Prevalence of *Dirofilaria repens* in dogs in Potchefstroom and Mahikeng, South Africa

By

Tatenda Roy Motsi

(Student number: u21734586)

Submitted in partial fulfilment of the requirement of the degree

Master of Science in Tropical Animal Health

in the Department of Veterinary Tropical Diseases,

Faculty of Veterinary Science, University of Pretoria

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

INSTITUTE OF TROPICAL MEDICINE ANTWERP

October 2023

Declaration

I, Tatenda Roy Motsi, declare that this dissertation hereby presented for the Master of Science in Tropical Animal Health degree to the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, is my own work and has not been previously submitted by me for degree purposes at any other tertiary institution.

Tatenda Roy Motsi — 30/10/2023

Date

Approved by:

Dr. E. V. Schwan — Date: 31/01/2024

Prof. M. C. Marufu — Date: 31/01/2024

List of Abbreviations

µm	Micrometres
AEC	Animal Ethics Committee
APA	Acid Phosphatase Activity
APS	Acid Phosphatase Staining
CCA	Creative Commons Attribution
COX 1	Cytochrome c oxidase subunit 1
DALRRD	Department of Agriculture, Land Reform and Rural Development
DARD	Department of Agriculture and Rural Development
DH	Definitive Host
DNA	Deoxyribonucleic acid
DVTD	Department of Veterinary Tropical Diseases
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EIP	Extrinsic Incubation Period
FVS	Faculty of Veterinary Science
IgG	Immunoglobulin G
IH	Intermediate Host
MF	Membrane Filtration
mm	Millimetres
ml	Millilitres
MRI	Magnetic Resonance Imaging
MT	Malpighian tubules
PAWS	Potchefstroom Animal Welfare Society
PCR	Polymerase Chain Reaction

REC	Research Ethics Committee
rpm	revolutions per minute
SEM	Scanning Electron Microscopy
SPCA	Society for the Prevention of Cruelty to Animals
UP	University of Pretoria

Summary

Prevalence of *Dirofilaria repens* in dogs in Potchefstroom and Mahikeng, South Africa

By

Tatenda Roy Motsi

Supervisor: Dr. Ernst Volker Schwan

Co-supervisor: Prof. Munyaradzi Christopher Marufu

Degree: MSc Tropical Animal Health

Department: Veterinary Tropical Diseases

Dirofilaria repens is a filarial nematode causing subcutaneous dirofilariosis in canids and felids, both domestic and wild. It is a mosquito-borne infection with zoonotic implications. Though previous studies have shown the presence of *D. repens* in domesticated dogs and cats in South Africa, there is less available evidence regarding its occurrence specifically in the North West Province, particularly among hunting and shelter dogs. The research was focused on North West Province on account of its geographical proximity to Gaborone, Botswana, where prior surveys have identified a significant prevalence of *D. repens* in dogs.

The aim of this study was to investigate the prevalence of *D. repens* in dogs in the towns of Potchefstroom and Mahikeng, located within North West Province. The study focuses on dogs, as they are considered to be more competent hosts and reservoirs of the infection, in comparison to cats, and thus are a major source of *D. repens* for mosquitoes which then transmit infection to humans. Human cases of dirofilariasis due to *D. repens* have been documented in South Africa.

A quantitative, correlational, cross-sectional study employing convenience and random sampling was conducted. Sheltered dogs at the Potchefstroom Animal Welfare Society (PAWS) were targeted in Potchefstroom, whereas hunting dogs kept in colonies were targeted in Mahikeng. A total of 157 animals were tested, 87 from Potchefstroom and 70 from Mahikeng. Venous blood drawn into EDTA tubes was screened for microfilariae using the membrane filtration test. No microfilariae were detected in samples from Potchefstroom. Microfilariae

were detected in 14 out of 70 samples from Mahikeng. Acid phosphatase staining confirmed these positive samples to be attributed to *Acanthocheilonema reconditum,* which is regarded as non-pathogenic. *Dirofilaria repens* was not detected in this survey. Based on our findings, it can be concluded that the presence of *D. repens* in hunting and shelter dogs in the towns of Mahikeng and Potchefstroom, North West Province, is either absent or exhibits a low prevalence, hence hindering its detection in the current study. Future research endeavours should prioritize the broadening of sample sizes and survey locations in the province, as well as the diversification of sampling populations to include cats and mosquitoes.

Acknowledgements

I wish to thank the following individuals and organizations for their various roles in making this research a success.

- The Department of Veterinary Tropical Diseases (DVTD) and Institute for Tropical Medicine, Antwerp, Belgium for the FA4 bursary that funded both my studies and the research.
- Dr. Ernst Volker Schwan for his insights, guidance and critical review of the research work and assistance with the laboratory work.
- Prof. Munyaradzi Christopher Marufu for his encouragement, guidance, and critical review of the research work.
- The Helminthology Laboratory team at DVTD, University of Pretoria, with mention of Mr. Louis Taljaard who assisted in the laboratory evaluation of the blood samples.
- Dr. Matthew Nyirenda and his team from North West University in the collection and transportation of blood samples from Mahikeng.
- Potchefstroom Animal Welfare Society (PAWS) in allowing the collection of blood samples from sheltered dogs in Potchefstroom.
- Sub-Directorate: Epidemiology of the Department of Agriculture, Land Reform and Rural Development (DALRRD) for the mapping software and Ms. Marna Laing, for her valuable support in mapping the survey areas.
- My family and friends for continued encouragement.
- Ultimately Yahweh, for granting me the grace and opportunity to conduct this research.

Dedication

"I was dull and muddied, and yet you picked me up, washed me, and polished me. When everything else was black, you saw the light within me. You are the reason I can stand out in this world."

Table of Contents

List of Figures

List of Tables

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Dirofilaria repens, the etiological agent responsible for subcutaneous dirofilariosis, is a filariid affecting canids and felids as final hosts, with mosquitoes serving as intermediate hosts and vectors (Bajer *et al.* 2016). Humans are susceptible to infection and regarded as incidental hosts (Capelli *et al.* 2018). Blood sucking mosquitoes get infected by ingesting microfilariae as they feed on the infected host. Microfilariae traverse the lining of the midgut and subsequently travel into the Malpighian tubules (MT), where they moult through two stages from first to third larvae (L1 – L3) and eventually migrating to the proboscis (Ferreira *et al.* 2015). The final hosts are infected by L3 through mosquito bites, which then undergoes two more moults into sexually mature adults, which reside in the subcutaneous tissue (Ferreira *et al.* 2015). Dogs are considered more competent hosts and reservoirs of infection, in comparison to cats (Simon *et al.* 2009). Humans are regarded as incidental hosts as infective larvae (L3) only develop over several months into solitary sexually immature adults (Moodley *et al.* 2015). The worm's anomalous movement results in subcutaneous and conjunctival/ocular nodules often confused with neoplastic tumours (Gad *et al.* 2002). Canine and feline dirofilariosis, and human dirofilariasis as caused by *D. repens* are known to occur in South Africa (Schwan *et al.* 2000, Moodley *et al.* 2015).

1.2 Problem statement

Dirofilariasis (*D. repens*) is a global emerging metazoonotic disease (Simón *et al*, 2012). However, it has garnered relatively little scientific scrutiny in comparison to the disease produced by *Dirofilaria immitis*. The significantly lower pathogenicity of *D. repens* compared to *D. immitis* results in the majority of infections remaining undetected. While human, canine, and feline infections due to *D. repens* have been documented in South Africa, the prevalence of canine dirofilariosis due to *D. repens* has not been fully researched in North West Province (Moodley, *et al.* 2015, Schwan 2009, Voigts 2018). Its occurrence in humans has been noted in KwaZulu-Natal Province, which interestingly has also recorded the highest prevalence of dirofilariosis in dogs and cats in previous studies (Moodley, *et al.* 2015, Schwan 2009). Despite the known endemicity of *D. repens* in South Africa, epidemiologic or published data on the prevalence of *D. repens* in hunting and sheltered dogs, in the peri-urban and rural areas is still limited. Establishing its prevalence will assist in determining the zoonotic risk of this filarial parasite in these communities. With the growth in the use of macrocyclic lactones in dogs for

the prevention and treatment of ecto- and endoparasites, it is important to continuously update our understanding regarding the occurrence of *D. repens,* as these treatments can potentially cause anaphylactic reactions in infected dogs.

1.3 Justification/Rationale

The study was conducted with the rationale of broadening our understanding of the occurrence of filarial helminths in domestic dogs. Based on broad surveys by Schwan (2009), we have reasonable information on the occurrence of filarial helminths in the various provinces of South Africa except for North West, Northern Cape, and Free State provinces. The primary impetus for examining the North West Province stems from the notable prevalence of *D. repens*, as observed in the vicinity of Gaborone, Botswana, which is in close proximity to Mahikeng, South Africa. Furthermore, the present study serves to broaden the survey area for *D. repens* in the North West Province. A previous study by Voigts (2018) only focused on Mahikeng, whereas our investigation will also encompass Potchefstroom.

Extrapolating from previous studies, dogs serve as a major reservoir of infection for mosquitoes and eventually other hosts, including humans. Thus, evaluating the occurrence of *D. repens* in dogs in these communities will provide valuable insights into the potential threat of anthropozoonosis. To diagnose a *D. repens* infected dog, drawn blood samples were subjected to two diagnostic tests that were conducted in series. The membrane filtration technique, which is the initial test employed, is a very sensitive test for detecting microfilariae (Cringoli *et al.* 2007, Desowitz *et al.* 1970, Schwan, 2009). Acid phosphatase staining was employed on microfilariae-positive samples as it is a specific test that allows for the differentiation of *D. repens* microfilariae (Kelly, 1973, Peribáñez *et al.* 2001, Cringoli *et al.* 2007, Schwan, 2009).

1.4 Aim

The study aspires to establish the prevalence of *D. repens* in dogs in Potchefstroom and Mahikeng, North West Province.

1.5 Objectives

a) To determine the prevalence of *D. repens* in sheltered dogs in Potchefstroom.

b) To determine the prevalence of *D. repens* in hunting dogs in villages in Mahikeng.

1.6 Hypotheses

H_o: *Dirofilaria repens* is prevalent in dogs in Potchefstroom and Mahikeng.

