Filariosis of domestic carnivores in Gauteng, KwaZulu-Natal and Mpumalanga provinces, South Africa, and Maputo province, Mozambique

By

Ernst Volker Schwan

Submitted in partial fulfillment of the requirements for the degree Doctor of Philosophy in the Department of Veterinary Tropical Diseases in the Faculty of Veterinary Science, University of Pretoria

Date submitted: July 2009
All things are subject to interpretation

whichever interpretation prevails at a given time

is a function of power and not truth

Friedrich Nietzsche
My sincerest gratitude goes to my family for the encouragement received, without it would not have been possible to do this work.

I am also grateful to Prof Joop Boomker for the supervision of the thesis.

My colleagues in the Department of Veterinary Tropical Diseases for their valuable contributions: Ms Dawn Durand and Mr Ryno Watermeyer for their assistance in the laboratory and Ms Rina Serfontein for her kind assistance in formatting of the thesis.

I would also like to express my gratitude to Ms Rina Owen from the Department of Statistics, University of Pretoria, for the statistical analysis.

The following persons I would like to thank for their active support by providing samples from various geographic localities in South Africa and Mozambique: Dr Dagwin Camby (Kolonnade Animal Hospital, Pretoria), Dr Corrie van Aardt (Pretoria North Veterinary Clinic, Pretoria), Dr Willi Cilliers (Florandia Animal Hospital, Pretoria), Dr Nico Degenaar (Overkruin Veterinary Clinic, Pretoria), Dr Lina Gerber (Akasia Veterinary Clinic, Pretoria), Dr Peter Kirchner (Petland Animal Hospital, Pretoria), Dr Heinrich van Niekerk (Bergsig Animal Clinic, Pretoria), Dr Heidi Schroeder (Willow Park Small Animal Medicine Specialist Hospital, Pretoria), Dr Stephan Vogel (Ridge Animal Hospital, Pretoria), Dr Mariana van der Vyver (Sinoville Veterinary Clinic, Pretoria), Dr Anita Schwan (Heatherdale Veterinary Clinic), Dr Heinz Kôhrs (Pongola Animal Clinic, Pongola), Dr Karl Aadnesgaard (Zululand Veterinary Hospital, Empangeni), Dr Peer Singery (Meerensee Veterinary Hospital, Meerensee), Dr Charles Pryke (Eshowe Veterinary Clinic, Eshowe), Dr Trevor Viljoen (Mtubatubu Veterinary Clinic, Mtubatubu), Dr Rick Mapham (Veterinary House Hospital, Pietermaritzburg), Dr Allan Hancox (Longmarket Veterinary Clinic, Pietermaritzburg), Dr Brian Longmore (Hayfields Veterinary Hospital, Pietermaritzburg), Dr Barry Hyman (Novartis SA), Drs Francois Malan and Neil Fourie (Malelane Research Unit, Intervet SA), Drs Albertus Coetzee and Johan Viljoen (West Acres Animal Clinic, Nelspruit), Dr Carlos Lopes Pereira (Direcção Nacional de Pecuária, Maputo) and last Dr Luis Neves (Eduardo Mondlane University, Maputo).

Special thanks to Ms Antoinette Lourens for her relentless efforts to track numerous publications from journals difficult to access.

This work was financially supported by Merial France, Merial SA, Novartis SA, Fort Dodge SA, Pfizer SA, the Research Committee of the Faculty of Veterinary Science, University of Pretoria and the Department of Veterinary Tropical Diseases, University of Pretoria.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS .................................................................................................. III

TABLE OF CONTENTS ................................................................................................ IV

LIST OF FIGURES ....................................................................................................... VIII

LIST OF TABLES ......................................................................................................... IX

SUMMARY ...................................................................................................................... X

CHAPTER 1  INTRODUCTION ....................................................................................1

CHAPTER 2  LITERATURE REVIEW OF THE DIFFERENT AETIOLOGIES OF FILARIOSIS IN DOMESTIC CARNIVORES IN AFRICA .................................................................4

