bernal, Sauri & Rodríguez-Vivas 2002; Schwan & Schröter 2006). Dermal clinical signs in a dog attributed to *A. dracunculoides* infection improved after treatment with ivermectin at a dose rate of 50 µg/kg (Rodríguez 1990). There are no reports of human infections (Chauve 1990).

2.4.7 Distribution on the African continent

Although Nelson (1963) claims that *A. dracunculoides* is widespread in the drier areas of Africa extending from the Mediterranean to South Africa, the parasite has only been reported from some countries. According to Nelson (1963) the distribution of the parasite coincides with the distribution of *Hippobosca longipennis*.

2.4.7.1 NORTHERN AFRICA

Acanthocheilonema dracunculoides has been reported from Algeria, Morocco, Sudan and Tunisia.

Algeria: The first report comes from a dog in the Alger region (Rioche 1960), who found numerous adult worms at necropsy in the peritoneal cavity. The author provides a detailed description of the adult male and female worms and the microfilaria. Montaron (1975) reports on a survey in Alger in which 49 (22.8 %) out of 215 dogs were found positive for microfilariae on examination of blood. In 48 dogs microfilariae were identified as those of *A. drancunculoides* based on motility and appearance of the tail. The diagnosis was confirmed at necropsy were adult worms were found in the abdominal and thoracic cavities.

Morocco: Bouin (1921) reports on a necropsy survey conducted in southern Morocco with no exact locality given. The survey involved 109 dogs of which 19 tested positive for microfilariae prior to necropsy. The microfilariae were identified morphometrically as those of *A. dracunculoides*. However, adult filarial worms were found in the thoracic, peritoneal and pelvic cavities of 1 dog only. In a later necropsy survey, *A. dracunculoides* was reported in 6 out of 57 stray dogs from Rabat and the nearby towns of Temara, Sidi Yahiya des Zaers and Ain Aouda (Pandey *et al.* 1987). Giemsa-stained blood films were prepared prior to euthanasia. The authors do not provide any information on what specimens (microfilariae or adult worms) and what criteria the diagnosis was based.

Sudan: Specimens were obtained from the peritoneal cavity of a dog from an undisclosed locality in the country (Baylis 1929).

Tunisia: Railliet *et al.* (1912) report on a microfilaraemic dog from Tunis. At necropsy adult worms were recovered from the peritoneal cavity. The microfilariae found in the blood were identical to those isolated from the posterior parts of the female uterus. The authors, however, do not provide any description of the microfilariae. In a later survey involving 348 dogs in Tunis, 17.54 % were found to be infected at necropsy, and adult worms were recovered from the peritoneal and pleural cavities (Bernard *et al.* 1967).

2.4.7.2 WESTERN AFRICA

Reports come from Mali and Nigeria.

Mali: Leger (1911) isolated adult worms from the peritoneal cavity of a spotted hyaena in the outskirts of Bamako. Railliet & Henry (1911b) suspected the relation of Leger's filarial species to *A. dracunculoides*. Railliet *et al.* (1912) examined Leger's specimens and confirmed that they were *A. dracunculoides*. In a collation of data on helminth infection of the former French West Africa, the specimens collected by Leger (1911) are listed and the subsequent identification by Railliet *et al.* (1912) is mentioned (Joyeux, Gendre & Baer 1928).

Nigeria: *Acanthocheilonema dracunculoides* was first reported from dogs in the Zaria area (Schillhorn van Veen 1974). The diagnosis was based on the demonstration and morphometrical analysis of microfilariae, with, however, no details being provided other than the length (234-264 µm). Subsequently, Schillhorn van Veen & Blotkamp (1975) report on 'short-type microfilariae' found in dogs during a survey in the Zaria area, which, based on morphometrical analysis, were similar to those of *A. reconditum* and *A. dracunculoides*. In a host-parasite checklist of helminth parasites of domestic animals in Northern Nigeria, *A. dracunculoides* is listed as recorded in the Zaria area in dogs but is said to be rare (Schillhorn van Veen, Shonekan & Fabiyi 1975).

2.4.7.3 CENTRAL AFRICA

The only report comes from the Democratic Republic of the Congo where it was recovered from the abdominal cavity of a dog and from the pleural cavity of another dog from Katanga by Rodhain (Gedoelst 1916).

2.4.7.4 EASTERN AFRICA

Reports on the occurrence of the parasite come from Kenya, Tanzania, Uganda and Zimbabwe.

Kenya: *Acanthocheilonema dracunculoides* was first discovered in hyaenas (species not specified) near Nairobi and in dogs from the Northern Province (Nelson *et al.* 1962). The authors also report that the parasite had not been seen in dogs at the coast. Another report comes from Lokitaung in northern Turkana where adult specimens were recovered from the peritoneal cavity of a dog (Nelson 1963). Another report on a survey involving 63 dogs of which 79 % were found to be infected on blood examination and necropsy came from the same locality (Lightner & Reardon 1983). According to the authors, the morphology and dimensions of the microfilariae corresponded with descriptions given by Nelson (1963) and Wolfe *et al.* (1971).

Tanzania: Sachs (1976) reports on *A. dracunculoides* from the abdominal cavity of spotted haenas in the Serengeti.

Uganda: Carmichael & Bell (1943) report on a 6-year-old Alsation which presented with multiple non-pruritic skin lesions. Unsheathed microfilariae, 240 µm long and 4.5 µm wide, were found in the blood but no further information was provided. Necropsy did not reveal any adult worms. The authors claim that the microfilariae were close to *A. dracunculoides*.

Zimbabwe: *Acanthocheilonema dracunculoides* in the spotted hyaena is listed in a check list of helminth parasites of domestic and wild mammals of Zimbabwe (Jooste 1990).

2.4.7.5 SOUTHERN AFRICA

Reports come from Namibia and South Africa.

Namibia: Microfilariae found in two dogs from Windhoek were identified by acid phosphatase staining as those of *A. dracunculoides* (Schwan & Schröter 2006).

South Africa: The country, where the species was first discovered and described from specimens recovered from an aardwolf caught at an undisclosed locality (Cobbold 1870).

2.5 *Cercopithifilaria grassii*

2.5.1 Taxonomy

Cercopithifilaria grassii (Noé, 1907) has no vernacular name and has been known in the literature by the names *Filaria grassii* (Noé, 1907), *Acanthocheilonema grassii* (Baylis, 1929) and the still widely used previous name *Dipetalonema grassii* (Noé, 1907) (Sonin 1985).

2.5.2 Morphology

The genus *Cercopithifilaria* is used to accommodate filarial species considered as specialized *Acanthocheilonema* (Bain *et al.* 1982a). Differentiating features are a short and undivided oesophagus, a very small buccal capsule, a stumpy right spicule without a distinct sheath, and the caudal papillae reduced in number and situated close to the cloaca (Bain *et al.* 1982a).

The morphology of the adult stages of *C. grassii* is described by Noè (1907); Costa & Freitas (1962) and Balasubramaniam, Anandan & Alwar (1975) According to these authors females are 24-27.2 mm long and 56-114 µm wide whereas males are 10-11.2 mm long and 40-45 µm wide. The left spicule is 200-215 µm and the right spicule 50-65 µm long.

Microfilariae are sheathed. According to the only detailed descriptions by Noè (1907, 1908) the microfilariae have a squat, blunt tail that terminates in three papillae. The cephalic end is slightly distended and there are transversal cuticular ridges along the rest of the body. Compared to the other canine and feline filarial species, the microfilariae are huge, measuring 567 µm in length and 12.25 µm in width (Noè 1907).

The infective larva in *Rhipicephalus sanguineus* has been described by Noè (1908), Bain, Aeschlimann & Chatelanat (1982b) and Bain & Chabaud (1986).

2.5.3 Life cycle

Cercopithifilaria grassii microfilariae are found mainly in the skin (Noè 1907), the lymph (Pampiglione & Canestri Trotti 1990) and occasionally in the blood (Casarosa 1985). *Rhipicephalus sanguineus* is the only vector identified (Noè 1908; Bain *et al.* 1982b).