H_1: *Dirofilaria repens* is not prevalent in dogs in Potchefstroom and Mahikeng.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Filarial worms belong to the superfamily Filarioidea. Their occurrence is documented worldwide. They are viviparous nematodes with an indirect life cycle that includes an arthropod vector and a vertebrate host (Schwan 2009). Infections resulting from these parasites in their definitive or vertebrate hosts are known as filariosis. According to a survey done by Schwan (2009), *Acanthocheilonema reconditum*, *Acanthocheilonema dracunculoides* and *Dirofilaria repens* microfilariae were identified in South Africa, suggesting autochthonous infections. In the same study, *Dirofilaria immitis and Brugia patei* were only identified in regular tests for filarial infections in pets imported into South Africa (Schwan 2009). In terms of pathogenicity, *D. immitis* is the most significant infection as it is the etiological agent responsible for heartworm disease in canines and felines (Simón *et al.* 2012). *Dirofilaria repens* and *Acanthocheilonema* species both typically mature into adult worms in the subcutaneous tissue, resulting in skin lumps, whereas the adults of *Brugia* species are typically found in the lymphatic system draining the head and neck (Megat Abd Rani *et al.* 2010). Apart from *D. immitis* most filarial infections do not result in debilitating infections in animals (Cringoli *et al.* 2007). Nonetheless, both *D. repens and D. immitis* are of significant veterinary and public health importance due to the zooanthropophilic nature of some of the mosquito vectors responsible for the spread of infections to humans (Simón *et al.* 2012). Human pulmonary and subcutaneous/ocular dirofilariosis are caused by *D. immitis* and *D. repens*, respectively (Simón *et al.* 2012). This chapter reviews the literature pertaining to the morphology, occurrence and distribution, life cycle, pathogenesis, clinical manifestations, diagnosis, treatment, and prevention of *D. repens* in dogs, cats, and humans.

2.2 Genus *Dirofilaria*

The nematodes of the genus *Dirofilaria* belong to the order Spirurida , superfamily Filarioidea, family Onchocercidae and subfamily Dirofilariinae (Cringoli *et al.* 2007). There are two distinct subgenera within the genus *Dirofilaria*. *Dirofilaria repens* is a member of the subgenus *Nochtiella*, while *D. immitis* is a member of the subgenus *Dirofilaria* (Cringoli *et al.* 2007). Filarial worms of the *Dirofilaria* subgenus are relatively large; they lack cuticular longitudinal ridges, and the males exhibit a pronounced asymmetry in the number and distribution of the caudal papillae. The reverse is true for nematodes falling under *Nochtiella* (Cringoli *et al.* 2007).

2.3 *Dirofilaria* (*Nochtiella*) *repens*

Dirofilaria repens is further defined as a mosquito borne parasitic filarial helminth that infects dogs, cats, and other carnivores (Bajer *et al.* 2016). Due to its poor host specificity, it is also known to parasitize humans (Simón *et al.* 2012). More than 60 mosquito species have been implicated in the transmission of this filarial worm (Genchi *et al.* 2011). The parasite has a complex life cycle involving both mosquitoes and mammals, particularly dogs, as definitive hosts. *Dirofilaria repens* has gained considerable attention due to an alarming increase in the number of human cases being detected worldwide (Simon *et al.* 2009). Understanding the biology, epidemiology, clinical manifestations, and prevention strategies of *D. repens* from a veterinary and public health perspective are fundamental to the control of this infection.

2.3.1 Morphology

The adult worm has a white cuticle that has conspicuous longitudinal and inconspicuous transverse striations as depicted in Figure 2.1 below (Cringoli *et al.* 2007). In a literature review done by Schwan (2009), the females are 84 to 170 mm long and 3.8 to 6.5 mm wide whereas the males are 39 to 70 mm long and 2.7 to 4.5 mm wide. These morphometric dimensions fairly compare to those stated by Cringoli *et al.* (2007) in which the adult females are 100 to 170 mm long and 4.6 to 6.5 mm wide and males are 50 to 70 mm long and 3.7 to adult 4.5 mm wide. The worm is characterised by unequal copulatory spicules; the left spicule 0.43 – 0.54 mm in length and the right spicule measures 0.15 – 0.18 mm in length (Cringoli *et al.* 2007, Demiaszkiewicz *et al.* 2011).

The unsheathed microfilariae produced by the adult female worms have an obtuse anterior end and a posterior end that is thin and pointed, ending in the form of an umbrella handle (crook shaped) which is conspicuous in the unstained microfilariae (Schwan 2009, Cringoli *et al.* 2007). They measure between 207 – 385 µm in length and 5 – 9 µm in width. Characteristics are further elucidated by Cringoli *et al.* (2007), Schwan (2009) and Demiaszkiewicz *et al.* (2011).

Figure 2.1: The conspicuous longitudinal and inconspicuous transverse striations or ridges of the cuticle of *D. repens* under SEM.
(Source: Capelli *et al.* 2018, p. 10). CCA 4.0 License.

2.3.2 Occurrence and distribution

Dirofilaria repens is unique to the Old World (Simón *et al.* 2012). Its distribution is reported in Europe, Africa, and Asia; however, it is notably absent from the continents of America and Australasia.

Animal dirofilariosis due to D. repens

In Africa, its occurrence has been documented in animals in Egypt, Sudan, Tunisia, Nigeria, the Central African Republic, Kenya, Uganda, Zambia, Botswana, and Zimbabwe (Schwan 2009, Siwila *et al.* 2015, Ntesang 2016). In a survey by Schwan (2009) between 1994 and 2008, the prevalence rate of *D. repens* in selected South African provinces were: 12.47 % (52/417) in KwaZulu-Natal, 1.5 % (5/333) in Mpumalanga, and 0 % (1/316) in Gauteng, where the only positive dog had been moved from KwaZulu-Natal Province one month before being sampled. The same survey extended to Maputo Province in Mozambique, with the prevalence of *D. repens* established as 5.08 % (70/1379). In a survey done by Voigts (2018), between August 2016 and August 2017 in 100 dogs from Mahikeng, no evidence of filarial infections were noted. In the most recent published surveys for filarial infections in Zambia (2013) and

Botswana (2014), the prevalence of *D. repens* was 0 % (0/272) and 14.67 % (22/150), respectively (Siwila *et al.* 2015, Ntesang 2016).

Its occurrence in Europe is widespread. It has been documented in Russia, Poland, Ukraine, Lithuania, Estonia, Belarus, Germany, Austria, Hungary, the Netherlands, France, Italy, Greece, Portugal, Spain, Slovenia, Slovakia, Romania, Serbia, Bulgaria, the Czech Republic, and Switzerland (Ferreira *et al.* 2015, Kurucz *et al.* 2016, Genchi *et al.* 2011, Alsarraf *et al.* 2021, Tarello 2003, Capelli *et al.* 2018, Simón *et al.* 2012).

In Asia, its occurrence is documented in China, India, Iran, Iraq, Israel, Saudi Arabia, Dubai, Kuwait, Turkey, and Sri Lanka. (Capelli *et al.* 2018, Genchi and Kramer, 2020).

Human dirofilariasis due to D. repens

Its occurrence in humans generally follows its endemicity in animals, particularly in dogs (Capelli *et al.* 2018). In the past three decades, there have been many more recorded cases of dirofilariasis in humans around the world, of which numerous are attributed to *D. repens*, with many reported cases from the European Union, Russia, and Sri Lanka (Simón *et al.* 2012).

Moodley *et al.* (2015) documented the first cases of human *D. repens* infections in South Africa that presented as subcutaneous and ocular infections. Both case reports were suggestive of autochthonous transmission in South Africa, with a correlation to the KwaZulu-Natal Province, which is known to have a relatively high reported incidence of *D. repens* in dogs and cats in South Africa (Schwan 2009).

The conventional view that the parasite is poorly adapted to humans appears to be evolving. In a case review done by Simón *et al.* (2022), it wasn't rare for the nematode to fully develop, with the females bearing microfilariae in their uterus. Additionally, microfilariae have been noted in the blood of humans (Simón *et al.* 2022), disputing previous assertions by Moodley *et al.* (2015) that microfilariae in blood is not a feature of human infections.

2.3.3 Host range

Domestic and wild carnivores (felids, canids) are definitive hosts of *D. repens*. The nematode is more adapted to domesticated and wild dogs in comparison to felids, and it has been shown that domestic dogs serve as significant reservoirs of the infection (Simón *et al.* 2012). Infections have been noted in foxes, wolves, coyotes, lions, large-spotted genets, and non-human primates (Schwan 2009). The nematode does not fully develop in humans, and as such, humans are conventionally regarded as dead-end hosts.

2.3.4 Vectors and transmission

Various mosquito species belonging to the genera *Aedes, Armigeres, Anopheles, Culex, Mansonia, Mansonoides,* and *Coquillettidia* have been identified as intermediate hosts and vectors of *D. repens* (Schwan 2009, Simón *et al.* 2012, Cringoli *et al.* 2007). Male mosquitoes are not hematophagous and do not participate in transmission. The behavior of the host seems to play a key role in mosquito feeding preferences. Most mosquitoes are more active from sunset. Cats, by nature, are more active after sunset as opposed to dogs, which generally sleep during the night. Cringoli *et al.* (2007) relates the activity of the cat during the nocturnal periods as likely disruptive to mosquitoes, which need ample contact time to feed with the host, thus dogs are preferred by mosquitoes, which explains the relative higher prevalence of *D. repens* compared to cats. While this might be the case, it is important to acknowledge the results of a study by Schwan (2009) in the KwaZulu-Natal Province, which revealed a prevalence of *D. repens* in cats (10.98%) which was nearly as high as that in dogs (12.47%), suggesting that cats and dogs are both equally susceptible to infections.

The presence of competent vectors and *D. repens* infected animals harbouring adult worms that produce microfilariae are important in maintaining circulation of the infection. Anthropogenic factors influencing the care of dogs and cats and climatic factors influencing the presence of competent vectors affect the completion of the worm's life cycle (Capelli *et al.* 2018, Cringoli *et al.* 2007, Cimpan *et al.* 2022). Of over 3000 culicid mosquitoes, there are scant field studies in Africa that serve to demonstrate vector competence, that is, the development of the infective third stage larvae in the mosquito vector (Simón *et al.* 2012). The vectoral capacity of the various geographical strains of mosquito species from Africa is not well reviewed in literature. Some of the factors that would influence vectorial capacity include vector competence, mosquito density and seasonality, extrinsic incubation period (EIP), infective lifetime,

mosquito survival/lifespan, host preferences and availability of infected hosts (Capelli *et al.* 2018, Cringoli *et al.* 2007).

2.3.5 Life cycle

Dirofilaria repens adult females are viviparous and typically lay microfilariae which eventually appear in the blood stream of the definitive vertebrate host (Cringoli *et al.* 2007, Schwan 2009). The peripheral circulation of microfilariae in dogs and cats is variable depending on physiological changes in the host, with higher levels of microfilariae noted during the night (Cringoli *et al.* 2007).

The female mosquito ingests/acquires microfilariae after feeding on the blood of infected animals. Microfilariae traverse through the throat to reach the mid-gut, where they linger for 24 hours and subsequently travel to the MT and infiltrate the distal end cells to transform into the L1 (sausage stage), L2, and finally L3 in the competent vector (Cringoli *et al.* 2007). Perforating the MT distal end, the L3 migrates through the haemocele to the labium (Cringoli *et al.* 2007).