2.1  Dirofilaria immitis ..................................................................................................4
  2.1.1 Taxonomy ........................................................................................................4
  2.1.2 Morphology ......................................................................................................5
  2.1.3 Life cycle ......................................................................................................... 6
  2.1.4 Host range ....................................................................................................... 8
  2.1.5 Vectors ............................................................................................................ 8
  2.1.6 Laboratory diagnosis in live animals ............................................................... 9
    2.1.6.1 MORPHOMETRICAL IDENTIFICATION OF MICROFILARIAE .......................10
    2.1.6.2 MORPHOLOGICAL IDENTIFICATION OF MICROFILARIAE .......................10
    2.1.6.3 PHYSIOLOGICAL CHARACTERISTICS OF MICROFILARIAE .....................11
  2.1.6.4 HISTOCHEMICAL IDENTIFICATION OF MICROFILARIAE .........................11
  2.1.6.5 SEROLOGY ....................................................................................................12
  2.1.6.6 MOLECULAR DIAGNOSIS ..............................................................................13
  2.1.7 Veterinary and medical importance ..................................................................13
  2.1.8 Distribution on the African continent and its islands ...................................... 16
    2.1.8.1 NORTHERN AFRICA .....................................................................................16
    2.1.8.2 WESTERN AFRICA .....................................................................................18
    2.1.8.3 CENTRAL AFRICA .....................................................................................20
    2.1.8.4 EASTERN AFRICA .....................................................................................20
    2.1.8.5 SOUTHERN AFRICA ...................................................................................23

2.2  Dirofilaria repens ..................................................................................................23
  2.2.1 Taxonomy ....................................................................................................... 23
  2.2.2 Morphology .....................................................................................................23
  2.2.3 Life cycle ......................................................................................................... 24
  2.2.4 Host range ....................................................................................................... 25
  2.2.5 Vectors ............................................................................................................ 25
  2.2.6 Laboratory diagnosis in live animals ............................................................... 26
  2.2.7 Veterinary and medical importance ..................................................................26
  2.2.8 Distribution on the African continent ...............................................................29
    2.2.8.1 NORTHERN AFRICA .....................................................................................29
CHAPTER 3  MATERIALS AND METHODS ..............................................57
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 .......................................................... 57

3.1.1 Description of survey areas ........................................................................ 57
3.1.1.1 GAUTENG PROVINCE .................................................................................. 57
3.1.1.2 KWAZULU-NATAL PROVINCE ................................................................. 58
3.1.1.3 MPUMALANGA PROVINCE ....................................................................... 59
3.1.1.4 MAPUTO PROVINCE .................................................................................. 59

3.1.2 Survey animals, sample size and selection criteria ........................................... 60

3.1.3 Filarial diagnostic techniques ......................................................................... 60
3.1.3.1 MEMBRANE FILTRATION ........................................................................... 60
3.1.3.2 ACID PHOSPHATASE STAINING ................................................................. 61
3.1.3.3 ANTIGEN CAPTURE ELISA ......................................................................... 61

3.1.4 Statistical analysis .......................................................................................... 62

3.2 Survey on the occurrence and prevalence of filarial helminths of cats in KwaZulu-Natal province ........................................................................................................ 62

3.2.1 Description of survey areas ........................................................................... 62

3.2.2 Survey animals, sample size and selection criteria ........................................... 62

3.2.3 Filarial diagnostic techniques ......................................................................... 63

3.3 Routine examinations for filarial infections of dogs and cats from South Africa between 1994 and 2008 .................................................................................. 63

3.3.1 Filarial diagnostic techniques ......................................................................... 63

3.4 Routine examinations for filarial infections of dogs and cats imported from African countries between 1992 and 2008 .................................................. 63

3.4.1 Filarial diagnostic techniques ......................................................................... 64

CHAPTER 4 RESULTS .......................................................................................... 65

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 .......................................................... 65

4.1.1 Dirofilaria immitis ........................................................................................... 65
4.1.2 Dirofilaria repens ........................................................................................... 66
4.1.3 Acanthocheilonema reconditum ..................................................................... 67
4.1.4 Acanthocheilonema dracunculoides ................................................................. 68

4.2 Survey on the occurrence and prevalence of filarial helminths of cats in KwaZulu-Natal province ........................................................................................................ 68

4.3 Routine examinations for filarial infections of dogs and cats from South Africa between 1994 and 2008 .................................................................................. 69