2.5.4 Host range

Domestic dogs and cats are the only definitive hosts reported for *C. grassii* (Tarello 2004). Adult worms are found in the subcutaneous and intermuscular connective tissue as well as in the abdominal cavity (Costa & Freitas 1962; Chauve 1990).

2.5.5 Laboratory diagnosis in live animals

The laboratory diagnosis is based on the demonstration of sheathed microfilariae in skin snips or blood, taking the unique dimensions of the microfilaria into consideration (Tarello 2004).

2.5.6 Veterinary and medical importance

Although, infection with *C. grassii* is rarely reported and considered to be harmless (Nelson 1966; Bain *et al.* 1982b; Chauve 1990), there is a report by Tarello (2004) of an infected cat that presented with pruritic dermatitis and multifocal alopecia. Following treatment with melarsomine and ivermectin the lesions and clinical signs resolved. There are no reports of human infections (Chauve 1990).

2.5.7 Distribution on the African continent

In the only documentation from Africa, Heisch *et al*. (1959) and Nelson *et al.* (1962) report on finding a *Dipetalonema* species in a dog in Faza, Kenya and a dog in Dar-es-Salaam, with adults recovered from the subcutaneous tissues and under the abdominal muscles. The microfilariae, which are only described as 300 µm long on average, were confined to the skin. The authors suggest that this might be *C. grassii*. The authors do not provide any information on the adults.

2.6 *Brugia patei*

2.6.1 Taxonomy

Brugia patei (Buckley, Nelson & Heisch, 1958) is referred to in the literature also as *Wuchereria patei* Buckley, Nelson & Heisch, 1958 (Sonin 1985). The genus name *Brugia,* chosen in honour of the discoverer of *Microfilaria malayi*, Dr SL Brug, was proposed by Buckley in 1960 for a distinct group of filarial helminths which is parasitic in the lymphatic system of primates, carnivores and insectivores. The species was named after Pate Island where it was first discovered by Nelson & Heisch (1957).

2.6.2 Morphology

The genus *Brugia* accommodates a small group of morphologically very similar filarial helminths with affinities to *Wuchereria bancrofti* (Sonin 1975). The subtle morphological features that characterize the genus and differentiate it from *Wuchereria* have been described by Buckley (1960).

A description of the adult stages is given by Buckley, Nelson & Heisch (1958). According to these authors females are 34.5-50.7 mm long and 135-190 µm wide whereas males are 14-25.4 mm long and 75-100 µm wide. The left spicule is 255-295 µm and the right spicule 110-130 µm long.

The microfilariae are sheathed and very similar to those of *Brugia malayi* with the only constant difference in the length of the nuclei-free cephalic space which averages 4.8 µm as compared with 6.9 µm in *B. malayi* (Feng 1933; Buckley *et al.* 1958). Measurements regarding width and length range from 165-260 and 5-6 µm respectively and there are characteristic terminal tail nuclei (Feng 1933; Laurence & Simpson 1971).

The infective larva in mosquitoes has been described by Nelson (1959) and Bain & Chabaud (1986).

2.6.3 Life cycle

Brugia patei microfilariae are found in the blood of the definitive host. A non-periodic and nocturnal subperiodic strain have been identified in Kenya (Nelson *et al.* 1962).

Mosquitoes act as intermediate hosts. The incubation period in *Mansonia uniformis* takes 6-9 days at 28 °C (Laurence & Pester 1960; Nelson *et al.* 1962). There is no information on the prepatent and patent period in definitive hosts.

2.6.4 Host range

Apart from the domestic dog and cat, the large-spotted genet and the greater bushbaby (*Galago crassicaudatus*) have been identified as natural hosts (Nelson 1959). Infection rates in cats are reported to be higher than in dogs and large-spotted genets and adult worms are most commonly found in the lymphatics of the hind legs (Heisch *et al*. 1959).

2.6.5 Vectors

Brugia patei infective larvae were found in *Aedes pembaensis*, *Mansonia uniformis* and *Mansonia africanus* (Nelson *et al*. 1962). The vector *A. pembaensis* has an obligatory phoretic association with salt-water crabs (Goiny, Van Someren & Heisch 1957).

2.6.6 Laboratory diagnosis in live animals

The diagnosis is based on the demonstration of sheathed microfilariae in the blood, taking into consideration the characteristic tail nuclei as a differentiating morphological feature (Buckley 1960).

2.6.7 Veterinary and medical importance

Although *B. patei* is regarded as largely non-pathogenic (Nelson 1966), there is a report from Kenya where two cats with ascitis had adult worms in the abdominal lymphatics and numerous sheathed microfilariae in the ascitic fluid (Heisch *et al*. 1959).

Microfilariae of *B. patei* have never been seen in the blood of man (Heisch *et al.* 1959). However, they may be an important aetiology of tropical pulmonary eosinophilia which is not uncommon on the Kenya coast (Nelson *et al*. 1962). This assumption is based on observations of Buckley (1958) who provided experimental proof by inoculating himself with infective larvae of *Brugia* spp. from animals.

2.6.8 Distribution on the African continent and its islands

Brugia patei was first discovered in domestic dogs and cats on Pate Island in Kenya and has not been reported from any other country in Africa (Nelson & Heisch 1957). The discovery was based on finding a sheathed microfilaria of the *Brugia malayi*-type. Buckley *et al*. (1958) report on a 56 % prevalence (14 out of 24 animals) of microfilarial infection in cats from Pate Island and also mention infection in 2 out of 5 dogs and three out of seven large-spotted genets. In a subsequent survey on the island, infection was reported again in dogs, cats and large-spotted genets with a similar higher prevalence in cats (21 out of 29) than in dogs (3 out of 12) (Heisch *et al*. 1959). The diagnosis was made by demonstration of adults at necropsy and microfilariae in day bloods. The microfilariae found in the blood were identical to those isolated from the gravid uteri of adult worms. The authors also found the parasite in cats on the Island of Lamu and in villages on the Tana River. However, *B. patei* could not be demonstrated in day bloods from cats and dogs at Mombasa, Pemba, other unidentified villages along the southern Kenya coast, Zanzibar and Dar-es-Salaam. In another survey on filarial infections in man, animals and mosquitoes on the Kenya coast from Somalia to the former Tanganyika, 15 out of 252 dogs, 38 out of 240 cats, 3 out of 9 large-spotted genets and 1 out of 10 greater bushbabies were found infected on day blood films (Nelson *et al*. 1962).

2.7 Other reported species

2.7.1 Microfilaria auquieri

Foley (1921) reports on a new microfilaria found in the blood of dogs in Beni-Ounif de Figuig on the Algerian-Moroccan border which was named in memory of Dr Auquier, a former physician in Figuig. The microfilaria was described as unsheathed, without a cephalic hook and characterized by its remarkable shortness (58-102 µm) and relative large width (6-8 µm). The only other report comes from the Région de Palestro in Algeria, where the microfilaria was found in one dog and is described as 89 µm long and 7 µm wide on average (Rioche 1960). Although a systematic necropsy was conducted, adult worms could not be found.

2.7.2 Filaria ochmanni

A new microfilaria described as sheathed and 320 µm long on average in haematoxylin-stained thin blood films was found in a dog from Dar-es-Salaam in Tanzania (Fülleborn 1908b). The microfilaria was named after the state veterinarian Ochmann in Dar-es-Salaam who supplied the material. Buckley *et al*. (1958) suggest that this microfilaria belongs to the '*malayi*' group of microfilariae.