The incubation period for larval development depends on the mosquito species and environmental temperature (Schwan 2009, Cringoli *et al.* 2007). Larval development takes 8 – 10 days at 28 –30 °C, 11 – 12 days at 24 °C, and 16 – 20 days at 22 °C (Cringoli *et al.* 2007). The development of larvae in the mosquito hibernates when temperatures drop below 18 °C (Cringoli *et al.* 2007).

The vertebrate hosts are infected during mosquito probing and blood feeding. The infective L3, which emerges from the folded labium in haemolymph is dropped onto the host's skin and infects the host when the mosquito pierces the skin (Cringoli *et al.* 2007). The metacyclic, infectious larvae (L 3) typically follow a straightforward movement in the subcutaneous tissue. Aberrant migration has been reported, rarely resulting in significant clinicopathological effects on the host (Schwan *et al.* 2000, Cringoli *et al.* 2007). The prepatent period of *D. repens is* 6 ½ to 9 months and the patent period is at least 2 – 3 years (Schwan 2009, Cringoli *et al.* 2007). Macrofilariae (adult worms) have been found in connective tissue of most parts of the body (Cringoli *et al.* 2007). Because adult worms can persist for years at high parasite loads and produce microfilariae in the majority of infections, dogs are regarded as reservoirs of infection, as opposed to cats, which have been implied to be rarely microfilaraemic (Simon *et al.* 2009, Trotz-Williams and Trees 2003).

2.3.6 The function of the symbiotic bacteria of the genus *Wolbachia* in the life cycle of *Dirofilaria repens*

There is evidence of an endosymbiotic relationship between nematodes of the genus *Dirofilaria* and bacteria of the genus *Wolbachia* (Cringoli *et al.* 2007, Simón *et al.* 2012). It has been demonstrated that these intracellular bacteria play an essential role in the moulting, development, and survival of dirofilariae. In exchange, dirofilariae provide amino acids for the growth of bacteria (Simón *et al.* 2012). *Wolbachia* bacteria are maternally transmitted and present in all *D. repens* developmental stages in the various hosts. The direct and indirect identification of *Wolbachia* bacteria provides excellent supplementary data that can be utilized in epidemiological studies and research. Due to the role of *Wolbachia* in the inflammatory effects caused by the sudden death of macrofilariae and the massive release of bacteria after anti-filarial drug treatment, it has been suggested that dirofilariosis be treated with a combination of antibiotics (Simon *et al.* 2009).

2.3.7 Clinical manifestations

Animal D. repens infection

Subcutaneous dirofilariosis in dogs and cats is frequently linked to resident *D. repens* adult worms in subcutaneous tissue (Cringoli *et al.* 2007). Additionally, nematodes can inhabit the conjunctiva of the eye. Many infections are asymptomatic for a considerable period of time, which is variable depending on various host parasite factors (Simón *et al.* 2012). Aberrant migrations of dirofilariae can result in infections of internal organs like the liver and kidneys (Simón *et al.* 2012, Schwan *et al.* 2000).

The pathological effects of the parasite have been attributed to the migration and immunopathological response to the micro- and macrofilariae (Simón et al., 2012, Cringoli *et al.* 2007). The seasonality of some of these dermatological symptoms has been inferred (Tarello 2011). A myriad of dermatological signs has been identified in dogs by Tarello (2002a), Tarello (2003), (Alsarraf *et al.* 2021) as pruritus, erythema, alopecia, hyperkeratosis, crusting, nodules, acanthosis, eczema, pyoderma, and oedema. In cats, pruritus, alopecia, erythema, papillae, crusting, and lichenification were noted as presenting dermatological signs (Tarello 2002b). Non-specific clinical symptoms such as anorexia, vomiting, fever, lethargy, conjunctivitis, liver failure, and lymphadenopathy have been reported in animals (Tarello 2002b) (Schwan *et al.* 2000).

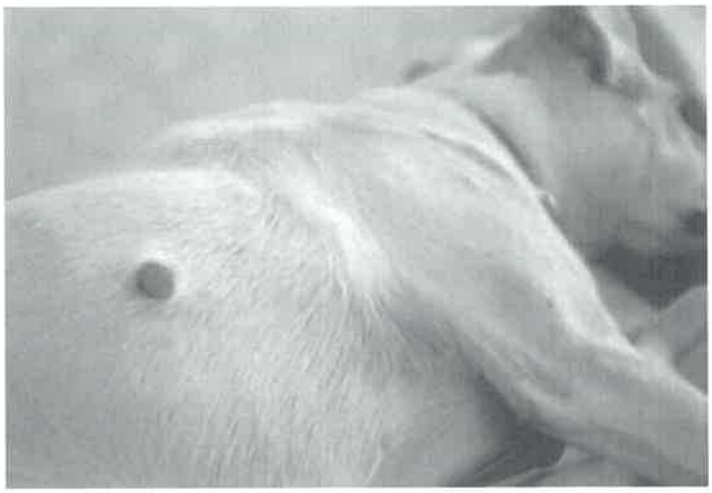


Figure 2.2: Nodule on the flank of a dog due to *D. repens* macrofilariae.
(Source: Tarello 2011, p. 3) CCA 4.0 License.

Human D. repens infection

Dirofilaria repens is the causative agent of both subcutaneous and ocular dirofilariasis (Figure 2.3) in humans (Simón *et al.* 2022). The subcutaneous form of the disease manifests as lumps in the skin in different parts of the body that may be mistakenly perceived to be tumours. In a systemic review of human clinical dirofilariosis cases by Simón *et al.* (2022), people who sought medical attention described symptoms such as a nodule, a discrete redness or irritation, pain or discomfort, itching, swelling, a feeling of a foreign or moving object, and difficulty seeing.

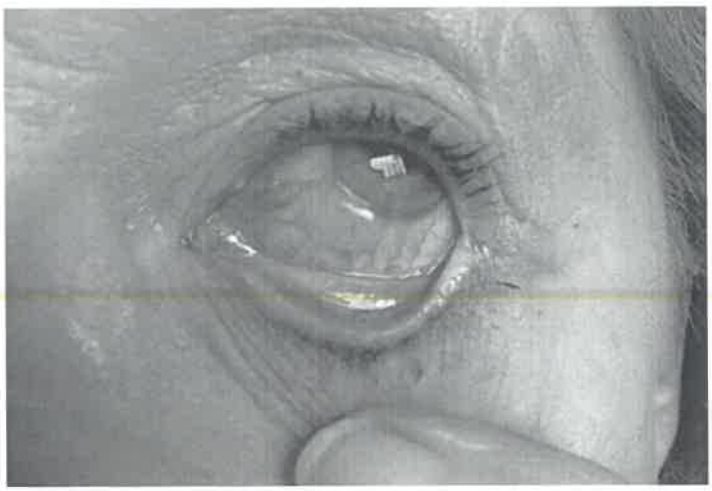


Figure 2.3: Cystic lesion due to *D. repens* on the left eye.
(Source: Moodley *et al.* 2015, p.8) CCA Non-Commercial 3.0 License.

2.3.8 Diagnosis

Diagnosis in dogs and cats

In order to diagnose *D. repens*, the circulating microfilariae or localized macrofilariae must be identified or detected. This can be achieved through morphological, morphometric, histochemical, serological, and molecular techniques. Diagnosis has traditionally been based on the presence of microfilariae during the patent period (Ciuca *et al.* 2020), notwithstanding the challenge that most infections are asymptomatic. The demonstration of adult *D. repens* from a subcutaneous lump/ nodule by fine needle sampling or biopsy examination is also diagnostic (Capelli *et al.* 2018).

Concentration techniques

A fresh blood smear is of little use in demonstrating microfilariae particularly when there are few circulating microfilariae (Cringoli *et al.* 2007). Standardized concentration techniques are usually employed to improve the sensitivity and specificity of diagnostic techniques in detecting microfilariae.

- Modified Knott's test

  The conventional modified Knott's test entails centrifuging a mixture of 1 ml of venous blood and 10 ml of 2 % buffered formalin at 1500 rpm for 3-5 minutes (Cringoli *et al.* 2007, Knott 1939). The supernatant is expelled, the sediment is stained with methylene blue stain at a mixing ratio of 1:1000, and the stained wet preparation is evaluated under a microscope (Cringoli *et al.* 2007). Morphological identification of microfilariae can be achieved through determination of the size, tail morphology, and shape of the anterior extremity (Magnis *et al.* 2013, Cringoli *et al.* 2007, Genchi *et al.* 2021). In a recent study by Magnis *et al.* (2013), the Knott test could distinctly differentiate between *D. immitis, D. repens*, and *Acanthocheilonema* species on the basis of morphometric characteristics. Distinguishing between *A. dracunculoides* and *A. reconditum* requires the use of further tests as their size ranges overlap (Magnis *et al.* 2013). It is noteworthy that, though the morphometrical differentiation of microfilariae is possible by evaluating the cephalic and caudal morphologies, these characteristics are often difficult to differentiate, making the modified Knott test a time-consuming and impractical process when it comes to differentiating the specific microfilariae. (Schwan 2009, Simón *et al.* 2012).

- Filter test

  In a literature review by Schwan (2009), it was noted that the sensitivity of the modified Knott's technique is comparatively lower than that of the membrane filtration technique. In this technique, sampled blood preserved with an anticoagulant is added to a lysate preparation (Cringoli *et al.* 2007). And pressed through a Millipore filter chamber, after which the filter is removed, set on a glass slide, stained, and observed under a microscope for microfilariae (Desowitz *et al.* 1970). While it is a sensitive and rapid test, the lysate and staining alter the morphology of the microfilariae, and measurement standards would need to be ideally validated in each laboratory setting to differentiate species (Cringoli *et al.* 2007). Nonetheless, it would still be difficult to differentiate between microfilariae species of overlapping size seeing that the general morphology of microfilariae could become distorted in the processing of the specimen.

Histochemical stain – Acid phosphatase staining

This technique differentiates microfilariae on the basis of variable acid phosphatase activity (APA) throughout the various regions of microfilariae. The foundation of all approaches to this technique is the Barka method described by Kelly (1973). In the standard technique 10 products are used to prepare 5 reagents that are directly used in the test method substrate *(Peribáñez et al.* 2001). The standard methodology as described by Cringoli *et al* (2007) involves the injection of 1 ml of an EDTA blood sample into 10 ml of deionized water, followed by centrifugation, discarding of the supernatant, fixing of the sediment onto a slide using acetone and staining with the acid phosphatase substrate (Cringoli *et al.* 2007). The microfilariae of *D. repens* exhibit a distinct APA that is confined to the vicinity of the anal pore or to the anal pore and inner body (Schwan 2009). Conversely, the microfilariae of *D. immitis* display two acid phosphatase active spots that are limited to the anal and excretory pores, while the microfilariae of *A. reconditum* exhibit APA throughout their body (Cringoli *et al.* 2007, Schwan 2009, Peribáñez *et al.* 2001). This methodology is employed in tandem with concentration methodologies to ascertain the species of microfilariae. The test exhibits a high degree of specificity and serves as a valuable tool for confirming the presence of specific microfilariae *(Cringoli et al.* 2007). The application of histochemical staining for the identification of APA is a reliable and pragmatic method to validate the presence of *D. repens* or other microfilarial infections in canines and felines. (Kelly 1973, Peribáñez *et al.* 2001). The

drawbacks associated with the test include its high cost, the requirement for specialized skills to prepare reagents and conduct the test, as well as the labour-intensive nature of the procedure (Cringoli *et al.* 2007). Test kits designed for commercial use like the Leucognost-SP®, which contain ready-to-use reagents, have produced reproducible test results comparable to the conventional Barka method (Peribáñez *et al.* 2001).