4.4 Routine examinations for filarial infections of dogs and cats imported from African countries between 1992 and 2008 .................................................. 69

4.5 Literature review on filariosis of dogs and cats in Africa ........................................... 71

4.5.1 Dirofilaria immitis ........................................................................................... 71
4.5.2 Dirofilaria repens ........................................................................................... 72
4.5.3 Acanthocheilonema reconditum ..................................................................... 72
4.5.4 Acanthocheilonema dracunculoides ................................................................. 73
4.5.5 Brugia patei ..................................................................................................... 74
CHAPTER 5 DISCUSSION .................................................................................................91

5.1 *Dirofilaria immitis* ..............................................................................................92

5.2 *Dirofilaria repens* ..............................................................................................96

5.3 *Acanthocheilonema reconditum* .....................................................................97

5.4 *Acanthocheilonema dracunculoides* .................................................................98

5.5 *Brugia patei* .......................................................................................................98

CHAPTER 6 REFERENCES ............................................................................................99
LIST OF FIGURES

Figure 4.1: Microfilariae on a Giemsa-stained membrane filter ........................................75
Figure 4.2: *Dirofilaria immitis* microfilaria showing acid phosphatase activity at the excretory pore (E) and anal pore (A) ...........................................................................................................75
Figure 4.3: *Dirofilaria repens* microfilaria showing acid phosphatase activity at the inner body (IB) and anal pore (A) ........................................................................................................76
Figure 4.4: *Acanthocheilonema reconditum* microfilaria showing diffuse acid phosphatase activity in the area of the excretory pore, inner body and anal pore ...........................................................................................................76
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) ...........................................................................................................77
Figure 4.6: *Brugia patei* microfilaria with sheath (S) stained with Giemsa ..................77
Figure 4.7: Tail end of *Brugia patei* microfilaria with typical sub-terminal (ST) and terminal (T) tail nuclei ...........................................................................................................78
Figure 4.8: *Brugia patei* microfilaria showing acid phosphatase activity at the cephalic vesicle (CV), excretory pore (E) and tail ...........................................................................................................78
Figure 4.9: Geographical distribution of *Dirofilaria immitis* in dogs in Africa ...............79
Figure 4.10: Geographical distribution of *Dirofilaria repens* in dogs, cats and other carnivores in Africa ...........................................................................................................80
Figure 4.11: Geographical distribution of *Acanthocheilonema reconditum* in dogs and other carnivores in Africa ...........................................................................................................81
Figure 4.12: Geographical distribution of *Acanthocheilonema dracunculoides* in dogs and other carnivores in Africa ...........................................................................................................82
Figure 4.13: Geographical distribution of *Brugia patei* in dogs, cats and other carnivores and primates in Africa ...........................................................................................................83
LIST OF TABLES

Table 2.1: Filarial helminths described from dogs and cats and their geographical distribution................................................................................................51

Table 2.2: Length and width of *Dirofilaria immitis* microfilariae from dogs according to geographical origin and technique of processing ........................................52

Table 2.3: Natural culicine vectors of *Dirofilaria immitis* in Africa ........................................53

Table 2.4: Length and width of *Dirofilaria repens* microfilariae from dogs, cats and other carnivores according to geographical origin and technique of processing..............................................................54

Table 2.5: Length and width of *Acanthocheilonema reconditum* microfilariae from dogs according to geographical origin and technique of processing .................................................................55

Table 2.6: Length and width of *Acanthocheilonema dracunculoides* microfilariae from dogs and other carnivores according to geographical origin and technique of processing ........................................................................................................56

Table 4.1: Overall filarial prevalence in dogs by locality in Gauteng, KwaZulu-Natal, Mpumalanga and Maputo provinces .................................................................................................84

Table 4.2: Overall prevalence of *Dirofilaria repens* in dogs by age ........................................85

Table 4.3: Overall prevalence of *Acanthocheilonema reconditum* in dogs by age ....85

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 .................................................................86

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 .................................................................................................88
SUMMARY

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

By

ERNST VOLKER SCHWAN

Promoter: Prof JDF Boomker
Department: Veterinary Tropical Diseases
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.