Table 2.1: Filarial helminths described from dogs and cats and their geographical distribution

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

Table 2.2: Length and width of *Dirofilaria immitis* microfilariae from dogs according to geographical origin and technique of processing

Length [µm] Range ± SD or Mean	Width [µm] Range or Mean	Geographical origin/ Technique of processing	Source
Africa			
264 ± 5.8	5 ± 0.2	Algeria Technique not specified	Rioche (1960)
250		Kenya Technique not specified	Heisch *et al.* (1959)
232-260	4-6	Mozambique Methanol fixation and Giemsa staining	Schwan & Durand 2002
America			
327.46 ± 2.36		USA Knott's technique	Acevedo *et al.* (1981)
281.32 ± 4.24		USA Membrane filtration	Acevedo *et al.* (1981)
326.15 ± 3.27		USA Membrane filtration with subsequent methanol fixation and Giemsa staining	Acevedo *et al.* (1981)
269.5 ± 3.72		USA Membrane filtration with subsequent formalin fixation and methylene blue staining	Acevedo *et al.* (1981)
233-270 (253)	5.4-6.5 (5.96)	USA Microfilariae isolated from formalinized female worms	Fülleborn (1912)
285.6 – 339.8	6.1-7.2	USA Knott's technique	Lindsey (1961)
307-322	-	USA Knott's technique	Newton & Wright (1956)
Asia			
260-280	5.7-7.5	Vietnam Giemsa staining	Mathis & Léger (1911)
180-285	5	Vietnam Technique not specified	Railliet & Henry (1911b)
210-253 (233.7)		China Microfilariae isolated from female worms preserved in 70% ethanol	Fülleborn (1912)
245-333	7-8.5	Taschkent Technique not specified	Yakimoff (1917)
260 ± 5	4.5	China Bouin's fixative and Giemsa staining	Taylor (1960a)
Australia			
256.7 ± 16.6	-	Membrane filtration	Watson *et al.* (1973)
301.3 ± 22.6	-	Knott's test	Watson *et al.* (1973)
Europe			
220-340	5-6.5	France Technique not specified	Ducos de Lahitte *et al.* (1993)
290-330	6-6.5	Italy Unfixed and unstained microfilariae	Marconcini *et al.* (1996)
306.83 ± 22.41	5.9 ± 0.69	Spain Knott's technique	Valcárcel *et al.* (1990)

Table 2.3: Natural culicine vectors of *Dirofilaria immitis* in Africa

Species	Locality	Reference
Aedes aegypti	Kenya	Nelson *et al.* (1962)
Aedes pembaensis	Kenya Tanzania	Nelson *et al.* (1962) Mosha & Magayuka (1979)
Anopheles pharoensis	West Africa	Brengues & Nelson (1975)
Anopheles tenebrosus	Tanzania	Gillies (1964); Magayuka (1973); Mosha & Magayuka (1979)
Culex quinquefasciatus	Mauritius	Halcrow (1954)
Mansonia africana	Tanzania	Magayuka (1973); Mosha & Magayuka (1979)
Mansonia uniformis	Madagascar Tanzania West Africa	Brunhes, Rajaonarivelo & Nelson (1972) Magayuka (1973), Mosha & Magayuka (1979) Brengues & Nelson (1975)

Table 2.4: Length and width of *Dirofilaria repens* microfilariae from dogs, cats and other carnivores according to geographical origin and technique of processing

Length [µm] Range ± SD or Mean	Width [µm] Range or Mean	Geographical origin/ Technique of processing	Source
Africa			
328	-	Kenya (dog/cat/large-spotted genet)	Heisch *et al.* (1959)
300-369	-	Nigeria (dog) Brilliant cresylblue staining	Schillhorn van Veen (1974)
340-353 320-360	7.5-8 7.5-8	Nigeria (dog) Live/Stubbs technique Nigeria (dog) Live/Stubbs technique	Kamalu (1986) Kamalu (1991)
320-350	6.25-7.5	South Africa (cat) Membrane filtration and Giemsa staining	Schwan *et al.* (2000)
240-350	7-9	Tunisia (cat) Technique not specified	Chatton (1918)
Asia			
300-360	6.5-8	Vietnam (dog) Technique not specified	Railliet & Henry (1911b)
300-375	6.5-7	Vietnam (dog) Technique not specified	Bernard & Bauche (1913)
290 ± 15	6-8	Sri Lanka (dog) Giemsa staining	Gunewardene (1956)
337	8	Vietnam (fishing cat) Giemsa staining	Le-Van-Hoa & Le Thi-Ty (1971)
Europe			
315 ± 22	6 ± 0.5	Sardinia (dog) Unfixed and unstained	Webber & Hawking (1955)
290 ± 10	6	Italy (dog) Bouin's fixative + Giemsa staining	Taylor (1960a)
340 377	- -	Italy (cat) Italy (dog)	Cancrini & Iori (1981)
207-360	5-8	France Technique not specified	Chauve (1990)
290-360	6-8	France Technique not specified	Ducos de Lahitte & Ducos de Lahitte (1990)
345-385	6.5-7	Italy (red fox) Unfixed and unstained	Marconcini *et al.* (1996)
345.27 ± 19.3	6.4 ± 0.78	Spain (dog) Knott's technique	Valcárcel *et al.* (1990)

Table 2.5: Length and width of *Acanthocheilonema reconditum* microfilariae from dogs according to geographical origin and technique of processing

Length [µm] Range ± SD or Mean	Width [µm] Range or Mean	Geographical origin/ Technique of processing	Source
Africa			
270	4.5	Kenya Giemsa-stained blood films	Nelson (1962)
227.6-259.7 (239.3)	4.4-6.7	Liberia Haematoxylin/Ethanol fixation	Laub (1988)
239.8-273.1 (257.1)	4.4-6.7	Liberia Methylene blue/Formaldehyde fixation	Laub (1988)
225-282 (263)	4.93-5.86 (5.02)	Nigeria Knott's technique	Bobade *et al.* (1981)
200-204	4	Mozambique Giemsa-stained blood films	Schwan *et al.* (2002)
America			
262.09 ± 3.36	-	USA Knott's technique	Acevedo *et al.* (1981)
241.06 ± 2.34	-	USA Membrane filtration	Acevedo *et al.* (1981)
246.4-291.6	4.7-5.8	USA Technique not specified	Lindsey (1961)
276	-	USA Technique not specified	Newton & Wright (1956)
Asia			
230-290 (263.6) 167.8-228.5 (204.34)	4-5 2.9-5	Lebanon Knott's technique Brilliant cresyl blue staining	Korkejian & Edeson (1978)
230-285 (262)	3.2-5.8 (4.7)	Okinawa Knott's technique	Pennington & Phelps (1969)
Australia			
226.6 ± 13.4	-	Membrane filtration	Watson *et al.* (1973)
255.2 ± 24.8	-	Knott's technique	Watson *et al.* (1973)
Europe			
269-283	4	France Technique not specified	Chauve (1990)
200-230	4-5	France Technique not specified	Ducos de Lahitte (1990)
210-215	-	France Technique not specified	Euzéby (1961)
270-280	5-5.5	Italy in Vulpes vulpes Unfixed and unstained	Marconcini *et al.* (1996)
261.96 ± 14.28	-	Spain Knott's technique	Valcárcel *et al.* (1990)

Table 2.6: Length and width of *Acanthocheilonema dracunculoides* microfilariae from dogs and other carnivores according to geographical origin and technique of processing

Length [µm] Range ± SD or Mean	Width [µm] Range or Mean	Geographical origin/ Technique of processing	Source
Africa			
121-218	4.5-5.2	Algeria (dog) May-Grünwald-Giemsa staining	Rioche (1960)
255	4.5	Kenya (dog) Knott's technique	Lightner & Reardon (1983)
195-230	5-5.5	Mali (spotted hyaena) Technique not specified	Railliet *et al*. (1912)
240-260	5-6	Morocco (dog)	Bouin (1921)
Asia			
212-265 213	3.1-5.7 4.4	Pakistan (dog) Knott's test Haematoxylin-stained blood films	Wolfe *et al*. (1971)
Europe			
185-230	5-6	France Technique not specified	Chauve (1990)
199-230	5-6	France Technique not specified	Ducos de Lahitte & Ducos de Lahitte (1990)
237-247	4.2-4.4	Italy (red fox) Unfixed and unstained	Marconcini *et al*. (1996)
145-233	5.3-7.4	Portugal (dog) Giemsa-stained blood films	Fraga de Azevedo (1943)
233-277	4.5-6	Spain (dog) Knott's technique	Ortega-Mora *et al*. (1989)
263.51	5.04	Spain (dog) Knott's technique	Valcárcel *et al*. (1990)

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).