Serological techniques

In general, these methods are aimed at either the *D. repens* antigen or the humoral reaction (antibodies) to infection.

Antigen Assays

There is wide application of commercial point-of-care serological tests (qualitative, multilevel, and semi-quantitative ELISAs) being employed for the diagnosis of *D. immitis* and not *D. repens* in dogs (Cringoli *et al.* 2007). These tests target the antigen of the adult female worm. Even though they are easy to use, they are not sensitive enough to detect infections during the prepatent period, infections with very few worms, and infections where only male adult worms are present (Hoch and Strickland, 2008, Cringoli et al., 2007, Schwan, 2009). Furthermore, the tests that are available for *D. immitis* also have challenges with cross reactivity with other filarial species like *D. repens* (Ciuca *et al.* 2020).

Antibody Assays

In general, the clinical application of antibody serological tests has been limited based on the complexity of interpreting test results. A positive result could imply prior exposure (aborted infections or treated infections) and not necessarily an active or patent infection (Cringoli *et al.* 2007, Ciuca *et al.* 2020). Nonetheless, it remains an invaluable tool for epidemiological investigations.

There is continued research and several publications on the application of serological tests that target antibody responses to somatic antigens and phage specific surface antigens for *D. repens* (Ciuca *et al.* 2020, Pękacz *et al.* 2022, Cancrini *et al.* 2000). Serodiagnostics may be a preferable option since the antibody response begins during the prepatent period and IgG levels exhibit a consistent increase throughout the progression of the infection (Pękacz *et al.* 2022, Ciuca *et al.* 2020). Most of the ELISA tests are not commercially available. In a study by Pękacz *et al.* (2022), a *D. repens* Somatic Antigen ELISA and Phage ELISA was utilized with promising reproducible and comparable tests results for application as screening tests. In order to reduce the likelihood of cross-reactivity with antibodies targeting other parasite molecules,

the study centered on short peptides through the utilisation of phage display technology (Pękacz *et al.* 2022). These tests are being designed to address the challenges in diagnosing 'occult' amicrofilaraemic and pre-patent infections as seen in concentration and histochemical techniques. Cross reactions between the somatic antigens of various helminths (filarial and non-filarial) that may be prevalent within a canine and feline population still poses a challenge to a diagnosis (Ciuca *et al.* 2020). The simultaneous application of antibody assays targeting *Wolbachia* species that are specific to filarial nematodes seems to offer a more promising prospect for limiting the serological methods' cross-reactivity solely to filarial helminths, thus excluding non-filarial helminths (Simón *et al.* 2012, Ciuca *et al.* 2020). IgG-type antibodies, which are targeted towards *Wolbachia* surface protein, have been found in samples from dogs, cats, and humans exhibiting diverse clinical manifestations of dirofilariosis (Simón *et al.* 2012).

Molecular techniques

The extended prepatent period of *D. repens*, in conjunction with a wide variation in the pre-patency period in dogs and cats, makes the detection of microfilariae an insensitive tool for detecting prepatent infections (Capelli *et al.* 2018, Simón *et al.* 2012). The polymerase chain reaction (PCR) is a highly sensitive and specific technique capable of detecting early infections and differentiating *D. repens* from various filarial infections in dogs and cats (Cringoli *et al.* 2007). Several molecular techniques have been designed for the diagnosis of *D. repens*, which include conventional and real-time PCRs, multiplex PCR, probe-based methods, and high-resolution melting analysis methods (Capelli *et al.* 2018, Tahir *et al.* 2017, Albonico *et al.* 2014, Simsek and Ciftci, 2016). In a study by Latrofa *et al.* (2012), it was demonstrated that a single-step multiplex-PCR based technique was quick and accurate in the simultaneous detection of the most common filarioids infecting dogs, namely *D. immitis, D. repens, Acanthocheilonema. reconditum*, and *Cercopithifilaria* spp. This technique targeted various fragments of COX 1 using a mix of species-specific forward primers (Latrofa *et al.* 2012). PCR has also been utilized in epidemiological studies to identify potential mosquito vectors for *D. repens*, owing to its remarkable sensitivity in detecting minute quantities of genomic DNA in body, tissue, and fluid samples (Ferreira *et al.* 2015, Capelli *et al.* 2018, Kurucz *et al.* 2016, Tahir *et al.* 2017, Albonico *et al.* 2014).

The downfall in the application of PCR for molecular diagnostics is that it requires specialized laboratories and skilled technicians (Ciuca *et al.* 2020, Cringoli *et al.* 2007). It is also noteworthy that PCR results should be interpreted carefully, particularly in clinical diagnosis,

as noted in a case review by Manzocchi *et al.* (2017), in which a positive *D. repens* PCR was insufficient to establish the cause of subcutaneous lesions due to atypical cytological findings.

Diagnosis in humans

While dogs infected with *D. repens* generally do not display noticeable symptoms, the initial examination in humans is typically initiated due to the detection of a growth or lump, which causes concern for the affected individual (Capelli *et al.* 2018). The identification of a mass or cystic lesion in the subconjunctiva of the eye is highly suggestive of an infection. Microfilaraemia and eosinophilia are typically absent in human cases (Cringoli *et al.* 2007). The diagnosis typically relies on the examination of the intact worm's morphology and the identification of specific histopathological findings (Cringoli *et al.* 2007, Capelli *et al.* 2018). Most often, infection in humans is attributable to the presence of a single immature worm in human tissues (Cringoli *et al.* 2007). In moderating the previous statement, 42.95 % were noted as adult worms in a systematic literature review by Simón *et al.* (2022). Surgical excision is opted for in most cases relating to a subcutaneous lump or growth, which proves to be both therapeutic and curative, provided further tests are done on the lump to confirm the presence of an adult worm. Freshly frozen samples and biopsy samples allow for molecular diagnostics and histopathology, respectively, in cases where morphological identification of the excised worm is not possible (Capelli *et al.* 2018, Cringoli *et al.* 2007). Ultrasound and magnetic resonance imaging (MRI) are other reported diagnostic tools in humans (Joseph *et al.* 2023, Simón *et al.* 2022). The utility of serodiagnostics in human cases is limited due to the fact that filarial infections primarily trigger an immunological response that is primarily initiated by microfilariae (Capelli *et al.* 2018).

2.3.9 Treatment and prevention

Dogs and cats

According to a literature review by Capelli *et al.* (2018), it is recommended to administer chemoprophylactic treatment to dogs and cats on a routine basis due to the anthropozoonotic risk and the apathogenic clinical manifestations of *D. repens*. Contrastingly, in a literature review by Gad *et al.* (2002) and Schwan (2009), therapy should only be administered to dogs displaying clinical manifestations of the disease, like pruritus and dermal swelling. It is crucial to acknowledge that routine utilization of chemotherapeutic drugs will result in the development of drug resistance, as observed in the routine chemoprophylaxis strategy for *D. immitis* (Prichard 2021). Moreso, the anthropozoonotic risk of *D. repens* is minor, with mostly benign subcutaneous nodules and a few complicated ocular cases (Cringoli 2007).

The majority of chemoprophylaxis or chemotherapeutic protocols in use were derived from the knowledge and research developed in the field of heartworm disease prevention (Capelli *et al.* 2018). These protocols primarily involve the routine administration of macrocyclic lactones as either tablets or subcutaneous injections (Cringoli *et al.* 2007). The oral ivermectin formulation and topical, oral, or injectable moxidectin formulation have been experimentally evaluated for their preventive efficacy against *D. repens* microfilariae (L3, L4). All moxidectin preparations showed full efficacy, while the oral ivermectin showed 87 % – 93 % efficacy (Cancrini *et al.* 1989, Genchi *et al.* 2013, Rossi *et al.* 2004, Rossi *et al.* 2002). Additional macrocyclic lactones that have demonstrated efficacy in the therapy of *D. repens* by inhibiting the maturation of microfilariae include doramectin, milbemycin, and selamectin (Capelli *et al.* 2018). Diethylcarbamazine, a piperazine derivative, taken orally at a dose of 5.5 mg/kg once daily for a month, has been documented for use in the treatment of microfilaremia caused by *D. repens* (Gad *et al.* 2002).

A new way to treat cardiovascular dirofilariosis targets the *Wolbachia* endosymbionts of dirofilariae with doxycycline in addition to the regular macrocyclic lactone treatments (Capelli *et al.* 2018). A few protocols claim adulticidal activity for this filariid. Suggested protocols include the use of imidacloprid and moxidectin for six consecutive months and use of the adulticide arsenical melarsomine in combination therapy (Capelli *et al.* 2018, Gad *et al.* 2002).

Another aspect of prevention in periods of high mosquito activity involves using contact-repellent insecticides, such as veterinary products with pyrethroids, with a specific label to prevent mosquito bites (Capelli *et al.* 2018).

Humans

The therapy of choice remains surgery. While chemotherapy is not recommended in humans (Simón *et al.* 2012), diethylcarbamazine, ivermectin, levamisole, albendazole, thiabendazole, and cortisones have only occasionally been used in humans, with very doubtful results (Cringoli *et al.* 2007). Due in large part to incorrect clinical diagnosis, chemotherapeutics are never considered in these cases, contributing to their limited use in humans (Cringoli *et al.* 2007). Histopathological analysis of the removed tumour or growth serves to inform the diagnosis in the majority of these cases. The use of filaricides with a reasonable level of toxicity and adverse reactions appears inappropriate in cases of subcutaneous infection, when the problem can be effectively treated with a minor outpatient surgical procedure (Cringoli *et al.* 2007).

CHAPTER 3: MATERIALS AND METHODS

3.1: Study area

The survey was conducted in the metropolitan regions of the two cities within the North West Province, namely Mahikeng (25° 51' 11.3796" S, 25° 38' 24.6516" E) and Potchefstroom (26° 43' 0.01" S, 27° 06' 0.00" E), with a geographical separation of roughly 200 km. The spatial disposition of the survey area in relation to Botswana and other provinces in South Africa are depicted in Figure 3.1. Further subdivisions of the survey area in Mahikeng include Ramatlabama, Mogosane, Mathlonyane, Disaneng, and Rooigrond. The survey in Potchefstroom covered the entire city, which includes all neighborhoods that PAWS provides service to. All impounded dogs were placed at the animal welfare center for PAWS, located in Miederpark. The province has a relatively warm and arid climate, a characteristic that may be ascribed to its geographical position in the southern region of the Kalahari Desert. Potchefstroom exhibits relatively cooler and more humid climatic conditions compared to Mahikeng (Figures 3.2, 3.3 and 3.4).