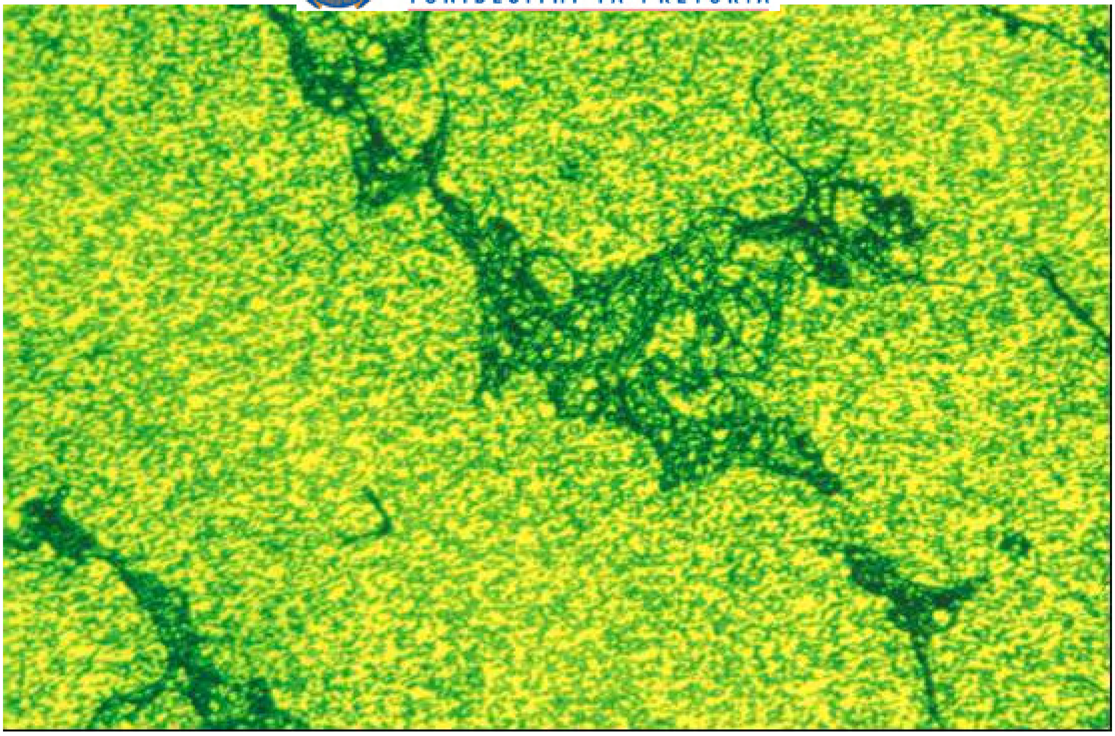

Figure 4.1: Microfilariae on a Giemsa-stained membrane filter

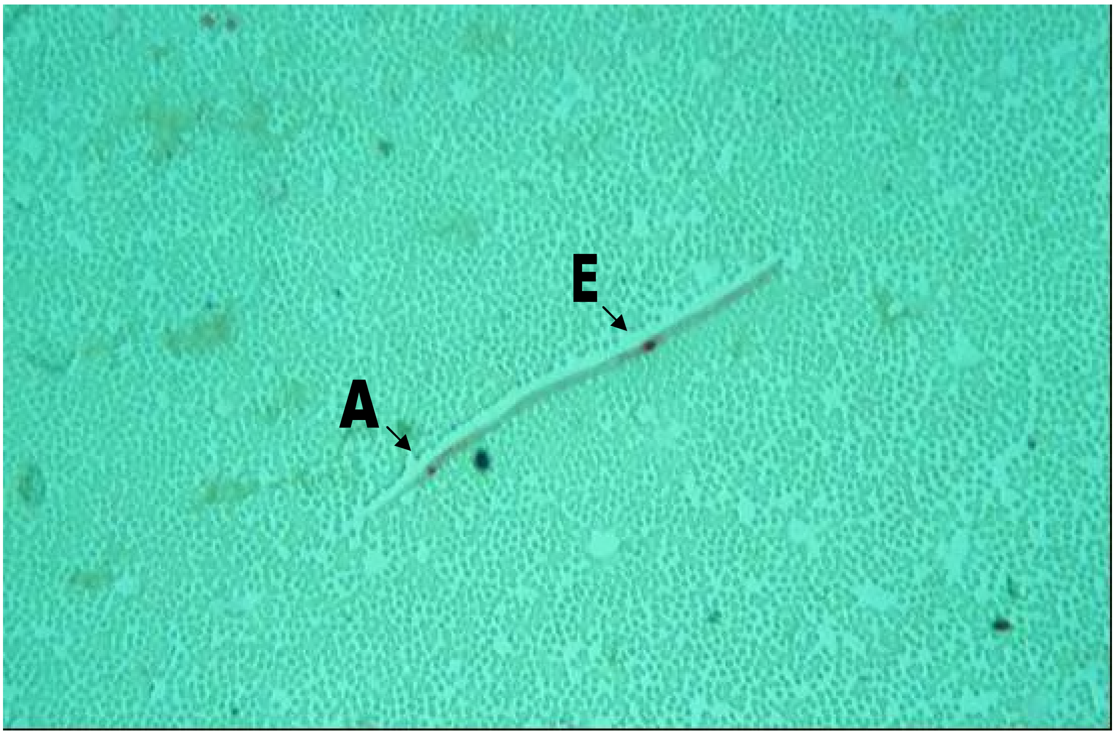

Figure 4.2: *Dirofilaria immitis* microfilaria showing acid phosphatase activity at the excretory pore (E) and anal pore (A)

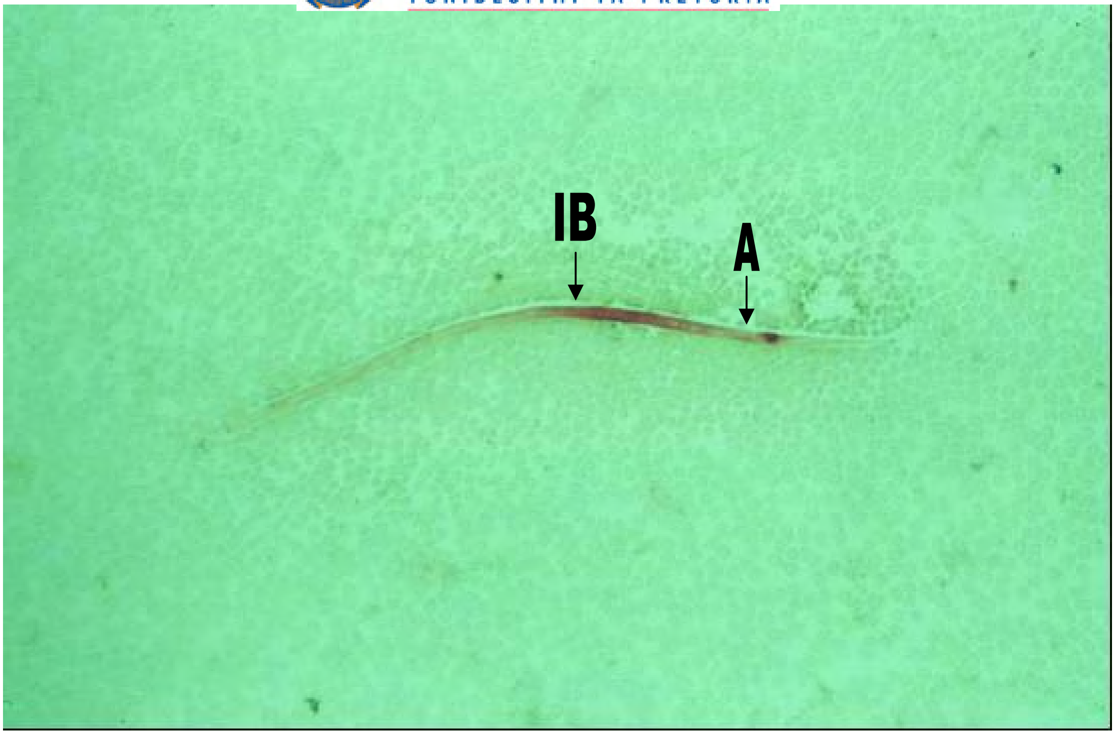

Figure 4.3: *Dirofilaria repens* microfilaria showing acid phosphatase activity at the inner body (IB) and anal pore (A)

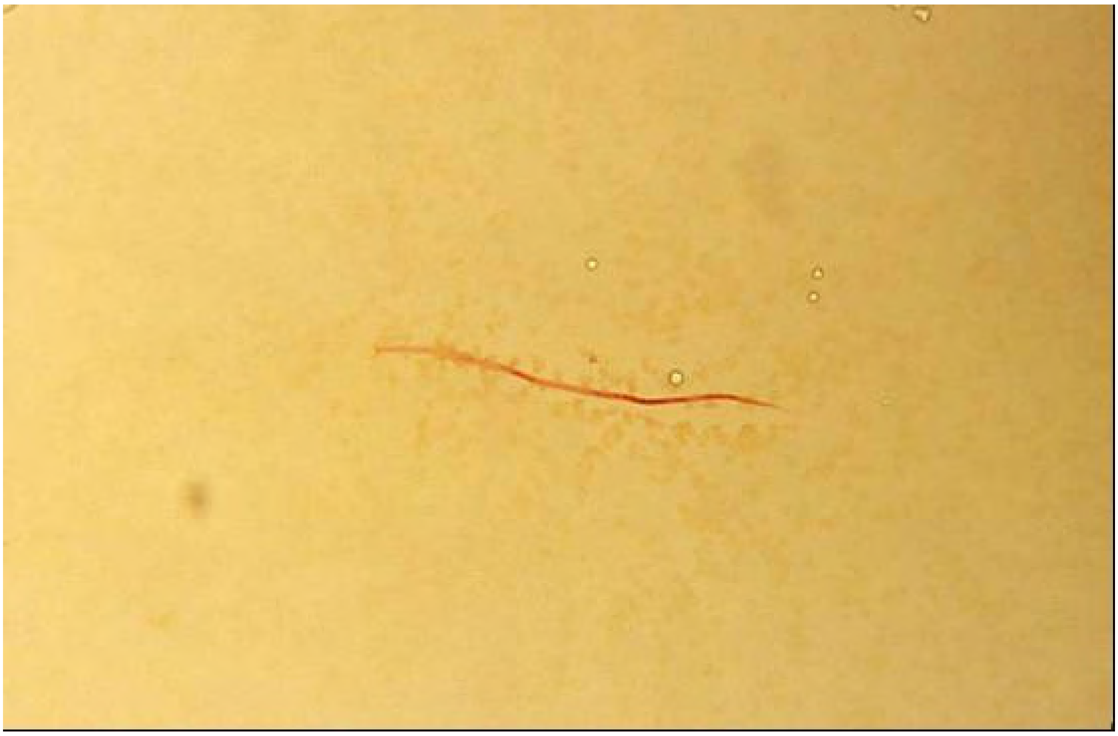

Figure 4.4: *Acanthocheilonema reconditum* microfilaria showing diffuse acid phosphatase activity in the area of the excretory pore, inner body and anal pore

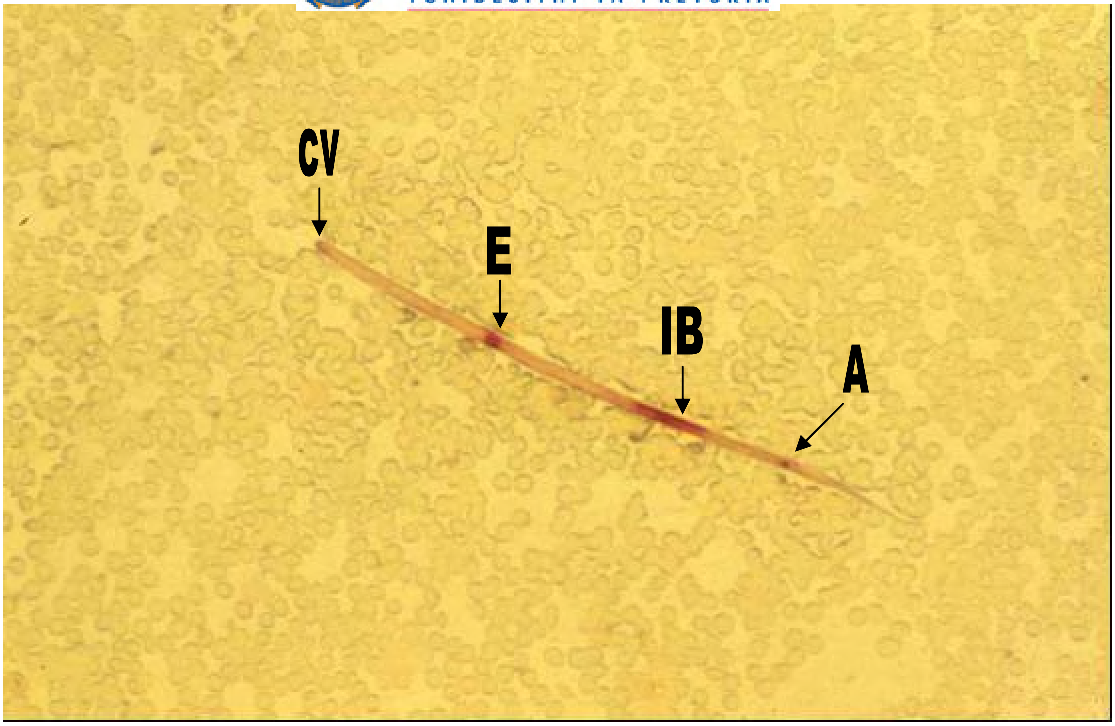

Figure 4.5: *Acanthocheilonema dracunculoides* microfilaria showing acid phosphatase activity at the cephalic vesicle (CV), excretory pore (E), inner body (IB) and anal pore (A)