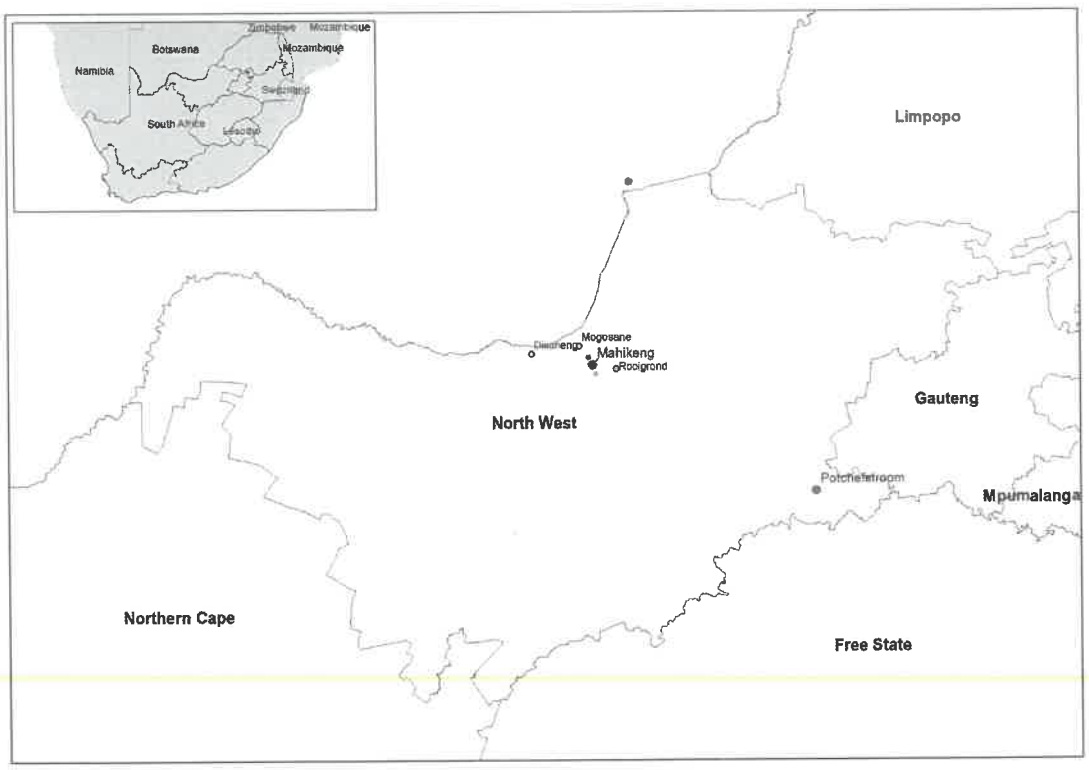
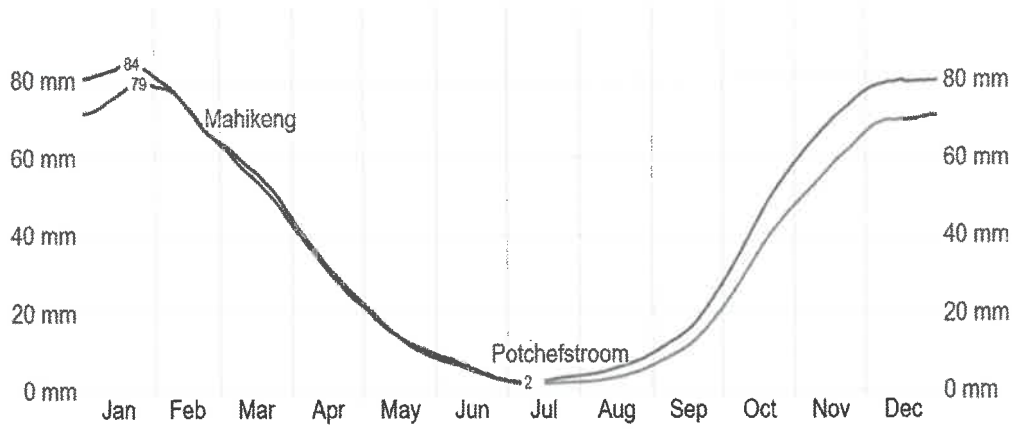
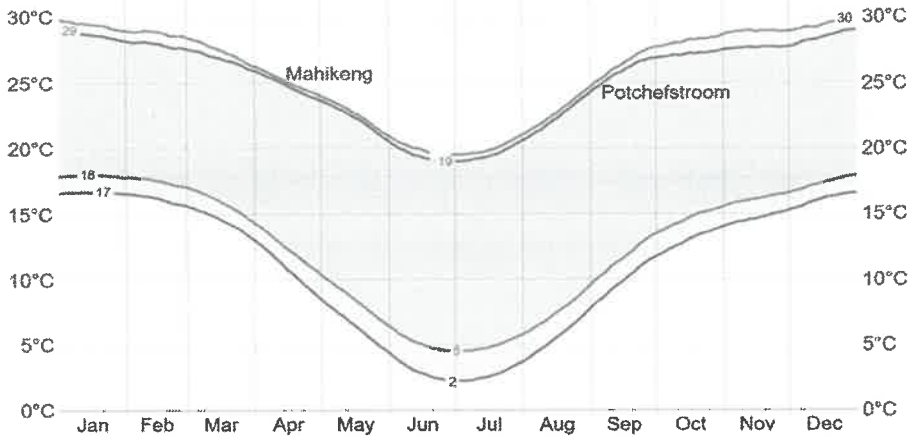


Figure 3.1: Locations of the survey area where samples were collected in North West Province, South Africa between September – November 2022. Gaborone (black dot), Mathlonyane (green dot), and Ramatlabama (blue dot). Map courtesy of the Sub-Directorate of Epidemiology, DALRRD.



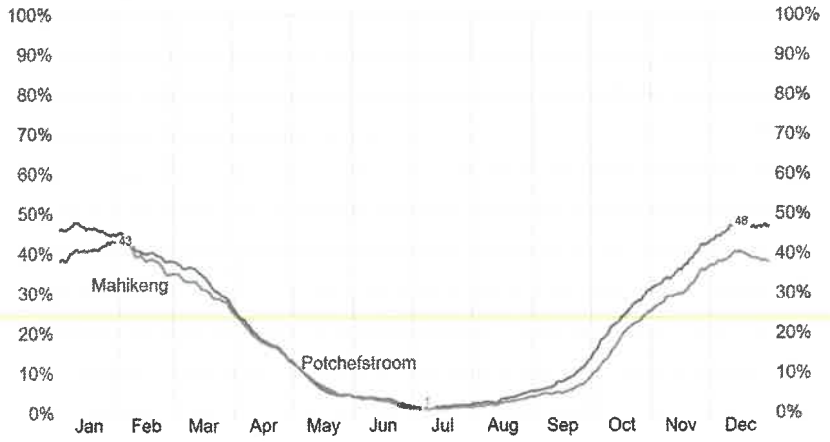
© WeatherSpark.com

Figure 3.2: A comparison of the average monthly rainfall in Potchefstroom and Mahikeng.



© WeatherSpark.com

Figure 3.3: A comparison of the average high and low temperature in Potchefstroom and Mahikeng.



© WeatherSpark.com

Figure 3.4: A comparison of the daily chance of precipitation in Potchefstroom and Mahikeng.

3.2: Ethics approval

The research was approved by the Faculty of Veterinary Science (FVS) Research Ethics Committee (REC) and the University of Pretoria Animal Ethics Committee (AEC) with reference number REC117-21 (Annexures 1 and 2). Permission to do research in terms of Section 20 of the Animal Disease Act, 1984 (Act 35 of 1984) was received from the Department of Agriculture Land Reform and Rural Development (DALRRD), with the reference number 12/11/1/1/6 (2230 AC) (Annexure 3).

3.3: Study animals and design

The subjects of the study were domestic dogs. One cohort of dogs (n = 87) comprised of sheltered dogs under the care of PAWS in Potchefstroom. A second group of dogs (n = 70) comprised of dogs that were predominantly bred and maintained for hunting in villages in Mahikeng. The study comprised of dogs of both sexes and diverse breeds.

The survey exclusively included dogs that were at least one year old, as this age threshold was deemed necessary due to the extended prepatent periods associated with *D. repens*. Canines that had been given a macrocyclic lactone in the preceding twelve months were not included in the survey on account of the drug's microfilaricidal activity. The examiner bleeding the dogs recorded the signalment (breed, age, and sex) of the dogs included in the study. In cases in which the age could not be derived, an age or age group was assigned to the dogs based on their dentition using guidelines provided by Barton (1939).

The research design employed in this study is in accordance with a quantitative, correlational, and cross-sectional framework.

3.4: Sample size

According to Schwan (2009), the KwaZulu Natal Province of South Africa had a *D. repens* prevalence of 12.47 % in dogs. Based on this information, the minimum sample size required for the study was calculated to be 168 dogs, which was to be evenly distributed between Mahikeng and Potchefstroom.

Sample size formulation (Pourhoseingholi *et al.* 2013)

$n = Z^2P\ (1 - P) \div d^2$

n = *size of sample*

Z = Z statistic for a level of confidence = 1.96 for 95 % CI

P = *expected prevalence*

d = *precision* = *0.05*

A total of 165 dogs were sampled in the survey, a slight deviation from the intended sample size of 168. Out of the 165 dogs sampled, 87 from Potchefstroom and 78 from Mahikeng, only 157 blood samples were included in the study, as 8 samples from Mahikeng were unusable in the diagnostic tests. Thus, a resultant sample size of 157 dogs is recorded for this survey.

3.5: Sample collection process

A hybrid approach utilizing both random and convenience sampling methods was employed. Canines exhibiting fractious and skittish behaviour and those under one year old were excluded from the survey. Blood was aseptically drawn from either the cephalic or jugular veins. A minimum of approximately 2 ml of blood per sampled dog was drawn into an EDTA tube. Each tube was gently rolled or inverted to facilitate thorough mixing with the anticoagulant before being placed into a sampling tube rack. Subsequently, the sampling tube rack was placed inside a cooler box that was packed with ice packs. Considerable care was taken to ensure that the EDTA blood tubes did not directly contact ice packs, as this could freeze the blood samples causing the microfilariae to rupture and become nonviable for microfilariae detection diagnostic tests. The breed, sex, and age group of the sampled animals were recorded. The blood collection tubes were assigned unique identifiers to distinguish between individual dogs or groups of dogs from which samples were obtained. During collection and transportation, samples were kept in cooler boxes packed with ice packs and stored overnight under refrigeration at temperatures ranging from 2 ˚C to 8 ˚C.