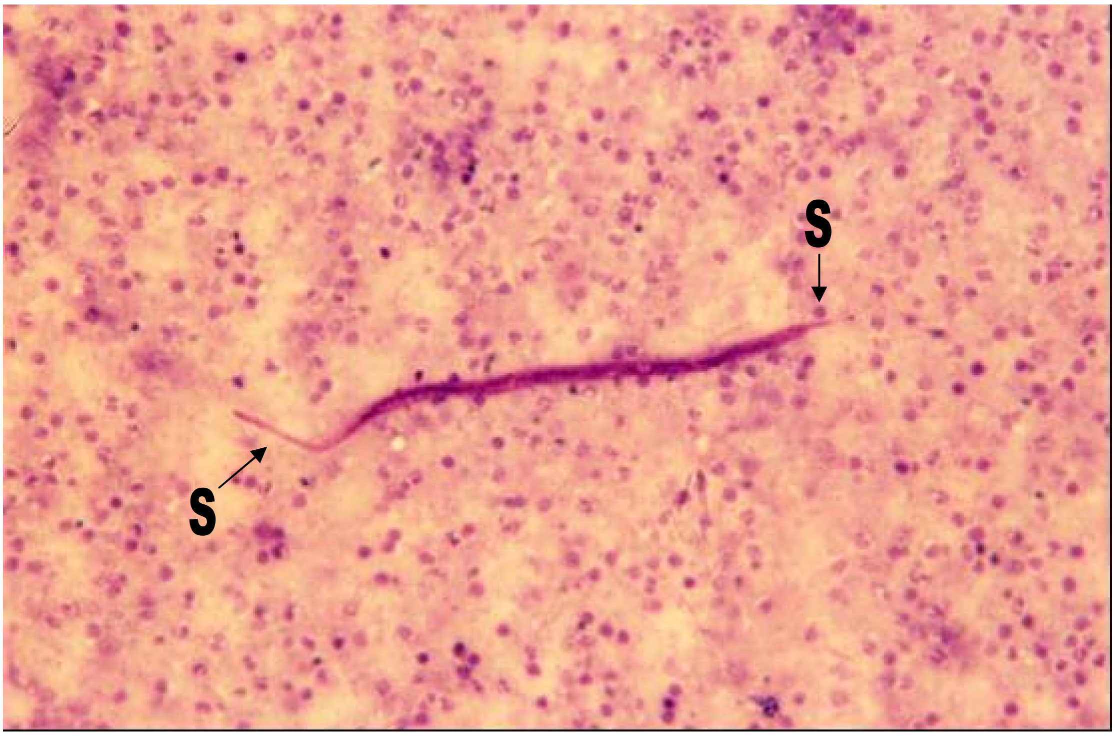

Figure 4.6: *Brugia patei* microfilaria with sheath (S) stained with Giemsa

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

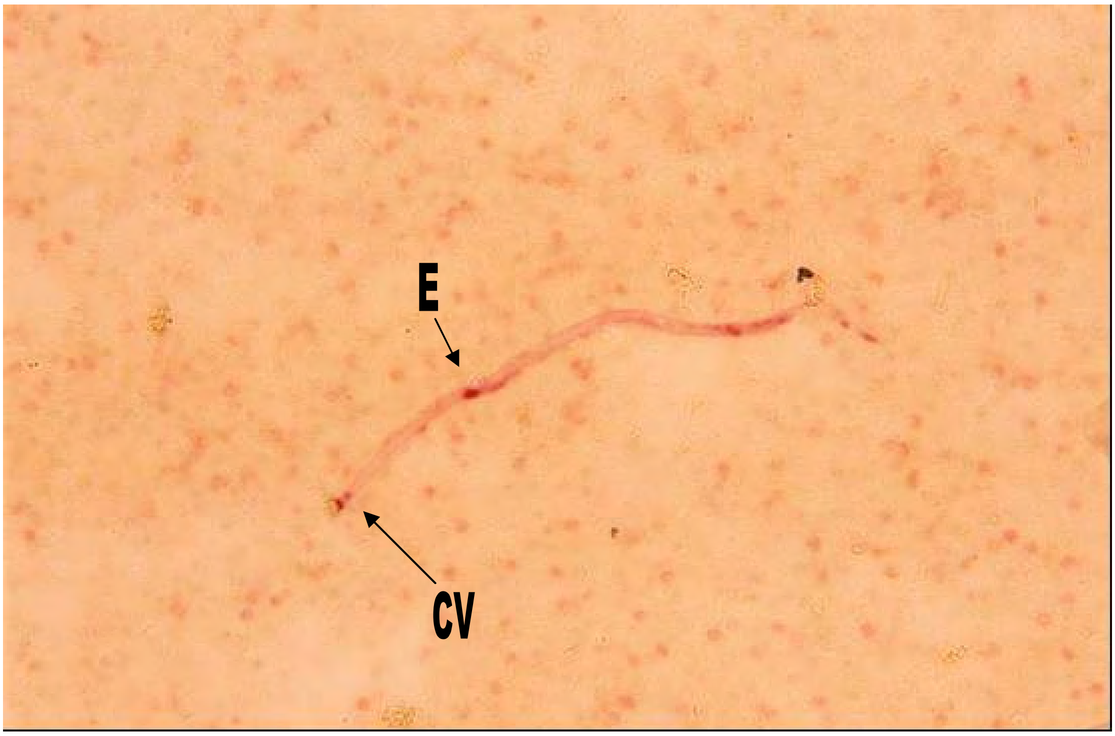

Figure 4.8: *Brugia patei* microfilaria showing acid phosphatase activity at the cephalic vesicle (CV), excretory pore (E) and the tail

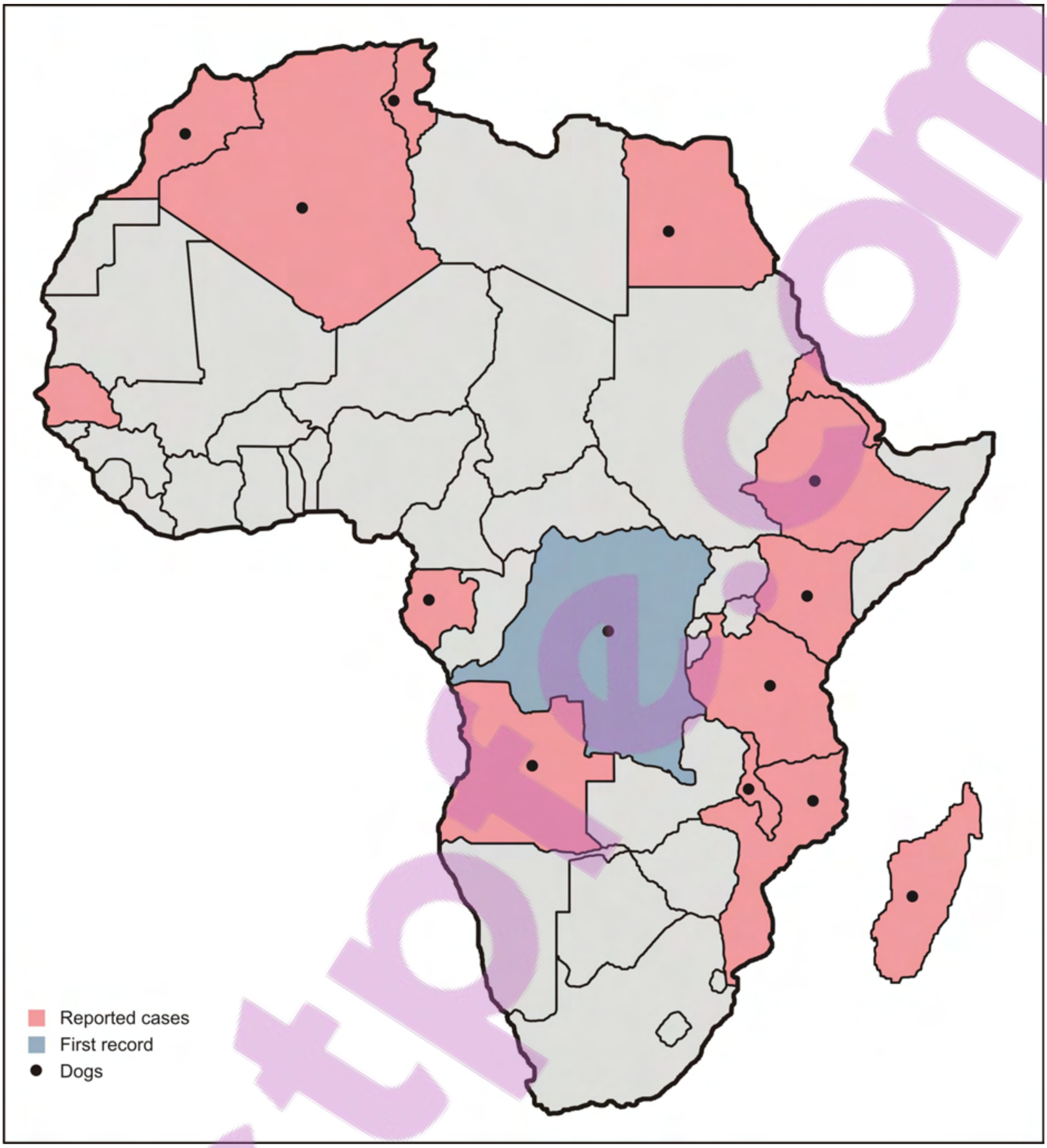

Figure 4.9: Geographical distribution of *Dirofilaria immitis* in dogs in Africa

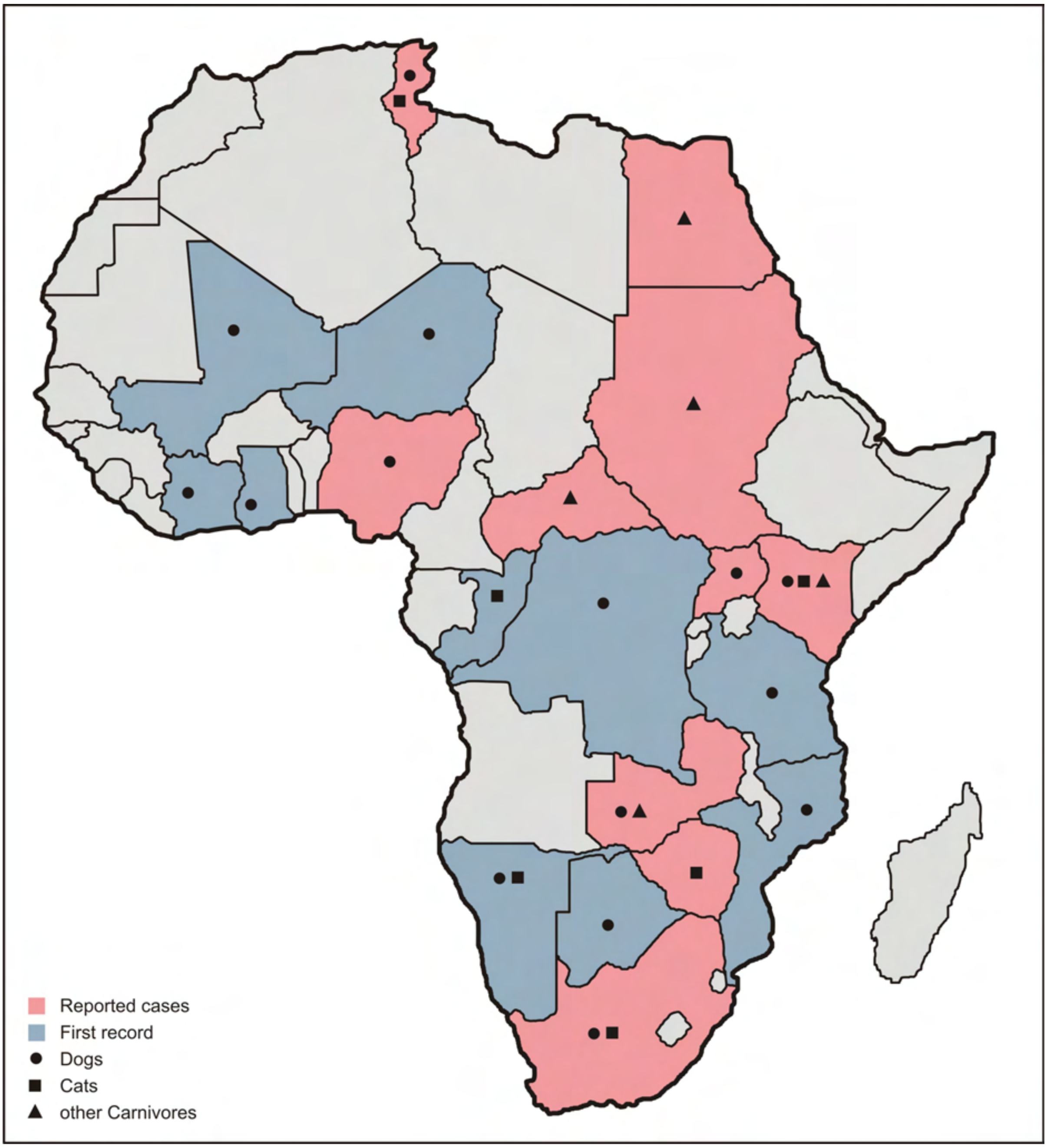

Figure 4.10: Geographical distribution of *Dirofilaria repens* in dogs, cats and other carnivores in Africa

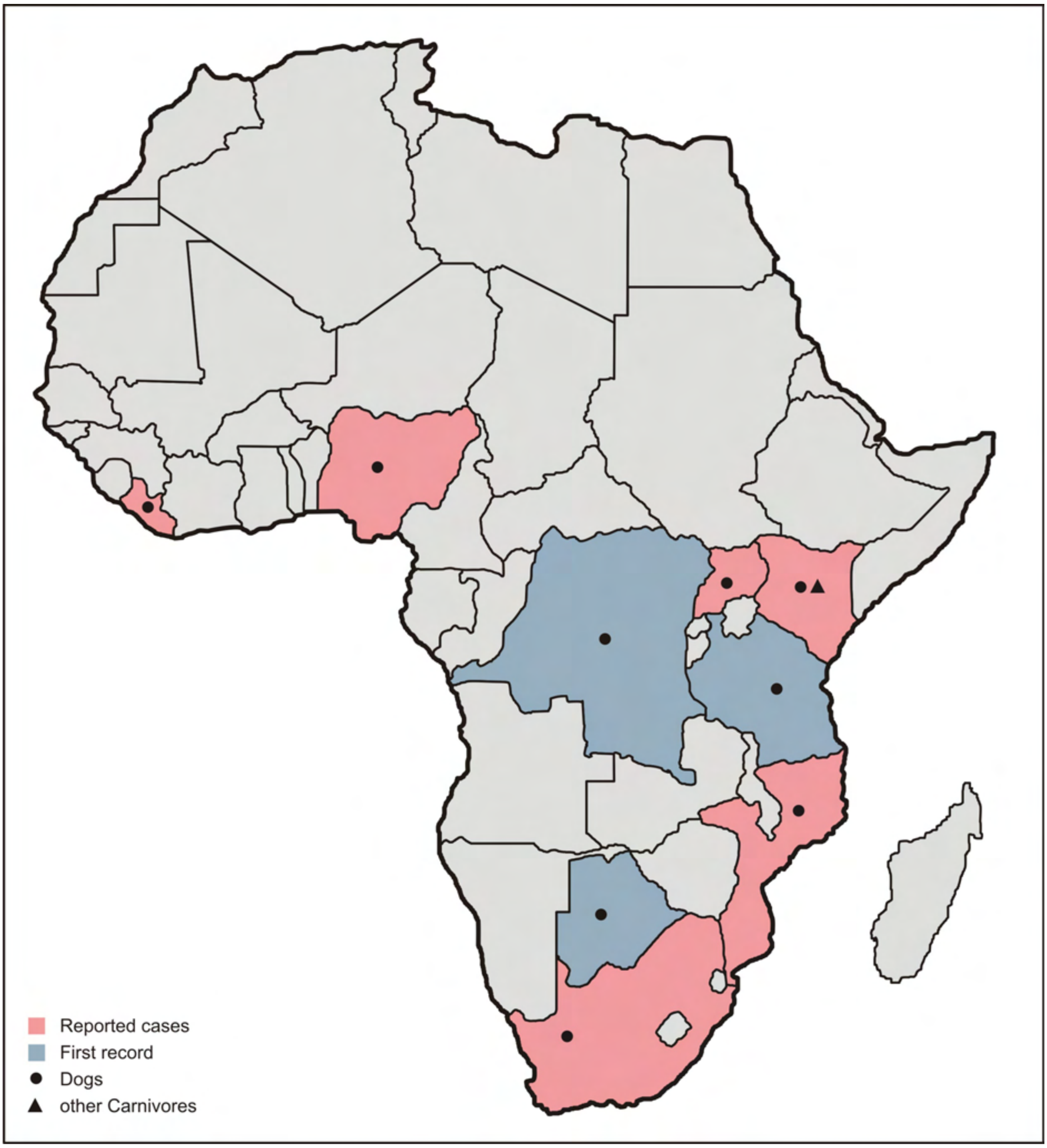

Figure 4.11: Geographical distribution of *Acanthocheilonema reconditum* in dogs and other carnivores in Africa