In Potchefstroom, dogs were bled on 26 and 27 September 2022. Informed consent was provided by PAWS to bleed the group of sheltered dogs. These dogs were housed in outdoor kennels, either individually or in small groups. In Mahikeng, dogs were bled on 18 and 21 November 2022, during community extension services offered by North West University. With the owner's consent, blood was collected and submitted for further testing. Most of these dogs

were kept as colonies and maintained for the specific objective of hunting. These dogs were kept in outdoor shelters. Blood samples from Mahikeng were submitted in two batches.

Samples were transported to the Helminthology Laboratory, DVTD, within 9 days from sampling. The samples were subsequently placed in refrigerated storage at the laboratory, adhering to the recommended temperature guidelines. The assessment of both the quality and volume of the submitted samples was conducted prior to testing, and any samples that had formed clots and had a volume of less than 1 ml were discarded. As a result, 8 of the 165 submitted blood samples, were discarded.

3.6: Diagnostic procedures

The testing of samples was conducted in accordance with the Helminthology Laboratory's standard operating procedures at the DVTD, University of Pretoria.

3.6.1 Screening test - Membrane Filtration (MF) Test

The blood samples were subjected to the MF technique, as outlined by Dennis & Kean (1971), to detect the presence of microfilariae. A 3 µm Whatman ® Nucleopore (Merck) Track-Etch membrane filter was installed into a Whatman® Swin-Lok (Merck) 25 mm filter holder. A 5 ml syringe was used to draw up 0.5 ml of EDTA blood and 3 ml of air. The syringe was then positioned vertically, with the assembled filter system facing downward into a beaker. The mixture in the syringe was plunged down, and the filtrate was collected into the beaker to be discarded. Using the same syringe, 5 ml of normal saline (0.9 % NaCl) was drawn and washed through the assembled filter system. After repeating the process twice, the remaining fluid was taken out by plunging down air loaded syringes through the membrane filter. The filter holder was disassembled, and the filter was taken out of the holder and set down on a slide. It was then air-dried, fixed with methanol for one minute and stained for 28 min with a Giemsa solution (Merck) diluted 1:9 with a pH 7.2 buffer. The stained slide was then washed, dried, and mounted in Entellan® (Merck). The slides were examined for microfilariae using a compound microscope at 40x magnification under high power.

3.6.2 Confirmatory test – Acid Phosphatase Staining (APS) Test

Prior to identifying the species with the aid of acid phosphatase staining, the blood samples that yielded positive results for microfilariae were subjected to a microfilariae concentration technique, the Modified Knott's technique (Cringoli *et al.* 2007, Knott 1939, Genchi *et al.* 2021). A 1 ml volume of the microfilariae positive EDTA blood sample was mixed with 9 ml of a 2% formalin solution and subjected to centrifugation at a force of 500 g for a duration of

5 minutes (Cringoli *et al.* 2007). The supernatant of the sample was disposed of, and a few drops of the sediment was carefully placed onto a microscope slide and treated with acetone for fixation (Knott 1939). The method described by Yen & Mak (1978) was utilized for acid phosphatase staining. The microfilariae on the slides were observed using a compound microscope at magnifications of 40x and 100x to identify any species-specific variations in the somatic staining patterns and/or APA of the microfilariae, as outlined by Cringoli *et al.* (2007), Kelly (1973) and Peribáñez *et al.* (2001).

3.7 Data analysis

The data on the dog signalments and their owners or carers was entered into a Microsoft Excel spreadsheet.

CHAPTER 4: RESULTS

4.1 Characteristics of dogs

A resultant sample size of 157 dogs was obtained. Due to clotting or insufficient quantity of blood, 8 of 165 blood samples submitted to the laboratory were excluded from the study. The age of sampled dogs was categorized into three distinct physiological groups: young adult (1 – 3 years of age), mature adult (4 – 7 years of age), and senior adult (over 7 years of age) as depicted in table 4.1. The majority of dogs included in the survey fell within the age range of 4 to 7 years and the mean age was 4.7 years assuming an upper age limit of 10 years. The Potchefstroom sample population (n = 83) consisted of sheltered dogs at PAWS, with 41 males and 46 females. In this dog subpopulation, cross-bred dogs (n = 73) constituted an excessively large proportion. Additional breeds observed within this category included Jack Russell Terriers (n = 11), Fox Terrier (n = 1), Boerboel (n = 1), and Labrador Retriever (n = 1). The sub-population of dogs (n = 70) from Mahikeng consisted exclusively of male dogs belonging to the Greyhound breed, specifically kept for hunting purposes.

Table 4.1: Age groups, sex and breeds of dogs sampled in the survey.

Town	Age group in years			Sex				Breeds					
	1 – 3	4 – 7	> 7	M	NM	F	SF	Boerboel	Fox Terrier	Jack Russell	Labrador	Greyhound	Mixed
Potchefstroom	40	41	6	34	7	43	3	1	1	11	1	0	73
Mahikeng	0	70	0	70*	0	0	0	0	0	0	0	70	0

* Refers to male dogs that were not further characterized as either intact or neutered.
M – Intact male dogs, unless otherwise specified. NM – Neutered male dogs.
F – Intact female dogs. SF – Spayed female dogs

4.2 Microfilariae in blood samples

Of the 157 samples that were examined, a total of 14 (8.9 %) from Mahikeng were detected as positive for microfilariae using the membrane filtration technique. *Acanthocheilonema reconditum* was distinctly identified on all positive microfilariae samples using the acid

phosphatase staining technique. No other microfilariae, including *D. repens*, were detected or identified.

4.3 *Acanthocheilonema reconditum*

Both microfilariae-positive and *A. reconditum*-positive samples were only found in Mahikeng, resulting in a prevalence of 20% for this city (Table 4.2). No further inferences on *A. reconditum* prevalence by sex, breed or age group could be made as the signalments of the individual dogs in the survey were similar. The overall prevalence of *A. reconditum* in the study was 8.9% (14/157).

Table 4.2: Test results for *D. repens* and *A. reconditum* in the survey

Test results	Potchefstroom (n = 87)	Mahikeng (n = 70)	Overall Prevalence (%)
D. repens - positive	0	0	0
A. reconditum - positive	0	14	8.9
Town prevalence (%)	0	20	

CHAPTER 5: DISCUSSION

5.1 *Dirofilaria repens*

This research was designed to resolve a critical informational gap about the prevalence of *D. repens* in specific canine populations, like sheltered and hunting dogs. Our study expands on the current body of literature regarding the prevalence of *D. repens* and other microfilarial infections in canines within South Africa and Southern Africa, as a region. With respect to previous studies done in the North West Province, this study was able to broaden the scope of the survey for *D. repens,* considering the notable prevalence of the filarial nematode in past surveys done in Gaborone.

With a 95 % level of confidence, no evidence of *D. repens* infection was observed in a sample size of 157 dogs which was deducted from an assumed/ expected *D. repens* prevalence of at least 12.47 %. The absence of *D. repens* in the current study agrees with the outcomes of a survey conducted by Voigts (2018), wherein no indications of infection were observed in sampled dogs in Mahikeng, North West Province. Conversely, the absence of *D. repens* in the current study differs from a previous study that reported a significant prevalence of *D. repens* in the metropolitan region of Gaborone, Botswana (14.67 %), which is located less than 150 km from Mahikeng, South Africa (Ntesang 2016). Moreover, surveys done by Schwan (2009) have reported the existence of autochthonous *D. repens* infections in dogs and cats in the KwaZulu-Natal and Mpumalanga Provinces of South Africa.

The present investigation yields several implications based on the notable observation that there was no evidence of *D. repens* microfilariae in the North West Province. There is either a potential lack of competent vectors (mosquitoes) in the investigated regions/localities or insufficient competent vector densities to support the circulation of *D. repens*. Vector abundance may well be influenced by the variations in the altitude, climate, and vegetation cover amongst the various surveyed areas (Google Earth 2023, WeatherSpark 2023a). Additionally, it is possible to infer that the persistent absence of *D. repens*, as seen in the current investigation and in a study by Voigts (2018), indicates that the actual prevalence of *D. repens* in the area may actually be lower than the expected/assumed prevalences used in determining the sample sizes in those surveys. Therefore, conducting broader surveys with a larger sample size could provide valuable insights into the prevalence of this filariid infection in the province, further bearing in mind that a case of *D. repens* has been reported in a dog from Rustenburg, North West Province (Schwan 2009).

Notwithstanding that the researchers investigated treatment history of the sampled dogs with respect to the use of macrocyclic lactones, it is not impossible that dogs included in the survey might have been treated with these drugs within the 12 months prior to being sampled. This can be particularly argued for dogs that were bled at the shelter. The investigators were unable to evaluate the history of treatments given to the dogs before the animal welfare organisation impounded and/or admitted them into the shelter. Based on dog admission records at PAWS, the sampled dogs had been admitted to the shelter sometime between February 2021 and September 2022. Hence, the treatment history with respect to macrocyclic lactones administered within the preceding 12-month period, could not be verified for dogs that had been recently impounded and/or admitted. The credibility of this assertion may be called into question regarding the dogs surveyed in Mahikeng, as these dogs were under collective ownership and primarily received veterinary care through community outreach clinics associated with North West University, North West DARD, and SPCA Mahikeng. It is highly improbable that the dogs would have been administered macrocyclic lactones without the knowledge of the investigators. Moreover, the owners of the dogs also verified that the dogs had not received any form of macrocyclic lactones within the preceding 12 months of the study. It is worth noting the varied formulations (oral, spot-on, and injectable) of macrocyclic lactones (ivermectin, selamectin, doramectin and milbemycin) that are readily available over the counter in South Africa. Examples of such products include Milbemax®, Milpro®, Revolution®, Advocate®, Ivomec®, Dectomax®, and Nexgard Spectra®. Other over-the-counter products on the South African market are labelled as mosquito repellants for dogs. Many of such products are permethrin (synthetic pyrethroid) based, like Advantix® and Shoo-Fly®. Treatment history with such products was also not eliminated in this study, and it is possible that previous use of such products in the study group would have prevented or reduced vector (mosquito) transmission.

5.2 *Acanthocheilonema reconditum*

The occurrence of *A. reconditum* in this survey was an accidental yet anticipated outcome, given its extensive geographical dispersion. Reported cases of *A. reconditum* have been documented in various African countries, including Liberia, Nigeria, Kenya, Mozambique, Uganda, Botswana, Zambia, and South Africa (Ntesang 2016, Schwan 2009, Siwila *et al.* 2015). Based on previous surveys conducted in the region, the prevalence rates of *A. reconditum* have been documented as follows: 9.56% (102/1066) in South Africa, including the provinces of KwaZulu-Natal, Mpumalanga, and Gauteng; 8.85% (122/1379) in

Mozambique, specifically in the province of Maputo; 2.67% (4/150) in Botswana, specifically in Gaborone; and 4.7% (13/272) in Zambia, specifically Lusaka (Ntesang 2016, Schwan 2009, Siwila *et al.* 2015). The current study builds on a previous one by Voigts (2018), which was unsuccessful in finding this filariid in Mahikeng.