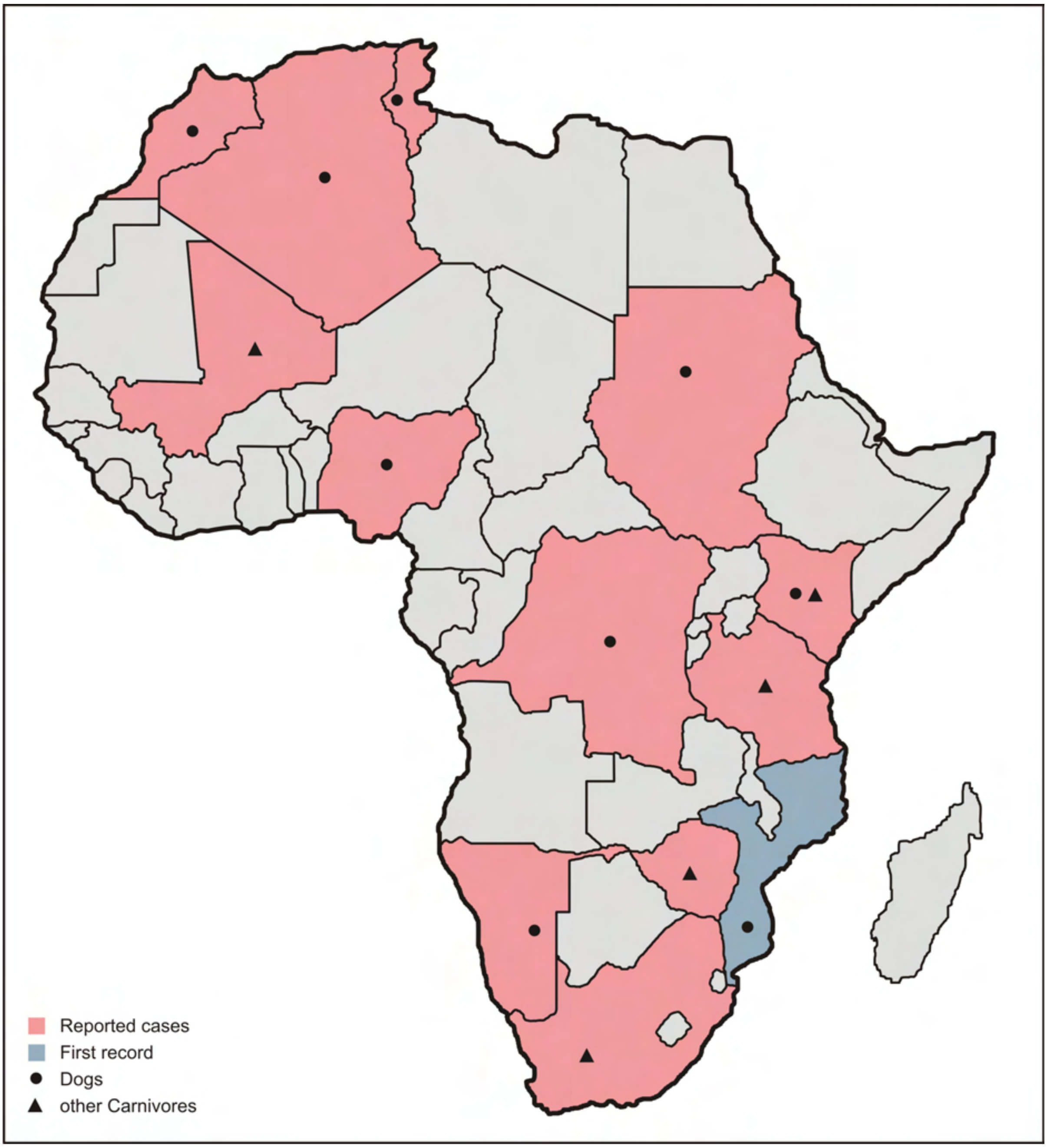

Figure 4.12: Geographical distribution of *Acanthocheilonema dracunculoides* in dogs and other carnivores in Africa

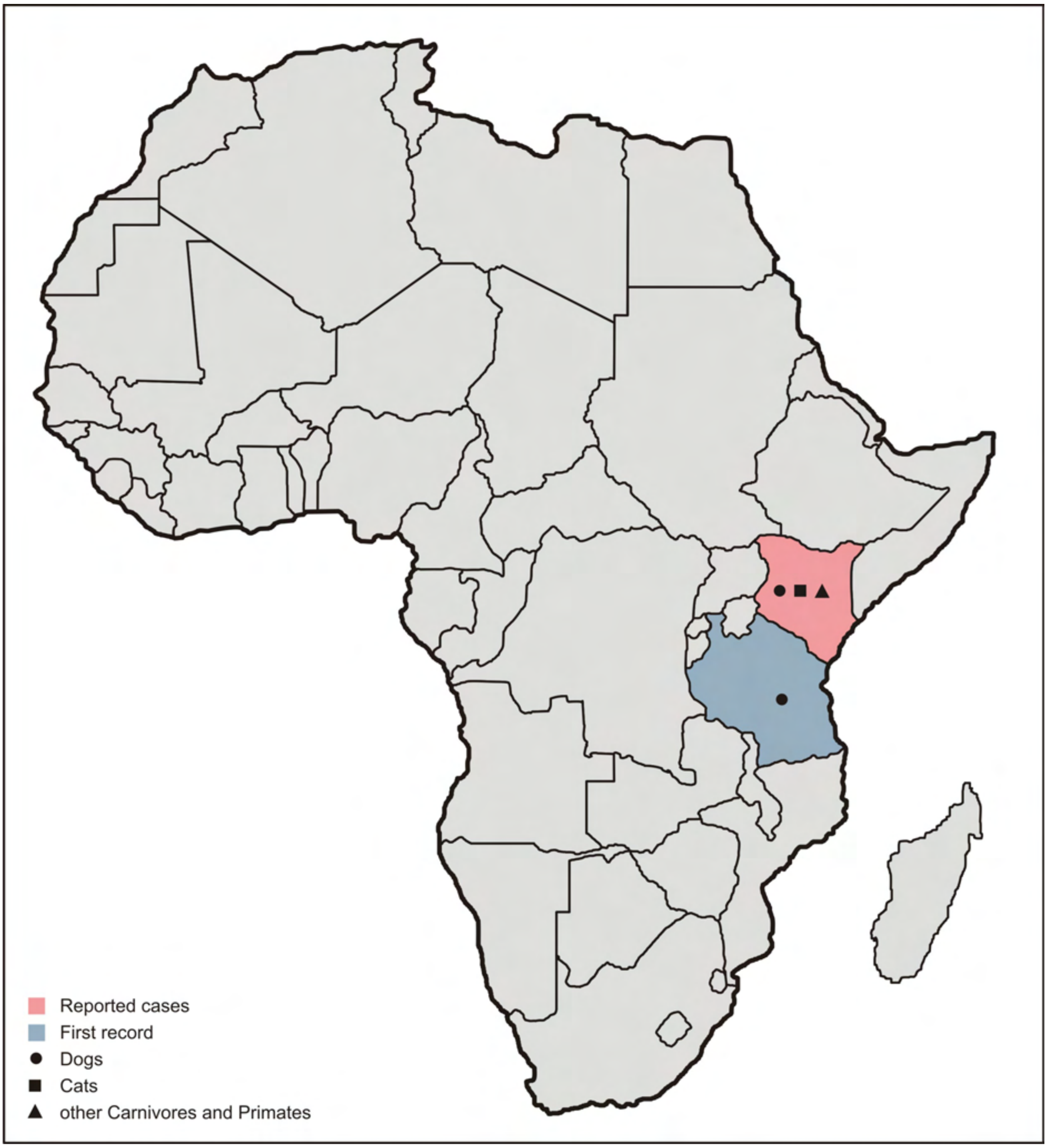

Figure 4.13: Geographical distribution of *Brugia patei* in dogs, cats and other carnivores and primates in Africa

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	1[a]	1.49[a]
	≥ 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. record.*)
	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. record.*)
	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.

Chapter 6
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