The observed disparity in the prevalence of *A. reconditum* in dogs between Mahikeng and Potchefstroom can be attributed to the general care and management of the two cohorts, particularly as it pertains to ectoparasite prevention and control. The present literature, as reviewed by Schwan (2009) and Pacifico *et al.* (2021), indicates that fleas, lice, and ticks are the intermediate hosts and vectors for *A. reconditum.* The animal shelter had a relatively higher standard of nutrition, housing, ecto- and endoparasite management, and general veterinary care for the dogs in their care compared to that of the hunting dogs.

CHAPTER 6: CONCLUSION

Based on the surveys conducted in Potchefstroom and Mahikeng, it can be concluded that the presence of *D. repens* in hunting and shelter dogs in the North West Province is either absent or exhibits a low prevalence, hence hindering its detection in the current study. To better understand the epidemiology of *D. repens* in North West Province, future research endeavours should prioritize increasing sample sizes and survey locations, as well as the diversification of sampling populations to include cats. There is a paucity of information concerning the specific mosquito species accountable for the transmission of *D. repens* within South Africa and future research ought to be directed towards this particular trajectory. To produce comparative test results for the Gaborone *D. repens* survey (Ntesang 2016), focus should be shifted to locations further north of Mahikeng, closer to Gaborone and the border with Botswana.

CHAPTER 7: REFERENCES

ALBONICO, F., LOIACONO, M., GIOIA, G., GENCHI, C., GENCHI, M. & MORTARINO, M. 2014. Rapid differentiation of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood by real-time PCR coupled to high resolution melting analysis. *Veterinary Parasitology,* 200, 128-132.

ALSARRAF, M., LEVYTSKA, V., MIERZEJEWSKA, E. J., POLIUKHOVYCH, V., RODO, A., ALSARRAF, M., KAVALEVICH, D., DWUŹNIK-SZAREK, D., BEHNKE, J. M. & BAJER, A. 2021. Emerging risk of *Dirofilaria* spp. infection in Northeastern Europe: high prevalence of *Dirofilaria repens* in sled dog kennels from the Baltic countries. *Scientific Reports,* 11, 1068.

BAJER, A., RODO, A., MIERZEJEWSKA, E. J., TOŁKACZ, K. & WELC-FALECIAK, R. 2016. The prevalence of *Dirofilaria repens* in cats, healthy dogs and dogs with concurrent babesiosis in an expansion zone in central Europe. *BMC Veterinary Research,* 12, 183.

BARTON, A. 1939. Age determination in dogs. Vol. 2. Available: https://dr.lib.iastate.edu/server/api/core/bitstreams/f012940c-d237-4f7d-9bf7-74aafec7118a/content

CANCRINI, G., ALLENDE, E., FAVIA, G., BORNAY, F., ANTÓN, F. & SIMÓN, F. 2000. Canine dirofilariosis in two cities of southeastern Spain. *Veterinary Parasitology,* 92, 81-86.

CANCRINI, G., TASSI, P. & COLUZZI, M. 1989. Ivermectin against larval stages of *Dirofilaria repens* in dogs. *Parassitologia,* 31, 177-82.

CAPELLI, G., GENCHI, C., BANETH, G., BOURDEAU, P., BRIANTI, E., CARDOSO, L., DANESI, P., FUEHRER, H.-P., GIANNELLI, A., IONICĂ, A. M., MAIA, C., MODRÝ, D., MONTARSI, F., KRÜCKEN, J., PAPADOPOULOS, E., PETRIĆ, D., PFEFFER, M., SAVIĆ, S., OTRANTO, D., POPPERT, S. & SILAGHI, C. 2018. Recent advances on *Dirofilaria repens* in dogs and humans in Europe. *Parasites & Vectors,* 11, 663.

CIUCA, L., VISMARRA, A., LEBON, W., BEUGNET, F., MORCHON, R., RINALDI, L., CRINGOLI, G., KRAMER, L. & GENCHI, M. 2020. New insights into the biology, diagnosis and immune response to Dirofilaria repens in the canine host. *Veterinary Parasitology,* 277, 100029.

CRINGOLI, G., GENCHI, C. & RINALDI, L. (eds.) 2007. *Biology of filarial worms parasitizing dogs and cats,* Università degli Studi di Napoli Federico II, Naples, Italy: Mappe Parassitologiche.

DEMIASZKIEWICZ, A., POLAŃCZYK, G., OSIŃSKA, B., PYZIEL, A., KULIGOWSKA, I. & LACHOWICZ, J. 2011. Morphometric characteristics of *Dirofilaria repens* Railliet et Henry, 1911 parasite of dogs in Poland 1. *Wiadomości parazytologiczne,* 57, 253-256.

DENNIS, D. T. & KEAN, B. H. 1971. Isolation of microfilariae: report of a new method. *Journal of Parasitology,* 57, 1146-7.

DESOWITZ, R. S., CHULARERK, P. & PALUMBO, N. E. 1970. Evaluation of a simplified membrane filtration technique for the diagnosis of canine filariasis. *Southeast Asian Journal of Tropical Medicine and Public Health,* 1, 231-232.

FERREIRA, C. A. C., MIXAO, V. D. P., NOVO, M. T. L. M., CALADO, M. M. P., GONCALVES, L. A. P., BELO, S. M. D. & ALMEIDA, A. P. G. D. 2015. First molecular identification of mosquito vectors of *Dirofilaria immitis* in continental Portugal. *Parasites & Vectors,* 8, (3 March 2015).

GAD, B., ZAFRIR, V., YIGAL, A., FAVIA, G., BAIN, O., GOLDSTEIN, R. E. & HARRUS, S. 2002. *Dirofilaria repens* infection in a dog: diagnosis and treatment with melarsomine and doramectin. *Veterinary Parasitology,* 105, 173-178.

GENCHI, C., GENCHI, M., PETRY, G., KRUEDEWAGEN, E. M. & SCHAPER, R. 2013. Evaluation of the efficacy of imidacloprid 10 % / moxidectin 2.5 % (Advocate®, Advantage® Multi, Bayer) for the prevention of *Dirofilaria repens* infection in dogs. *Parasitology Research,* 112 Suppl 1, 81-9.

GENCHI, C. & KRAMER, L. H. 2020. The prevalence of *Dirofilaria immitis* and *D. repens* in the Old World. *Veterinary Parasitology,* 280, 108995.

GENCHI, C., KRAMER, L. H. & RIVASI, F. 2011. Dirofilarial infections in Europe. *Vector Borne Zoonotic Diseases,* 11, 1307-17.

GENCHI, M., CIUCA, L., VISMARRA, A., CICCONE, E., CRINGOLI, G., KRAMER, L. & RINALDI, L. 2021. Evaluation of alternative reagents on the performance of the modified Knott's test. *Veterinary Parasitology,* 298, 109555.

GOOGLE EARTH. 2023. *Mahikeng* [Online]. Available: https://earth.google.com/web [Accessed 29 October 2023].

HOCH, H. & STRICKLAND, K. 2008. Canine and feline dirofilariasis: life cycle, pathophysiology, and diagnosis. *Compendium on Continuing Education for the Practising Veterinarian,* 30, 14151.

JOSEPH, M., KRISHNA, M. M. & VIJAYAN, A. 2023. Human subcutaneous dirofilariasis. *Cureus,* 15, e35879.

KELLY, J. D. 1973. DETECTION AND DIFFERENTIATION OF MICROFILARIAE IN CANINE BLOOD. *Australian Veterinary Journal,* 49, 23-27.

KNOTT, J. 1939. A method for making microfilarial surveys on day blood. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 33, 191-196.

KURUCZ, K., KEPNER, A., KRTINIC, B., ZANA, B., FÖLDES, F., BÁNYAI, K., OLDAL, M., JAKAB, F. & KEMENESI, G. 2016. First molecular identification of *Dirofilaria* spp. (Onchocercidae) in mosquitoes from Serbia. *Parasitology Research,* 115, 3257-3260.

LATROFA, M. S., WEIGL, S., DANTAS-TORRES, F., ANNOSCIA, G., TRAVERSA, D., BRIANTI, E. & OTRANTO, D. 2012. A multiplex PCR for the simultaneous detection of species of filarioids infesting dogs. *Acta Tropica,* 122, 150-4.

MAGNIS, J., LORENTZ, S., GUARDONE, L., GRIMM, F., MAGI, M., NAUCKE, T. J. & DEPLAZES, P. 2013. Morphometric analyses of canine blood microfilariae isolated by the Knott's test enables *Dirofilaria immitis* and *D. repens* species-specific and *Acanthocheilonema* (syn. *Dipetalonema*) genus-specific diagnosis. *Parasites & Vectors,* 6, 48.

MANZOCCHI, S., VENCO, L., PISEDDU, E., PANTCHEV, N., MASSERDOTTI, C., BONFANTI, U., ALBANESE, F., KRAMER, L. H. & BERTAZZOLO, W. 2017. Positive PCR alone should not be considered sufficient to establish *Dirofilaria repens* as the cause of subcutaneous nodular lesions in the absence of a clear cytologic picture. *Veterinary Clinical Pathology,* 46, 389-390.

MEGAT ABD RANI, P. A., IRWIN, P. J., GATNE, M., COLEMAN, G. T., MCINNES, L. M. & TRAUB, R. J. 2010. A survey of canine filarial diseases of veterinary and public health significance in India. *Parasites & Vectors,* 3, 30.

MOODLEY, K., GOVIND, C. N., PEER, A. K., VAN DER WESTHUIZEN, M., PARBHOO, D., SUN, L. M., DU PLESSIS, D. C. & FREAN, J. A. 2015. First detection of human dirofilariasis in South Africa. *Infectious Disease Reports,* 7, 5726.

NTESANG, K. 2016. *Gastrointestinal and filarial helminth infections of domestic dogs in Gaborone Botswana.* MSc dissertation, University of Pretoria.

PACIFICO, L., FERRARI, N., ROMEO, C., BUONO, F., VARUZZA, P., SGROI, G., NEOLA, B., BUCH, J., BEALL, M., BREITSCHWERDT, E. B., CHANDRASHEKAR, R., VENEZIANO, V. & PIANTEDOSI, D. 2021. Haematological and biochemical abnormalities in hunting dogs infected with Acanthocheilonema reconditum, associated risk factors, and a European overview. *Parasitol Res,* 120, 2109-2124.

PĘKACZ, M., BASAŁAJ, K., KALINOWSKA, A., KLOCKIEWICZ, M., STOPKA, D., BĄSKA, P., DŁUGOSZ, E., KARABOWICZ, J., MŁOCICKI, D., WIŚNIEWSKI, M. & ZAWISTOWSKA-DENIZIAK, A. 2022. Selection of new diagnostic markers for *Dirofilaria repens* infections with the use of phage display technology. *Scientific Reports,* 12, 2288.

PERIBÁÑEZ, M. A., LUCIENTES, J., ARCE, S., MORALES, M., CASTILLO, J. A. & GRACIA, M. A. J. 2001. Histochemical differentiation of *Dirofilaria immitis*, *Dirofilaria repens* and *Acanthocheilonema dracunculoides* microfilariae by staining with a commercial kit, Leucognost-SP®. *Veterinary Parasitology,* 102, 173-175.

POURHOSEINGHOLI, M. A., VAHEDI, M. & RAHIMZADEH, M. 2013. Sample size calculation in medical studies. *Gastroenterology and Hepatology from Bed to Bench,* 6, 14-7.

PRICHARD, R. K. 2021. Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. *Int J Parasitol,* 51, 1121-1132.

ROSSI, L., FERROGLIO, E. & AGOSTINI, A. 2002. Use of moxidectin tablets in the control of canine subcutaneous dirofilariosis. *Veterinary Record,* 150, 383.

ROSSI, L., FERROGLIO, E. & AGOSTINI, A. 2004. Use of an injectable, sustained-release formulation of moxidectin to prevent canine subcutaneous dirofilariosis. *Veterinary Record,* 154, 26-7.

SCHWAN, E. V. 2009. *Filariosis of domestic carnivores in Gauteng, KwaZulu-Natal and Mpumalanga provinces, South Africa, and Maputo province, Mozambique.* PhD thesis, University of Pretoria.

SCHWAN, V., MILLER, D. B., KOCK, D. & HEERDEN, A. 2000. *Dirofilaria repens* in a cat with acute liver failure. *Journal of the South African Veterinary Association,* 71, 197-200.

SIMÓN, F., DIOSDADO, A., SILES-LUCAS, M., KARTASHEV, V. & GONZÁLEZ-MIGUEL, J. 2022. Human dirofilariosis in the 21st century: A scoping review of clinical cases reported in the literature. *Transboundary and Emerging Diseases,* 69, 2424-2439.

SIMON, F., MORCHON, R., GONZALEZ-MIGUEL, J., MARCOS-ATXUTEGI, C. & SILES-LUCAS, M. 2009. What is new about animal and human dirofilariosis? *Trends in Parasitology,* 25, 404-409.

SIMÓN, F., SILES-LUCAS, M., MORCHÓN, R., GONZÁLEZ-MIGUEL, J., MELLADO, I., CARRETÓN, E. & MONTOYA-ALONSO, J. A. 2012. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. *Clinical Microbiology Reviews,* 25, 507-44.

SIMSEK, S. & CIFTCI, A. T. 2016. Serological and Molecular Detection of *Dirofilaria* species in stray dogs and investigation of *Wolbachia* DNA by PCR in Turkey. *Journal of Arthropod Borne Diseases,* 10, 445-453.

SIWILA, J., MWASE, E. T., NEJSUM, P. & SIMONSEN, P. E. 2015. Filarial infections in domestic dogs in Lusaka, Zambia. *Veterinary Parasitology,* 210, 250-254.

TAHIR, D., BITTAR, F., BARRÉ-CARDI, H., SOW, D., DAHMANI, M., MEDIANNIKOV, O., RAOULT, D., DAVOUST, B. & PAROLA, P. 2017. Molecular survey of *Dirofilaria immitis* and *Dirofilaria repens* by new real-time TaqMan® PCR assay in dogs and mosquitoes (Diptera: Culicidae) in Corsica (France). *Veterinary Parasitology,* 235, 1-7.

TARELLO, W. 2002a. Dermatitis associated with *Dirofilaria (Nochtiella) repens* microfilariae in dogs from central Italy. *Acta Veterinaria Hungarica,* 50, 63-78.

TARELLO, W. 2002b. *Infestation with the zoonotic nematode Dirofilaria repens in cats from central Italy* [Online]. Perugia, Italy: Vet On-Line. Available: https://www.priory.com/vet/filiarisis.htm [Accessed 17 June 2023].

TARELLO, W. 2003. Dermatitis associated with *Dirofilaria repens* microfilariae in three dogs in Saudi Arabia. *Journal of Small Animal Practice,* 44, 132-4.

TARELLO, W. 2011. Clinical aspects of dermatitis associated with *Dirofilaria repens* in pets: A review of 100 canine and 31 feline cases (1990-2010) and a report of a newclinic case imported from Italy to Dubai. *Journal of Parasitology Research,* 2011, 578385.

TROTZ-WILLIAMS, L. A. & TREES, A. J. 2003. Systematic review of the distribution of the major vector-borne parasitic infections in dogs and cats in Europe. *Veterinary Record,* 152, 97-105.

VOIGTS, B. 2018. *Survey for filarial helminth infections of domestic dogs in Mahikeng North West Province South Africa.* MSc dissertation, University of Pretoria.

WEATHERSPARK. 2023a. *Comparison of the Average Weather in Durban Mpumalanga Gaborone and Mahikeng*[Online].Available:https://weatherspark.com/compare/y/96783~96295~91720~91710/Comparison-of-the-Average-Weather-in-Durban-Mpumalanga-Gaborone-and-Mahikeng#Figures-PrecipitationProbability [Accessed 29 October 2023].

WEATHERSPARK. 2023b. *Comparison of the Average Weather in Potchefstroom and Mahikeng Figures* [Online]. Available: https://weatherspark.com/compare/y/94200~91710/Comparison-of-the-Average-Weather-in-Potchefstroom-and-Mahikeng#Figures-Temperature [Accessed 28 October 2023].

YEN, P. K. F. & MAK, J. W. 1978. Histochemical differentiation of *Brugia*, *Wuchereria*, *Dirofilaria* and *Breinlia* microfilariae. *Annals of Tropical Medicine & Parasitology,* 72, 157-162.

ANNEXURE 1: RESEARCH ETHICS COMMITTEE APPROVAL

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee

14 March 2022

CONDITIONALLY APPROVAL

Ethics Reference No	**REC117-21**
Protocol Title	**The prevalence of Dirofilaria repens in dogs in North-West province of South Africa**
Principal Investigator	**Dr TR Motsi**
Supervisors	**Dr EV Schwan**

Dear Dr TR Motsi,

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC117-21) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

NOTES: Conditionally approved pending the following (and to ensure that rerouting to AEC is not delayed):
1. Obtaining ALL other relevant approvals.
2. Please note for researcher agreements: Only a postgraduate student can be granted the rights to publish a dissertation/thesis.

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

Room 6-6, Arnold Theiler Building
University of Pretoria, Faculty of Veterinary Science
Private Bag X04, Onderstepoort, 0110, South Africa
Tel +27 (0)12 529 8090
Email mare.watson-kriek@up.ac.za
www.up.ac.za

Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa

ANNEXURE 2: ANIMAL ETHICS APPROVAL

Faculty of Veterinary Science
Animal Ethics Committee

19 May 2022

Approval Certificate
New Application

AEC Reference No.:	REC117-21
Title:	The prevalence of Dirofilaria repens in dogs in North-West province of South Africa
Researcher:	Dr TR Motsi
Student's Supervisor:	Dr EV Schwan

Dear Dr TR Motsi,

The New Application as supported by documents received between 2022-02-14 and 2022-05-03 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-05-03.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Dogs - All breeds	168
Samples	**Number**
Canine - Blood sample (Samples from live animals)	168

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-05-19.
3. Please remember to use your protocol number (REC117-21) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. All incidents must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-12, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel: +27 12 529 8434
Fax: +27 12 529 8324
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha le Disaense tša Bongakadimelwa

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

08 June 2023

Approval Certificate
Annual Renewal
(EXT1)

AEC Reference No.:	REC117-21 Line 1
Title:	The prevalence of Dirofilaria repens in dogs in North-West province of South Africa
Researcher:	Dr TR Motsi
Student's Supervisor:	Dr EV Schwan

Dear Dr TR Motsi,

The Annual Renewal as supported by documents received between 2023-04-26 and 2023-05-29 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2023-05-29.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Approved
Dogs - All breeds	168
Samples	Approved
Canine - Blood sample - South Afri - Live	168

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2024-06-08.
3. Please remember to use your protocol number (REC117-21) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. All incidents must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 0-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadimelo

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

ANNEXURE 3: SECTION 20 APPROVAL

agriculture, land reform
& rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development Private Bag X138, Pretoria 0001
Enquiries: Ms Mama Laing • Tel: +27 12 319 7442 • Fax: +27 12 319 7470 • E-mail: MamaL@dalrrd.gov.za
Reference: 12/11/1/1/6 (2230 AC)

Dr Tatenda Roy Motsi

Department of Veterinary Tropical Diseases

Faculty of Veterinary Science

University of Pretoria

Onderstepoort

Tel: 071 887 0233

Email: troymotsi@gmail.com ; TatendaM@Dalrrd.gov.za

Dear Dr Motsi,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Your application received on 22 November 2021 requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1) This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2) The research project is approved as per the application form received 22 November 2021 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this research project under this Section 20 permit. Please apply in writing to MamaL@dalrrd.gov.za;
3) The study must be conducted in compliance with the Veterinary and Para-Veterinary Professions Act 1982 (Act No. 19 of 82);
4) EDTA blood samples may be collected from:
   a) Impounded stray dogs at the Potchefstroom Animal Welfare Society, within the Potchefstroom area of the North West province, for which a state veterinary letter has been received;

b) Privately owned canine patients presented to participating community outreach animal clinics, within the rural Mahikeng area of the North West province, for which a state veterinary letter has been received;

Owners of the patients must provide full consent, as described;

5) It is the researcher's responsibility to remain in contact with the responsible State Veterinarian regarding the disease status of the area from which dogs will be sampled. Records must be kept for five years for auditing purposes;
6) Samples must be packaged and transported in accordance with the National Road Traffic Act, 1996 (Act No. 93 of 1996);
7) The EDTA blood samples may only be sent to the Parasitology Laboratory (Room 1-41) of the Department for Veterinary Tropical Diseases, University of Pretoria, for Membrane Filtration Testing and Acid Phosphatase Staining for *Dirofilaria repens*, as described;
8) Any results communicated needs to be clearly labelled and identified as research results;
9) All potentially infectious material utilised, collected or generated during the study is to be destroyed at the completion of the study using the specified waste contractor as indicated;
10) Records must be kept for five years for auditing purposes;
11) If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 permit. Please apply in writing to Mamal@dalrrd.gov.za.

Title of research/study: *"The prevalence of Dirofilaria repens in dogs in the North-West province of South Africa"*

Researcher: Dr Tatenda Roy Motsi

Institution: Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort

Our ref Number: 12/11/1/1/6 (2230 AC)

Your ref: REC117-21

Expiry date: February 2024

Kind regards,

Name: Dr Mpho Maja
Reason: .
Date: 2021.12.10 16:38:11 CAT

DR. MPHO MAJA

DIRECTOR: ANIMAL HEALTH

Date: