UNIVERSITY OF KWAZULU-NATAL

Trichinella infections in wildlife in the Greater Kruger National Park, South Africa: Unravelling epidemiological gaps with special emphasis on infectivity of *Trichinella zimbabwensis* in selected tropical fishes

by

LOUIS J. LA GRANGE

Submitted in partial fulfillment of the academic requirements of

Doctor of Philosophy

Discipline of Biological Sciences
School of Life Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Durban
South Africa

Supervisor: Professor S. Mukaratirwa
April 2020

As the candidate's supervisor I have~~/have not~~ approved this thesis for submission.

Signed: [redacted] Name: **Prof. S. Mukaratirwa** Date: **21 April 2020**

*"...public-health researchers and practitioners, and those in the political and public realms with whom they interact, must take a broad view of the determinants and, indeed, the sustainability of population health. This is an ecological view of health; an awareness that shifts in the ecology of human living, in relation to both the natural and social environments which account for much of the ebb and flow of diseases over time."**

*McMichael, A.J., Beaglehole, R. (2000) "The changing global context of public health" *The Lancet*, 356, 2000

Dedicated to:

My loving wife, Twanette La Grange and sons, Conrad and Schalk La Grange

PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Biology (Parasitology), School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, South Africa. The research was financially supported by the Mpumalanga Department of Agriculture, Rural Development, Land and Environmental Affairs, Chief Directorate Veterinary Services and Wildlife Pharmaceuticals Pty Ltd.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

_____

Signed: **Prof. S. Mukaratirwa**

Date: **21 April 2020**

DECLARATION 1: PLAGIARISM

I, Louis Jacobus La Grange, declare that:

(i) the research reported in this thesis, except where otherwise indicated or acknowledged, is my original work;

(ii) this thesis has not been submitted in full or in part for any degree or examination to any other university;

(iii) this thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this thesis is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed: Louis J. La Grange

Date: 16 April 2020

DECLARATION 2: PUBLICATIONS

My role in each paper and presentation is indicated. The * indicates corresponding author.

1. *Mukaratirwa, S., **La Grange**, L.J., Malatji, M.P., Reininghaus, B., Lamb, J., (2019) Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga provinces of South Africa. *Journal of Helminthology*, 93, 50-56.

I herewith declare that I was responsible for collection of most of the field samples and initial screening on some samples, literature research of *Trichinella* surveillance conducted for the period 1964-2016,
drafting of Introduction and the section discussing the distribution and prevalence of *Trichinella* spp. in wildlife, compilation and drafting of tables, assisting with final editing and layout of manuscript prior to submission and review and responding to comments received from manuscript Reviewers.

2. **La Grange**, L.J., *Mukaratirwa, S., (2020) Epidemiology and hypothetical transmission cycles of *Trichinella* infections in the Greater Kruger National Park of South Africa: an example of host-parasite interactions in an environment with minimal human interactions. *Parasite*, 13, 1-12.

I herewith declare that I was responsible for literature search for the review, initial drafting of the manuscript in its entirety, effecting changes as suggested and discussed with co-author, assisting with final editing and layout prior to submission and reviewed and responded to comments as received from manuscript reviewers. The journal formatting (number format) for in-text referencing was retained and considered preferable to ensure full referencing in the tables without decreasing font sizes. The number format of citation allows proper fit of the tables within page margins.

3. **La Grange**, L.J., *Mukaratirwa, S., (2020) Experimental infection of Tigerfish (*Hydrocynus vittatus*) and African sharptooth catfih (*Clarias gariepinus*) with *Trichinella zimbabwensis*. *Onderstepoort Journal of Veterinary Research*, 87(1), a1876. https://doi.org/ 10.4102/ojvr.v87i1.1876

I herewith declare that I was responsible for the acquisition of fish used in this study as well as the establishment of research sites, securing of sponsorships and overall oversight of field experiments. I was also responsible for the fish analyses, initial drafting of the manuscript in its entirety, effecting changes as suggested and discussed with co-author, assisting with final editing and layout prior to submission. I was additionally responsible for preparation of response to comments a received from manuscript reviewers.

Signed: Louis J. La Grange Date: 16 April 2020

GENERAL ABSTRACT

Trichinella species are widely distributed on all continents with the exception of Antarctica, although the full spectrum of *Trichinella* species found in sub-Saharan African countries and their hosts has not been fully documented. This study was conducted to review reports on *Trichinella* infections in wildlife in the Kruger National Park and also to identify species and/or genotypes of *Trichinella* larvae isolated from muscle tissues of wildlife from Kruger National Park and adjacent areas of the Limpopo and Mpumalanga provinces, South Africa referred to as the Greater Kruger National Park using molecular techniques. A review of *Trichinella* spp. isolates and their wildlife hosts from the Greater Kruger National Park covering the period 1964–2011 was conducted and the results were compared with recent findings where isolates collected between 2012 and 2016 were identified to genotype/species level using molecular techniques. In the first 15 years the prevalence of infection was only reported twice in scientific publications and the reports included only four carnivorous mammal species and one rodent species. However, since the last report of *Trichinella* in an African civet (*Civettictis civetta*) other wildlife species were tested in the KNP and one new host was identified. Advances in molecular techniques allowed scientists to identify two isolates, collected in 1966 and 1988 respectively as *Trichinella* T8. Another isolate collected in 1991 was described as *T. nelsoni*. All of the other isolates found before 1991 were erroneously identified as *T. spiralis*. Ninety samples collected during the 2012–2016 period representing 15 mammalian, two avian- and three reptilian species were screened for *Trichinella* infection using artificial digestion. Isolates detected were identified using a multiplex polymerase chain reaction amplification of the ITS1, ITS2 and ESV regions of ribosomal DNA followed by molecular analysis of the sequences. Twenty (20) samples from seven wildlife species were positive for *Trichinella* spp. larvae with an overall prevalence of 21.1% (20/90). The prevalence was higher in carnivores (18.9%, 18/90) than in omnivores (2.2%, 2/90). Analysis of sequences showed that eight of the isolates; two from spotted hyaena (*Crocuta crocuta*) (2/8), three from lion (*Panthera leo*) (3/13), one from leopard (*Panthera pardus*) (1/6), one from small spotted genet (*Genetta genetta*) (1/2) and one Nile monitor lizard (*Varanus niloticus*) (1/2) conformed to *Trichinella zimbabwensis*. One isolate from a hyaena was grouped under the encapsulated species clade comprising *T. nelsoni* and genotype *Trichinella* T8 reported to be present in South Africa. This is the first report confirming natural infection of *T. zimbabwensis* in hyaena, leopard, genet and Nile monitor lizard, adding to the body of knowledge on the epidemiology of *Trichinella* infections in the Greater Kruger National Park, South Africa. Ten *Trichinella*-like larvae recovered after digestion from four

wildlife species in this study (2012–2016) revealed inconclusive results due to DNA degradation from poor storage or too few larvae for analysis in comparison to 20 isolates from five wildlife species not identified to species during the 1964–2011 period.

Knowledge on factors influencing the infectivity, epidemiology and survival of *Trichinella* spp. in different climatological environments is scanty. Availability of this knowledge will allow for the elucidation of epidemiology of *Trichinella* infections and the prediction of probable host-parasite cycles within specific ecological niches. The recent identification of new host species infected with three *Trichinella* taxa within the Greater Kruger National Park prompted a revision of previously published hypothetical transmission cycles for these species. Using data gathered from surveillance studies spanning the period 1964–2016, and the recently obtained data from molecular identification of isolates from the Greater Kruger National Park, the previously hypothesized transmission cycles were revised. The new hypothesized transmission cycles were established in consideration of epidemiological factors and prevalence data gathered from both the Greater Kruger National Park and similar wildlife protected areas in Africa where the same host- and parasite species are known to occur. The anecdotal nature of some of the presented data in the hypothesized transmission cycles confirms the need for more intense epidemiological surveillance in the rest of South Africa and continued efforts to unravel the epidemiology of *Trichinella* infections in this unique and diverse protected landscape.

Furthermore, to determine the role of fish in the epidemiology of *T. zimbabwensis* in the Greater Kruger National Park, experimental infections were conducted to assess the infectivity of this species to catfish (*Clarias gariepinus*) and tigerfish (*Hydrocynus vittatus*). Twenty-four catfish (581.7 ± 249.7 g) were randomly divided into 5 groups and experimentally infected with 1.0 ± 0.34 *T. zimbabwensis* larvae per gram (lpg) of fish. Results showed no adult worms or larvae in the gastrointestinal tract and body cavities of catfish euthanized at day 1, 2 and 7 post-infection (p.i.). These results suggest that African sharp tooth catfish does not play a role in the epidemiology of the parasite irrespective of the fact that the fish cohabit with crocodiles and Nile monitor lizards in the Greater Kruger National Park.

Forty-one tigerfish (298.6 ± 99.3 g) were randomly divided into three separate trials (T). Each trial (T) was divided into groups (G) as follows; Trial 1 (T_1G_1); Trial 2 (T_2G_1, T_2G_2) and Trial 3 (T_3G_1, T_3G_2, T_3G_3) infected with 2.12 ± 1.12 lpg of fish. An additional 7 tigerfish were assessed for the presence of natural infection.

Two tigerfish from T_1G_1 yielded *T. zimbabwensis* larvae in muscle tissues on day 26 p.i. (0.1 lpg) and 28 p.i. (0.02 lpg), respectively. No adult worms or larvae were detected in the fish from trials 2 or 3 on days 7, 21, 28, 33 or 35 p.i. or from the control group.

Results from this study suggest tigerfish to be generally unsuitable hosts for *T. zimbabwensis*. However, results from this study suggest that some individuals could, under very specific, and as yet to be elucidated circumstances, maintain the larvae of *T. zimbabwensis* but it could not be confirmed whether the parasite can fully develop and reproduce in this host.

These results preclude any definitive conclusion in respect of the potential of African sharp tooth catfish and tiger fish to serve as potential hosts for *T. zimbabwensis*. The influence of temperature on *T. zimbabwensis* larval development and survival in fish remains inconclusive. It is possible that these fish could only become infected during warmer seasons and in warmer climates. It is also not clear whether potentially infected fish would retain the infection in subsequent colder seasons. Variability of temperatures between different geographic regions may additionally influence the susceptibility of these fish to *T. zimbabwensis* infection.

However, the plethora of biological-, geographical- and climatic factors that could potentially influence the infectivity of *T. zimbabwensis* to certain fish host species precludes any definitive conclusion on the role of fish in the parasite's natural ecosystem. Results from this study do suggest that tigerfish could, under very specific and as yet unknown circumstances, sustain the development and establishment of *T. zimbabwensis*.

ACKNOWLEDGMENTS

All the glory to God the Father for providing me with the opportunity and ability to study and report on this small part of His infinite creation.

Thank you to:

My wife and children for their continued support and assistance with the project; their encouragement and faith in my abilities throughout this journey. The countless hours you selflessly devoted and spent collecting research specimens, construction of pond cages and assistance with experimental infections and laboratory analyses- I love you more than I could ever express in words.

To my family and friends for the unwavering support.

My supervisor, Professor S. Mukaratirwa for your patience, assistance, friendship and professional support without which I would never even have been able to start my journey.

Dr. J.P. Raath and all the staff of Wildlife Pharmaceuticals and Wildlifevets.com for the kind financial support, assistance with acquiring study animals and the use of their facilities to conduct the field work. I also wish to acknowledge the students and staff who assisted with experimental infection of cat fish.

Professor W. Luus-Powell and staff of the University of Limpopo for providing catfish for this study. Drs. G. O'Brien and K.D.A. Huchzermeyer for the technical advice on fish husbandry.

Dr F. Roux for sharing his expertise on tigerfish and providing material for tigerfish collection. Department of Agriculture, Rural Development, Land and Environmental Affairs, the Chief Directorate Veterinary Services and my work colleagues and supervisors for the logistical and financial support.

Dr. L. Williams and Mr. R. Williams for providing a collection site for tigerfish and housing facilities as well as the care-, monitoring and feeding of my study subjects, Dr S. Pfitzer and Messrs. W Selgobela and J. Breedt for taking care of-, monitoring and feeding my study subjects. Dr. F. Stroud for the many hours spent collecting tiger fish and especially for the ideas on- and assistance with experimental infection of tigerfish. Dr. J. Springfield, Messrs. E. Grové and D. Uys for the assistance in collecting tigerfish. Dr. B. Reininghaus for the field samples from terrestrial animals collected over many years. Staff of the veterinary laboratory, Skukuza for the use of laboratory facilities. Students and staff of the University of KwaZulu-Natal for the assistance with fish and rat analyses and the provision of infective material. Me. E. Umo for the assistance with analyses. Prof. J. Lamb for her contribution with the molecular analyses.

Management of Riverside farms and TSB Komatidraai farms for providing collection areas for tigerfish. Dr. J. Kotze for assistance with data analysis.

OUTLINE OF THESIS STRUCTURE

The thesis consists of a total of 6 chapters. Each chapter contains its own references section. Chapters 2 and 3 were published in peer reviewed journals. Chapters 4 and 5 were combined and submitted for publication in a peer reviewed journal.

Chapter 1 provides an introduction and literature review on trichinellosis with specific reference to *Trichinella zimbabwensis.* The rationale for the research, justification, aims and objectives of the study are discussed.

Chapter 2 summarises the molecular characterisation of *Trichinella* isolates found in different host species from the Greater Kruger National Park of South Africa from 1964-2016. The chapter was published in Journal of Helminthology, 2019, 93, 50-56.

Chapter 3 provides an update on the epidemiology and hypothetical transmission cycles of *Trichinella* infections in the Greater Kruger National Park, South Africa: an example of host-parasite interactions in an environment with minimal human interactions. This chapter was published in Parasite, 27, 1-11.

Chapter 4 is an experimental infection of the African sharp tooth catfish (*Clarias gariepinus*) with *T. zimbabwensis*. This chapter was combined with chapter 5 and submitted for publication in the Onderstepoort Journal of Veterinary Research.

Chapter 5 is an experimental infection of the tigerfish (*Hydrocynus vittatus*) with *T. zimbabwensis*.This chapter was combined with chapter 4 and submitted for publication in the Onderstepoort Journal of Veterinary Research.

Chapter 6 consists of synthesis of of study findings, significance, research gaps and suggestions for future studies in respect of *Trichinella* infections in Africa.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Table **Page**

LIST OF PLATES

Plate **Page**

LIST OF ABBREVIATIONS

AJOL - African Journals Online
bp - Base Pair
BRU - Biological Resource Unit
˚C - Degrees Celcius
DAFF - Department of Agriculture, Forestry and Fisheries
DC/AC - Direct Current/Alternating Current
DNA - Deoxyribonucleic acid
EBSCO - Elton B. Stephens Co.
ELISA - Enzyme Linked Serological Assay
ESV - Expansion Segment V
EU - European Union
FAO - Food and Agriculture Organization of the United Nations
GKNP - Greater Kruger National Park
ITRC - International *Trichinella* Reference Centre
ITS - Internal Transcriber Spacer
KNP - Kruger National Park
Kg - Kilogram
kGy - KiloGray
kw - Kilowatt
l - Litres
l/hour - Litres per hour
L_1 - First stage larva(e)
L_3 - Third stage larva(e)
lpg - Larvae per gram
lpg of fish – larvae per gram of fish
m - metres
MEGA6 - Molecular Evolutionary Genetics Analysis version 6
min - Minute
ml - Millilitre
ml/l - Millilitre per litre
NBL - Newborn larva(e)
OIE - World Organization for Animal Health
Paup - Phylogenetic analysis using parsimony
PCR - Polymerase Chain Reaction
p.i. - Post Infection
SANPARKS - South African National Parks
SD – Standard deviation
TFCA - Trans Frontier Conservation Area
µm - Micrometer
UKZN - University of KwaZulu- Natal
UV - Ultraviolet
W - Watt

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The history of *Trichinella* infections in wildlife in South Africa is brief and spans just over 60 years with the first documented reports from the Kruger National Park (KNP) dating back to 1966 (Marucci *et al*., 2009). The Greater Kruger National Park (GKNP) of South Africa represents a protected area where the abundance of sylvatic host species ensures both the *Trichinella* spp. survival and transmission (Marucci *et al*., 2009). Scholtz *et al*. (2013) reported that 1982 plant, 517 bird, 147 mammal and 21 reptile species exist in the approximate 20 000 km^2 of the Kruger National Park (KNP) of South Africa. Several pieces of private land are additionally included by proclamation as part of the protected area, adding approximately another 374.3 km^2 which, collectively is known as the GKNP (Scholtz *et al*., 2013). In the first 15 years since its initial report, the prevalence of infection was only reported twice in scientific publications and the reports included only four carnivorous mammal species and one rodent species (Young and Kruger, 1967; Young and Whyte, 1975). However, since the last report of *Trichinella* in an African civet (*Civettictis civetta*) (Young and Whyte, 1975) other wildlife species were tested in the KNP and one new host, the side-striped jackal (*Canis adustis*), was identified (Marucci *et al*., 2009). In all reports, the species reported were erroneously identified as *T. spiralis* (Marucci *et al*., 2009).

Advances in molecular techniques allowed scientists to identify two isolates, collected in 1966 and 1988 respectively as *Trichinella* T8 (Pozio *et al*., 1992). Another isolate collected in 1991 was described as *T. nelsoni* (La Rosa *et al*., 1992). Unfortunately, *Trichinella* surveillance in KNP was abandoned after 1991 and no information is available for the period 1991–2005. In 2006 the Mpumalanga Department of Agriculture through its Chief Directorate: Veterinary Services approved a proposal for the revival of wildlife surveillance for *Trichinella* by the Veterinary Public Health and Food Safety sub-directorate.

This has led to the description of the first report of a mixed infection of *Trichinella* T8 and *T. nelsoni* in a lion (Marucci *et al*., 2009) and the report of natural infection of *T. zimbabwensis* in wild Nile crocodiles (*Crocodylus niloticus*) from South Africa (La Grange *et al*., 2009; 2013) and a naturally infected lion (*Panthera leo*) (La Grange *et al*., 2010). A natural mixed infection of *Trichinella* T8 and *T. nelsoni* was also later described in a leopard (*Panthera pardus*) (La Grange *et al*., 2014).

1.2 Justification of the study

Three species of *Trichinella* (*T. nelsoni*, *Trichinella* T8 and *T. zimbabwensis*) have previously been reported in wild animals of the KNP of South Africa (Mukaratirwa *et al*., 2013). Mukaratirwa *et al*. (2013) emphasized, among others, the need for studies aimed at elucidating the occurrence and distribution of *Trichinella* species in wildlife protected areas in sub-Saharan Africa including KNP as well as identification of isolates using molecular techniques.

Among the most prevalent *Trichinella* species reported in sub-Saharan countries is *T. zimbabwensis* which is known to naturally infect Nile crocodiles in South Africa (La Grange *et al*., 2009; 2013) and was also reported in a naturally infected lion from the KNP (La Grange *et al*., 2010). These findings were included in a review paper and hypothetical transmission cycles for *T. nelsoni* and *T. zimbabwensis* in east- and southern Africa proposed by Mukaratirwa *et al*. (2013) (Figure 1.1 and 1.2). Many of the earlier *Trichinella* isolates could not be identified to species level due to limited molecular identification capability during that period. In order to improve on the hypotheses presented by Mukaratirwa *et al*. (2013) and to expand current knowledge on the prevalence and host distribution of *Trichinella* spp. infections in wildlife in KNP of South Africa, identification of recent isolates and the prevalence of *Trichinella* spp. in each host was required. This prompted further investigation into the molecular characterization of isolates collected between 2012 and 2016 to include the Mpumalanga- and Limpopo provinces of South Africa, parts of which forms part of the Greater Kruger National Park (GKNP).

Several unidentified isolates from various probable host species were obtained through passive surveillance during the period 2012–2016 and required molecular characterization to determine the species infecting different hosts. Subsequently, a review of previously reported *Trichinella* spp. was required with inclusion of more recent isolates to establish the prevalence of each of the *Trichinella* taxa.

La Grange *et al*. (2013) reported an 83.3 % prevalence of *T. zimbabwensis* in wild Nile crocodile populations of the KNP. Pozio *et al*. (2004) attributed the successful experimental infection of crocodiles and varans to the carnivorous and scavenging behaviour of members of these two species. A subsequent report of natural infections of Nile monitor lizards (*Varanus niloticus*) with *T. zimbabwensis* in Zimbabwe confirmed the involvement of Nile monitor lizards in the epidemiology of *T. zimbabwensis* (Pozio *et al*., 2007).

To date, no reports of *T. zimbabwensis* infection has been published in Nile monitor lizards from the KNP or adjacent areas, however, it is generally accepted that they also play a role in the epidemiology of *T. zimbabwensis* in the KNP. Previous reports concluded the high

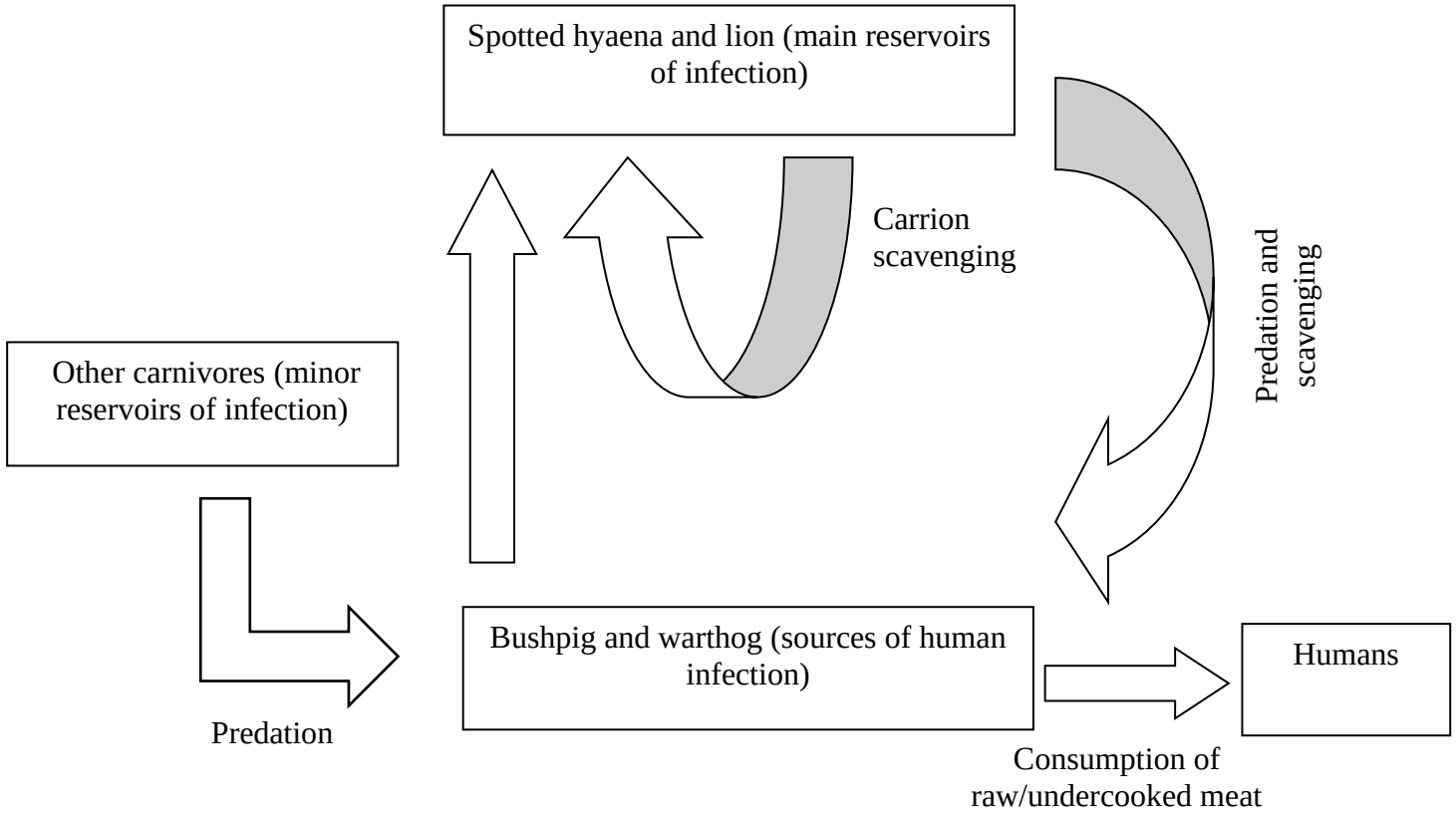


Fig. 1.1 Hypothetical sylvatic cycle of *Trichinella nelsoni* in East and Southern Africa (Adapted from Mukaratirwa *et al.* (2013)

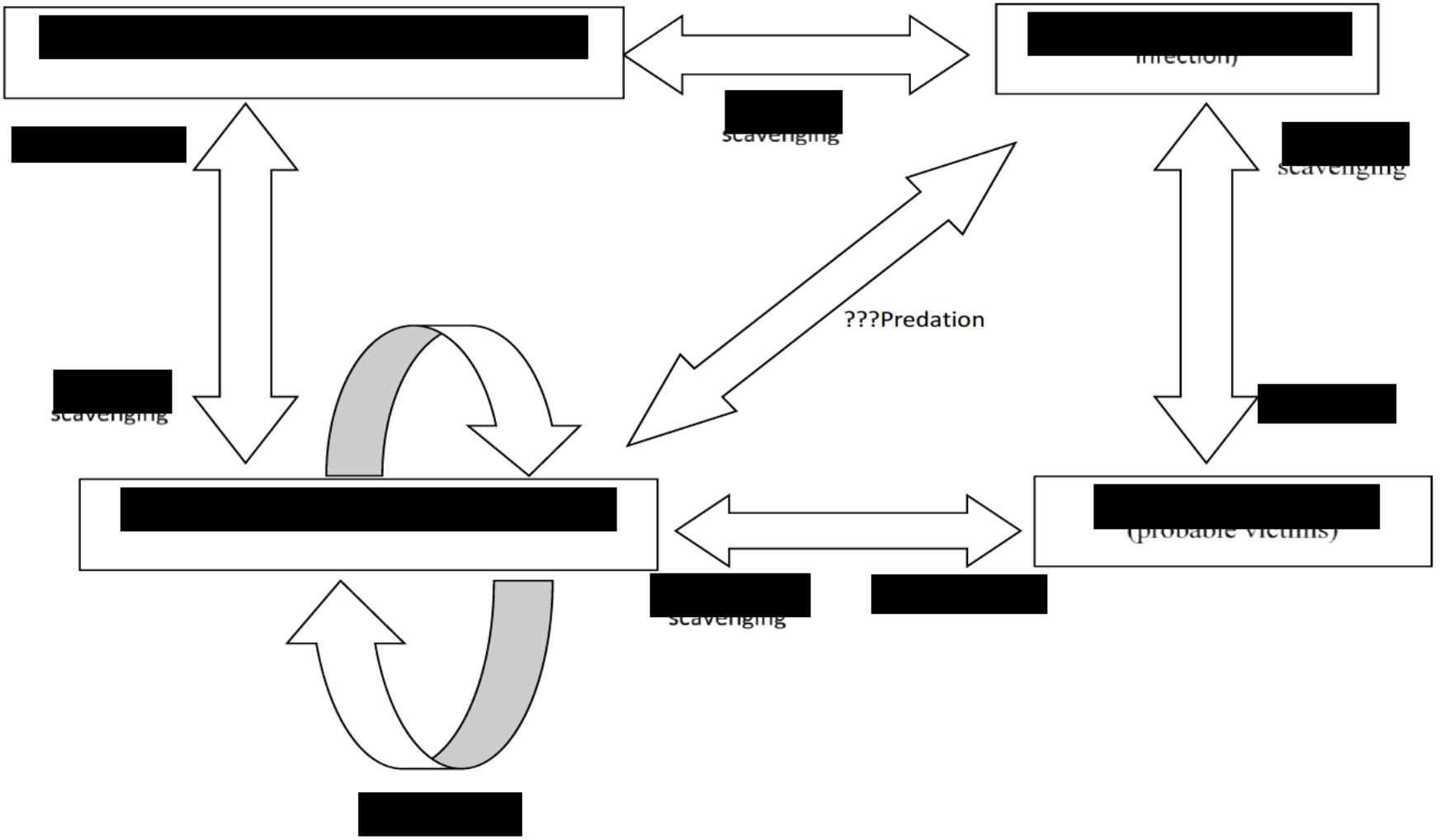


Figure 1.2 Hypothetical sylvatic cycle of *T. zimbabwensis* in East and Southern Africa (Adapted from Mukaratirwa *et al*. (2013)

prevalence of *T. zimbabwensis* among crocodiles from the KNP to be a natural phenomenon (La Grange *et al*., 2013) and the need for more epidemiological surveys to elucidate the role of other carnivorous and omnivorous mammals and reptiles cohabiting with crocodiles was expressed (Mukaratirwa *et al*., 2013).

Apart from additional reptilian and mammalian hosts, further investigation into the infectivity of *T. zimbabwensis* to other poikilothermic animals, especially omnivorous and scavenger fish and predatory fish which cohabit with Nile crocodiles is required to elucidate their potential role as reservoirs or propagators of the parasite.

Host characteristics are an important determinant for muscle predilection and infectivity of different *Trichinella* species to different hosts (Soule *et al*., 1989; Kapel *et al*., 1994; Kapel, 1995; Reina *et al*., 1996; La Grange *et al*., 2013). Experimental infection of fish with *T. britovi* showed migration of larvae to muscles and body cavity of the common carp (*Cyprinus carpio*) and catfish (*Ictalerus melas*) (Moretti *et al*., 1997). A similar study involving *T. pseudospiralis* and *T. spiralis* showed that *T. pseudospiralis* larvae migrated in unaltered form from the gastrointestinal tract to the body cavity, organs and muscles of fish while *T. spiralis* larvae were only found in the body cavity of fish (Tomašovičová, 1981). Tomašovičová (1981) included the European ruffe (*Gymnocephalus cernuus*), European perch (*Perca fluviatilis*), the common bleak (*Alburnus alburnus*) and the common carp in the experimental study but did not indicate in which of these species *Trichinella* spp. were found.

Current knowledge suggests that fish do not play any significant role in the epidemiology of *T. zimbabwensis* (Pozio and La Rosa, 2005). However, fish species cohabiting with known natural hosts of *T. zimbabwensis* have not been investigated.

Interspecies differences between *Trichinella* spp. also influences both muscle predilection and infectivity (Hurníková *et al*., 2004; Kapel *et al*., 2005). Several predatory fish species including tigerfish (*Hydrocynus vittatus*), smallmouth yellowfish (*Labeobarbus aeneus*), large scale yellowfish (*Labeobarbus marequensis*), African sharp tooth catfish (*Clarias gariepinus*) and largemouth bass (*Micropterus salmoides*) overlap with both Nile crocodiles and Nile monitor lizards in their natural habitat (Skelton, 2001). The carnivorous and/or scavenger behaviour of some species makes them more likely to act as potential reservoir hosts of *Trichinella* spp., especially *T. zimbabwensis* (Pozio and La Rosa, 2005).

Due to the impracticality of assessing the infectivity of *T. zimbabwensis* to all predatory fish cohabiting with crocodiles and varans in the GKNP, the criteria for selection

of fish species for experimental infection must be based on abundance and overlapping distribution and dietary habits, especially the ability to scavenge on flesh of known *T. zimbabwensis* hosts. The predatory tigerfish and the omnivorous African sharp tooth catfish are known to share their habitat with both crocodiles and Nile monitors in KNP, South Africa (Pienaar, 1968). Both these fish species are also known to overlap with crocodiles and Nile monitors in their natural range in the neighbouring countries of Zimbabwe and Mozambique where *T. zimbabwensis* is known to occur (Skelton, 2001). Based on the above, these species can, hypothetically, be considered as some of the most likely fish species to play a role in the epidemiology of *T. zimbabwensis*. The fact that these species also serve as source of food for humans further validates their investigation.

1.3 Aim of the study

To contribute to the understanding of the epidemiology of *Trichinella* infections in the GKNP with emphasis on the infectivity of *T. zimbabwensis* to selected fish species which cohabit with known hosts of the parasite.

1.4 Objectives of the study

The specific objectives of this study were to;

a. Determine the prevalence of *Trichinella* spp. in sylvatic hosts by reviewing previously published literature and molecular characterisation of more recent isolates found in the GNKP South Africa.

b. To synthesize published reports on *Trichinella* spp. isolated from wildlife in the KNP and elsewhere in Africa and infer inter- and intraspecies interaction and feeding behaviour of host species and; re-construct updated hypothetical life cycles for the *Trichinella* taxa known to circulate in GKNP.

c. Determine the infectivity of *T. zimbabwensis* to selected predatory fish which cohabit or share food and feeding habits with documented hosts of the parasite.

1.5 Literature Review

1.5.1 Historic overview of the genus *Trichinella*

At least half of the global population is estimated to be affected by food- and water-borne zoonoses (Macpherson *et al*., 2000). Trichinellosis is a food-borne parasitic disease that poses a global zoonotic threat (Murrell and Pozio 2011, Mukaratirwa *et al*., 2013) with often severe and sometimes fatal consequences for infected patients.

Human trichinellosis was first described by Owen (1835) and named *Trichina spiralis* following the discovery of "specks" in the diaphragm muscle of a deceased man during an autopsy by a first year medical student, James Paget (Campbell, 1979). However, the first discovery of the parasite has been attributed to Friedreich Triedemann who described "stony concretions" in human muscle of a cadaver as early as 1821 (Campbell, 1983) despite the absence of evidence that Triedemann's "concretions" actually described *Trichinella* larvae (Blancou, 2001). The first successful experimental infection involved dogs infected with larvae from badger meat in 1850 by Ernest Herbst followed by successful infection in mice by Leuckart in 1857 and subsequent experimental infection of a dog by Virchow in 1859 (Blancou, 2001). However, it was only after Leuckart observed the adult parasites in the small intestine of a deceased woman in 1860 that the life cycle of the parasite was elucidated by Leuckart and Virchow and the name altered from *Trichina spiralis* to *Trichinella spiralis* (Blancou, 2001). Herbst described the badger as the first naturally infected host and the parasite was described as enzootic in domestic pigs in Europe by the end of the 19th century (Blancou, 2001). At the same time infections were also reported in the United States of America and Chile (Blancou, 2001). By this time there was no doubt that pork was the principle source of infection to humans but another domestic source, the horse, was reported in 1975 (Touratier, 2001). By 2001 approximately 3000 cases of human infection in France and Italy could be attributed to the consumption of horse meat (Blancou, 2001).

Until recently all *Trichinella* isolates were considered to be *T. spiralis* but the recent advances in molecular techniques have led to the identification of 13 taxa including ten species and three genotypes (Pozio and Zarlenga, 2013; Sharma *et al*., 2019). Species/genotypes in the genus are considered to have a cosmopolitan distribution (Pozio and Murrell 2006; Pozio *et al*., 2009; Mukaratirwa *et al*., 2013) and naturally infect both endothermic and exothermic sylvatic carnivores and omnivores (Pozio, 2005; 2007; Pozio *et al*., 2009). A single species, *T. pseudospiralis* is capable of completing its life cycle in birds (Pozio, 2007). Today the natural hosts of *Trichinella* include well over 150 mammalian species, 13 bird species and at least three reptilian species (Pozio, 2005).

More importantly though is the zoonotic potential of these parasites, often causing mild to severe disease syndromes and even fatalities in humans (Gottstein *et al*., 2009). Several *Trichinella* spp. cause morbidity and even mortality in humans with *T. spiralis* and *T. britovi* being responsible for most human infections (Gottstein *et al*., 2009).

More than 65 000 human cases including 42 deaths were reported globally between 1986–2011 (Murrell and Pozio, 2011). However, only a small percentage (0.04%) of these infections were reported from sub-Saharan Africa (Murrell and Pozio, 2011). Although a variety of reasons to explain the low incidence of human infections in sub-Saharan Africa have been put forward (Pozio, 2005; 2007; Mukaratirwa *et al*., 2013), several other factors may preclude the determination of actual incidences (Bengis and Veary, 1997; Dupoy-Camet 2000; Pozio, 2007; Gottstein *et al*., 2009; Mukaratirwa *et al*., 2013; La Grange, 2013).

1.5.2 Characteristics and classification of *Trichinella* species

The genus *Trichinella* belongs to the family Trichinellidae and the Order Trichuridae, class Nematode (Pozio *et al*., 2009). It is composed of at least 10 species (*Trichinella spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. murelli*, *T. nelsoni*, *T. papuae*, *T. zimbabwensis*, *T. patagoniensis*, *Trichinella* T13) and 3 additional genotypes (*Trichinella* T6 related to *T. nativa* and T8 and T9 related to *T. britovi*) (Pozio and Zarlenga, 2013, Sharma *et al*., 2019). The *Trichinella* genus completes both its intermediate and definitive life cycle stages in a single host with larvae being intracellular parasites of striated muscle tissue and adult nematodes parasitizing the intestinal epithelium (Gottstein *et al*., 2009). Two clades (encapsulated and non-encapsulated) are recognized within the genus. The encapsulated clade includes *Trichinella britovi, T. murrelli, T. nativa, T. spiralis, T. nelsoni, T. patagoniensis*, *Trichinella* T6, *Trichinella* T8, *Trichinella* T9 and *Trichinella* T13, while the non-encapsulated clade consists of *T. pseudospiralis T. zimbabwensis* and *T. papuae* (Pozio and Zarlenga, 2013).

Host species serves as both definitive and intermediary hosts with the first stage larvae (L_1) representing the infective stage (Pozio, 2007, Pozio *et al*., 2009). Adult males average 1066 µm and females 1096 µm in length (Pozio *et al*., 2002). *Trichinella* establishes in the host when larvae contained in raw or undercooked meat is consumed (Dupoy-Camet, 2000).

1.5.3 Life cycle and predilection sites of first stage larvae in the host

Larvae are released during the host's normal digestive processes, maturing in the small intestine within 36-48 hours where they undergo four rapid moults before developing into adults after four to five days (Fabre *et al*., 2009, Gottstein *et al*., 2009). Newborn larvae (NBL) are released within five to seven days following infection (Gottstein *et al*., 2009). The first stage larvae (L_1) enter the circulatory system and establish in muscle cells where they may potentially survive for many years (Bruschi, 2012).

Through the evaluation of predilection muscles in different hosts, recommendations on suitable sampling sites, sample size and appropriate methods for detection of larvae have been developed (Gottstein *et al*., 2009). Wright *et al*. (1989) hypothesized that in light infections, larval distribution may be attributed to the passive transportation in the bloodstream and that larval survival is dependent on their ability to establish themselves in myofibres surrounded by venous capillary networks. Muscular blood supply is positively correlated with the frequency and intensity of movement required from the muscle (Folkow and Halicka, 1968; Andersen and Henriksson, 1977) and the most active muscles usually harbour the most larvae (Reina *et al*., 1996).

Previous studies have shown that predilection patterns of different *Trichinella* species vary with infection intensity in different hosts (Serrano and Pérez-Martín, 1999; La Grange and Mukaratirwa, 2014a). Additionally, anatomic and metabolic differences of host species may also affect parasite establishment in host musculature and differences have even been observed between hosts of the same family as shown by La Grange and Mukaratirwa (2014a) where predilection muscles in Nile crocodiles differed from those observed in a study involving Caimans (*Caiman crocodilus*) (Pozio *et al*., 2004). Studies involving Arctic foxes (*Alopex lagopus*) further showed predilection to be more dependent on the muscle's potential to move rather than the actual frequency of movement (Kapel *et al*., 1994; Kapel, 1995).

Despite the impact of host characteristics on muscle predilection, differences between parasite species also influence larval establishment in different hosts. These include differences in predilection (Hurníková *et al*., 2004; Kapel *et al*., 2005), larvae development sites (Wright *et al*., 1989) and differences in resistance to host immunity (Kociecka *et al*., 1980).

The geographic distribution and, to a certain extent, species specificity of different *Trichinella* taxa suggest that some environmental factors also influence the ability of the parasite to infect potential hosts. Most notably, temperature tolerance of the different taxa is a significant determinant for both geographic distribution and infectivity. Pozio *et al*. (2009) summarized the infectivity of different *Trichinella* species according to the temperature ranges preferred by their respective hosts. Climatological factors also directly impact on the survival of these parasites and the association between infectivity, geographic distribution, freezing tolerance and survival in decaying flesh have been discussed previously (Hurníková *et al*., 2004; Pozio *et al*., 2009).

Important as the above factors may be, results from several studies have suggested host characteristics to be the most important determinant for predilection selection (Soule *et al*., 1989; Kapel, 1995; Reina *et al*., 1996; La Grange *et al*., 2013). Kapel (1995) reported on the predilection of *Trichinella* larvae on herbivorous, carnivorous and omnivorous hosts and concluded that differences exist between carnivorous and herbivorous hosts.

1.5.4 Diagnosis

Several studies involving mammals have been conducted to find alternative methods for the detection of *Trichinella* infection. A number of serological assays were evaluated including enzyme immunoassay tests (Soule *et al*., 1989; Gamble *et al*., 1996), indirect immunofluorescence assays (Soule *et al*., 1989) and ELISA techniques (Soule *et al*., 1989; Reina *et al*., 1996; Pozio *et al*., 2002; Nöckler *et al*., 2009; Ludovisi *et al*., 2013). However, specific antibodies against *Trichinella* were only detectable for a short period of time following infection subsequently precluding their practical application (Ludovisi *et al*., 2013; La Grange and Mukaratirwa, 2014b). Pigs however, appear to be the exception since antibody titres reportedly persisted over extended periods and presumably remain detectable indefinitely except in wild boars which are less susceptible to certain *Trichinella* species (Gottstein *et al*., 2009).

Gamble *et al*. (1996) additionally indicated that the interval between infection and seroconversion in the host added to the impracticality as a surveillance tool. The rapid development of larvae into adults and subsequent rapid expulsion of adult parasites following reproduction does not allow the host to launch an effective immune response (Fabre *et al*., 2009). Antigenic heterogeneity of larvae and adult parasites further exacerbate this problem (Fabre *et al*., 2009).

Globally, efforts to eradicate the parasite from the human food chain and mitigate the risk of human infection have seen varying degrees of success and *Trichinella* still remains a notable zoonosis affecting communities in both developing and developed countries (Murrell and Pozio 2011; Mukaratirwa *et al*., 2013). Several authors have cited different reasons for the failure of control measures. The most notable reasons range from cosmopolitan distribution of species of the genus *Trichinella* (Pozio, 2007; Mukaratirwa *et al*., 2013), cultural eating habits favouring parasite transmission (Dupoy-Camet, 2000; Pozio, 2007; Mukaratirwa *et al*., 2013), poor animal husbandry (Pozio, 2000; 2001), globalization (Dupoy-Camet, 2000), reduced veterinary control resulting from changing political environments (Dupoy-Camet, 2000; Pozio, 2001; Gottstein *et al*., 2009), physicians' unfamiliarity with the clinical manifestations of infection (Dupoy-Camet,

2000; Gottstein *et al*., 2009), ecological changes (Dupoy-Camet, 2000) that includes the establishment of Trans Frontier Conservation Areas (TFCAs) (Mukaratirwa *et al*., 2013), poor communication and reporting between countries (Dupoy-Camet, 2000), inaccessibility of natural hosts for testing due to protective legislation (Pozio, 2005), physical and monetary challenges (La Grange, 2013) and sensitivity of direct testing methods in the absence of observable clinical manifestations of disease in animals (Gottstein *et al*., 2009). Additionally, the interspecies differences of host species and differences among parasite species hampers control efforts and successful detection of the parasite (Gottstein *et al*., 2009; La Grange and Mukaratirwa, 2014a).

A more positive indication in the control and prevention of *Trichinella* infections are the advances in biotechnology which have led to improved diagnosis, identification and reporting of outbreaks, explaining the emergence of new infection patterns that previously went unnoticed (Pozio, 2001; Pozio and Zarlenga, 2005; Pozio and Murrell, 2006; Pozio *et al*., 2009).

More than 65 000 cases of trichinosis in humans, including 42 fatalities were confirmed between 1986 and 2009 (Murrell and Pozio, 2011; Mukaratirwa *et al*., 2013). Despite differences in the biological and molecular structure of the species, clinical manifestations of the disease in humans follow a specific pattern with varying intensity depending on the infection dose and species of *Trichinella* involved (Kociecka, 2000).

Symptoms vary in accordance with the stage of the parasite and include those associated with gastrointestinal disease during the enteral phase of the parasite in the gut as well as muscular myositis in the systemic phase (Gottstein *et al*., 2009). The disease may manifest itself as an acute or chronic infection, but patients can remain asymptomatic depending on the initial infection dose (Gottstein *et al*., 2009).

In animals, the detection of muscle larvae in host tissue is the most common and widely accepted diagnostic method (European Commission, 2005). This is done by trichinoscopy or artificial digestion with additional serological methods such as ELISA employed as supplementary diagnostic tools in some instances (Nöckler *et al*., 2000). The efficacy and accuracy of diagnostic procedures in animals however, are dependent on several factors including the sample size, site and detection methods used for direct detection; as well as the time from infection to seroconversion and the persistence of antibodies in host species (Nöckler *et al*., 2000; La Grange, 2013). Identification to species level however, requires molecular techniques (Gottstein *et al*., 2009).

1.5.5 Treatment

The relatively low number of deaths reported for the period 1986-2009 (Mukaratirwa *et al*., 2013) suggests that current treatment regimens for humans are effective. However, the treatment costs still exceed that of preventative control measures (Gottstein *et al*., 2009).

Diagnostic protocols for the detection of human infection are well described and rely on the assessment of clinical symptoms, laboratory findings and epidemiological investigation (Gottstein *et al*., 2009). Following a positive diagnosis, treatment regimens involves the use of anthelmintics including albendazole, mebendazole, pyrantel (Kociecka, 2000; Gottstein *et al*., 2009) or thiabendazole (Kociecka, 2000). Glucocorticosteroids and protein and electrolyte replacement preparations (Kociecka, 2000; Gottstein *et al*., 2009) as well as immune-modulating drugs (Kociecka, 2000) are recommended.

Despite effective diagnostic and treatment regimens for human infections and the small number of infections reported from sub-Saharan countries (Murrell and Pozio, 2011), the disease has the potential to go as a misdiagnosis among many people on the African continent where the risk of infection is considerable. Possible reasons for the low incidence of human infections in sub-Saharan Africa (Pozio, 2005; 2007; Mukaratirwa *et al*., 2013) and factors precluding the determination of actual incidences (Bengis and Veary, 1997; Dupoy-Camet 2000; McGgregor, 2005; Pozio, 2007; Gottstein *et al*., 2009; Mukaratirwa *et al*., 2013; La Grange, 2013) have been put forward and may be summarized as follows: 1) the remote and vast nature of wildlife protected areas preclude access to proper slaughter facilities, diagnostic tools and education of rural communities; 2) the dependency of resource poor communities on local wildlife populations as a source of food; 3) cultural beliefs and practises in respect of food preparation that may not be aligned with preventative strategies; 4) preference to- and dependency on traditional healers and medicine; 5) misdiagnosis by physicians; 6) religious laws that prohibit the consumption of pork

There are treatment options aimed at inactivating or killing the parasite in meat and meat products such as cooking (> 71°C core temperature), freezing (-15°C for three to four weeks) and irradiation (0.3kGy) (Gottstein *et al*., 2009). Cooking of infected meat at high temperatures and sourcing meat from reputable sources are key measures that consumers can implement to protect themselves from meat-borne zoonoses (Sithole *et al*., 2020).

1.5.6 Prevention and control

Prevention of human infections relies on adequate control measures to curb potential transmission from both sylvatic and domestic hosts. Knowledge of potential sylvatic reservoirs, their potential epidemiological role in the life cycle and probable risks are of key importance to facilitate the design and implementation of control measures.

1.5.7 Overview of *Trichinella zimbabwensis*

Trichinella zimbabwensis is a non-encapsulating species and, unlike the encapsulated species, lacks a collagen capsule that surrounds the larva (Pozio *et al*., 2002; Pozio and Zarlenga 2005; Pozio *et al*., 2009). (Figure 1.3). It is known to naturally infect crocodiles in Zimbabwe, Ethiopia, Mozambique and South Africa (Pozio *et al*., 2007; La Grange *et al*., 2009) and Nile monitor lizards in Zimbabwe (Pozio *et al.*, 2007). Apart from its reptilian hosts, natural infections have also been found in mammalian hosts such as lions in South Africa (La Grange *et al*., 2010) and its potential as a zoonotic threat has been proven in successful experimental infection of primates (Mukaratirwa *et al*., 2008).

Figure 1.3 Non-encapsulated *Trichinella* larva (light microscope 1000x magnification) From: La Grange (2013). Image taken by L La Grange

Experimental studies with *T. zimbabwensis* in mammals and reptiles showed a protracted period of larval development in poikilothermic hosts resulting in larger larvae compared to isolates from mammalian hosts (Pozio *et al*., 2004). Both adult and larval stages of *T. zimbabwensis* display not only morphological similarities with *T. papuae*, but

adult males and females of both species can cross breed resulting in fewer and less viable F_2 larvae (Pozio *et al*., 2002). Genetic comparisons between the species confirmed that the three non-encapsulated species are more inter-related to each other than to any of the encapsulated species (Pozio *et al*., 2002). First-stage larvae of *T. zimbabwensis* are less resistant to freezing and lose their infectivity after 10 days at -10°C (Pozio *et al*., 2002). In rats, *T. zimbabwensis* can be transmitted from mother to offspring (Mukaratirwa *et al*., 2001) with both transplacental and transmammary infection routes having been confirmed (Matenga *et al*., 2006).

Genetic heterogeneity have been observed between isolates from different geographical locales (Pozio *et al*., 2009; La Grange *et al*., 2009) but *T. zimbabwensis* has a unique 264 base pair (bp) protein band (Pozio *et al*., 2009) making molecular distinction of this species possible through multiplex PCR. No human infections with *T. zimbabwensis* have been reported to date and there is little information on the distribution and epidemiology of this parasite.

Nile crocodiles and Nile monitor lizards are known natural hosts of *T. zimbabwensis* (Pozio, 2005). These species are voracious predators and equally accomplished scavengers in their natural aquatic environments where they coexist. A study of *T. zimbabwensis* in Nile crocodiles in the KNP revealed a prevalence of more than 80% of the animals studied and raised questions as to possible sources of infection (La Grange *et al*., 2013). Based on the above, it is widely accepted that a natural maintenance cycle for *T. zimbabwensis* exist between crocodiles and Nile monitor lizards based on interspecies predation and scavenging (Mukaratirwa *et al*., 2013). *Trichinella zimbabwensis* was also confirmed in a naturally infected lion in South Africa (La Grange *et al*., 2010). This finding supports the hypothetical life cycle proposed by Mukaratirwa *et al*. (2013) but also suggest that other hosts may exist in nature.

To date, no natural infections with *T. zimbabwensis* has been reported in fish and they are probably involved in the maintenance of the life cycle together with crocodiles and Nile monitor lizards. The most obvious fish species likely to be infected are those that not only share habitat with the known natural hosts, but serve as a source of food to both species. Moreover, if the suspected fish species are also accomplished predators and scavengers, their potential as hosts for *Trichinella* is considerably increased (Pozio and La Rosa, 2005).

A previous study suggested that selected equatorial fishes, specifically piranha (*Serrasalmus nattereri* and *S. rhombeus*), were not susceptible to *T. zimbabwensis* (Pozio

and La Rosa, 2005). These species were chosen based on their equatorial distribution and carnivorous nature (Pozio and La Rosa, 2005). However, he screened fish species are not indigenous to sub-Saharan Africa and do not cohabit with either Nile crocodiles or Nile monitor lizards in nature. Previous studies have shown that host characteristics play an important role in determining not only muscle predilection but also the infectivity of different *Trichinella* species to different hosts (Soule *et al*., 1989; Kapel *et al*., 1994; Kapel, 1995; Reina *et al*., 1996; La Grange *et al*., 2013). Studies with fish experimentally infected with encapsulated *T. britovi* and non-encapsulated *T. pseudospiralis* did show that the larvae, even though they did not develop into adults, migrated to the muscles and body cavity of some hosts and retained their infectivity for a limited period of time (Moretti *et al*., 1997; Tomašovičová, 1981). However, interspecies differences between *Trichinella* spp. have also been shown to influence both muscle predilection and infectivity in the same host (Kocieska *et al*., 1980; Hurníková, *et al*., 2004; Kapel *et al*., 2005). In order to fully understand the parasite epidemiology, the correct selection of a probable host species and *Trichinella* species based on their natural occurrence is of utmost importance.

Commercial farming of crocodiles is well established in several countries, including South Africa (La Grange *et al*., 2009; Botha, 2010) and skin and meat products destined for export to the European Union must undergo compulsory testing for *Trichinella* (European Commission, 2005). This compulsory testing has led to the detection of *Trichinella* spp. infection on two commercial crocodile farms in South Africa (Personal communication, DAFF, 2017).

Previous studies have shown that a gap exists between the testing of commercially slaughtered animals and breeding stock on farms (La Grange, 2013). Although meat from breeding stock is not considered for commercial purposes, improper feeding practises have been implicated in the transmission of the parasite from infected crocodiles to slaughter stock in Zimbabwe (Pozio *et al*., 2005).

Efforts to develop a serological test for the detection of *T. zimbabwensis* infection in crocodiles have to date been unsuccessful (Ludovisi *et al*., 2013; La Grange and Mukaratirwa, 2014b) and despite some promise shown in the use of biopsy sampling in breeding stock and wild populations (La Grange and Mukaratirwa 2014a), the practical application of this method presents several challenges: Apart from the obvious physical risk to handlers, the removal of a minimum required sample size of 10 grams of muscle tissue also presents an ethical dilemma since the post-surgical monitoring and care of the

animals is not practical. As a result, the information on *T. zimbabwensis* infection status on commercial crocodile farms in South Africa remains scanty.

Monitor lizards are considered to be the second most commercially exploited species of varanid (Pernetta, 1994; Jenkins and Broad 1994; Dowell *et al*., 2016). Eventhough they are mostly commercialised for the leather and pet trade, they are also a source of food in certain cultures (de Buffrenil and Hemery, 2002). The importance of varanids as a potential source of *Trichinella* infection to humans have been demonstrated previously with a report of human infection associated with the consumption of meat from a monitor lizard (*V. nebolosus*) in Thailand (Khamboonruang, 1991).

The commercial value of crocodiles and varanids combined with their close natural association and overlapping diets provides the incentive for studies aimed at identification of other probable sources of infection to these reptiles. Elucidating the potential role of synanthropic animals associated with crocodiles and varanids is important in unravelling the epidemiology of the parasite. In the absence of effective and practical methods to assess the actual status of commercial breeding stock and other animals not destined for meat export, it is necessary to at least assess the potential sources of infection to these animals to aid in the formulation of control strategies to mitigate such a risk.

1.6 References

Andersen P, Henriksson J. 1977. Capillary supply of the Quadriceps femoris muscle of man: Adaptive response to exercise. Journal of Physiology, 270, 677-690.

Bengis RG, Veary CM. 1997. Public health risks associated with the utilisation of wildlife products in certain regions of Africa. Scientific and Technical Review of the International Office of Epizootics, 16, 586-593.

Blancou J. 2001. History of Trichinellosis surveillance. Parasite, 8, S16-S19.

Botha PJ. 2010. The distribution, conservation status and blood biochemistry of Nile crocodiles in the Olifants river system, Mpumalanga, South Africa. Thesis (Ph.D.) Centre for Wildlife Management, Department of Animal and Wildlife Services, University of Pretoria, South Africa. 321 pages.

Bruschi F. 2012. Trichinellosis in developing countries: is it neglected? Journal of Infection in Developing Countries, 6, 216-222.

Campbell WC. 1979. History of trichinosis: Paget, Owen and the discovery of *Trichinella spiralis*. Bulletin of the History of Medicine, 53, 520-552.

Campbell WC. 1983. Historical introduction, in '*Trichinella* and Trichinosis'. Plenum Press. NY and London, 1983, 258 pages.

De Buffrenil V, Hemery G. 2002. Variation in longevity, growth and morphology in exploited Nile monitors (*Varanus niloticus*) from Sahelian Africa. Journal of Herpetology, 36, 419-426.

Dowell SA, Portik DM, de Buffrenil V, Ineich I, Greenbaum E, Kolokotronis S, Hekkala ER. 2016. Molecular data from contemporary and historical collections reveal a complex story of cryptic diversification in the *Varanus* (*Polydaedalus) niloticus* species group. Molecular Phylogenetics and Evolution, 94, 591-604.

Dupouy-Camet J. 2000. Trichinellosis: a worldwide zoonosis. Veterinary Parasitology, 93, 191–200.

European Commission. 2005. Commission Regulation (EC) No. 2075/2005 of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. Official Journal of the European Union, L338/60- L338/82.

Fabre MV, Beiting DP, Bliss SK, Appleton JA. 2009. Immunity to *Trichinella spiralis* muscle infection. Veterinary Parasitology, 159, 245-248.

Folkow B, Halicka HD. 1968. A comparison between “Red” and “White” muscle with respect to blood supply, capillary surface area and oxygen uptake during rest and exercise. Microvascular Research, 1, 1-14.

Gamble HR, Gajadhar AA, Solomon, MB. 1996. Methods for the detection of trichinellosis in horses. Journal of Food Protection, 59, 420-425.

Gottstein B, Pozio E, Nöckler K. 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clinical Microbiology Review, 22, 127–145.

Hurníková Z, Dubinsky S, Mukaratirwa S, Foggin CM, Kapel, CMO. 2004. Infectivity and temperature tolerance of non-encapsulating *Trichinella zimbabwensis* in experimentally infected Red foxes (*Vulpes vulpes*). Helminthologia, 41, 189–192.

Jenkins M, Broad S. 1994. International trade in reptile skins: A review and analyses of the main consumer markets, 1983-91. Traffic International, Cambridge. 75 pages.

Kapel C. 1995. *Trichinella* infections in Arctic foxes from Greenland: studies and reflections on predilection sites of muscle larvae. Journal of Helminthology, 69, 325-330.

Kapel C, Webster P, Gamble H. 2005. Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife. Veterinary Parasitology, 132, 101–105.

Kapel CM, Henriksen SA, Dietz HH, Henriksen P, Nansen P. 1994. A study on the predilection sites of *Trichinella spiralis* muscle larvae in experimentally infected foxes (*Alopex lagopus, Vulpes vulpes*). Acta Veterinaria Scandinavica, 35, 125-132.

Khamboonruang C. 1991. The present status of Trichinellosis in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health, 22, 312–315.

Kocieska, W. 2000. Trichinellosis: Human disease, diagnosis and treatment. Veterinary Parasitology, 93, 365-383.

Kociecka W, van Knapen F, Ruitenberg EJ. 1980. *Trichinella pseudospiralis* and *T. spiralis* infections in monkeys, I: Parasitological aspects. In: Kim CW, Ruitenberg EJ, Teppema JS. (Eds.), Proceedings of the Fifth International Conference on Trichinellosis, September 1-5, 1980, Noordwijkaan Zee, the Netherlands, 199-202.

La Grange LJ. 2013. Influence of *Trichinella zimbabwensis* infection intensity on predilection sites, blood biochemical values and humoral immune response in experimentally infected Nile crocodiles (*Crocodylus niloticus*). Dissertation (M.Sc.), University of KwaZulu-Natal, South Africa, 91 pages.La Grange LJ, Marucci G, Pozio E. 2009. *Trichinella zimbabwensis* in wild Nile crocodiles (*Crocodylus niloticus*) of South Africa. Veterinary Parasitology, 161, 88-91.

La Grange LJ, Govender D, Mukaratirwa S. 2013. The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. Journal of Helminthology, 87, 91-96.

La Grange LJ, Mukaratirwa S. 2014a. Distribution patterns and predilection muscles of *Trichinella zimbabwensis* larvae in experimentally infected Nile crocodiles (*Crocodylus niloticus* Laurenti). Onderstepoort Journal of Veterinary Research, 81, Art #652, 7 pages.

La Grange LJ, Mukaratirwa S. 2014b. Assessment of selected biochemical parameters and humoral immune response of Nile crocodiles (*Crocodylus niloticus*) experimentally infected with *Trichinella zimbabwensis*. Journal of the South African Veterinary Association, 85, Art#1085, 10 pages.

La Grange LJ, Reininghaus B, Mukaratirwa S. 2014. First report of a mixed infection of *Trichinella nelsoni* and *Trichinella* T8 in a leopard (*Panthera pardus*) from the Greater Kruger National Park, South Africa. Onderstepoort Journal of Veterinary Research, 81, 8-10.

La Grange LJ, Marucci G, Pozio E. 2010. *Trichinella zimbabwensis* in a naturally infected mammal. Journal of Helminthology, 84, 35-38.

La Grange LJ, Marucci G, Pozio E. 2009. *Trichinella zimbabwensis* in wild Nile crocodiles (*Crocodylus niloticus*) of South Africa. Veterinary Parasitology, 161, 88-91.

La Rosa G, Pozio E, Rossi P, Murrell KD. 1992. Allozyme analysis of *Trichinella* isolates from various host species and geographic regions. Journal of Parasitology 78, 641–646.

Ludovisi A, La Grange LJ, Gómez-Morales MA, Pozio E. 2013. Development of an ELISA to detect the humoral immune response to *Trichinella zimbabwensis* in Nile crocodiles. Veterinary Parasitology, 194, 189-192.

Macpherson CNL, Gottstein B, Geerts S. 2000. Parasitic food-borne and water-borne zoonoses. Scientific and Technical Review of the Office International des Epizooties (Paris), 19, 240-258.

Matenga E, Mukaratirwa S, Bhebhe E, Willingham AL. 2006. Evidence of congenital and transmammary transmission of *Trichinella zimbabwensis* in rats (*Rattus norvegicus*) and its epidemiological implications. International Journal for Applied Research in Veterinary Medicine, 4, 307-312.

Marucci G, La Grange LJ, La Rosa G, Pozio E. 2009. *Trichinella nelsoni* and *Trichinella* T8 in a lion (*Panthera leo*) of the Kruger National Park (South Africa). Veterinary Parasitology, 159, 225-228.

McGregor J. 2005. Crocodile crimes: people versus wildlife and the politics of post-colonial conservation on Lake Kariba, Zimbabwe. Geoforum, 36, 353-369.

Moretti A, Piergili-Fioretti D, Pasquali P, Mechelli L, Rossodivita ME, Polidori GA. 1997. Experimental infection of fish with *Trichinella* britovi: Biological evaluations. In: Ortega-Pierres G, Gamble HR, van Knapen F, Wakelin D. (Eds.), Trichinellosis. Centro de Investigacion y Estudios Avanzados IPN, Mexico City, 135-142.

Mukaratirwa S, La Grange LJ, Pfukenyi D. 2013. *Trichinella* infections in animals and humans in sub-Saharan Africa: A review. Acta Tropica, 125, 82-89.

Mukaratirwa S, Dzoma BM, Matenga E, Ruziwa SD, Sacchi L, Pozio E. 2008. Experimental infections of baboons (*Papio* spp.) and vervet monkeys (*Cercopithecus aethiops*) with *Trichinella zimbabwensis* and successful treatment with ivermectin. Onderstepoort Journal of Veterinary Research, 75, 173-180.

Mukaratirwa S, Magwedere K, Matenga E, Foggin CM. 2001. Transmission studies on *Trichinella* species isolated from *Crocodylus niloticus* and efficacy of fenbendazole and levamisole against muscle L1 stages in Balb C mice. Onderstepoort Journal of Veterinary Research, 68, 21–25.

Murrell KD, Pozio E. 2011. The worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerging Infectious Diseases, 17, 2194-2202.

Nöckler K, Reckinger S, Broglia A, Mayer-Scholl A, Bahn P. 2009. Evaluation of a Western Blot and ELISA for the detection of anti-*Trichinella*-IgG in pig sera. Veterinary Parasitology, 163, 341–347.

Nöckler K, Pozio E, Voigt WP, Heidrich J. 2000. Detection of *Trichinella* in food animals. Veterinary Parasitology, 93, 335-350.

Owen R. 1835. Description of a microscopic entozoon infesting the muscles of the human body. Transactions of the Zoological Society of London 1, 315–324.

Pernetta AP. 1994. Monitoring the trade: Using the CITES database to examine the global trade in live monitor lizards (*Varanus* spp.). Biawak, 3, 37-45.

Pienaar, U. de V., 1968, 'The freshwater fishes of the Kruger National Park:' Pretoria, National Parks Board, 1-20.

Pozio E. 2007. World distribution of *Trichinella* spp. infections in animals and humans. Veterinary Parasitology, 149, 3–21.

Pozio E. 2005. The broad spectrum of *Trichinella* hosts: From cold- to warm-blooded animals. Veterinary Parasitology, 132, 3-11.

Pozio E. 2001. New patterns of *Trichinella* infection. Veterinary Parasitology, 98, 133-148.

Pozio E. 2000. Factors affecting the flow among domestic, synanthropic and sylvatic cycles of *Trichinella*. Veterinary Parasitology, 93, 241-262.

Pozio E, Zarlenga DS. 2013. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. International Journal for Parasitology, 35, 1191- 1204.

Pozio E, Hoberg E, La Rosa G, Zarlenga DS. 2009. Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. Infection, Genetics and Evolution, 9, 606–616.

Pozio E, Foggin CM, Gelanew T, Marucci G, Hailu A, Rossi P, Gomez-Morales MA. 2007. *Trichinella zimbabwensis* in wild reptiles of Zimbabwe and Mozambique and farmed reptiles of Ethiopia. Veterinary Parasitology, 143, 305–310.

Pozio E, Murrell K. 2006. Systematics and epidemiology of *Trichinella*. Advances in Parasitology, 63, 372-445.

Pozio E, La Rosa G. 2005. Evaluation of the infectivity of *Trichinella papuae* and *Trichinella zimbabwensis* for equatorial freshwater fishes. Veterinary Parasitology, 132, 113-114.

Pozio E, Zarlenga DS. 2005. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. International Journal for Parasitology, 35, 1191-1204.

Pozio E, Marucci G, Casulli A. 2004. *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles. Parasitology, 128, 333-342.

Pozio E, Foggin CM, Marucci G, La Rosa G, Sacchi L, Corona S, Rossi P, Mukaratirwa S. 2002. *Trichinella zimbabwensis* n.sp. (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. International Journal for Parasitology, 32, 1787–1799.

Pozio E, La Rosa G, Rossi P, Murrell KD. 1992. Biological characterizations of *Trichinella* isolates from various host species and geographic regions. Journal of Parasitology, 78, 647–653.

Reina D, Munoz-Ojeda M, Serrano F. 1996. Experimental trichinellosis in goats. Veterinary Parasitology, 62, 125–132.

Serrano FJ, Pérez-Martín J. 1999. Influence of infection intensity on predilection sites in swine trichinellosis. Journal of Helminthology, 73, 251–254.

Scholtz M, Kruger M, Saayman M. 2013. Understanding the reasons why tourists visit the Kruger National Park during a recession. Acta Commercii, 13, 9 pages.Serrano FJ, Pérez-Martín J. 1999. Influence of infection intensity on predilection sites in swine trichinellosis. Journal of Helminthology, 73, 251–254.

Sharma R, Konecsni K, Scandrett B, Thompson P, Hoberg EP, Bouchard E, Buhler K, Harms NJ, Kukka PM, Jung TS, Elkin B, Mulders R, Larter NC, Branigan M, Pongracz J, Wagner B, Fenton H, Rosenthal BM, Jenkins E. 2019. Distribution and genetic diversity of *Trichinella* in Canadian wildlife: A previously undescribed species (T13) and an unexpected discovery of *T. spiralis*. Scientia Parasitologica, 20, 145-148.

Sithole M, Bekker J, Mukaratirwa S. 2020. Consumer knowledge and practices to pork safety in two *Taenia solium* cysticercosis endemic districts in Eastern Cape Province of South Africa. BMC Infectious Diseases, 20, 1-8.

Skelton P. 2001. Complete guide to freshwater fishes of South Africa. Struik Publishers (Pty) Ltd; 2nd edition, ISBN 1868726436, 395 pages.

Soule C, Dupoy-Camet J, Georges P, Ancelle T, Gillet JP, Vaissaire J, Delvigne A, Plateau E. 1989. Experimental trichinellosis in horses: Biological and parasitological evaluation. Veterinary Parasitology, 31, 19-36.

Tomašovičová O. 1981. The role of fresh water fish in transfer and maintenance of Trichinellae under natural conditions. Biolŏgia, 36, 115-125.

Touratier L. 2001. A challenge of veterinary public health in the European Union: Human trichinellosis due to horse meat consumption. Parasite, 252-256.

Wright KA, Matta I, Hong HP, Flood N. 1989. *Trichinella* larvae and the vasculature of the murine diaphragm. In: Tanner CE, Martinez-Fernandez AR, Bolas-Fernandez F. (Eds.), Trichinellosis. Madrid, CSIC Press. 70–75.

Young E, Whyte IJ. 1975. Trichinosis (*Trichinella spiralis* infestations) in wild animals of the Kruger National Park. Journal of the South African Veterinary Association, 46, 233–234.

Young E, Kruger SP. 1967. *Trichinella spiralis* (Owen, 1935) Railliet 1895. Infestation of wild carnivores and rodents in South Africa. Journal of the South African Veterinary Association, 38, 441–443.

CHAPTER 2: PREVALENCE AND MOLECULAR IDENTIFICATION OF *TRICHINELLA* SPECIES ISOLATED FROM WILDLIFE ORIGINATING FROM LIMPOPO AND MPUMALANGA PROVINCES OF SOUTH AFRICA

Published as:

Mukaratirwa S, La Grange LJ, Malatji MP, Reininghaus B, Lamb J. 2019. Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga Provinces of South Africa. Journal of Helminthology, 23, 1-7.

2.1 Abstract

Trichinella species are widely distributed on all continents with the exception of Antarctica, although the full spectrum of *Trichinella* species found in sub-Saharan African countries, and their hosts, has not been fully documented. This study was conducted to determine the prevalence of *Trichinella* spp. in wildlife from the Greater Kruger National Park and adjacent areas located in the Limpopo and Mpumalanga provinces of South Africa, and to identify the species and/or genotypes of *Trichinella* larvae isolated from muscle tissues, using molecular techniques. A review of *Trichinella* spp. and their wildlife hosts reported during 1964–2011 was also conducted and the results were compared with our current study. Two hundred and nine samples representing 20 mammalian and 1 reptilian species were screened during this period. Of these 17 conformed to *T. zimbabwensis* (16 crocodiles and 1 lion), three isolates conformed to *T. nelsoni* (lion) and 5 conformed to *Trichinella* T8 (4 lions and 1 hyaena). Twenty isolates were not identified to species level (6 lion, 11 hyaena, 1 jackal, 1 civet and 1 rodent). The remaining 164 samples were all negative for *Trichinella* spp. infection. Ninety samples representing 15 mammalian, two avian- and three reptilian species were screened for *Trichinella* infection during 2012–2016, using artificial digestion. Isolates detected were identified using a multiplex polymerase chain reaction amplification of the internal transcriber spacers ITS1, ITS2, and expansion segment V (ESV) regions of ribosomal DNA, followed by molecular analysis of the sequences. Twenty samples from seven wildlife species were positive for *Trichinella* spp. larvae, with an overall prevalence of 21.1% (20/90). The prevalence was higher in carnivores (18.9%, 18/90) than in omnivores (2.2%, 2/90). Analysis of sequences showed that eight of the isolates – two from spotted hyaena (*Crocuta crocuta*) (2/8), three from lion (*Panthera leo*) (3/13), one from leopard (*Panthera pardus*) (1/6), one from small

spotted genet (*Genetta genetta*) (1/2) and one Nile monitor lizard (*Varanus niloticus*) (1/2) conformed to *Trichinella zimbabwensis*. One isolate from a hyaena was grouped under the encapsulated species clade comprising *T. nelsoni* and genotype *Trichinella* T8 reported to be present in South Africa. This is the first report confirming natural infection of *T. zimbabwensis* in hyaena, leopard, genet and Nile monitor lizard, adding to the body of knowledge on the epidemiology of *Trichinella* infections in the GKNP of South Africa. Ten *Trichinella*-like larval isolates recovered after digestion from four wildlife species in this study (2012–2016) revealed inconclusive results due to DNA degradation from poor storage or too few larvae for analysis, in comparison to 20 unidentified isolates from five wildlife species during the 1964–2011 period.

2.2 Introduction

Trichinellosis is an important zoonotic disease caused by the infectious nematodes of the genus *Trichinella* (Fu *et al.,* 2009; Gottstein *et al.,* 2009; Pozio *et al.,* 2009; Krivokapich *et al.,* 2012). The parasite belongs to the family Trichinellidae, phylum Nematoda, and there are currently nine encapsulated species and genotypes, namely *Trichinella britovi, T. murrelli, T. nativa, T. spiralis, T. nelsoni, T. patagoniensis*, *Trichinella* T6, *Trichinella* T8 and *Trichinella* T9, with three additional non-encapsulated species: *T. pseudospiralis T. zimbabwensis T. papuae* (Pozio and Zarlenga, 2013).

Trichinella spp. have a direct life cycle characterized by completing both intermediary and definitive stages in a single host (Pozio, 2007; Gottstein *et al.,* 2009; Pozio *et al.,* 2009). Unlike other nematodes, *Trichinella* spp. are also characterized by an infective first larval stage (L_1) in contrast to the typical infective third-stage larvae (L_3) found in most nematode genera (Gajadhar *et al.,* 2009; Pozio *et al.,* 2009). Vertebrates, including humans, are infected through the ingestion of raw or undercooked meat infected with *Trichinella* larvae in the muscle tissue (Gottstein *et al.,* 2009). Newborn larvae (NBL) are transported passively to the striated muscles (Dupouy-Camet, 2000; Gottstein *et al.,* 2009) via the host lymphatic and blood vessels (Gottstein *et al.,* 2009). Based on the findings by Gottstein *et al.* (2009), *Trichinella* larvae may remain viable in the nurse muscle for many years after encysting. This however, may depend on the *Trichinella* species/genotype and the host's immune response.

Cases of trichinellosis have been reported worldwide with the exception of Antarctica (Pozio *et al.,* 2009; Mukaratirwa *et al.,* 2013). *T. zimbabwensis, T. britovi, T. nelsoni* and genotype T8 have been reported in sub-Saharan Africa and occur mainly in carnivorous and omnivorous sylvatics with *T. zimbabwensis* being the most prevalent

species (Mukaratirwa *et al.,* 2013). According to Blaga *et al.* (2009), 10 000 cases of human trichinellosis have been reported globally, with an annual mortality rate as low as 0.2% (Pozio, 2007). Generally, animals remain asymptomatic and, in the absence of clinical disease manifestation, infection with *Trichinella* spp. is referred to as *Trichinella* infection rather than trichinellosis (Gottstein *et al.,* 2009).

T. zimbabwensis, *T. nelsoni* and *Trichinella* T8 have been reported in wildlife from South Africa during epidemiological investigations in the Greater Kruger National Park (GKNP) and adjacent areas of the Limpopo and Mpumalanga Provinces (Marucci *et al.,* 2009; La Grange *et al.,* 2010; Mukaratirwa *et al.,* 2013; La Grange *et al.,* 2014). The main reservoirs for *Trichinella* spp. in the KNP are carnivorous wildlife with scavenging and cannibalistic behaviour (Mukaratirwa *et al.,* 2013). The spotted hyaena (*Crocuta crocuta*) and the lion (*Panthera leo*) appear to be the major reservoirs for *Trichinella* infections as they currently have the highest documented prevalence in South Africa (Marucci *et al.,* 2009; Mukaratirwa *et al.,* 2013). Mixed infections of *T. nelsoni* and *Trichinella* T8 have been reported in both a lion and a leopard (Marucci *et al.,* 2009; La Grange *et al.,* 2014) and *T. zimbabwensis* has also been reported in a lion (La Grange *et al.,* 2010). To date, no report of human infections or cases involving domestic animals have been documented in South Africa, despite this country having the highest documented prevalence of *Trichinella* infections in wildlife in sub-Saharan Africa (Mukaratirwa *et al.,* 2013).

There is paucity of information on *Trichinella* infections in humans, domestic and wild animals in most of sub-Saharan Africa (Dupouy-Camet, 2000; Pozio, 2007) including South Africa. Hence, the aim of this study was to close the gap by determining the prevalence of *Trichinella* infection in convenience samples collected from wildlife species from the GKNP and identifying the *Trichinella* spp. larvae isolates using molecular techniques.

2.3 Materials and methods

2.3.1 Sample collection and processing

Muscle samples were conveniently collected from carcasses of wildlife either culled from nature reserves, killed by hunters or animals that died of natural causes or as a result of vehicular accidents and/or poisoning in the GKNP, private nature reserves and towns neighbouring the GKNP in Limpopo and Mpumalanga provinces. Ninety samples representing 15 mammal, three reptile and two bird species were collected and tested during the period 2012–2016 (Table 2.1 and Table 2.2). Samples were digested as described by Nöckler and Kapel (2007) as preliminary screening for presence of *Trichinella* larvae in

the muscle sample. From the positive samples, *Trichinella* larvae were collected in small vials containing 70% ethanol and later used for DNA extraction. Due to logistic constraints, some of the muscle samples were either frozen and kept for extended periods of time prior to testing, or in other instances sample collection was delayed, resulting in larvae being degraded and not suitable for molecular analysis.

2.3.2 DNA extraction from *Trichinella* larvae

Genomic DNA was extracted from at least five larvae of each positive sample whenever possible, using the genomic DNA™ tissue mini-prep kit (Zymo Research Corporation, Irvine, California) according to manufacturer's instructions.

2.3.3 Polymerase chain reaction and sequencing

DNA was subjected to multiplex polymerase chain reaction using primers ESVIF + ESVIR, ITS1AF + ITS1AR, ITS1BF + ITS1BR, ITS2AF + ITS2AR and ITS2BF + ITS2BR as described by Zarlenga *et al.,* (1999) (Table 2.3). The amplifications were performed in reactions of 50 µl volume containing 20 µl TopTaq master mix, 2 µl of each primer (forward and reverse) and 10 µl of DNA. Thermal cycling was carried out at 94˚C for 3 min; followed by 40 cycles of 94˚C for 1 min, 60˚C for 1 min and 72˚C for 2 min; followed by a final extension at 72˚C for 7 min. Amplicons were separated by electrophoresis in 2% agarose gels, and visualised by staining with ethidium bromide. A laboratory-maintained reference strain of *T. zimbabwensis* was used as control. Polymerase Chain Reaction (PCR) amplification products of the expansion segment V (ESV) region were sent for sequencing by the Sanger dideoxy method at Inqaba Biotechnical Industries (Pty) Ltd., South Africa.

2.3.4 Data and molecular analysis

The number of wildlife species with positive isolates from digestion of muscle were tabulated and compared with the data reported in the period of 1964–2011. Sequence alignments were analysed using the maximum parsimony method in PAUP 4.0b10 (Swofford, 2002) and Bayesian inference as implemented in MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001). For parsimony analyses, starting trees were obtained by stepwise addition. The addition sequence was random, with one tree held at each step and with ten replicates. Node support was estimated using 1000 bootstrap replicates. Bayesian analyses were run using four Markov chains, sampling every 100 generations, for 500,000 generations, or until the standard deviation of the split frequencies was less than 0.01. The chains were heated with the temperature scaling factor $T = 0.02$. I discarded the first 2000

trees as burn-in, in each case having checked in a preliminary run that this was more than sufficient to achieve stationarity.

Bayesian inference trees were presented, with node support indicated as Bayesian posterior probabilities and maximum parsimony bootstrap values.

Analyses included *T. zimbabwensis, T. nativa, T. papuae, T. spiralis, T. britovi, T. pseudospiralis,* genotype T12 and genotype T8 as in-groups and *Paratrichosoma* spp. and *Trichuris arvicolae* as outgroups. Individual pairwise genetic p-distances between the sequences were determined using MEGA6 (Tamura *et al*., 2013).

2.4 Results

2.4.1 Prevalence of *Trichinella* spp. from muscle digestion

Extrapolated data from previously published reports (1964–2011) were combined with the findings of this study (2012–2016) on the screening of *Trichinella* larvae and identification of *Trichinella* spp. in wildlife carnivores (Table 2.1) and omnivores (Table 2.2) from South Africa. Results show that *T. zimbabwensis* was the most prevalent species recorded to date. The species has been reported in five wildlife species (lion, hyaena, leopard, genet and Nile monitor lizard (Tables 2.1 and 2.2).

Trichinella nelsoni was reported in a leopard and *Trichinella* T8 was reported once in a leopard as a mixed infection with *T. nelsoni* (Table 2.1). A similar mixed infection was also reported previously in a lion (Marucci *et al.,* 2009) (Table 2.1).

Ninety muscle samples from 20 wildlife species (15 mammals, 3 reptiles and 2 birds) were screened. Twenty (20) samples from seven wildlife species were positive for *Trichinella* spp. through digestion (six mammals and one reptile) (Table 2.1 and 2.2) and *Trichinella* prevalence was higher in carnivores (18.9%, 18/90) than in omnivores (2.2%, 2/90). In the period from 1964–2011, 45 samples from nine wildlife species were positive for *Trichinella* spp. through digestion (seven mammals, and one reptile) (Table 2.1) with a prevalence of 21.4% (43/210). The prevalence of *Trichinella* was 20.5% (43/210) in carnivores and 0.9% (2/210) in omnivores.

Table 2.1 Wild carnivores from the Greater Kruger National Park, South Africa, screened for *Trichinella* spp. larvae and the prevalence of *Trichinella* spp. in each species for the period 2012–2016 and previous studies (1964–2011)

Animal species	Current study (2012-2016)						Previous study (1964-2011)						
	No Positive/Tested	Tz	Tn	T8	NID	Total prevalence (%)	No Positive/Tested	Tz	Tn	T8	NID	Total prevalence (%)	**References**
Panthera leo	8/13	3	-	-	5	61	14*/85	1	3	4	6	16.5	Young & Kruger (1967), Pozio et al. (1994), La Rosa & Pozio (2000), Marucci et al., 2009, La Grange et al. (2010)
Panthera pardus	2*†/6	1	1	1	0	33.3	0/1	-	-	-	-	NC	Young & Whyte (1975), Marucci et al. (2009)
Varanus niloticus	1/2	1	-	-	-	-	0/0	-	-	-	-	NC	
Crocuta crocuta	5/8	2	-	-	3	62.5	12/18	-	-	1	11	67	Young & Kruger (1967); Marucci et al., 2009
Lycaon pictus	0/4	-	-	-	-	0	0/0	-	-	-	-	NC	
Necrosyrtes monachus	0/1	-	-	-	-	NC	0/0	-	-	-	-	NC	
Manis temminckii	0/1	-	-	-	-	NC	0/0	-	-	-	-	NC	
Varanus albigularis	0/1	-	-	-	-	NC	0/0	-	-	-	-	NC	
Asio capensis	0/1	-	-	-	-	NC	0/0	-	-	-	-	NC	
Naja annulifera	0/1	-	-	-	-	NC	0/0	-	-	-	-	NC	
Felis silvestris lybica	1/1	-	-	-	1	NC	0/0	-	-	-	-	NC	
Canis mesomelas	0/1	-	-	-	-	NC	0/0	-	-	-	-	NC	
Canis adustus	0/0	-	-	-	-	NC	1/3	-	-	-	1	NC	Marucci et al., 2009
Crocodylus niloticus	0/0	-	-	-	-	NC	16/43	16	-	-	-	37	La Grange et al. (2009; 2013)

Tz = *Trichinella zimbabwensis*, Tn = *Trichinella nelsoni*, T8 = *Trichinella* genotype T8, * One animal represents a mixed infection of *Trichinella nelsoni* and *Trichinella* T8, NC = Not calculated due to sample size < 4, † Four animals reported by La Grange et al (2014) included in present work. Adapted from Mukaratirwa et al. (2019)

Table 2.2 Wild omnivores from the Greater Kruger National Park, South Africa, screened for *Trichinella* spp. larvae and the prevalence of *Trichinella* spp. in each species for the period 2012–2016 and previous studies (1964–2011)

Animal species	Current study (2012-2016)						Previous study (1964-2011)						References
	No Positive/Tested	Tz	Tn	T8	NID	Total prevalence (%)	No Positive/Tested	Tz	Tn	T8	NID	Total prevalence (%)	
Civettictis civetta	0/1	-	-	-	-	NC	1/1	-	-	-	1	100	Young & Whyte (1975), Marucci et al., 2009
Genetta genetta	1/2	1	-	-	0	50	0/0	-	-	-	-	NC	
Phacocoerus africa	0/35*	-	-	-	0	0	0/12	-	-	-	-	0	Young & Kruger (1967); Marucci et al., 2009
Mellivora capensis	0/2	-	-	-	0	NC	0/0	-	-	-	-	NC	
Papio ursinus	1/6	-	-	-	1	17	0/0	-	-	-	-	NC	
Chlorocebus pygerythrus	0/1	-	-	-	1	NC	0/0	-	-	-	-	NC	
Potamochoerus larvatus	0/2	-	-	-	2	NC	0/0	-	-	-	-	NC	
Mungos mungo	0/1	-	-	-	1	NC	0/2	-	-	-	-	NC	Young & Whyte (1975), Marucci et al., 2009
Praomys natalensis	0/0	-	-	-		0	1/44	-	-	-	1	0.02	Young & Kruger (1967); Marucci et al., 2009

Tz = *Trichinella zimbabwensis*, Tn = *Trichinella nelsoni*, T8 = *Trichinella* genotypte T8, NC = Not calculated due to sample size < 4, NID = not identified, *17 Warthogs reported by La Grange et al, (2014) included in present work. Adapted from Mukaratirwa et al. (2019)

2.4.2 Multiplex polymerase chain reaction analysis and phylogenetic analysis

Electrophoresis of multiplex PCR amplicons of putative *Trichinella* isolates produced two general types of amplification pattern. One of these contained two main bands, with sizes of approximately 270 and 350 bp, and was shared by isolates from the hyaena, lion, monitor lizard and a *T. zimbabwensis* laboratory reference strain. This is generally consistent with the identification of these isolates as *T. zimbabwensis*. A contrasting amplification pattern, comprising two smaller main bands of approximately 150 and 250 nucleotides was shared by a genet, lion and leopard.

These isolates remain unidentified. It is interesting that a similar main banding pattern (bands of 127 and 253 nucleotides) was exhibited by *Trichinella* isolate T3 (Zarlenga *et al.*, 1999). Isolates from the African wildcat (*Felis silvestris lybica*), chacma baboon (*Papio ursinus*) and marsh owl (*Asio capensis*) did not produce amplification patterns. Alignment of ESV DNA sequences was created, based on 105 nucleotides (Fig. 2.1) as the sequences contained areas of microsatellite repeats, and did not all yield good quality sequence for the entire length. The alignment resolved phylogenetic relationships with support values as shown in *Trichinella* isolates formed a monophyletic clade (A) with reference to the outgroups (Fig. 2.1). There was strong support for clade (F) which included *T. papuae* and *T. zimbabwensis* from GenBank and experimental isolates from our study. The *T. papuae* clade (G) was strongly supported and sister to *T. zimbabwensis* (Fig. 2.1), a well-supported clade (H) comprising GenBank *T. zimbabwensis* samples and experimental *Trichinella* isolates from two hyaenas, lion, leopard, monitor lizard, genet and *T. zimbabwensis* reference isolate. Based on the phylogenetic species concept (Cracraft, 1983), these experimental isolates are *T. zimbabwensis.* Further, genetic distances separating these isolates from the *T. zimbabwensis* reference sample are small (0.00 to 0.02), and consistent with those separating other GenBank samples of *T. zimbabwensis* from the reference strain (also 0.00 to 0.02). This is consistent with the identification of the experimental isolates as *T. zimbabwensis* based on the genetic species concept (Baker and Bradley, 2006). In contrast, and as would be expected, genetic distances between *T. zimbabwensis* isolates and other *Trichinella* species are considerably higher, consistent with a greater level of taxonomic separation from *T. papuae* (0.11 to 0.16), *T. pseudospiralis* (0.26 to 0.31), *T. nativa* (0.36 to 0.39), *T. britovi* (0.38 to 0.41) and *T. spiralis* (0.43 to 0.47).

A third hyaena isolate was present in a strongly supported unresolved clade (D) containing GenBank samples of the encapsulated species *T. spiralis*, *T. britovi* and *T. nativa* (figure not shown), although it could not be identified to species level (Fig. 2.1).

Table 2.3 Nucleotide sequences amplified during multiplex polymerase chain reaction with respective forward and reverse oligonucleotide sequences

Amplified sequence	Oligonucleotide sequence
ESVF	5'-GTTCCATGRGAACAGCAGT-3'
ESVR	5'-CGAAAACATAGCACAACTGC-3'
ITS1AF	5'-GCTACATCCTTTTGATCTGTT-3'
ITS1AR	5'-AGACACAATATCAACCACAGTACA-3'
ITS1BF	5'-GCGGAAGGATCATTATCGTGTA-3'
ITS1BR	5'-TGGATTACAAAGAAAACCATCACT-3'
ITS2AF	5'-GTGAGCGTAATAAAGGTGCAG-3'
ITS2AR	5'-TTCATCACACATCTTCCACTA-3'
ITS2BF	5'-CAATTGAAAACCGCTTAGCGTGTTT-3'
ITS2BR	5'-TGATCTGAGGTCGACATTTCC-3'

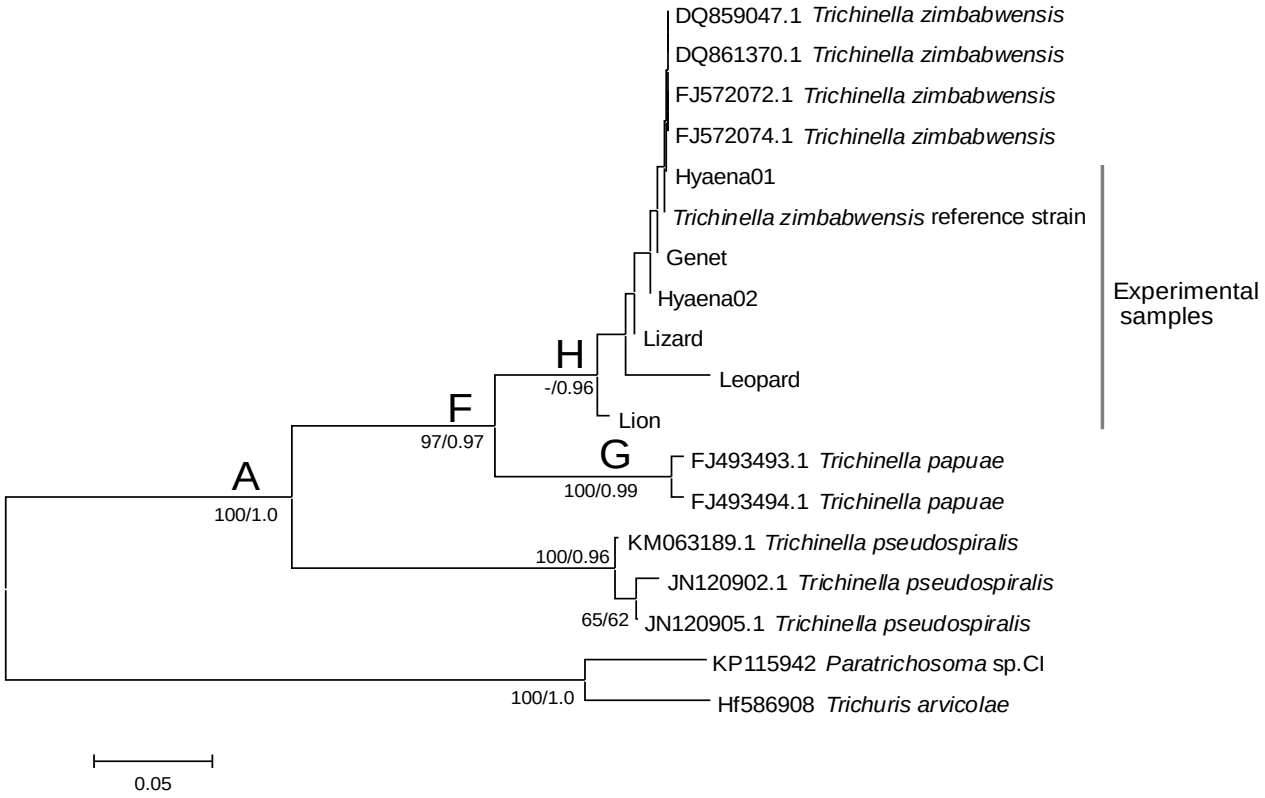


Fig. 2.1 Bayesian Inference tree based on 105 nucleotides of the ESV DNA region depicting relationships between experimental samples and sequences downloaded from the NCBI GenBank. Nodal support from maximum parsimony and Bayesian analyses is shown in that order

2.4.3 Distribution and prevalence of *Trichinella* spp. in wildlife species

Trichinella spp. larvae were isolated from eight lions during the course of this study (8/13, prevalence 61.5%). Of these, three were *T. zimbabwensis* (37.5%) and five isolates were notidentified to species level (Table 2.1). Previous studies (1964–2011) reported isolates from 14 lions (14/85, prevalence 16.5%). Of these, one was *T. zimbabwensis*, three were *T. nelsoni*, four were *Trichinella* T8 and six were notidentified to species level (Table 2.1).

Of eight hyaenas tested in this study, five were found to harbour *Trichinella* spp. larvae (5/8, prevalence 62.5 %) (Table 2.1). Based on ESV sequence analysis, two were *T. zimbabwensis* (40%) (Fig. 2.1), and a third fell under the encapsulated clade, although it was not possible to identify this isolate to species level. The other two isolates were also notidentified to species level. Twelve isolates from hyaena have been reported from 18 screened during the period from 1964–2011 (12/18, prevalence 66.7%). Of these, one was *Trichinella* T8 and the remaining 11 were not identified.

Two *Trichinella* spp. isolates were recovered from six screened leopards (2/6, prevalence 33.3%). One leopard had a mixed infection of *T. nelsoni* and T8 the results of which were previously published (see La Grange *et al*., 2014.). The remaining isolate from this study was *T. zimbabwensis.* This is the first report of a natural infection with *T. zimbabwensis* in this host. From the previous studies (1964–2011) only one leopard was screened and was found to be negative (Table 2.1).

Two Nile monitor lizards were screened in our study and one was positive for *T. zimbabwensis* (1/2, prevalence 50%). Isolates from a single African wildcat in this study and from a side-striped jackal in the period (1964–2011) were not identified.

Of the two small spotted genets tested, only one (1/2, prevalence 50%) tested positive. The isolate was identified as *T. zimbabwensis* and this is the first report of natural infection of *T. zimbabwensis* infection in this host.

Six chacma baboons were screened during the course of this study; one was positive (1/6, prevalence 16.7%) and a single larva recovered. The sample was in an advanced state of autolysis and the identification to species level was unresolved.

Samples from one African wildcat and one marsh owl were positive from screening. Only a few larvae were detected and, similar to that of baboon, the samples were in an advanced state of autolysis and no species identification was possible.

2.5 Discussion

Results from this study are consistent with the commonly accepted postulate (Mukaratirwa *et al*., 2013) that the most important route for *Trichinella* transmission in wild

animals appears to be via predation, cannibalism and scavenging. In a review by Mukaratirwa *et al.* (2013), *Trichinella* spp. prevalence was reported to be high in wild carnivores, which is consistent with results of the present study, which revealed a high prevalence in carnivores with predatory and scavenging behaviour compared to omnivorous animals.

The ability of *T. zimbabwensis* to infect mammalian hosts has been demonstrated in several experimental studies (Mukaratirwa and Foggin, 1999; Pozio *et al*., 2004; Mukaratirwa *et al*., 2008) and proven to occur in nature (La Grange *et al*., 2010). This species has previously been documented in Nile crocodiles of Zimbabwe, Mozambique, Ethiopia and South Africa, in Nile monitor lizards of Zimbabwe and in a lion from the Kruger National Park, South Africa (Mukaratirwa and Foggin, 1999; Pozio *et al*., 2002; La Grange *et al*., 2009, 2010, 2013). These results represent the second report of *T. zimbabwensis* natural infection in a lion and this confirms the previous report by La Grange *et al*. (2010) that the lion is an exceptional host for all the three *Trichinella* taxa (*Trichinella* T8, *T. nelsoni* and *T. zimbabwensis*) circulating in South Africa (La Grange *et al*., 2010).

Results from this study confirms that hyaenas are equally important hosts for at least two *Trichinella* taxa known to circulate in South Africa. Mukaratirwa *et al.*, (2013) suggested the existence of a maintenance cycle for *T. nelsoni* and *Trichinella* T8 between lions and hyaenas.

Results from this study suggest a similar maintenance cycle for *T. zimbabwensis* between these two carnivorous species. One isolate formed a well-supported association with a clade of encapsulated species and was unidentified to species level. Most likely this isolate was *T. nelsoni* or *Trichinella* T8 since these are the only two encapsulated species which have been reported in lions and hyaenas in South Africa to date (Marucci *et al*., 2009; Mukaratirwa *et al*., 2013).

An important finding from this study is that of *T. zimbabwensis* infection in the small spotted genet. Lariviere and Calzada (2001) reported the diet of the small spotted genet to be euryphagous. The diet of this opportunist omnivore consists of small mammals, amphibians, reptiles, fruits and birds (Lariviere and Calzada, 2001) and the genet may have acquired infection from feeding on infected reptiles or small mammals such as rodents.

Mukaratirwa *et al*. (2013) postulated that carnivorous reptiles from the families Crocodylidae and Varanidae are likely to be the main reservoir hosts for *T. zimbabwensis*. This study reports, for the first time, a natural infection of *T. zimbabwensis* in a Nile monitor lizard from South Africa. Results from reports to date show a high prevalence of *Trichinella* spp. in

carnivores with cannibalistic and scavenging behaviour (Mukaratirwa *et al*., 2013). This study also reports for the first time the occurrence of *T. zimbabwensis* in a leopard.

Trichinella spp. larvae were detected for the first time in a baboon, although with a significantly low number of larvae. Species identification was not possible due to the small number of larvae and degradation of larval DNA. For the first time a *Trichinella* spp.-like infection was reported in a bird from South Africa. *Trichinella pseudospiralis* is the only *Trichinella* taxon known to infect birds and have been reported in birds and mammals from Asia, North America, Europe and Tasmania (Pozio and Murrell, 2006). This parasite species has not previously been reported in Africa and, despite preliminary data from this study suggesting the existence of this parasite in South Africa, the absence of conclusive molecular evidence precludes a definitive report.

Previous reports of natural infections involving *T. zimbabwensis* may suggest a propensity of this parasite species towards infecting reptiles, but results from this study clearly show that *T. zimbabwensis* infect a variety of ecto- and endothermic host species indiscriminately. Additionally, previous reports of a lion (La Grange *et al*., 2010) and the current report of spotted hyaena, leopard, lion and a small spotted genet naturally infected with *T. zimbabwensis* confirm previous suggestions of the significant epidemiological role of mammals in the parasite epidemiology (La Grange *et al*., 2010; Mukaratirwa *et al*., 2013).

The infection in a small spotted genet may additionally suggest the existence of a large biomass of this parasite maintained in a number of smaller rodent and/or reptile species. Similarly, the infection in a leopard suggests that smaller carnivores, such as the genet, are infected frequently in nature and supports the previous hypothesis. Leopards predate small carnivorous mammals (Hayward *et al*., 2006) and their potential to serve as sources of infection to leopards has previously been alluded to (La Grange *et al*., 2014). This is a cause of concern from a veterinary public health perspective, since the potential risk of transmission of the parasite from the natural sylvatic cycle to domestic animals can be through rodent infestations.

The presence of *Trichinella* spp. in at least four different omnivorous species in GKNP certainly suggest they act as maintenance hosts, although results from this study together with reports by Young and Whyte (1975) and Young and Kruger (1967) represent the only four cases of *Trichinella* infections reported in omnivores in the GKNP and adjacent areas.

Epidemiological investigations on *Trichinella* species have been carried out mostly on wild animals from the GKNP and surrounding areas in South Africa. More surveys aimed at elucidating the prevalence and species richness of this parasite genus in the rest of South Africa is required. Such surveys will certainly prove invaluable in adding to the body of knowledge

on *Trichinella*. However, more importantly, they will prove crucial in determining the risk of human infection, which will inevitably increase alongside the expansion of the game industry, population growth and the search for alternative food sources to ensure food security for the country's inhabitants.

2.6 Acknowledgements

Our sincere appreciation to the Mpumalanga Department of Agriculture, Rural Development, Land and Environmental Affairs for logistical and financial support provided through the Chief Directorate of Veterinary Services, Dr. J.P. Raath and staff of Wildlifevets.com and Wildlife Pharmaceuticals for financial, logistical and technical support provided.

2.7 Financial Support

Financial support was provided by the National Research Foundation of South Africa from the Incentive for Rated Researchers scheme for S.M. for the laboratory work, and by the Chief Directorate of Veterinary Services, Mpumalanga Department of Agriculture, Rural Development, Land and Environmental Affairs and Wildlife Pharmaceuticals for collection of samples.

2.8 Conflict of Interest

None.

2.9 References

Baker RJ, Bradley RD. 2006. Speciation in mammals and the genetic species concept. Journal of Mammology, 87, 643- 662.

Blaga R, Gherman C, Cozma V, Zocevic A, Pozio E, Boireau P. 2009. *Trichinella* species circulating among wild and domestic animals in Romania. Veterinary Parasitology, 159, 218-221.

Cracraft J. 1983. Species concept and speciation analysis. Current Ornithology, 1, 159-187.

Dupouy-Camet J. 2000. Trichinellosis: a worldwide zoonosis. Veterinary Parasitology, 93, 191–200.

Fu BQ, Liu MY, Yao CY, Li WH, Li YG, Wanga YH, Wub XP, Zhang DL, Cai XP, Blaga R, Boireau P. 2009. Species identification of *Trichinella* isolates from China. Veterinary Parasitology, 159, 214–217.

Gajadhar AA, Pozio E, Gamble HR, Nöckler K, Maddox-Hyttel C, Forbes LB, Vallee I, Rossi P, Marinculic A, Boireau P. 2009. *Trichinella* diagnostics and control: Mandatory and best practices for ensuring food safety. Veterinary Parasitology, 159, 197–205.

Gottstein B, Pozio E, Nöckler K. 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clinical Microbiology Review, 22, 127–145.

Hayward MW, Henschel P, O'Brien J, Hofmeyr M, Balme G, Kerley GIH. 2006. Prey preferences of the leopard (*Panthera pardus*). Journal of Zoology, 270, 298-313.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: a Bayesian inference of phylogeny. Bioinformatics, 17, 754–755.

Krivokapich SJ, Pozio E, Gatti GM, Gonzales Prous CL, Ribicich M, Marucci G, La Rosa C, Confalonieri V. 2012. *Trichinella patagoniensis* n. sp. (Nematoda), a new encapsulated species infecting carnivorous mammals in South America. International Journal for Parasitology, 42, 903-910.

La Grange LJ, Reininghaus B, Mukaratirwa S. 2014. First report of a mixed infection of *Trichinella nelsoni* and *Trichinella* T8 in a leopard (*Panthera pardus*) from the Greater Kruger National Park, South Africa. Onderstepoort Journal of Veterinary Research, 81, 8-10.

La Grange LJ, Govender D, Mukaratirwa S. 2013. The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. Journal of Helminthology, 87, 91-96.

La Grange LJ, Marucci G, Pozio E. 2010. *Trichinella zimbabwensis* in a naturally infected mammal. Journal of Helminthology, 84, 35–38.

La Grange LJ, Marucci G, Pozio E. 2009. *Trichinella zimbabwensis* in wild Nile crocodiles (*Crocodylus niloticus*) of South Africa. Veterinary Parasitology, 161, 88–91.

Lariviere S, Calzada J. 2001. Mammalian species, *Genetta genetta*. American Society of Mammalogists, 680, 1-6.

La Rosa G, Pozio E. 2000. Molecular investigation of African isolates for *Trichinella* reveals genetic polymorphism in *Trichinella nelsoni*. International Journal for Parasitology, 30, 663-667.

Marucci G, La Grange LJ, La Rosa G, Pozio E. 2009. *Trichinella nelsoni* and *Trichinella* T8 mixed infection in a lion (*Panthera leo*) of the Kruger National Park (South Africa). Veterinary Parasitology, 159, 225–228.

Mukaratirwa S, La Grange LJ, Pfukenyi DM. 2013. *Trichinella* infections in animals and humans in sub-Saharan Africa: A review. Acta Tropica, 125, 82– 89.

Mukaratirwa S, Dzoma BM, Matenga E, Ruziwa SD, Sacchi L, Pozio E. 2008. Experimental infections of baboons (*Papio* spp.) and vervet monkeys (*Cercopithecus aethiops*) with *Trichinella zimbabwensis* and successful treatment with ivermectin. Onderstepoort Journal of Veterinary Research, 188, 181-188.

Mukaratirwa S, Foggin, CM. 1999. Infectivity of *Trichinella* sp. isolated from *Crocodylus niloticus* to the indigenous Zimbabwean pig (Mukota). International Journal for Parasitology, 29, 1129–1131.

Nöckler K, Kapel CMO. 2007. Detection and surveillance for *Trichinella*: Meat inspection hygiene, and legislation. In: J. Dupouy-Camet & KD. Murrell (eds.), FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis, pp. 69-97, World Organisation for Animal Health Press, Paris.

Pozio E. 2007. World distribution of *Trichinella* spp. infections in animals and humans. Veterinary Parasitology, 149, 3–21.

Pozio E, Zarlenga DS. 2013. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. International Journal for Parasitology, 35, 1191- 1204.

Pozio E, Hoberg E, La Rosa G, Zarlenga DS. 2009. Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. Infection, Genetics and Evolution, 9, 606–616.

Pozio E, Murrell KD. 2006. Systematics and epidemiology of *Trichinella*. Advances in Parasitology, 63, 371-445.

Pozio E, Marucci G, Casulli A. 2004. *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles. Parasitology, 128, 333-342.

Pozio E, Foggin CM, Marucci G, La Rosa G, Sacchi L., Corona S, Rossi P, Mukaratirwa S. 2002. *Trichinella zimbabwensis* n.sp. (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. International Journal for Parasitology, 32, 1787–1799.

Pozio E, Verster A, Braack L, De Meneghi D, La Rosa G. 1994. Trichinellosis south of the Sahara. In: Campbell CW, Pozio E, Bruschi F. (Eds.), Trichinellosis. Istituto Superiore di Sanità Press, Rome, Italy, 527–532.

Swofford DL. 2002. Paup*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sunderland, MA: Sinauer Associates.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 30, 2725- 2729.

Young E, Whyte IJ. 1975. Trichinosis (*Trichinella spiralis* infestations) in wild animals of the Kruger National Park. Journal of the South African Veterinary Association, 6, 233–234.

Young E, Kruger SP. 1967. *Trichinella spiralis* (Owen, 1935) Railliet 1895, infestation of wild carnivores and rodents in South Africa. Journal of the South African Veterinary Association, 38, 441–443.

Zarlenga DS, Chute MB, Martin A, Kapel CMO. 1999. A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. International Journal for Parasitology, 29, 1859-1867.

CHAPTER 3: EPIDEMIOLOGY AND HYPOTHETICAL TRANSMISSION CYCLES OF *TRICHINELLA* INFECTIONS IN THE GREATER KRUGER NATIONAL PARK OF SOUTH AFRICA: AN EXAMPLE OF HOST-PARASITE INTERACTIONS IN AN ENVIRONMENT WITH MINIMAL HUMAN INTERACTIONS

Published as:

La Grange LJ, Mukaratirwa S. (2020). Epidemiology and hypothetical transmission cycles of *Trichinella* infections in the Greater Kruger National Park of South Africa: an example of host-parasite interactions in an environment with minimal human interactions. Parasite, 27, 1-12.

3.1 Abstract

Knowledge on the epidemiology, host range and transmission of *Trichinella* infections in different ecological zones in southern Africa including areas of wildlife-human interface is limited. The majority of reports on *Trichinella* infections in sub-Saharan Africa were from wildlife resident in protected areas. Elucidation of the epidemiology of the infections and the prediction of hosts involved in the sylvatic cycles within specific ecological niches is critical. Parasites of the genus *Trichinella* are known to primarily infect sylvatic carnivores with cannibalistic and/or scavenger behaviour. The subsequent maintenance of the flow of parasites between sylvatic, synanthropic and domestic environments relies on parasite and ecological characteristics, human behaviour and availability of synanthropes. This would ultimately result in unique life cycles for each taxon within a specific ecological niche. Of recent, there have been reports of *Trichinella* infections in several wildlife species within the Greater Kruger National Park (GKNP) of South Africa, which has prompted the revision and update of published hypothetical transmission cycles including the hypothetical options based previously on the biology and feeding behaviour of wildlife hosts confined to the GKNP. At least two species and one genotype have been confirmed across six mammalian and 2 reptilian hosts from the GKNP. *Trichinella*-like infections have additionally been reported in six other mammalian hosts but species confirmation of the parasite was not possible. The unidentified isolates, for the most part, were generally believed to be one or more of the parasite species known to circulate in the area. A *Trichinella*-like infection has additionally been reported in a marsh owl (*Asio capensis*). Using data gathered from surveillance studies and reports spanning the period 1964–2019, confirmed transmission cycles and revised hypothesized transmission cycles of three known *Trichinella* species (*T. zimbabwensis*, *Trichinella* T8 and *T. nelsoni*) are

presented. These were formulated based on the epidemiological factors, feeding habits of hosts and prevalence data gathered from the GKNP. We presume that the formulated sylvatic cycles may be extrapolated to similar national parks and wildlife protected areas in sub-Saharan Africa where the same host and parasite species are known to occur. The anecdotal nature of some of the presented data confirms the need for more intense epidemiological surveillance in national parks and wildlife protected areas in the rest of sub-Saharan Africa to unravel the epidemiology of *Trichinella* infections in these unique and diverse protected landscapes.

Keywords: Trichinella, Kruger National Park, South Africa

3.2 Introduction

Nematodes of the genus *Trichinella* are zoonotic and have a cosmopolitan distribution and infect an array of hosts ranging from cold-blooded reptiles to birds and mammals [53, 65, 70, 81]. Ten species are known to exist within the genus: *Trichinella murrelli* Pozio and La Rosa, 2000 [71], *T. papuae* Pozio *et al*., 1999, [77], *T. nativa* Britov and Boev, 1972 [6], *T. britovi* Pozio *et al*., 1992 [73], *T. spiralis* Owen, 1835 [59], *T. pseudospiralis* Garkavi, 1972 [17], *T. nelsoni* Britov and Boev, 1972 [6], *T. patagoniensis* Krivokapich *et al*., 2012 [34], *T. zimbabwensis* Pozio *et al*., 2002 [69] and *Trichinella* T13 Sharma, 2019 [89]; Sharma *et al*., 2019 [90] as well as three additional genotypes, *Trichinella* T6 Pozio *et al*., 1992 [73], *Trichinella* T8 Pozio *et al*., 1992 [73] and *Trichinella* T9 Nagano *et al*., 1999 [55]. At least four species of *Trichinella* are known to circulate in sub-Saharan Africa, including *T. nelsoni*, *Trichinella* T8, *T. britovi* and *T. zimbabwensis* [53]. Of these all except *T. britovi*, have been reported in the GKNP [53, 54].

Mukaratirwa *et al*. [53, 54] confirmed lions (*Panthera leo*) and hyaenas (*Crocuta crocuta*) to be the major reservoirs for *Trichinella* infections in (GKNP), based on reported prevalence data. However, of late, *Trichinella* spp. infections have been confirmed in at least six mammalian and two reptilian species from the GKNP [38, 40, 43, 54, 79] as well as *Trichinella*-like infections in at least six additional mammalian hosts [43, 54, 100, 101]. Despite the diverse host range and the fact that South Africa has the highest reported prevalence of *Trichinella* in sub-Saharan Africa [54], no human cases have been reported from South Africa to date.

Trichinella spp. infection is notifiable and listed in the Terrestrial Animal Health Code of the World Organization for Animal Health [58]. Owing not only to its potential economic and public health impact as a food-borne parasitic zoonosis, the diverse nature of the genus and subsequently diverse host range has led to a myriad of investigations aimed at elucidating not

only its evolutionary expansion [80] but also the host-parasite relationships that exist within different ecological niches [64, 66, 70, 75, 78]. Factors influencing these relationships, however, are equally diverse and preclude any definitive report on the epidemiology of any one *Trichinella* species, especially where the natural sylvatic cycles are concerned.

The GKNP of South Africa represents a protected area where the abundance of sylvatic host species ensures survival and transmission of *Trichinella* spp. [43]. Scholtz *et al*. [88] reported that 1982 plant, 517 bird, 147 mammal and 21 reptile species exist in the approximate 20 000 km^2 of the Kruger National Park (KNP) of South Africa. Several pieces of private land are additionally included by proclamation as part of the protected area, adding approximately another 374.3 km^2 which, collectively is known as the GKNP [88]. This species-rich and diverse habitat is maintained by intricate prey-predator-scavenger interactions, all of which are well protected within its borders. This creates an optimal system for *Trichinella* species to thrive.

However, the situation in the GKNP is not unique and similarly optimal conditions may be expected in other national protected areas in sub-Saharan Africa such as the Serengeti (Tanzania), Kafue (Zambia), Hwange (Zimbabwe), Masaai Mara (Kenya) and Gorongoza (Mozambique).

In this study, we reviewed published information on *Trichinella* infection in wildlife in the GKNP of South Africa from 1964–2019 and based on the results, the authors constructed complete hypothetical transmission cycles for the three taxa known to circulate in the GKNP. In justifying the hypotheses, the factors which may be influencing the establishment of these cycles are discussed, together with the potential of spillage into domestic environments and risk for human infections.

Pozio [65] noted differences in infection between host species as a result of unique host characteristics including diet, life span, distribution, behaviour and human interaction. Gottstein *et al*. [19] additionally noted that the survival of encysted larvae in host musculature is also influenced by the host immunity, ultimately influencing the overall epidemiology of infection. Similarly, specific evolutionary adaptations among individual species of the genus affect their infectivity to specific hosts as well as epidemiology and survival in specific environments [64, 80]. These factors cannot be considered as constant either and are continually changing; most notably as a result of human activity and interaction, which influences environments, host species and parasites alike [12, 85]. However, these changes are, for the most part, slow in development, allowing at least some consistency as far as parasite transmission cycles are concerned. This allows for the elucidation of current epidemiology of

Trichinella infections and more importantly, the prediction of probable host-parasite cycles within a set ecological niche.

These host-parasite interactions are likely to be more constant in environments such as national parks and wildlife protected areas where established relationships remain relatively unchanged through minimal human interference. This is especially true for *Trichinella* infections which evidently have a larger biomass in sylvatic animals compared to domestic animals [75].

3.3 Material and Methods

3.3.1 Study area

The KNP (Figure 3.1) is situated in the North-Eastern corner of South Africa and is bordered by Zimbabwe to the north and Mozambique to the east [87]. This protected area covers approximately 20000 km^2 and boasts a diverse fauna comprising among others more than 150 mammal, 500 bird and 116 reptile species inhabiting its diverse tropical to sub-tropical "Biological Environment" [87]. The western- and south-western borders of the park are flanked by large communal areas and several private nature reserves while the southern border is mainly flanked by private agricultural- and game farms. The impressive size of the GKNP allows for interactions between large predator and prey species which can be considered "near-natural" [87]. These conditions have undoubtedly favoured, specifically in respect of *Trichinella* spp. infections, the establishment and maintenance of unique parasite-host relationships.

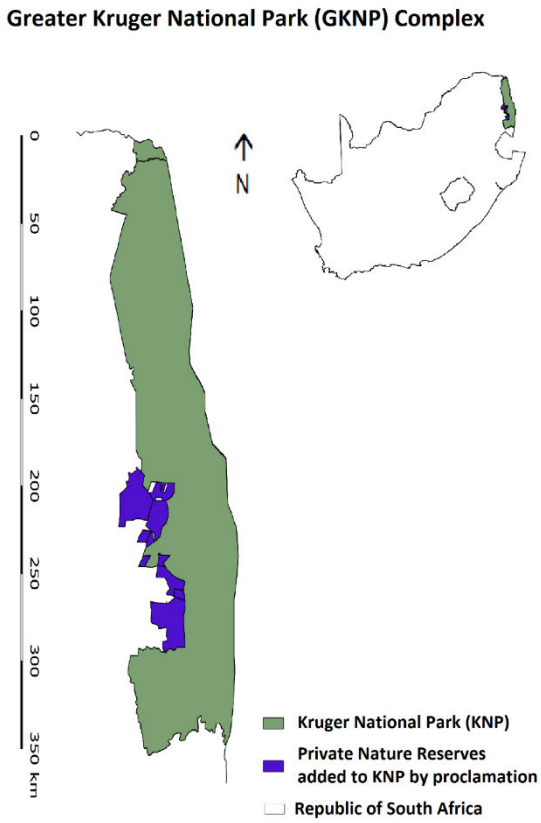


Figure 3.1 Map showing the Greater Kruger National Park (GKNP) of South Africa with Kruger National Park (KNP) depicted in green and GKNP depicted in green and blue

3.3.2 Search strategy

A search in Google Scholar, PubMed, AJOL and EBSCO Host database was conducted using the following terms and Boolean operators (AND, OR): *Trichinella* AND Wildlife; *Trichinella* infections in wildlife AND Kruger National Park; *Trichinella* spp, *Trichinella zimbabwensis*, *Trichinella* T8, *Trichinella nelsoni* AND Kruger National Park. Search results were carefully scrutinized and the relevant articles were selected for inclusion in the study. Some of the references of identified articles were additionally used to check for other relevant articles. The inclusion criteria included "all published peer-reviewed articles reporting on *Trichinella* infection in wildlife/livestock/humans in the GKNP from 1964–2019".

3.4 Construction of hypothetical transmission cycles

The probability of *T. nelsoni*, *Trichinella* T8 and *T. zimbabwensis* parasites being transmitted among different wildlife hosts present in the GKNP was inferred from published literature on dietary habits of specific host species (Table 3.1 and 3.2). No absolute or

quantitative values could be attributed to these probabilities by any statistical means. The multifactorial and constantly changing nature of the multitude of ecological factors that may influence host interactions and subsequent parasite epidemiology precludes such an analysis. Furthermore, reports and publications from other sub-Saharan countries involving similar host species were also reviewed to provide supplementary data for the information portrayed in the hypothetical transmission cycles (Table 3.1).

Based on the available prevalence data, lions are proposed to be the main reservoirs for both *T. nelsoni* and *Trichinella* T8 while crocodiles are considered to be main reservoirs for *T. zimbabwensis*. However, there is limited information on additional and other potential reservoirs, and in most cases the numbers of animals screened for *Trichinella* spp. infection are very low. It is also important to consider the overall biomass of each potential host species within the ecological framework being assessed. Species representing a larger biomass will require a higher number of individuals to be tested compared to species with a smaller biomass in order to reach conclusive evidence in respect of identifying main reservoirs. In the case of rodents, this problem is further compounded by the fact that vertical parasite transmission is possible via both the transmammary and transplacental routes [45].

Table 3.1 Predation/scavenging habits of wildlife species reported to harbour *Trichinella* spp. in sub-Sahara Africa (Events of predation/scavenging among species depicted below are not indicative of any degree of probability but merely suggest a possibility of such events occurring based on the literature cited).

Animal species	Common Name	Species predated/scavenged	References
Panthera leo	Lion	warthog rodents baboon hyaena leopard, wild dog, cheetah crocodile lion	[10, 21, 61] [10] [7, 21] [63, 84] [63] [57, 92]
Panthera pardus	Leopard	hyaena, lion, wild dog, cheetah crocodiles baboon rodents	[63] [63, 92] [7, 27] [26]
Varanus niloticus	Nile monitor	rodents, juvenile crocodiles	[95]
Crocuta crocuta	Spotted hyaena	warthog, baboon lion, leopard, cheetah	[20] [63, 84] [63]
Felis silvestris lybica	African Wildcat	rodents, carrion (unspecified)	[24]
Canis mesomelas	Black-backed jackal	cheetah rodents, carrion (unspecified)	[63] [3, 5, 23]
Civettictis civetta	African civet	rodents, carrion (unspecified)	[5]
Genetta genetta	Small spotted genet	rodents	[39]
Papio ursinus	Chacma baboon	baboon rodents	[62] [1]
Praomys natalensis	Multimammate mouse	multimammate mouse	[26]
Crocodylus niloticus	Nile crocodile	leopard crocodile Nile monitor lion, hyaena, warthog, baboon	[63] [92] [92] [16]
Potamochoerus larvatus [a]	Bushpig	carrion (unspecified)	[91]
Phacochoerus africanus[a]	Warthog	hyaena civet, carrion (unspecified)	[84] [9]
Canis adustis[a]	Side-striped jackal	rodents	[3, 5]
Acinonyx jubatus[a]	Cheetah	rodents, carrion (unspecified)	[47]
Leptailurus serval[a]	Serval	warthog rodents, small spotted genet	[82]
Otocyon megalottis[a]	Bat-eared fox	rodents	[96]
Ichneumia albicauda[a]	White-tailed mongoose	rodents	[11]
Hyaena hyaena[a]	Striped hyaena	rodents, carrion (unspecified)	[98]

[a] = Species native to GKNP and known host of *Trichinella* spp. elsewhere

3.5 Results

3.5.1 *Trichinella* species reported in the Greater Kruger National Park

In the GKNP, the prevalence of *T. nelsoni* in lions and hyaenas was reported to be 3.06% (3/98) and 0% (0/26) respectively [54]. It is important to note that the majority of these isolates (11/21) and (14/17) found in lions and hyaenas respectively, were not identified to species level [54] and thus the actual prevalence in GKNP could be higher than reported. In the case of hyaenas, only three isolates were identified to species level but none conformed to *T. neloni*. Based on the overall prevalence of *T. nelsoni* in sub-Saharan Africa (Table 3.3) and GKNP (Table 3.2), hyaenas and lions are considered to be the main reservoirs for this parasite species in GKNP, as may be the general case with similar habitats in other African countries [43, 53].

Table 3.2 Occurrence of *Trichinella* spp. in wildlife species from the Greater Kruger National Park, South Africa from 1964–2019.

Animal species	Common Name	No Positive/Tested	Total Prevalence (%)	Tz	Tn	T8	NID	References
Panthera leo	Lion	22*/98	22.4	4	4	4	11	[36, 40, 43, 54, 79, 100]
Panthera pardus	Leopard	2*/7	28.6	1	1	1	0	[38, 54, 101]
Varanus niloticus	Nile monitor	1/2	NC	1	-	-	-	[54]
Crocuta crocuta	Spotted hyaena	17/26	65.4	2	-	1	14	[54, 100]
Felis silvestris lybica	African Wildcat	1/1	NC	-	-	-	1	[54]
*Canis mesomelas***	Black-backed jackal	1/2	NC	-	-	-	1	[100]
Civettictis civetta	African civet	1/2	NC	-	-	-	1	[54, 101]
Genetta genetta	Small spotted genet	1/2	NC	1	-	-	0	[54]
Papio ursinus	Chacma baboon	1/6	16.7	-	-	-	1	[54]
Praomys natalensis	Multimammate mouse	1/44	2.3	-	-	-	1	[100]
Crocodylus niloticus	Nile crocodile	16/43	37.2	16	-	-	-	[35, 37, 54]
Asio capensis	Marsh owl	1/1	NC	-	-	-	1	[54]
TOTAL		**65/234**		**25**	**5**	**6**	**31**	

Tz = *Trichinella zimbabwensis*, Tn = *Trichinella nelsoni*, T8 = *Trichinella* genotypte T8, NID = Not identified to species level, * One animal represents a mixed infection of *Trichinella nelsoni* and *Trichinella* T8, ** Incorrectly reported as Side striped jackal (*Canis adustis*) by Marucci et al. (2009) and Mukaratitwa et al. (2013), NC = Not calculated due to sample size < 5

In the GKNP, only a single leopard (1/7, 14%) tested positive for *T. nelsoni* [54] and the same species has previously been isolated from leopards in Kenya [66] and Tanzania [67]. La Grange *et al*. [38] described a mixed infection of *T. nelsoni* and *Trichinella* T8 in a leopard from the GKNP and based on the dietary habits of the species [4, 20, 42, 94], I hypothesize that other small mammalian carnivores may serve as an important source of infection to these animals in the GKNP.

Similar to *T. nelsoni*, genotype *Trichinella T8* has been found in low prevalence in lions (4/98, 4%), hyaenas (1/26, 3.8%) [43, 54, 76] and leopards (1/7, 14%) from the GKNP [38, 54]. Again, as in the case of *T. nelsoni*, many *Trichinella* spp. isolates found were reported prior to the advent of molecular characterisation techniques and thus the parasite species involved remain unknown. Data on the actual distribution and prevalence of *Trichinella* T8 are still fragmented. Although closely related to *Trichinella* T8, *Trichinella britovi* has never been isolated from South African wildlife. Pozio and Murrell [75] confirmed the geographical distribution of *T. britovi* to include amongst others Northern and Western Africa, whereas *Trichinella* T8 is confined to the South Western and South Eastern parts of Africa. Pozio *et al*. [78] hypothesized that large natural barriers such as the Zaire lake basin and river Cross of Nigeria together with environmental changes, may have contributed to the evolution of these two unique taxa.

Trichinella zimbabwensis was previously isolated from wild Nile crocodiles in the KNP and just beyond its north western- and southern boundaries [35, 37, 54] and in a Nile monitor lizard (*Varanus niloticus*) from the city of Nelspruit located close to the south-western border of the KNP [54]. Furthermore, it has also been detected in farmed crocodiles in South Africa (Department of Agriculture, Forestry and Fisheries (DAFF), (personal communication, 2017). The locale of infected farm(s) was not provided. This species is infective to mammals and reptiles [51, 52, 53, 74]. Results from passive surveillance in the GKNP further revealed that *T. zimbabwensis* has the highest prevalence in crocodiles and carnivores, of the three species known to circulate in South Africa [54].

Table 3.3 Occurrence of *Trichinella* spp. in wildlife species from sub-Sahara Africa other than Kruger National Park, South Africa.

Country of origin	Animal species	Common Name	No Positive/ Tested	Total Prevalence (%)	Tz	Tn	T8	Tb	NID	References
Tanzania	*Panthera leo*	Lion	3/24	12.5	-	3	-	-	-	[67]
Namibia			1/1	NC	-	-	1	-	-	[40, ITRC]
Tanzania	*Panthera pardus*	Leopard	1/3	NC	-	1	-	-	-	[67]
Kenya			1/4	NC	-	1	-	-	-	[56, 66]
Zimbabwe	*Varanus niloticus*	Nile monitor	6/29	20.7	6	-	-	-	-	[53, 68]
Tanzania	*Crocuta crocuta*	Spotted hyaena	3/13	23	-	3	-	-	-	[67]
Congo			1/1	NC	-	-	-	-	1	[101]
Kenya			1/1	NC	-	1	-	-	-	[66, ITRC]
Namibia			1/?	NC	-	-	-	-	1	[79]
Senegal	*Canis adustis*	Side-striped jackal	1/10	10	-	-	-	-	1	[18]
Kenya			?	NC	-	-	-	-	-	[66]
Namibia	*Canis mesomelas*	Black-backed jackal	1/?	NC	-	-	-	-	1	[79]
Tanzania			1/11	9	-	-	-	-	1	[86]
Senegal	*Ichneumia albicauda*	White-tailed mongoose	6/17	35.3	-	-	-	-	6	[18]
Guinea	*Civettictis civetta*	African civet	1/19	5.3	-	-	-	1	-	[78]
Guinea	*Nandinia binotata*	African palm civet	2/45	4.4	-	-	-	1	-	[78]
Kenya	*Potamochoerus larvatus*	Bush pig	1/40	2.5	-	-	-	-	1	[56]
Kenya	*Phacochoerus africanus*	Warthog	18/450	4	-	-	-	-	18	[18]
Tanzania			1/1	NC	-	1	-	-	-	[38, ITRC]
Zimbabwe	*Crocodylus niloticus*	Nile crocodile	256/648	39.5	256	-	-	-	-	[15]
Mozambique			8/40	20	8	-	-	-	-	[68]
Tanzania	*Acinonyx jubatus*	Cheetah	1/5	20	-	1	-	-	-	[67]
Kenya	*Leptailurus serval*	Serval	1/9	11	-	1	-	-	-	[56, 66]
Tanzania	*Otocyon megalottis*	Bat-eared fox	1/6	17	-	1	-	-	-	[67]
Kenya	*Hyaena hyaena*	Striped hyaena	1/2	50	-	1	-	-	-	[56, 66]
Nigeria	*Cricetomys gambianus*	African giant rat	16/100	16	-	-	-	-	16	[46]
Nigeria	*Sus domesticus*	Domestic pigs	42/883	4.8	-	-	-	-	42	[2]
TOTAL			**379/2362**	**16**	**270**	**15**	**1**	**2**	**88**	

Tz = *Trichinella zimbabwensis*, Tn = *Trichinella nelsoni*, T8 = *Trichinella* genotype T8, Tb = *Trichinella britovi*, NID = Not identified to species level, NC = Not calculated/reported due to sample size < 5, ITRC = International *Trichinella* Reference Centre, ? = Actual numbers not reported in cited literature.

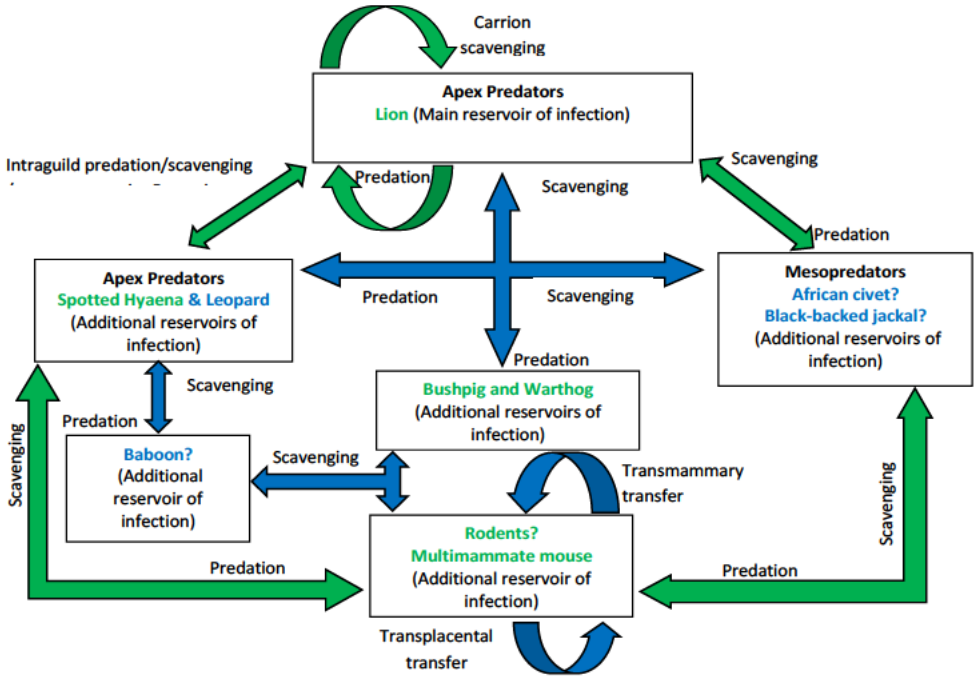
3.5.2 Hypothetical transmission cycles of *Trichinella* spp. in GKNP

Previous findings have prompted speculation concerning the epidemiology of *T. nelsoni*, *Trichinella* T8 and *T. zimbabwensis* including hypothetical transmission cycles as proposed by Mukaratirwa *et al*. [53]. Since the publication of these hypotheses, new host species have been confirmed [54], prompting a revision of the proposed hypotheses. Unravelling the enigmatic epidemiology of these potentially zoonotic species from the genus *Trichinella* is important from a public health perspective as it aids in establishing not only the potential risk for human infection [63], but ultimately proper control and prevention measures [53, 64, 80].

New additions to the knowledge on the prevalence of *Trichinella* spp. isolated from wildlife hosts in the GKNP and other surrounding areas outside the park and elsewhere in Eastern- and Southern Africa provides for an update of the previously hypothesized transmission cycles for the three taxa known to circulate in this area. Considering the potential epidemiological drivers based on host species richness and interspecies interaction in the GKNP and the region discussed earlier, hypothetical transmission cycles for the three *Trichinella* taxa are proposed in Figures 3.2 and 3.3

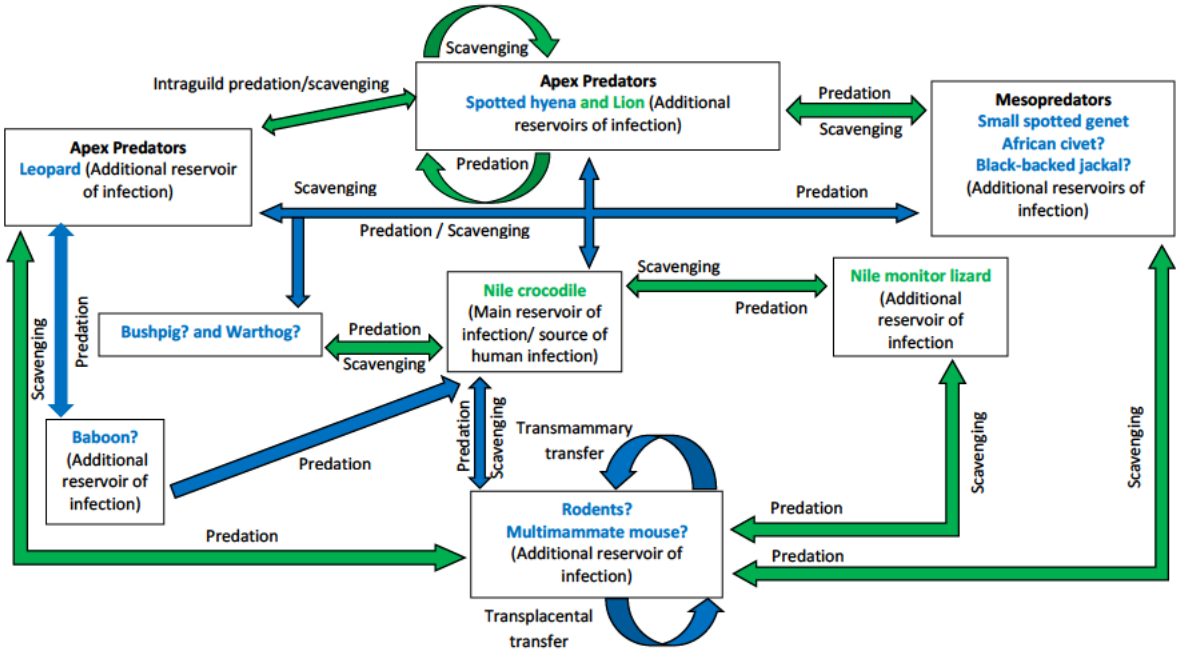
Based on the sympatric existence of *T. nelsoni* and *Trichinella* T8, we hypothesize a transmission cycle applicable to both these species (Figure 3.2). The hypothetical cycle previously presented by Mukaratirwa *et al*. [53] was updated to include recent findings presented by Mukaratirwa *et al*. [54].

A separate hypothetical cycle is presented for *T. zimbabwensis* (Figure 3.3) and was similarly updated from Mukaratirwa *et al*. [53] to include recent findings [54]. Two apex predators (hyaena and leopard) and a mesopredator, the small spotted genet (*Genetta genetta*) have been confirmed as new host species and included in the hypothetical transmission cycle. Additionally, rodents and in particular the multimammate mouse (*Praomys natalensis*) [100] and three mesopredators, the African civet (*Civettictis civetta*) [101], black-backed jackal (*Canis mesomelas*) [100] and African wild cat (*Felis sylvestris lybica*) [54] which were previously found to be infected by unidentified species of *Trichinella* have been added as probable host species in both hypothetical cycles.



? = Species involvement is yet to be confirmed; Arrows indicate direction of transmission; Arrows in green colour = Previously hypothesized mode of transmission (Mukaratirwa et al., 2013); Arrows in blue colour = Additional hypothesized mode of transmission (current hypothesis); Host species in green text = Previously hypothesized host species (Mukaratirwa et al., 2013); Host species in blue text without ? = Additional host(s) species (current hypothesis).

Figure 3.2 Updated hypothetical sylvatic cycle of *Trichinella nelsoni* and *Trichinella* T8 in the Greater Kruger National Park (GKNP) of South Africa



? = Species hypothesized to be involved but yet to be confirmed; Arrows indicate direction of transmission; Arrows in green colour = Previously hypothesized mode of transmission (Mukaratirwa *et al*., 2013); Arrows in blue colour = Updated hypothesized mode of transmission (current hypothesis); Host species in green text = Confirmed host species (Mukaratirwa *et al*., 2013); Host species in blue text without ? = Updated confirmed host(s) species (current hypothesis).

Figure 3.3 Updated hypothetical sylvatic cycle of *Trichinella zimbabwensis* in the Greater Kruger National Park (GKNP) of South Africa.

Interspecies predation between hyaenas and lions has previously been presented as a contributing factor in the maintenance of the two encapsulated *Trichinella* species, *T. nelsoni* and *Trichinella* T8 found in GKNP [53]. Results reported by Mukaratirwa *et al*. [54] now also suggest that *T. zimbabwensis* could be similarly maintained between these species. The addition of a leopard as a host for *T. zimbabwensis*, however, compels its inclusion into this equation. However, several factors that drive intraguild predation as discussed by Palomares and Caro [60] need to be taken into account. The interactive role of leopards may be predominantly asymmetrical with leopards being more prone to predation by the other two species compared to a more symmetrical interaction between lions and hyaenas. However, as predators, leopards play a much more significant role in the *Trichinella* epidemiology when considering their interaction with smaller mesopredators, such

as the small spotted genet. Mukaratirwa *et al*. [54] also alluded to the importance of mesopredators as sources of infection to larger species and the possible existence of a large parasite biomass in rodents and reptiles that could act as a primary infection source.

3.6 Discussion

Parasites of the genus *Trichinella* are known to primarily infect sylvatic carnivores with cannibalistic and/or scavenger behaviour [9, 53, 75]. Domestic cycles involving some species, most notably *T. spiralis* are recognised [19, 64] and intrusion from the sylvatic cycle into the domestic environment usually results from human failure to properly manage the wildlife-domestic animal interface [75]. Pozio [64] noted that successful intrusion from the sylvatic cycle and the subsequent maintenance of the flow of parasites between sylvatic, synanthropic and domestic environments relies on parasite and ecological characteristics, human behaviour and availability of synanthropes. This would ultimately result in unique life cycles for each taxon within a specific ecological niche.

From the beginning of *Trichinella* surveillance studies in South Africa in 1964 to the end of 2016, at least two species and one genotype have been confirmed across six mammalian and 2 reptilian hosts from the GKNP [38, 40, 43, 54, 79]. *Trichinella*-like infections have additionally been reported in six other mammalian hosts but species confirmation of the parasite was not possible [43, 54, 100, 101]. The unidentified isolates, for the most part, were generally believed to be one or more of the parasite species known to circulate in the area. A *Trichinella*-like infection has additionally been reported in a marsh owl (*Asio capensis*), possibly suggesting the existence of an additional *Trichinella* specie not known to occur on the African continent or a different tissue-dwelling nematode/larva not related to *Trichinella*.

Trichinella nelsoni is known to occur in Eastern and Southern Africa [53, 78] and has been detected in Kenya, Tanzania and South Africa [38, 43, 54, 67, 79]. Carnivores appear to be the major reservoirs for *T. nelsoni* and the parasite has been found in high prevalence especially in lions (35.3%) and spotted hyaenas (29.4%) [53]. It has additionally been detected in leopards [38, 67], cheetah (*Actinonyx jubatus*) and bat eared fox (*Otocyon megalotis*) [67].

Despite its high infectivity to carnivores, members of the Suidae family are only moderately susceptible to *T. nelsoni* [28, 29]. These findings are supported by the fact that the actual prevalence of *Trichinella* spp. in warthogs (*Phacochoerus africanus*) and bushpigs (*Potamochoerus larvatus*) appears to be very low [53, 75]. Despite the reported low infectivity of wild Suidae to *T. nelsoni* [28, 29], previous reports of infections in these animals [18, 56, 75, 79, 86] suggest that they might, albeit to a lesser extent, play a role in the epidemiology of *Trichinella* spp. in the GKNP. Importantly, the aforementioned was discovered prior to the advent of molecular characterization techniques and thus the parasite species involved remain unknown although the involvement of *T.*

nelsoni cannot be ruled out. The exception might be the studies by Grétillat and Chevallier, [18] which were conducted in Senegal and the reported infections may have involved *T. britovi*.

In the Majete Wildlife Reserve, in Malawi, interspecies cannibalism among warthogs (*P. africanus*) were observed and predation by warthogs on hyaena cubs suggested as a contributing factor to the small hyaena population [84]. Apart from the incidences reported by Sachs [86], *Trichinella* spp. infection has never been reported in wild pigs from South Africa albeit that the numbers screened thus far have been very low [54].

Trichinella T8 has previously been isolated in a lion from the Etosha National Park in Namibia [75, 79], but surprisingly has never been positively identified in any other African country except South Africa [53, 66]. However, Marucci *et al*. [43] did observe that both *T. nelsoni* and *Trichinella* T8 appear to circulate among hyaenas and lions in the KNP with similar prevalence and hypothesized sympatric status. This sympatry is confirmed to extend to other host species and include leopards [38].

Results from passive surveillance in the GKNP revealed that *T. zimbabwensis* has the highest prevalence in Nile crocodiles and carnivores compared to the three species known to circulate in South Africa [54]. *Trichinella zimbabwensis* has also been detected in farmed crocodiles in South Africa (DAFF, personal communication, 2017). This parasite species was proved experimentally to be infective to mammals and reptiles [51, 52, 53, 70] and notably, domestic pigs [44, 52]. No known cases of human disease have been associated with *T. zimbabwensis*. However, its infectivity to pigs and other animal species utilized as human food sources provide the incentive to regard *T. zimbabwensis* as a high food safety risk concern [13].

Moleón *et al*. [48] suggested the risk of parasitic infection associated with conspecific and heterospecific carrion scavenging between carnivores to be a selective force preventing carnivores from eating each other. However, in the case of *Trichinella* infections, predation and scavenging among carnivores is the primary mode of transmission and carnivores are the primary hosts [8]. Carnivores like lions and hyaenas are considered apex predators [60] and have a dietary overlap of more than 68% [20, 61]. Additionally, within the GKNP, these two predators both prefer the same habitat [61], which may result in encounters where kleptoparasitism by both species frequently occurs [41]. These encounters can prove fatal to individuals of both species although mortality of hyaenas is usually higher [61]. There is a paucity of literature on the actual incidence of intraguild predation with consumption of the victim by these two predators. Palomares and Caro [60] have shown that carnivores sometimes consume or at least partially consume their victims. This would suggest intraguild scavenging between carnivores to be secondary to active predation where the transmission of *Trichinella* spp. is concerned. However, secondary carrion scavenging by both

apex- and mesopredators such as jackals (*Canis mesomelas* and *C. adustis*) on carnivore carcasses cannot be totally excluded.

Leopards are known to prey on smaller mesopredators [4, 22, 42] and a review by Palomares and Caro [60] showed that these opportunistic predators not only killed but consumed a variety of carnivore species including young hyaenas [4]. Similarly, lions and hyaenas frequently kill and sometimes consume smaller mesopredators [60].

A previous study showed that odours, specifically 2-phenylethylamine, from carnivore carcasses triggers an innate fear response that leads to avoidance of carnivore carrion by rodents [14]. However, multimammate mice (*Praomys natalensis*) are known to occur in the GKNP and their diet can include carrion [49]. This is also supported by the fact that a single case of *Trichinella* infection was previously reported in this species from the KNP [100]. Vertical transmission of *T. zimbabwensis* via the transmammary and transplacental routes have been experimentally proven in rodents (*Rattus norvegicus*) [45] which suggests that endemic rodent populations in the GKNP may play an integral role in the maintenance and transmission of the three *Trichinella* taxa known to circulate in the region.

In addition to the plethora of potential mammalian hosts, the GKNP is home to a high population of Nile crocodiles and predation between crocodiles and mammalian carnivores are known to occur [99]. Previous studies have shown a high prevalence of *T. zimbabwensis* in Nile crocodiles in the KNP [35]. This could probably be attributed to high levels of intraspecies predation and scavenging among crocodiles. However, a recent report by Mukaratirwa *et al*. [54] showed *T. zimbabwensis* not only to be the most prevalent, but also to infect the widest host range of all the *Trichinella* species isolated thus far from the GKNP. This would certainly suggest the general knowledge and perceptions of interspecies predation and scavenging to be incomplete.

3.7 Limitations of the review

Several factors preclude a co-ordinated surveillance effort to enable screening of all the potential host species in the GKNP and other nature reserves in the rest of South Africa and elsewhere. Access to a variety of samples is reliant on the acquisition of convenient samples from State Veterinary Services, reserve staff and private veterinarians. A more structured and co-ordinated approach such as the effective implementation of existing regulations [Regulation (EU) 2015/1375] employed in North America, Europe and Asia is required to maintain and improve wildlife surveillance for *Trichinella* infections in GKNP.

All potential stakeholders should be sensitized to the importance of surveillance through continued collaborative efforts. Many of the potential host species are also protected by national- and international legislation which further hampers sample acquisition. Overcoming legislative

barriers can only be attained through close collaboration with local authorities. Establishing effective communication between researchers and other stakeholders with the applicable authorities mandated to regulate the collection and transportation of samples is essential to future success. Lack of funding and other resources also precludes effective surveillance. Private and institutional funding opportunities should continually be sought and motivated through highlighting the potential impact of *Trichinella* on human health and the threat to commercial farming industries.

Indeed, the lack of data on human infections and cases involving domestic animals has resulted in *Trichinella* surveillance not being considered a public health priority by the controlling veterinary authority. This perception needs to be changed and emphasis must be placed on the marginal cost of surveillance compared to the cost of remedial action in the event of a human outbreak or the cost of control and eradication in the event of domestic spill-over.

3.8 Research gaps and future research

Maintenance of, and where possible, improving of collaborative efforts with GKNP staff and other stakeholders is crucial. Wildlife surveillance should also be encouraged in other African countries and the rest of South Africa, and hence there is need to employ current knowledge and expertise to establish a *Trichinella* Reference Centre for Africa to assist in the surveillance of infections and capacity building of expertise.

A study on the role of predatory fish as potential hosts for *T. zimbabwensis* is currently underway in South Africa. A previous study suggests that fish do not play any significant role in the epidemiology of *T. zimbabwensis* [72]. However, the potential host species used in the study are not associated with either Nile crocodiles or Nile monitor lizards in nature and do not co-exist with any of the predators in any of their respective natural habitats. Previous studies have shown that host characteristics play an important role in determining not only muscle predilection but also the infectivity of different *Trichinella* species to different hosts [30, 31, 37, 83, 93]. Studies with fish experimentally infected with encapsulated *T. britovi* and *T. spiralis* and non-encapsulated *T. pseudospiralis* did show that the larvae, even though they did not develop into adults, migrated to the body cavity and internal organs (*T. spiralis*) and also the muscles (*T. britovi* and *T. pseudospiralis*) of some fish species and retained their infectivity for a limited period of time [50, 97]. However, interspecies differences between parasites of the genus *Trichinella* have also been shown to influence both muscle predilection and infectivity in the same host [25, 32, 33]. In order to fully understand the parasite epidemiology the correct selection of a probable host species and parasite species based on their natural occurrence is of utmost importance.

Future surveillance efforts will also include more focused efforts on migratory carnivorous birds and targeted surveillance of rodents to elucidate their potential role as maintenance reservoirs for the different *Trichinella* taxa in GKNP.

3.9 Conclusion

The vast size and limited human interference combined with the species richness within the protected area of the GKNP provide an excellent setting for the establishment and maintenance of *Trichinella* spp. known to circulate in the area. As a testament to this, *T. zimbabwensis, T. nelsoni* and *Trichinella* T8 have all established very unique and diverse transmission and maintenance cycles consisting of a multitude of equally diverse host species. Results from surveys spanning more than 50 years suggest our knowledge of the actual incidence and epidemiology of *Trichinella* in this area to be curtailed at best. As such, the information presented here cannot, by any means, be considered complete but should rather be viewed as ongoing which undoubtedly will require future update as new evidence is presented. Despite much of the information presented being based on anecdotal evidence, this study confirms not only a need for more intense epidemiological surveillance in the rest of South Africa and beyond [53], but also the need for continued efforts to unravel the remaining gaps in the epidemiology of *Trichinella* spp. in these unique and diverse protected landscapes in eastern and southern Africa.

3.10 Author ORCIDs

LJ La Grange, 0000-0002-5981-0564
S Mukaratirwa, 0000-0001-6843-1497

3.11 Acknowledgements

Our sincere appreciation to the Mpumalanga Department of Agriculture, Rural Development, Land and Environmental Affairs for logistical and financial support provided through the Chief Directorate of Veterinary Services; Dr. J.P. Raath and staff of Wildlifevets.com and Wildlife Pharmaceuticals for financial, logistical and technical support provided. The staff and students of the School of Life Sciences, University of KwaZulu-Natal for their continued support and assistance in testing research samples, SANPARKS, KNP, adjoining Nature reserves staff for the continued collection of samples.

3.12 Statements

3.12.1 Financial support

This study was supported in part by the Mpumalanga Department of Agriculture, Rural Development, Land and Environmental Affairs as part of a continuous surveillance project and by Wildlife Pharmaceuticals Pty (Ltd).

3.12.2 Conflicts of interest

The authors declare that they have no conflict of interest.

3.13 References

1. Abu Baker M, Brown, JS. 2010. Islands of fear: effects of wooded patches on habitat suitability of the striped mouse in a South African grassland. Functional Ecology, 24, 1313–1322.

2. Akinboade OA, Awotoye A, Dipeolu OO. 1984. Prevalence of trichinosis in pigs in Oyo State of Nigeria. Annales de la Société Belge de Médecine Tropicale, 64, 315-318.

3. Atkinson RPD, Macdonald DW, Kamizola R. 2002. Dietary opportunism in side-striped jackal *Canis adustis* Sundevall. Journal of Zoology, 257, 129-139.

4. Bailey TN. 1993. The African leopard: ecology and behaviour of a solitary felid. 429 pages, Columbia University Press, New York.

5. Bothma J du P. 1971. Food habits of some carnivora (Mammalia) from southern Africa. Annals of the Transvaal Museum, 27, 15-26.

6. Britov VA, Boev SN. 1972. Taxonomic rank of various strains of *Trichinella* and their circulation in nature. Vestnik Akademii Nauk KSSR 28, 27–32.

7. Busse C. 1980. Leopard and lion predation upon chacma baboons living in the Moremi Wildlife Reserve. Botswana Notes & Records, 12, 15-21.

8. Campbell WC. 1988. Trichinosis revisited, another look at modes of transmission. Parasitology Today, 4, 83–86.

9. Cumming DHM. 1975. A field study of the ecology & behaviour of warthog. The Trustees of the National Museums and Monuments of Rhodesia Salisburv, Rhodesia, Museum Memoir No *7*, 179 pages.

10. Davidson Z, Valeix M, Van Kesteren F, Loveridge AJ, Hunt JE, Murindagomo F, Macdonald DW. 2013. Seasonal diet and prey preference of the African lion in a waterhole-driven semi-arid savanna. PLoS ONE, 8, e55182. https://doi.org/10.1371/journal.pone.0055182*Plos One*.

11. Do Linh San E, Stuart C, Stuart, M. 2016. A conservation assessment of *Ichneumia albicauda*. In: Child MF, Roxburgh L, Do Linh San E, Raimondo D, Davies-Mostert HT. (eds.), The Red List

of Mammals of South Africa, Swaziland and Lesotho. South African National Biodiversity Institute and Endangered Wildlife Trust, South Africa. 4 pages.

12. Engering A, Hogerwerf L, Slingenbergh J. 2013. Pathogen-host-environment interplay and disease emergence. Emerging Microbes and Infections, 2, 1-7.

13. FAO. 2013. Summary risk profile on *Trichinella* in meat. http://www.fao.org/fileadmin/user_upload/agns/pdf/Foodborne_parasites/RiskProfTrichinellaOct2013.pdf.

14. Ferrero DM, Lemon JK, Fluegge D, Pashkovski SL, Korzan WJ, Datta SR, Spehr M, Fendt M, Liberies SD. 2011. Detection and avoidance of a carnivore odor by prey. Proceedings of the National Acadamy of Sciences, 108, 11235-11240.

15. Foggin CM, Vassilev GD, Widdowson MA. 1997. Infection with *Trichinella* in farmed crocodiles (*C. niloticus*) in Zimbabwe. In: Abstract Book on the 16th International Conference of the World Association for the Advancement of Veterinary Parasitology, Sun City, South Africa, 10–15 August (Abstract no. 110).

16. Furstenburg D. 2008. Crocodile (*Crocodylus niloticus*). Game & Hunt, 14, 6-12.

17. Garkavi BL. 1972. Species of *Trichinella* isolated from wild animals. Veterinariya, 10, 90–91.

18. Gretillat S, Chevalier JL. 1970. Preliminary note on the epidemiology of trichinosis in wild animals in western Africa. Bulletin of the World Health Organization, 43, 749–757 (in French).

19. Gottstein B, Pozio E, Nöckler K. 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clinical Microbiology Reviews, 22, 127–145.

20. Hayward MW. 2006. Prey preferences of the spotted hyaena (*Crocuta crocuta*) and degree of dietary overlap with the lion (*Panthera leo*). Journal of Zoology, 270, 606–614.

21. Hayward M, Kerley IH. 2005. Prey preferences of the lion (*Panthera leo*). Journal of Zoology, 267, 309–322.

22. Hayward MW, Henschel P, O'Brien J, Hofmeyr M, Balme G, Kerley GIH. 2006. Prey preferences of the leopard (*Panthera pardus*). Journal of Zoology, 270, 298–313.

23. Hayward MW, Porter L, Lanszki J, Kamler JF, Beck JM, Kerley IH, Macdonald DW, Montgomery RA, Parker DM, Scott DM, O'Brien J, Yarnell RW. 2017. Factors affecting the prey preferences of jackals (Canidae). Mammalian Biology, 85, 70-82.

24. Herbst M, Mills MGL. 2010. The feeding habits of the African wild cat (*Felis silvestris*), a facultative trophic specialist, in the southern Kalahari (Kgalagadi Transfrontier Park, South Africa/Botswana). Journal of Zoology, 280, 403-413.

25. Hurníková Z, Dubinsky S, Mukaratirwa S, Foggin CM, Kapel CMO. 2004. Infectivity and temperature tolerance on non-encapsulating *Trichinella zimbabwensis* in experimentally infected Red foxes (*Vulpes vulpes*). Helminthologia, 41, 189–192.

26. Isaäcson M. 1975. The ecology of *Praomys* (Mastomys) *natalensis* in southern Africa. Bulletin of the World Health Organization, 52, 629-636.

27. Jooste E, Pitman RT, van Hoven W, Swanepoel LH. 2012. Unusually high predation on chacma baboons (*Papio ursinus*) by female leopards (*Panthera pardus*) in the Waterberg mountains, South Africa. Folia Primatologica, 83, 353–360.

28. Kapel CMO. 2000. Host diversity and biological characteristics of the *Trichinella* genotypes and their effect on transmission. Veterinary Parasitology, 93, 263–278.

29. Kapel CMO, Gamble HR. 2000. Infectivity, persistence, and antibody response to domestic and sylvatic *Trichinella* spp. in experimentally infected pigs. International Journal for Parasitology, 30, 215–221.

30. Kapel C, Henrikson SA, Dietz HH, Henrikson P, Nansen P. 1994. A study on the predilection sites of *Trichinella spiralis* larvae in experimentally infected foxes (*Alopex lagopus, Vulpes vulpes*). Acta Veterinaria Scandinavica, 35, 125-132.

31. Kapel C, Henrikson SA, Berg TB, Nansen P. 1995. *Trichinella* infections in Arctic foxes from Greenland: Studies and reflections on predilection sites of muscle larvae. Journal of Helminthology, 69, 325-330.

32. Kapel C, Webster P, Gamble H. 2005. Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife. Veterinary Parasitology, 132, 101–105.

33. Kocieska W, van Knapen F, Ruitenberg EJ. 1980. *Trichinella pseudospiralis* and *T. spiralis* infections in monkeys, I: Parasitological aspects. In: Kim CW, Ruitenberg EJ, Teppema, JS (Eds.), Proceedings of the Fifth International Conference on Trichinellosis, September 1-5, 1980, Noordwijk aan Zee, The Netherlands, 199-202.

34. Krivokapich SJ, Pozio E, Gatti GM, Gonzales Prous CL, Ribicich M, Marucci G, La Rosa G, Cofalonieri V. 2012. *Trichinella patagoniensis* n. sp. (Nematoda), a new encapsulated species infecting carnivorous mammals in South America. International Journal for Parasitology, 42, 903-910.

35. La Grange LJ, Marucci G, Pozio E. 2009. *Trichinella zimbabwensis* in wild Nile crocodiles (*Crocodylus niloticus*) of South Africa. Veterinary Parasitology, 161, 88–91.

36. La Grange LJ, Marucci G, Pozio, E. 2010. *Trichinella zimbabwensis* in a naturally infected mammal. Journal of Helminthology, 84, 35–38.

37. La Grange LJ, Govender D, Mukaratirwa S. 2013. The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. Journal of Helminthology, 87, 91-96.

38. La Grange LJ, Reininghaus B, Mukaratirwa, S. 2014. First report of a mixed infection of *Trichinella nelsoni* and *Trichinella* T8 in a leopard (*Panthera pardus*) from the Greater Kruger National Park, South Africa. Onderstepoort Journal of Veterinary Research, 81, 8-10.

39. Lariviere S, Calzada J. 2001. *Genetta genetta*. Mammalian Species, 680, 1-6.

40. La Rosa G, Pozio E. 2000. Molecular investigation of African isolates of *Trichinella* reveals genetic polymorphism in *Trichinella nelsoni*. International Journal for Parasitology, 30, 663–667.

41. Lehmann KDS, Montgomery TM, McLachlan SM, Parker JM, Spagnuolo OS, VandeWetering KJ, Bills PS, Holekamp KE. 2017. Lions, Hyenas and mobs (oh my!). Current zoology, 63, 313-322.

42. Le Roux PG, Skinner JD. 1989. A note on the ecology of the leopard (*Panthera pardus* Linnaeus) in the Londolozi Game Reserve, South Africa. African Journal of Ecology, 27, 167–171. http://dx.doi.org/10.1111/j.1365-2028.1989.tb00940.x

43. Marucci G, La Grange LJ, La Rosa G, Pozio E. 2009. *Trichinella nelsoni* and *Trichinella* T8 mixed infection in a lion (*Panthera leo*) of the Kruger National Park (South Africa). Veterinary Parasitology, 159, 225–228.

44. Matenga E, Mukaratirwa S, Bhebhe E, Willingham AL. 2006a. Comparison of the infectivity of *Trichinella zimbabwensis* in indigenous Zimbabwean pigs (Mukota) and exotic Large White pigs. International Journal of Applied Research in Veterinary Medicine, 4, 301-306.

45. Matenga E, Mukaratirwa S, Bhebhe E, Willingham AL. 2006b. Evidence of congenital and transmammary transmission of *Trichinella zimbabwensis* in rats (*Rattus norvegicus*) and its epidemiological implications. The International Journal of applied Research in Veterinary Medicine, 4, 307-312.

46. Mbaya W, Ahmed MI, Ibrahim UI, Larmude KJ. 2010. Trichinosis in African giant rats (*Cricetomys gambianus*) in the arid region of north eastern Nigeria. Journal of Medicine and Medical Sciences, 1, 460-464.

47. Mills MGL, Broomhall LS, du Toit JT. 2004. Cheetah *Acinonyx jubatus* feeding ecology in the Kruger National Park and a comparison across African savannah habitats: is the cheetah only a successful hunter on open grassland plains? Wildlife Biology, 10, 177-186.

48. Moleón M, Martinez-Carrasco C, Muellerklein OC, Getz WM, Muñoz-Lozano C, Sánchez-Zapata JA. 2017. Carnivore carcasses are avoided by carnivores. Journal of Animal Ecology, 86, 1179-1191.

49. Monadjem A, Taylor PJ Denys C, Cotterill FPD. 2015. Rodents of sub-Saharan Africa: A biogeographic and taxonomic synthesis. Berlin, Boston: De Gruyter, 2015, 1092 pages, ISBN 978-3-11-030191-5.

50. Moretti A, Piergili-Fioretti D, Pasquali P, Mechelli L, Rossodivita ME, Polidori GA. 1997. Experimental infection of fish with *Trichinella britovi*: Biological evaluations. In: Ortega-Pierres G, Gamble HR, van Knapen F, Wakelin D. (Eds.), Trichinellosis. Centro de Investigacion y Estudios Avanzados IPN, Mexico City, pp. 135-142.

51. Mukaratirwa S, Dzoma B. 2008. Experimental infections of baboons (*Papio spp*.) and vervet monkeys (*Cercopithecus aethiops*) with *Trichinella zimbabwensis* and successful treatment with ivermectin. Onderstepoort Journal of Veterinary Research, 188, 181-188.

52. Mukaratirwa S, Foggin CM. 1999. Infectivity of *Trichinella* sp. isolated from *Crocodylus niloticus* to the indigenous Zimbabwean pig (Mukota). International Journal for Parasitology, 29, 1129–1131.

53. Mukaratirwa S, La Grange L, Pfukenyi DM. 2013. *Trichinella* infections in animals and humans in sub-Saharan Africa: A review. Acta Tropica, 125, 82– 89.

54. Mukaratirwa S, La Grange LJ, Malatji MP, Reininghaus B, Lamb J. 2019. Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga Provinces of South Africa. Journal of Helminthology, 23, 1-7.

55. Nagano I, Wu Z, Matsuo A, Pozio E, Takahashi Y. 1999. Identification of *Trichinella* genotypes by polymerase chain reaction—restriction fragment length polymorphism of mitochondrial cytochrome c oxidase subunit I gene. International Journal for Parasitology, 29, 1113–1120.

56. Nelson GS, Guggisberg CWA, Mukundi J. 1963. Animal hosts of *Trichinella spiralis* in East Africa. Annals of Tropical Medicine and Parasitology, 57, 332–346.

57. Nishank SS, Swain S. 2019. Cannibalism in animals. Science Horizon, 4, 33-39.

58. OIE-World Organisation for Animal Health. 2019. https://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_diagnostic_tests.htm

59. Owen R. 1835. Description of a microscopic entozoon infesting the muscles of the human body. Transactions of the Zoological Society of London, 1, 315–324.

60. Palomares F, Caro TM. 1999. Interspecific killing among mammalian carnivores. American Naturalist, 153, 492-508.

61. Périquet S, Fritz H, Revilla E. 2015. The lion king and the hyaena queen: large carnivore interactions and coexistence. Biological Reviews, 90, 1197–1214.

62. Pienaar U de V. 1964. The small mammals of the Kruger National Park-A systematic list and zoogeography. Koedoe, 7, 1-25.

63. Polis GA, Myers CA, Holt RD. 1989. The ecology and evolution of intraguild predation: Potential competitors that eat each other. Annual Review of Ecology, Evolution and Systematics, 20, 297-330.

64. Pozio E. 2000. Factors affecting the flow among domestic, synanthropic and sylvatic cycles of *Trichinella*. Veterinary Parasitology, 93, 241-262.

65. Pozio E. 2005. The broad spectrum of *Trichinella* hosts: from cold- to warm-blooded animals. Veterinary Parasitology, 132, 3-11.

66. Pozio E. 2007. World distribution of *Trichinella* spp. infections in animals and humans. Veterinary Parasitology, 149, 3–21.

67. Pozio E, De Meneghi D, Roelke-Parker ME, La Rosa G. 1997. *Trichinella nelsoni* in carnivores from the Serengeti ecosystem, Tanzania. Journal of Parasitology, 83, 1195-1198.

68. Pozio E, Foggin CM, Gelanew T, Marucci G, Hailu A, Rossi P, Gómez-Morales M. 2007. *Trichinella zimbabwensis* in wild reptiles of Zimbabwe and Mozambique and farmed reptiles of Ethiopia. Veterinary Parasitology, 143, 305-310.

69. Pozio E, Foggin CM, Marucci G, La Rosa G, Sacchi L, Corona S, Rossi P, Mukaratirwa S. 2002. *Trichinella zimbabwensis* n.sp. (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. International Journal for Parasitology, 32, 1787–1799.

70. Pozio E, Hoberg E, La Rosa G, Zarlenga DS. 2009. Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. Infection, Genetics and Evolution, 9, 606–616.

71. Pozio E, La Rosa G. 2000. *Trichinella murrelli* n.sp.: Etiological agent of sylvatic trichinellosis in temperate areas of North America. Journal of Parasitology, 86, 134-139.

72. Pozio E, La Rosa G. 2005. Evaluation of the infectivity of *Trichinella papuae* and *Trichinella zimbabwensis* for equatorial freshwater fishes. Veterinary Parasitology, 132, 113-114.

73. Pozio E, La Rosa G, Murrell KD, Lichtenfels JR. 1992. Taxonomic revision of the genus *Trichinella*. Journal of Parasitology, 78, 654-659.

74. Pozio E, Marucci G, Casulli A, Sacchi L, Mukaratirwa S, Foggin CM, La Rosa G. 2004. *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, puthons and turtles. Parasitology, 128, 333-342.

75. Pozio E, Murrell KD. 2006. Systematics and epidemiology of *Trichinella*. Advances in Parasitology, 63, 371-445.

76. Pozio E, La Rosa G, Rossi P, Murrell KD. 1992. Biological characterizations of *Trichinella* isolates from various host species and geographic regions. Journal of Parasitology, 78, 647–653.

77. Pozio E, Owen IL, La Rosa G, Sacchi L, Rossi P, Corona S. 1999. *Trichinella papuae* n.sp. (Nematoda), a new non-encapsulated species from domestic and sylvatic swine of Papua New Guinea. International Journal for Parasitology, 29, 1825-1839.

78. Pozio E, Pagani P, Marucci G, Zarlenga DS, Hoberg EP, De Menighi D, La Rosa G, Rossi L. 2005. *Trichinella britovi* etiological agent of sylvatic trichinellosis in the Republic of Guinea (West Africa) and a re- evaluation of geographical distribution for encapsulated species in Africa. International Journal for Parasitology, 35, 955-960.

79. Pozio E, Verster A, Braack L, De Meneghi D, La Rosa G. 1994. Trichinellosis south of the Sahara. In: Campbell CW, Pozio E, Bruschi F. (Eds.), Trichinellosis. Istituto Superiore di Sanità Press, Rome, Italy, 527–532.

80. Pozio E, Zarlenga DS. 2005. Recent advances on the taxonomy, systematic and epidemiology of *Trichinella*. International Journal for Parasitology, 35, 1191-1204.

81. Pozio E, Zarlenga DS. 2013. New pieces of the *Trichinella* puzzle. International Journal for Parasitology, 43, 983- 997.

82. Ramesh T, Downs CT. 2015. Diet of serval (*Leptailurus serval*) on farmlands in the Drakensberg Midlands, South Africa. Mammalia, 79, 399-407.

83. Reina D, Munoz-Ojeda M, Serrano F. 1996. Experimental trichinellosis in goats. Veterinary Parasitology, 62, pp. 125–132.

84. Retief F. 2016. The ecology of spotted hyena, *Crocuta crocuta*, in Majete Wildlife Reserve, Malawi. Thesis (MSc) Stellenbosch University, 137 pages.

85. Rosenthal J. 2009. Climate change and the geographic distribution of infectious diseases. EcoHealth, 6, 489-495.

86. Sachs R. 1970. Zur Epidemiologie der Trichinellose in Afrika. *Zeitschrift fur* Tropenmedizin und Parasitologie, 20, 117–126.

87. SANPARKS. 2008. Kruger National Park Management Plan: Revised and updated December 2008. 151 pages.

88. Scholtz M, Kruger M, Saayman M. 2013. Understanding the reasons why tourists visit the Kruger National Park during a recession. Acta Commercii, 13, 9 pages.

89. Sharma R. 2019. *Trichinella* species and *Toxoplasma gondii* in wolverine (*Gulo gulo*) from north western Canada. Thesis (PhD) University of Saskatchewan, Saskatoon.

90. Sharma R, Konecsni K, Scandrett B, Thompson P, Hoberg EP, Bouchard E, Buhler K, Harms NJ, Kukka PM, Jung TS, Elkin B, Mulders R, Larter NC, Branigan M, Pongracz J, Wagner B, Fenton H, Rosenthal BM, Jenkins E. 2019. Distribution and genetic diversity of *Trichinella* in Canadian wildlife: A previously undescribed species (T13) and an unexpected discovery of *T. spiralis*. Scientia Parasitologica, 20, 145-148.

91. Skinner JD, Breytenbach GJ, Maberly, CTA. 1976. Observations on the ecology and biology of the bush pig (*Potamochoerus porcus) Linn* in the northern Transvaal. South African Journal of Wildlife Research, 6, 123-128.

92. Somaweera R, Brien M, Shine R. 2013. The role of predation in shaping crocodilian natural history. Herpetological Monographs, 27, 23–51.

93. Soule C, Dupouy-Camet J, Georges P, Ancelle T, Gillet JP, Vaissaire J, Delvigne A, Plateau E. 1989. Experimental trichinellosis in horses: Biological and parasitological evaluation. Veterinary Parasitology, 31, 19-36.

94. Stander PE. 1997. The ecology of lions and conflict with people in north-eastern Namibia. In: J. van Heerden (ed.), Proceedings of a Symposium on Lions and Leopards as Game Ranch Animals, Onderstepoort, Oct 1997, 10–17.

95. Szczepaniuk K. 2011. "*Varanus niloticus*" (On-line), Animal Diversity Web. Accessed October 18, 2019 at https://animaldiversity.org/accounts/Varanus_niloticus/b

96. Thomson P. 2002. *Otocyon megalotis* (On-line), Animal Diversity Web. Accessed December 30, 2019 at https://animaldiversity.org/accounts/Otocyon_megalotis/

97. Tomašovičová O. 1981. The role of fresh water fish in transfer and maintenance of Trichinellae under natural conditions. Biolŏgia, 36, 115-125.

98. Wagner AP. 2006. Behavioral ecology of the striped hyena (*Hyaena hyaena*). PhD thesis, Montana State University, Bozeman, Montana, 182 pages.

99. World Heritage Encyclopedia. 2017. Nile crocodile *World Public Library*, Article id WHEBN 001284973, Retrieved 4 December 2017.

100. Young E, Kruger SP. 1967. *Trichinella spiralis* (Owen, 1935) Railliet 1895. Infestation of wild carnivores and rodents in South Africa. Journal of the South African Veterinary Association, 38, 441–443.

101. Young E, Whyte IJ. 1975. Trichinosis (*Trichinella spiralis* infestations) in wild animals of the Kruger National Park. Journal of the South African Veterinary Association, 46, 233–234.

CHAPTER 4: EXPERIMENTAL INFECTION OF AFRICAN SHARP TOOTH CATFISH WITH *TRICHINELLA ZIMBABWENSIS*

Published in combination with Chapter 5 as:
La Grange LJ, Mukaratirwa S. (2020). Experimental infection of tigerfish (*Hydrocynus vittatus*) and African sharp tooth catfish (*Clarias gariepinus*) with *Trichinella zimbabwensis*. Onderstepoort Journal of Veterinary Research, 87, a1876. https://doi.org/ 10.4102/ojvr.v87i1.1876.

4.1 Abstract

African sharp tooth catfish (*Clarias gariepinus*) have a varied omnivorous diet and are accomplished scavengers and predators with food ranging from plankton to small mammals. The fish cohabit with Nile crocodiles (*Crocodylus niloticus*) and Nile monitor lizards (*Varanus niloticus*) and serve as food source to both these aquatic predators. This makes it a probable host for *T. zimbabwensis* despite the fact it has never been reported to naturally infect any fish species. Moreover, the value of *C. gariepinus* as a source of human food, and thus its potential to serve as a source of human infection, from a veterinary public health perspective, validates further investigation. A total of 24 *C. gariepinus* was randomly divided into 5 groups and each fish experimentally infected with *T. zimbabwensis* larvae. Group 1 (n = 5, average weight 354.9 ± 75.46 g) were infected with 1.26 ± 0.58 larvae per gram (lpg) of fish under anaesthesia; Group 2 (n = 5, average weight 531.0 ± 160.3 g) with 1.02 ± 0.29 lpg of fish without anaesthesia; Group 3 (n = 5, average weight 568.0 ± 109.7 g) with 0.92 ± 0.20 lpg of fish under anaesthesia; Group 4 (n = 5, average weight 499.6 ± 74.1 g) with 1.02 ± 0.14 lpg of fish without anaesthesia and Group 5 (n = 4, average weight 1048 ± 137.8 g) with 1.0 ± 0.11 lpg of fish. Groups 1 and 2 were infected using an orogastric tube. Groups 3 and 4 were infected using blank gelatin capsules and Group 5 infected through natural feeding. The fish were euthanized at day 1 post-infection (p.i.) (Group 4), day 2 p.i. (Group 3 and 5) and day 7 p.i. (Group 1 and 2). Results showed that *T. zimbabwensis* larvae did not establish or develop in the gastrointestinal tract as no larvae or adult *T. zimbabwensis* were observed at day 1 (Group 4); day 2 (Groups 3 and 5) and at day 7 p.i (Groups 1 and 2). Results from this experimental study suggests that *T. zimbabwensis* is not able to establish and develop in *C. gariepinus* which implies a less likelihood for the fish to play a role in the epidemiology of the parasite. The potential influence of temperature on *T. zimbabwensis* survival and development in African sharp tooth catfish preclude a definitive conclusion in respect of the fish's potential role in the epidemiology of *T. zimbabwensis.*

4.2 Introduction

Pienaar (1968) reported the Kruger National Park (KNP) of South Africa to be home to a total of 46 freshwater fish species including predators such as African sharp tooth catfish (*Clarias gariepinus*), tigerfish (*Hydrocynus vittatus*), large scale yellowfish (*Labeobarbus marequensis*), Madagascar mottled eel (*Anguilla marmorata*) and common long fin eel (*A. mossambicus*).

The African sharp tooth catfish is a large, air breathing fish with an elongated body devoid of scales and long dorsal and anal fins (Skelton, 2001). It is considered to probably have the widest distribution of all fish in Africa and is native to most of Africa (Skelton, 2001) including the KNP (Pienaar, 1968; Anchor Environmental, 2017) (Figure 4.1). Exceptions on geographical distribution include the Maghreb, Upper and Lower Guinea and the Eastern- and Western Cape provinces of South Africa (Picker and Griffiths, 2011). *Clarias gariepinus* can tolerate a wide array of habitats including estuaries but favours the slow flowing waters of floodplains, rivers and the more stagnant environments of lakes and dams (Skelton, 2001).

Figure 4.1 Distribution of the African sharp tooth catfish in South Africa depicting native (green) and introduced (red) habitats (Anchor Environmental 2017)

Catfish have an omnivorous diet and are accomplished scavengers and predators that can feed on diets ranging from plankton to small mammals (Bruton, 1979; Skelton, 2001). Although they prefer inactive food (Bruton, 1979; Skelton, 2001), pack hunting behaviour has also been described (Merron, 2003).

This fish is also commercially farmed for food internationally and by 2010 production yields in South Africa had exceeded 190 000 tonnes per annum (Anchor Environmental, 2017). Britz *et al*., (2009) reported the existence of two commercial catfish farms in South Africa which consistently produced 180 tonnes of fish between 2006 and 2010 (DAFF, 2012; Anchor Environmental, 2017). Britz (2007) projected that annual catfish production in South Africa

could reach 10 000 tonnes per annum. Although exact figures are not available for actual production volumes, the FAO reported the existence of 13 commercial farms in South Africa by 2010 (FAO, 2010).

However, catfish is an unknown commodity in South Africa and the inability to effectively market and sell the product locally has led to a collapse of the local catfish industry (FAO, 2014; Mahieu, 2015). Mahieu (2015) noted some potential for catfish to be marketed among African immigrants where catfish is considered a traditional dish in their native countries. Some predictions also indicated that the market value of catfish could potentially grow in excess of ZAR150 million within the next few years (DAFF, 2012). Apart from its commercial value as a source of protein, catfish is commonly consumed by subsistence fisherman in South Africa and is also frequently caught by recreational- and sport anglers.

Clarias gariepinus are known to inhabit the same environments where Nile crocodiles (*Crocodylus niloticus*) and Nile monitor lizards (*Varanus niloticus*) reside rendering them a potential food source to both these aquatic predators and this is the case in the Greater Kruger National Park (GKNP) (Pienaar, 1968; Skelton, 2001). Catfish are also known to be predated upon by leopards (*Panthera pardus*) (Skelton, 2001) which, in addition to their own feeding behaviour, further strengthens their candidature to potentially host *T. zimbabwensis*. La Grange *et al*. (2013) and La Grange and Mukaratirwa (2020) pointed out the need for surveillance studies to elucidate the epidemiological role of additional, probable host species that could serve as sources of infection to crocodiles. Moreover, *C. gariepinus* is a source of food for many resource-poor communities and thus it's potential to serve as a source of human infection for *T. zimbabwensis* needs to be clarified.

4.3 Materials and Methods

The study protocol was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ref: AREC/029/016M and 030/019D).

4.3.1 Sourcing and transportation of experimental fish

Twenty-four (24) *C. gariepinus* fish were randomly selected for the study. *Clarias gariepinus* were obtained from a research breeding and holding facility of the University of Limpopo, Polokwane, South Africa.

A custom-made plastic tank (capacity 500 litres) was used to transport the fish. The tank was equipped with a large screw-on lid able to comfortably accommodate a medium sized scoop net to easily transfer fish from- and into the tank. The tank was secured on the back of a light delivery vehicle and filled with approximately 250 litres of water from the source where the fish were housed. The tank was additionally custom-fitted with a small 12 volt circulating

pump powered from the battery of the vehicle (Attwood, Tsunami series, T800). The outlet from the pump was secured to the top of the tank allowing water from the pump to be dispensed freely from above the waterline to assist in the addition of oxygen to the water. 2-phenoxyethanol (Sigma-Aldrich, USA) was added to the water at a concentration of 0.3 ml/l as a sedative to reduce stress associated with handling and transportation of the fish (Ögretmen and Göçek, 2013; Husen and Sharma, 2014).

4.3.2 Fish husbandry

From the 51 fish which arrived in good health to the experimental station (Tipperary farm, Nelspruit, South Africa), 24 fish were randomly selected for the study and the remaining fish were donated to Wildlifevets.com for future research.

Fourteen days prior to the arrival of fish from source, three circular, artificial ponds (3 m diameter, 7000 litre capacity) were set up outdoor in a fenced area on the research facility at Tipperary farm. A 60% shade cloth roof was established approximately 2.5 metres above the artificial ponds for shade and each individual pond was covered with a bird net to prevent escape of fish and predation by birds (Plate 4.1). Ponds were filled with approximately 3500 litres of water and each equipped with a filter system comprising of a brush filter, biofilter, UV filter and a submersible pump (6500 l/ hour) to circulate and filter the water.

Plate 4.1 Artificial ponds used to house African sharp tooth catfish

The biofilters were primed with water from a small outside dam housing a few ornamental koi fish and the experimental ponds filled with potable water. The ponds were left

unoccupied for 14 days to allow aerobic bacteria to establish and colonize the biofilters and to ensure that any residual chlorine that may be present in the water would evaporate.

Fish were transferred from the transport tank to two of the experimental ponds and larger individuals were separated from the smaller fish to avoid potential competition for food. Fish were fed commercial koi pellets (Aqua Plus, Midfeeds, South Africa) on a daily basis. Brush filters and water surfaces were cleaned weekly.

4.3.3 Experimental infection of *Clarias gariepinus*

A total of 24 fish were used during the experiment and randomly divided into four groups of five fish and one group of 4 fish. Individual fish were randomly collected from the tank with a net on the relevant day of infection (Table 4.1). *Trichinella zimbabwensis* larvae previously derived from a Nile crocodile were maintained in a colony of rats housed at the Biological Resource Unit (BRU) at the Westville Campus of the University of KwaZulu-Natal, South Africa.

Infected stock rats were euthanized and larvae isolated by means of artificial muscle digestion according to a published protocol (Nöckler and Kapel, 2007). After digestion, larvae were washed three times with distilled water, counted and transferred to small glass vials in distilled water. 2 x 15 ml vials with approximately 100 larvae/ml were used. Vials with larvae were refrigerated during transportation from BRU to Nelspruit and on arrival were allowed to reach room temperature prior to infection. Larvae were examined under a stereo microscope prior to infection to confirm viabilty.

4.3.3.1 Infection via an orogastric tube with and without the use of anaesthesia

A total of ten fish were infected using an orogastric tube (Table 4.1). Group 1 fish (n = 5) with an average weight of 354.9 ± 75.46 g (Table 4.1) were individually caught and placed in a 20 litre container containing water from the dams with 0.3 ml/l of 2- phenoxyethanol added to the water. Fish were carefully observed until a suitable level of sedation (Stage 2 anaesthesia) was reached (Table 4.2). Once anaesthetized, the fish were removed by hand and a small polypropolene canine urinary catheter (Sovereign®, French Gauge 8, Sherwood Medical, St. Louis, United States of America) was inserted down the oesophagus into the stomach while holding the fish in an upright position. The other end of the catheter was attached to a 20 ml glass syringe filled with 5 ml of infective material (approximately 500 larvae per animal; 1.26 ± 0.58 lpg of fish). The total time of administering of the infective larvae to each anaesthetized fish was approximately 30 seconds per fish (Plate 4.2).

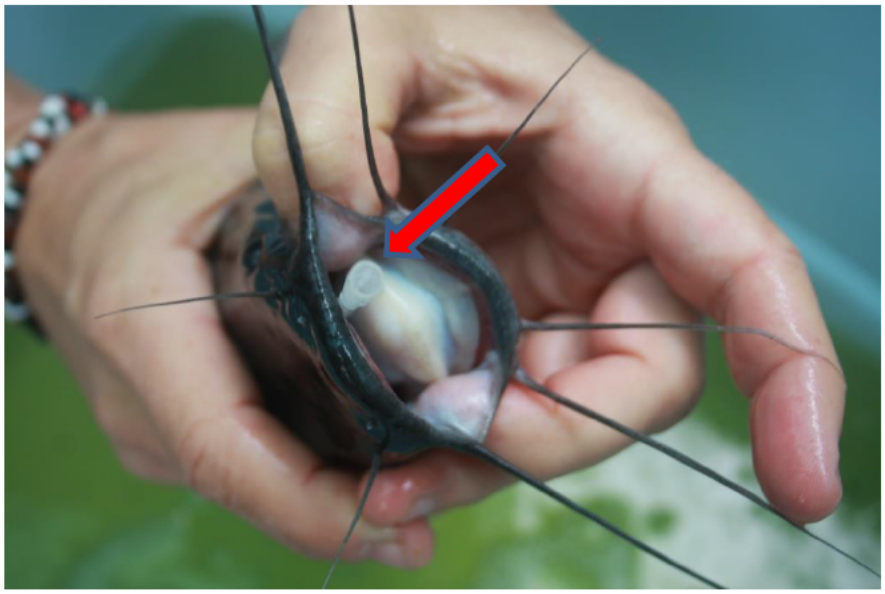


Plate 4.2 Position of orogastric tube inserted into the stomach of a catfish

Once the administration of larvae was completed, the fish were returned to a separate pond and allowed to recover. The procedure was repeated for Group 2 (n = 5) which had an average of 531.0 ± 160.3 g in weight (Table 4.1) and each fish was infected with approximately 500 larvae (1.02 ± 0.29 lpg of fish) without using anaesthetic (Table 4.1). This was done to rule out the possibility of negative effect of anaesthetic on the establishment and/or development of *T. zimbabwbensis* larvae in the fish.

4.3.3.2 Infection via gelatin capsules

A total of ten fish were infected using blank gelatin capsules. Blank gelatin capsules (size 0, Medisurge, South Africa) were obtained from a local pharmacy. Due to the limited size and volume of the capsules, the concentration of larvae per ml of water was increased to ensure that a similar infective dose was administered as for the first two trial groups. The procedure of capture and restraint was conducted as previously described in 4.3.3.1. Group 3 (n = 5) averaged 568.0 ± 109.7 g in weight and was infected under anaesthesia as described previously.

Infective material (approximately 500 larvae, 0.92 ± 0.20 lpg of fish) was transferred into one half of the empty capsule and closed. Once the fish were anaesthetized and restrained, the capsule was carefully inserted into the oral cavity and gently pushed down the oesophagus with an index finger. Fish were held upright for approximately 45 seconds to ensure that the

capsule had been completely swallowed. The procedure was repeated without anaesthesia for Group 4 (n = 5) averaged 499.6 ± 74.1 g in weight and approximately 500 larvae per fish (1.02 ± 0.14 lpg of fish) administered (Table 4.1).

4.3.3.3 Infection via natural feeding behaviour

Group 5 consisted of four fish and infective material was harvested from the muscle tissue of infected stock rats. Muscle tissue was dissected from euthanized rats and mixed into a homogenous, pooled sample. A portion of the sample was subjected to artificial digestion to determine the mean larvae per gram (lpg) of tissue (Nöckler and Kapel, 2007). The final sample was adjusted with the addition of non-infected material to a final concentration of 50 lpg. The sample was refrigerated until the day of use. In the two weeks prior to infection the fish diet was slowly changed from the commercial pellets and replaced with increasing amounts of chicken hearts (Mikon Farming abattoir, Mpumalanga, South Africa) until the fish were exclusively feeding on the chicken hearts offered. This was done to accustom the fish to fresh bait to ensure feeding on the day of infection.

Food was withheld from the experimental fish for 48 hours prior to infection and 10 grams of infective tissue was used to prepare infective boluses (approximately 500 larvae). Each 10 grams of infective tissue was mixed into a bolus using a small amount of maize flour to act as a binding agent. A total of 8 boluses were prepared and each fish offered two boluses (approximately 1000 larvae/fish, 1.00 ± 0.11 lpg of fish) since the fish had grown considerably by this stage in the study and averaged 1048 ± 137.8 g. The maize flour also served to prevent the infective material from dissipating in the water before being consumed by the fish. Boluses were offered as normal food on the day of infection and were readily consumed by all the experimental fish immediately on exposure.

4.3.4 Analysis of experimentally infected *Clarias gariepinus*

Fish were collected from the ponds on the relevant day post-infection as depicted in Table 4.1. Fish were euthanized by placing them in a container with water containing double the normal concentration of 2- phenoxyethanol (0.6 ml/ l). The fish were allowed to reach stage 4 anaesthesia (Table 4.2). After the fish had lost equilibrium and response to stimuli and breathing had ceased, they were decapitated.

The dead fish were placed in a mobile refrigerator powered from the 12 volt power supply of a vehicle and immediately transported to UKZN Parasitology laboratory in Durban, for further processing and analysis the following day.

Table 4.1 Results from experimental infection of African sharp tooth catfish (*Clarias gariepinus*) with *Trichinella zimbabwensis.*

Group	N	M/F	Weight(g) $\bar{x}$ ± SD	Infection Method	Larvae/g of fish ± SD	Water daily T °C $\bar{x}$ ± SD	Day (p.i.)	Infection results		
								Body cavity	Intestines	Muscle
Group 1	5	3/2	354.9 ± 75.46	Orogastric (anaesthesia)	1.26 ± 0.58	22.47 ± 1.28	7	Neg	Neg	Neg
Group 2	5	5/0	531.0 ± 160.3	Orogastric (no anaesthesia)	1.02 ± 0.29	15.59 ± 0.50	7	Neg	Neg	Neg
Group 3	5	5/0	568.0 ± 109.7	Gelatin capsules (anaesthesia)	0.92 ± 0.20	15.49 ± 0.36	2	Neg	Neg	Neg
Group 4	5	3/2	499.6 ± 74.1	Gelatin capsules (no anaesthesia)	1.02 ± 0.14	15.55 ± 0.41	1	Neg	Neg	Neg
Group 5	4	4/0	1048.0 ± 137.8	Natural Feeding	1.00 ± 0.11	21.58 ± 1.36	2	Neg	Neg	Neg

M/F = Male/Female; SD = Standard deviation; Neg = Negative for infection; p.i. = post-infection.

Each fish was dissected on the ventral aspect and open lengthwise with a pair of scissors from the cloaca to the head to expose internal organs. The body cavity was rinsed with 0.9% saline solution and the flush liquid collected in a large glass specimen dish. The intestinal tract was removed and the small intestine and stomach separated into specimen dishes containing 0.9% saline solution (Justine *et al*., 2012). The intestine and stomach were cut open and the mucosa was carefully scraped. The intestinal and stomach wash contents and the material collected during rinsing of the body cavity were examined under a stereo microscope for the presence of *T. zimbabwensis* larvae and/or adults (Plate 4.4). Additionally, 100 g of muscle tissue was collected from various sites to provide a representative sample from the whole body. Muscle tissues were subjected to artificial digestion according to a published protocol (Nöckler and Kapel, 2007) to detect the presence of *T. zimbabwensis* larvae in muscles.

Table 4.2 Stages of anaesthesia in fish (Adapted from Gardner (2017)).

Stage	Description	Response	Application
0	Normal	Normal motility, fully responsive to stimuli, normal muscle tone, full equilibrium, spontaneous ventilation.	Minimal
1	Light sedation	Sluggish response to stimuli	Transport, drug administration, gavage feeding
2	Deep sedation	Uncoordinated/ slow/ ceased motility, near complete loss of response to stimuli, decreased respiration, partial loss of equilibrium	Superficial sampling, drug administration, gavage feeding
3	Moderate anaesthesia	Loss of righting reflex, decreased muscle tone, partial response to noxious stimuli	Short, minimally painful surgical procedures
4	Deep anaesthesia	Unresponsive to noxious stimuli, very slow respiration, minimal/ no muscle tone, decreased heart rate	All surgical procedures
5	Death	Loss of respiration, cardiac arrest	Euthanasia

4.4 Results

No larvae or adults of *T. zimbabwensis* were observed in the stomach, intestinal tract, body cavity and muscle tissue of all the fish infected using different modes on day 7 p.i (Groups 1 and 2), day 2 p.i. (Groups 3 and 5) and day 1 p.i (Group 5) (Table 4.1).

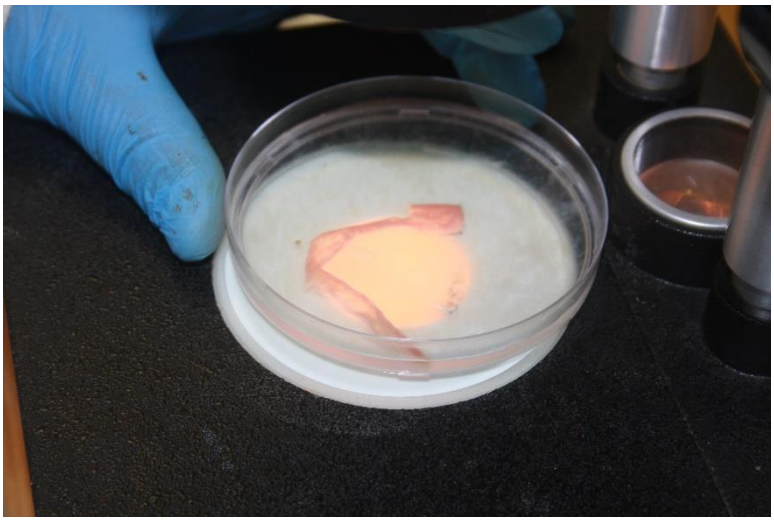


Plate 4.4 Examination of the intestine and its contents for adult *Trichinella zimbabwensis* under a stereo microscope at 400 x magnification

4.5 Discussion

Trichinella zimbabwensis has been reported to naturally infect both warm- and cold-blooded hosts, and of the cold-blooded hosts, Nile crocodiles (Mukaratirwa and Foggin 1999; Pozio *et al*., 2004; La Grange *et al*., 2009; 2013) and Nile monitor lizards (Pozio *et al*., 2004; Mukaratirwa *et al*., 2019) have been reported. Limited experimental studies to assess the infectivity of *Trichinella* spp. to fish have been conducted to date and most notable were the studies by Tomašovičová (1981); Moretti *et al*. (1997) and Pozio and La Rosa (2005).

In the study by Tomašovičová (1981), European ruffe (*Gymnocephalus cernuus*), European perch (*Perca fluviatilis*), the common bleak (*Alburnus alburnus*) and the common carp (*Cyprinus carpio*) were experimentally infected with *T. pseudospiralis* and *T. spiralis* larvae delivered per os at a rate of 500 larvae per fish. The size of fish used were not reported and hence the number of larvae administered per gram of fish is not known. Results showed that *T. pseudospiralis* larvae migrated in unaltered form from the gastrointestinal tract to the body cavity, organs and muscles of fish while *T. spiralis* larvae were only confined to the body cavity of fish (Tomašovičová, 1981). Larvae were isolated from the body cavity of fish after 2 days p.i. in the case of *T. pseudospiralis* and similarly in the gastrointestinal tract of fish in the case of *T. spiralis*.). However, the fish species in which larvae of *Trichinella* spp. were found was not indicated.

Similarly, in the study by Moretti *et al*., (1997), *T. britovi* larvae were isolated from the gastrointestinal tract and body cavity of common carp and from the gastrointestinal tract of

catfish (*Ictalurus melas*) after experimental infection. Fish were infected using an orogastric tube and larvae administered at dosages varying between 100, 200 and 100 larvae per fish (Moretti *et al*., 1997). The size of fish and inoculum per gram of fish was not calculated.

No *T. zimbabwensis* larvae or adults could be detected in the gastrointestinal tract and cavities of piranha (*Serrasalmus nattereri* and *S. rhombeus*) in studies by Pozio and La Rosa (2005). The non-encapsulated species of *T. zimbabwensis* and *T. papuae* requires a temperature range of 26˚C to 40˚C in order to develop in homeothermic and poikilothermic hosts (Pozio *et al*., 2004). In view of this, in the study by Pozio and La Rosa (2005) temperature was maintained between 25˚C–32˚C. Larvae were administered per os at a dosage of 1000 larvae per fish. However, the exact method of infection, size of fish and inoculum per gram of fish was not reported.

During the course of this study, water temperatures averaged between 15.5˚C–22.5˚C. (Table 4.1). Temperature ranges reported by Tomašovičová (1981) and Moretti *et al*. (1997) were warmer than most of those recorded during this study and this may have impacted on larval development and survival in this study. Evidence from previous studies involving poikilothermic hosts suggested temperature to play an integral role in larval development and survival (Asatrian *et al*., 2000; Cristeau and Perian 1999; Pozio *et al*., 2004). Temperatures recorded during this study were well below the temperature range required for *T. zimbabwensis* development. Previous studies have suggested the entozoic environment in the gastrointestinal tract of fish to be unsuitable for *Trichinella* spp. larvae (Moretti *et al*., 1997; Pozio and La Rosa, 2005).

It is intriguing that all of the previous studies focussed on either *Trichinella* spp. which are known not to be infective to ectothermic hosts except the study by Pozio and La Rosa (2005) or host species that do not occur in natural environments where *T. zimbabwensis* is known to occur. It should be sensible to argue that parasites co-existing with particular host species could adapt over time to establish new patterns of infection. This sentiment is supported by the diversification of *Trichinella* spp. alongside their contemporary hosts as hypothesized by Pozio *et al*. (2009). This appears not be the case with *T. zimbabwensis* and fish. To date, the number of experimental studies involving *T. zimbabwensis* and fish have been very few and the perceived failure of *T. zimbabwensis* to adapt to tropical fish may be proven otherwise in some, as yet to be identified fish species.

4.6 Conclusion

Results from this study are in agreement with previous studies conducted on experimental infection of fish with *T. spiralis* and *T. pseudospiralis* (Tomašovičová, 1981); *T.*

britovi (Moretti *et al*., 1997), *T. zimbabwensis* and *T. papuae* (Pozio and La Rosa, 2005) where infection failed to establish.

The fact that larvae of *T. britovi* (Moretti *et al*., 1997), *T. pseudospiralis* and *T. spiralis* (Tomašovičová, 1981) managed to survive in the gut of at least some of the experimental fish species examined, even at lower temperatures, further supports evidence that both host- (Soule *et al*., 1989; Kapel, 1995; Reina *et al*., 1996; La Grange *et al*., 2013) and parasite characteristics (Hurnikova *et al*., 2004; Kapel *et al*., 2005) are crucial in determining the infectivity of *Trichinella* spp.

Furthermore, previous studies by Tomašovičová (1981) and Moretti *et al*. (1997) showed the ability of some *Trichinella* species to survive in fish tissue for some time, albeit short periods, suggesting that some tropical fish may in fact act as paratenic hosts for the parasite. This could not be confirmed in the African sharp tooth catfish used in this study.

Results from this study and other similar studies suggest the entozoic environment in the gastrointestinal tract of fish may be unsuitable for the survival and development of *T. zimbabwensis*. Pozio and La Rosa (2005) similarly concluded the entozoic environment of the piranha used in their experiments might be unsuitable for the development and establishment of *T. zimbabwensis* and *T. pseudospiralis*. Moretti *et al*. (1997) reached a similar conclusion and postulated several probable reasons for the inability of *Trichinella* spp. to complete its life cycle in fish. The following was particularly noted: 1) lack of villi in the small intestine that would aid in anchoring the larvae and provide a means to resist peristaltic movements, 2) the copious amounts of bile and mucous excreted in the intestine of fish, 3) low body temperature of the host, 4) quick passage of larvae through the intestine and 5) the possible destruction of larvae by digestive processes of fish.

It is evident from their wide distribution that catfish tolerate and thrive in a wide range of temperatures. The potential influence of temperature on *T. zimbabwensis* survival and development in African sharp tooth catfish was not considered in the original study design.

Even though the average temperature recorded in this study was well within the natural, tolerable range of catfish, it was not aligned with the range required for *T. zimbabwensis* development. This precludes a definitive conclusion in respect of the fish's potential role in the epidemiology of *T. zimbabwensis.*

Future research efforts should focus on additional tropical fish such as large scale yellowfish, Madagascar mottled eel and common long fin eel that share aquatic habitats with other known hosts of *T. zimbabwensis* in the GKNP to elucidate the role of any specific fish species in the epidemiology of *T. zimbabwensis*. Future research should also consider the

influence of temperature on the development and/or survival of *T. zimbabwensis* in African sharp tooth catfish and other tropical fish species.

4.7 References

Anchor Environmental. 2017. African sharp tooth cat fish *Clarias gariepinus*. Available at: https://anchorenvironmental.co.za/sites/default/files/2017-1/BRBA%20C.%20gariepinus.pdf

Asatrian A, Movsessian S, Gevorkian A. 2000. Experimental infection of some reptiles with *T. spiralis* and *T. pseudospiralis*. Abstract book, p. 154. The Tenth International Conference on Trichinellosis, 20-24 August, Fontaineblue, France.

Britz PJ. 2007. A Study on the Status of Aquaculture Production and Trade in South Africa. Volume 2: Growth Potential of the South African Aquaculture Industry and Recommendations for Sector Development. A report for the Department of Trade and Industry produced by EnviroFish Africa (Pty.) Ltd. 24 pages.

Britz PJ, Lee B, Botes L. 2009. AISA 2009 Aquaculture Benchmarking Survey: Primary Production and Markets. AISA report produced by Enviro-Fish Africa (Pty) Ltd. 117 pages.

Bruton MN. 1979. The food and feeding behaviour of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of cichlids. Transactions of the Zoological Society of London, 35, 47-114.

DAFF. 2012. Aquaculture Yearbook 2012 South Africa. Compiled by Chief Directorate: Aquaculture and Economic Development Fisheries Branch, Department of Agriculture, Forestry and Fisheries, 41 pages.

Eco Travel Africa. (2000-2005). Travel guide to Southern African Safari Adventures. http://home.intekom.com/ecotravel/Guides/Reserves/KNP/Kruger_National_Park_Fish.htm Accessed March 2020.

FAO. 2014. The State of World Fisheries and Aquaculture Opportunities and Challenges. Food and Agriculture Organization of the United Nations, Rome, 2014, 223 pages.

FAO. 2010. National Aquaculture Sector Overview. South Africa. National Aquaculture Sector Overview Fact Sheets. Text by K. Halley and B. Semoli. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2010. [Cited 28 February 2019].FAO. 2014. The State of World Fisheries and Aquaculture Opportunities and Challenges. Food and Agriculture Organization of the United Nations, Rome, 2014, 223 pages.

Gardner BR. 2017. Anaesthesia of fresh water teleost fish for the private practitioner. http://www.cpdsolutions.co.za/Publications/org_publications_public.php?o=51, Copyright Reserved, South African Veterinary Association, 6 pages.

Hurníková Z, Dubinsky S, Mukaratirwa S, Foggin CM, Kapel CMO. 2004. Infectivity and temperature tolerance on non-encapsulating *Trichinella zimbabwensis* in experimentally infected Red foxes (*Vulpes vulpes*). Helminthologia, 41, 189–192.

Husen A, Sharma S. 2014. Efficacy of anaesthetics for reducing stress in fish during aquaculture practices- a review. Journal of Science, Engineering and Technology, 10, 104-123.

Justine J, Briand MJ, Bray RA. 2012. A quick and simple method, usable in the field, for collecting parasites in suitable condition for both morphological and molecular studies. Parasitology Research, 111, 341-351.

Kapel C. 1995. *Trichinella* infections in Arctic foxes from Greenland: studies and reflections on predilection sites of muscle larvae. Journal of Helminthology, 69, 325-330.

Kapel C, Webster P, Gamble H. 2005. Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife. Veterinary Parasitology, 132, 101–105.

La Grange LJ, Mukaratirwa S. 2020. Epidemiology and hypothetical transmission cycles of *Trichinella* infections in the Greater Kruger National Park of South Africa: an example of host-parasite interactions in an environment with minimal human interference. Parasite, 27, 1-12.

La Grange LJ, Govender D, Mukaratirwa S. 2013. The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. Journal of Helminthology, 87, 91-96.

La Grange LJ, Marucci G, Pozio E. 2009. *Trichinella zimbabwensis* in wild Nile crocodiles (*Crocodylus niloticus*) of South Africa. Veterinary Parasitology, 161, 88–91.La Grange LJ, Govender D, Mukaratirwa S. 2013. The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. Journal of Helminthology, 87, 91-96.

Mahieu A. 2015. Fish-farming in South Africa: A study of the market environment and the suitable species. Thesis (MSc), Faculty of Economic and Management Sciences, Stellenbosch University, 77 pages.

Merron GS. 1993. Pack-hunting in two species of cat fish, *Clarias gariepinus* and *C. ngamensis*, in the Okavango Delta, Botswana. Journal of Fish Biology, 43, 575–584.

Moretti A, Piergili-Fioretti D, Pasquali P, Mechelli L, Rossodivita ME, Polidori GA. 1997. Experimental infection of fish with *Trichinella britovi*: biological evaluations. In: Ortega-Pierres G, Gamble HR, van Knapen F, Wakelin D. (Eds.), Trichinellosis. Centro de Investigacion y Estudios Avanzados IPN, Mexico City, 135-142.

Mukaratirwa S, La Grange LJ, Malatji MP, Reininghaus B, Lamb J. 2019. Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga provinces of South Africa. Journal of Helminthology, 93, 50-56.

Mukaratirwa S, Foggin CM. 1999. Infectivity of *Trichinella sp*, isolated from *Crocodylus niloticus* to the indigenous Zimbabwean pig (Mukota). International Journal for Parasitology, 29, 1129–1131.

Nöckler K, Kapel CMO. 2007. Detection and surveillance for *Trichinella*: Meat inspection hygiene, and legislation. In: Dupouy-Camet J. & Murrell KD. (Eds.), FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis. Paris, World Organisation for Animal Health Press, 69–97.

Ögretmen F, Göçek K. 2013. Comparative efficacy of three anaesthetic agents on juvenile African sharp tooth cat fish, *Clarias gariepinus* (Burchell, 1822). Turkish Journal of Fisheries and Aquatic Sciences, 13, 51-56.

Picker M, Griffiths C. 2011. Alien & invasive animals, a South African perspective. Struik Publishers, Cape Town.

Pienaar U. de V. 1968. The freshwater fishes of the Kruger National Park: Pretoria, National Parks Board, 1-20.

Pozio E, Hoberg E, La Rosa G, Zarlenga DS. 2009. Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. Infection, Genetics and Evolution, 9, 606–616.

Pozio E, La Rosa G. 2005. Evaluation of the infectivity of *Trichinella papuae* and *Trichinella zimbabwensis* for equatorial freshwater fishes. Veterinary Parasitology, 132, 113-114.

Pozio E, Marucci G, Casulli A, Sacchi L, Mukaratirwa S, Foggin CM, La Rosa G. 2004. *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles. Parasitology, 128, 333–342.

Reina D, Munoz-Ojeda M, Serrano F. 1996. Experimental trichinellosis in goats. Veterinary Parasitology, 62, 125–132.

Skelton P. 2001. A complete guide to the freshwater fishes of Southern Africa. 2nd Edition, Struik Publishers, Cape Town.

Soule C, Dupoy-Camet J, Georges P, Ancelle T, Gillet JP, Vaissaire J, Delvigne A, Plateau E. 1989. Experimental trichinellosis in horses: Biological and parasitological evaluation. Veterinary Parasitology, 31, 19-36.

Tomašovičová O. 1981. The role of fish in transfer and maintenance of Trichinellae under natural conditions. Biologia, 36, 115-125.

CHAPTER 5: EXPERIMENTAL INFECTION OF TIGERFISH (*HYDROCYNUS VITTATUS*) WITH *TRICHINELLA ZIMBABWENSIS*

Published in combination with Chapter 4 as:
La Grange LJ, Mukaratirwa S. (2020). Experimental infection of tigerfish (Hydrocynus vittatus) and African sharp tooth catfish (Clarias gariepinus) with *Trichinella zimbabwensis*. Onderstepoort Journal of Veterinary Research, 87, a1876. https://doi.org/ 10.4102/ojvr.v87i1.1876.

5.1 Abstract

Trichinella zimbabwensis naturally infect a variety of reptilian and wild mammalian hosts in South Africa. Despite significant strides having been made to elucidate the epidemiology of the parasite, the search for additional natural hosts is still ongoing. Previous studies involving Piranha fish (*Serrasalmus nattereri* and *S. rhombeus)* experimentally infected with *T. zimbabwensis* and *T. papuae* suggested that fish were refractory to *T. zimbabwensis* infection. Tigerfish (*Hydrocynus vittatus*) are apex predators and inhabit fresh water systems in large parts of southern Africa. They often cohabit with known natural hosts of *T. zimbabwensis*, Nile crocodiles (*Crocodylus niloticus*) and Nile monitor lizards (*Varanus niloticus*), which are poikilotherms. To assess the potential role of tigerfish as hosts for *T. zimbabwensis,* forty-one tigerfish (298.6 ± 99.3 g) were used in three separate trials (T) and for each trial the fish were randomly allocated to groups (G) as follows; T_1G_1 (n = 8, average weight 301.3 ± 95.7 g) was experimentally infected with 2.2 ± 0.9 larvae per gram (lpg) of fish; T_2G_1 (n = 6, average weight 265.4 ± 103.5 g) with 2.7 ± 1.1 lpg of fish, T_2G_2 (n = 6 average weight 242.0 ± 105.4 g) with 2.9 ± 1.2 lpg of fish; T_3G_1 (n = 5, average weight 356.3 ± 71.9 g) with 0.6 ± 0.1 lpg of fish; T_3G_2 (n = 5, average weight 305.7 ± 103.3 g) with 2.2 ± 0.9 lpg of fish and T_3G_3 (n = 5, average weight 330.5 ± 56.7 g) with 1.9 ± 0.3 lpg of fish. An additional 7 tigerfish were assessed for the presence of natural infection. From T_1G_1 only two fish yielded *T. zimbabwensis* larvae in muscle tissues on day 26 p.i. (0.1 lpg) and day 28 p.i. (0.02 lpg), respectively. No adult worms or larvae were detected in body cavities, stomachs, intestinal tracts or muscle tissue of any of the experimentally infected fish from Trial 2 (n = 12) and Trial 3 (n = 15) on days 7, 21, 28, 33 or 35 post-infection (p.i.) and from the control group. Average temperature during Trial 1 was 26.9˚C ± 0.4˚C, Trial 2 was 25.1˚C ± 1.3˚C for T_2G_1 and 24.9˚C ± 1.2˚C for T_2G_2. Average water temperature for T_3G_1 was 26.9˚C ± 0.4˚C, T_3G_2 was 26.4˚C ± 0.9˚C and T_3G_3 was 26.4˚C ± 1.6˚C.

Results from this study are in general agreement with previous studies that suggest that fish might not be suitable hosts for *Trichinella* spp. However, the observation of larvae, although in low numbers do suggest that tigerfish could, under specific and as yet unknown circumstances, sustain the development and establishment of *T. zimbabwensis*.

5.2 Introduction

Trichinella zimbabwensis naturally infect a variety of reptilian and wild mammalian hosts in South Africa (Mukaratirwa *et al*., 2019, La Grange and Mukaratirwa, 2020). Results from passive surveillance in the Greater Kruger National Park (GKNP) showed that *T. zimbabwensis* has a higher prevalence compared to *T. nelsoni* and *Trichinella* T8 which are also known to circulate in the GKNP (Mukaratirwa *et al*., 2019). Significant strides have already been made in identifying natural hosts of *Trichinella* spp. in the GKNP (Mukaratirwa *et al*., 2019) and there is need for constant surveillance aimed at identifying additional hosts for *T. zimbabwensis* (La Grange *et al*., 2013; La Grange and Mukaratirwa, 2020). Previous studies involving experimental infection of fishes have shown that some *Trichinella* species could survive for short periods in some fish species (Tomašovičová, 1981; Moretti *et al*., 1997), suggesting that some tropical fish may act at least as paratenic hosts for the parasite.

Studies involving tropical fish such as tigerfish (*Hydrocynus vittatus*) that share aquatic habitats with known hosts of *T. zimbabwensis* such as Nile crocodiles (*Crocodylus niloticus*) and Nile monitor lizards (*Varanus niloticus*) are essential in order to determine their role in the epidemiology of the parasite.

5.2.1 Distribution of *Hydrocynus vittatus*

Tigerfish are generally found in tropical regions and also inhabit the southern waters of the inter-tropical region of South Africa (Roux, 2013). They occur from the Okavango, Zambezi and Lowveld coastal systems to the Phongolo River in KwaZulu-Natal Province of South Africa including the Kruger National Park (KNP). They are also found in the Democratic Republic of Congo, Lake Tanganyika in Tanzania, Rufigi and the larger Nilo-Sudanian rivers of North- and West Africa (Skelton, 2001) (Figure 5.1). They have a striking silver colour with black, parallel stripes extending along the length of the body from behind the operculum. Fins are yellow to red (Skelton, 2001). The most striking features are the 8 needle shaped, sharp teeth protruding from both jaws and the parallel stripes and large dog-like teeth provides the origin for their apt name (Jubb, 1967).

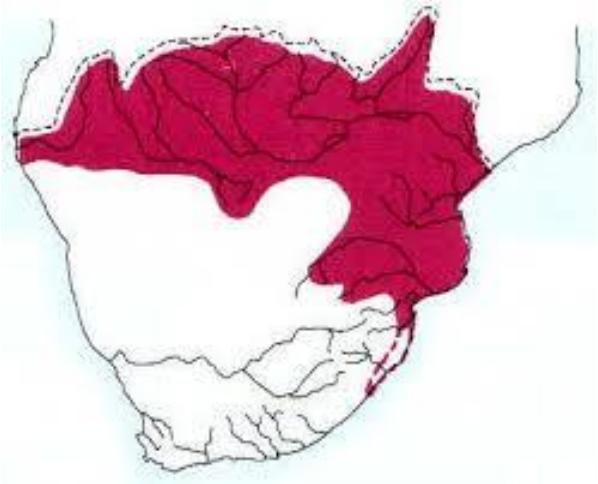


Fig. 5.1 Distribution of Tigerfish in Africa. (Image adapted from Skelton (2001)

5.2.2 Dietary habits of the tigerfish

Tigerfish is not commercially reared in South Africa and is not generally considered to be a table fish. However, it has a reputation for being one of the most sought after game fishes in the world (Skelton, 2001; Roux, 2013). Most sport anglers catch and release their quarry but some recreational fisherman sometimes consume or sell part of their catch (McCafferty *et al*., 2012). Furthermore, subsistence fishermen may also catch and consume tigerfish. In Lake Kariba of Zimbabwe, tigerfish is an important commercial fish and Skelton (2001) reported that 184 tonnes were harvested in 1977.

Tigerfish are considered apex predators and occupy the same trophic level as crocodiles in their shared environments (Roux *et al*., 2018). They have been described as both insectivores and piscivores (Bell-Cross, 1965). However, Skelton (2001) described the diet of *H. vittatus* to show an ontogenic shift from a predominantly insectivorous diet in juveniles to an increasingly piscivorous diet as the fish mature into adulthood. O'Brien *et al*. (2014) additionally described avivorous feeding behaviour of *H. vittatus*. Skelton (2001) noted that tigerfish would feed on whatever prey was most abundant and from the author's personal experience tigerfish can be caught using a variety of artificial lures ranging from simple spinning lures to those that resemble anything from frogs, lizards, snakes and even small rodents. They generally hunt in large schools with similarly sized fish congregating together but large individuals may be solitary hunters (Skelton, 2001).

Although they have been described as voracious, fierce predators (Skelton, 2001; Roux, 2013), scavenging behaviour cannot be excluded since tigerfish are easily caught with "still bait" such as chicken hearts (La Grange, personal observation.). In a study on the predation of crocodilians by other species, Somaweera *et al*. (2013) noted that predation by fish on crocodilians was not easily observable and cited Webb (1979) who considered that hatchling

Australian freshwater crocodiles (*C. johnstoni*) were possibly predated upon by the Black bream (*Hephaestus fuliginosus*) and Saratoga (*Scleropages leichardti*). Although no supporting published reports could be found, it may be possible for large tigerfish to similarly predate on smaller Nile crocodiles. Furthermore, scavenging behaviour of tigerfish on larger crocodile remains cannot be excluded.

The voracious carnivorous feeding habit and potential scavenging behaviour of this apex predator fish makes it a suitable contender as a host to *T. zimbabwensis.* This, combined with their known distributional overlap with crocodiles and monitor lizards in Africa compels for investigation of infectivity of *T. zimbabwensis* to tigerfish. Stable isotope and stomach content analyses of crocodiles in the Olifants River showed that crocodiles in this region depended largely on terrestrial prey as a source of protein and that an ontogenic dietary shift towards fish may only be seasonal (Woodborne *et al*., 2012).

General conclusions cannot however be drawn from a study on a limited number of a single crocodile population. Tigerfish are predated upon by crocodiles (Gagiano, 1997) and although the ability of large crocodiles might be limited in respect of catching agile tigerfish, old, morbid or dead fish would make an easy meal. In view of its marginal role as a commercial food source, there is limited potential risk of infection to humans from tigerfish.

5.3 Materials and Methods

The study protocol was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ref: AREC/029/016M and 030/019D).

5.3.1 Source and collection of tiger fish

Tigerfish were collected from TSB Komatidraai (31˚55’07.1’’E, 25˚29’41.4’’S) situated on the banks of the Komati river on a private farm and The Hippos (31˚58’02.3’’E, 25˚29’12.8’’S) situated on the banks of the Komati river further downstream and to the east of TSB Komatidraai. Crocodiles and Nile monitors naturally cohabit in both sites. The fish were collected using rods and reels.

A total of forty-one fish were collected for this study (TSB Komatidraai, n = 15; The Hippos, n = 26). All fish were bathed in a solution of benzalkonium chloride (BAC 50, Michem, Nelspruit) at a concentration of 1 ppm for 1 hour prior to transportation to shed the outer layer of mucus on their bodies and in the process removing any existing external parasites and superficial bacteria (Huchzermeyer KDA, 2018).

5.3.2 Transport of fish

Tigerfish caught at TSB Komatidraai were transported in a similar fashion described previously for catfish in Chapter 4. Tigerfish, in contrast to catfish however, are more

dependant on oxygen-rich habitats (Goodier *et al*., 2011). As a result water circulation and oxygen levels were increased by fitting a larger 220 V circulating pump with a flow rate of 2400 l/hour (Waterfall Flow 2400, Dubai) to the tank. The pump was powered by a 700 W DC/AC inverter from the vehicle's battery. Individual fish caught at The Hippos were immediately transported under anaesthesia in a 100 litre plastic container to the experiment ponds on the same premises.

5.3.3 Fish husbandry

Two circular, moveable ponds (3 m diameter, 7000 litre capacity) were set up on the research facility premises. Ponds were situated indoors on the The Hippos. Ponds were covered with a custom bird netting to prevent escape of fish and filled with approximately 3500 litres of water and each equipped with a filtration system comprising of a brush filter, biofilter, UV filter connected in line to a submersible pump (6500 l/hour) to circulate and filter the water. An in-line water heater (UltraZap, 3 kw) was additionally installed. Ponds were sub-divided into six "cages" using frames constructed from 50 mm polyvinyl chloride (PVC) pipes. The frames were covered with 40% shade netting to prevent movement of fish between the cages without obstructing water flow (Plate 5.1). This also allowed allocation of fish into groups for easy monitoring during experimental infections.

Plate 5.1 Experiment pond with six "cages" used to house the experimental tigerfish

Ponds were filled with water collected from the Komati River and were left unoccupied for 14 days to allow aerobic bacteria to establish and colonize the biofilters. Fish were

transferred from either the transport tank or container to the dams and placed together in groups of two fish per cage. Larger individuals were separated from the smaller fish to avoid potential competition for food and were fed fresh chicken hearts (Mikon Farming Abattoir, South Africa) on a daily basis. Brush filters and water surfaces were cleaned weekly.

Water temperature in the ponds was monitored using temperature loggers (Hobo Water temp pro V2, Onsetcomp) and an additional logger was deployed in the Komati River from 26 January 2019 until 26 January 2020 for comparison with the experimental ponds. Loggers were set to record water temperature on a 4 hour interval.

5.3.4 Infection of *Hydrocynus vittatus* through natural feeding

Trichinella zimbabwensis larvae previously derived from a Nile crocodile were maintained in a colony of rats housed at the Biological Resourse Unit (BRU) at the Westville Campus of the University of KwaZulu-Natal. Infected rats were euthanized and larvae isolated by means of artificial muscle digestion according to a published protocol (Nöckler and Kapel, 2007). After digestion larvae were washed three times with water, counted and transferred to small glass vials in sterile water. Larval concentration was adjusted to ensure approximately 200 larvae/ml.

Chicken hearts freshly collected from Mikon Farming, South Africa, were dried with absorbent paper towels and “loaded” with solution containing infective material using a 3 ml syringe and 25 gauge needle. The needle was inserted into the muscle tissue to reach the chambers and the solution was administered by slowly retracting the needle while injecting to ensure that the hearts would not rupture. The procedure was repeated at various sites around the heart until all the solution was exhausted before being presented to the fish. (Plate 5.2). Each fish received a single chicken heart with infected material.

Experimental infection was conducted over the course of three separate trials. Relevant data for all the trials are summarised and depicted in Table 5.1.

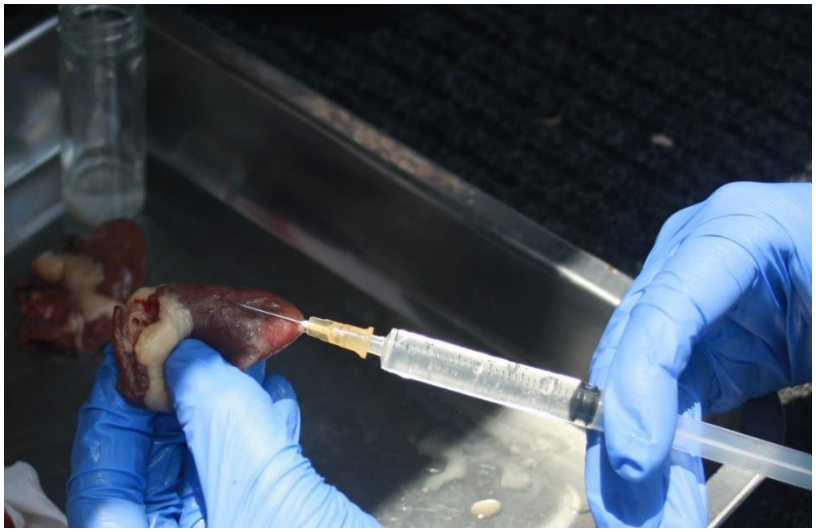


Plate 5.3 "Loading" of chicken hearts with *Trichinella zimbabwensis* larvae

5.3.4.1 Trial 1

For Trial 1 (T_1) only a single group of fish (T_1G_1) (n = 8) (301.3 ± 5.7 g), caught at TSB Komatidraai, was used. Each fish received an inoculum of approximately 600 larvae, administered via a single chicken heart (2.2 ± 0.9 larvae per gram (lpg) of fish). This trial was conducted in the summer between November and December 2018.

5.3.4.2 Trial 2

Trial 2 consisted of two groups (T_2G_1 and T_2G_2) (n = 12) of fish caught at The Hippos. T_2G_1 (n = 6) (265.4 ± 103.5 g) received approximately 600 larvae each, administered via a singe chicken heart (2.7 ± 1.1 lpg of fish). T_2G_2 (n = 6) averaged 242.0 ± 105.4 g and received a similar inoculum (2.9 ± 1.2 lpg of fish). This trial was conducted during the autumn between March and April 2019.

5.3.4.3 Trial 3

Trial 3 consisted of three groups (T_3G_1, T_3G_2 and T_3G_3) (n = 15). T_3G_1 (n = 5) (356.3 ± 71.9 g) was infected with an inoculum of 200 larvae/fish (0.6 ± 0.1 lpg of fish) administered via a single chicken heart. The inoculum was adjusted as a result of limited availability of infective material at the time. T_3G_2 (n = 5) (305.7 ± 103.3 g) received an inoculum of approximately 600 larvae/fish administered via a single chicken heart (2.2 ± 0.9 lpg of fish). T_3G_3 (n = 5) (330.5 ± 56.7 g) was infected with approximately 600 larvae/fish administered via a single chicken heart (1.9 ± 0.3 lpg of fish). Trial 3 was conducted in summer between November 2019 and January 2020. Average water temperature for T_3G_1 was artificially maintained at 26.9˚C ± 0.4˚C, for T_3G_2 at 26.4˚C ± 0.9˚C and for T_3G_3 at 26.4˚C ± 1.6˚C.

Fish collected and intended for T_1 (n =1), T_2 (n = 3) and T_3 (n = 3) died during the respective first weeks of acclimation due to stress. These fish were preserved (frozen) and retained as a control group.

5.3.5 Euthanazia of fish and screening for infection

Fish were caught from the experimental ponds using a medium sized hand net on the relevant day post infection (Table 5.1). All the experimentally infected fish were euthanized on the relevant day post infection and screened for infection as referred to in Chapter 4. The frozen specimens retained as a control group were screened in a similar fashion.

5.3.6 Data and statistical analysis

Fish were individually weighed and the average weight ± standard deviation (SD) expressed per group. Water temperature in the ponds was digitally recorded on a four hour interval using temperature loggers (Hobo Water temp pro V2, Onsetcomp) and expressed as average daily water temperature ± SD during each trial period. Average daily temperature in the Komati river was similarly recorded.

Mean temperature during each trial was compared with the mean temperature recorded in the river during the corresponding time frame using student T-tests.

5.4 Results

Results from the experimental trials conducted are summarised in Table 5.1.

5.4.1 Trial 1

Due to prolonged national power failures during the last two weeks of T_1G_1 (n = 8), four fish died at day 23 p.i and two at day 26 p.i. from oxygen depletion of the water during the power failures. Only the two fish that died at day 26 p.i. were suitable for analysis since they were observed soon after death and could be retrieved and preserved timeously. The other four fish died over the course of a weekend and were in an advanced state of autolysis at the time of retrieval and could not be analyzed. The two remaining live fish were euthanized on day 28 p.i.

Analysis of the two fish that died at day 26 p.i. yielded the presence of 10 dead larvae (0.1 lpg) in muscle tissue of one fish. Of the two fish euthanized on day 28 p.i., one live larva (0.02 lpg) was detected in muscle tissue. No larvae or adult parasites were however observed in the body cavity, stomach or intestinal tract of any of the fish.

Table 5.1 Results from experimental infection of tigerfish (*Hydrocynus vittatus*) with *Trichinella zimbabwensis.*

Trial (T) Group (G)	N	M/F	Weight(g) x̄ ± SD	Larvae/g of fish	Daily Temp (˚C) x̄ ± SD	Day (p.i.)	No Tested	Infection results		
								Body cavity	Intestines	Muscle
T_1G_1	8	5/3	301.3 ± 95.7	2.2 ± 0.9	25.6 ± 1.6	28	4	Neg	Neg	0.02*; 0.1** lpg
T_2G_1	5	4/1	265.4 ± 103.5	2.7 ± 1.1	25.1 ± 1.3	28	5	Neg	Neg	Neg
T_2G_2	6	4/2	242.0 ± 105.4	2.9 ± 1.2	24.9 ± 1.2	35	6	Neg	Neg	Neg
T_3G_1	5	4/1	356.3 ± 71.9	0.6 ± 0.1	26.9 ± 0.4	7	5	Neg	Neg	Neg
T_3G_2	5	1/4	305.7 ± 103.3	2.2 ± 0.9	26.4 ± 0.9	21	5	Neg	Neg	Neg
T_3G_3	5	3/2	330.5 ± 56.7	1.9 ± 0.3	26.4 ± 1.6	33	5	Neg	Neg	Neg

T_1G_1 = Trial 1 Group 1; T_1G_2 = Trial Group 2; T_3G_1 = Trial 3 Group 1; T_3G_2 = Trial 3 Group 2; T_3G_3 = Trial 3 Group 3. M/F = Male/Female; SD = Standard deviation; Neg = Negative for infection; p.i. = post-infection; Results for control fish (n =7) not included in the above. *Muscle larvae observed on day 28 p.i. after death of fish; ** Muscle larvae observed on day 26 p.i. after death of fish, All controls were negative

Water temperature was not adjusted during T_1 and averaged 25.6˚C ± 1.6˚C (Table 5.1). Data on water temperature in the Komati river was not available for this period. However, the recorded water temperature of the pond was compared to water temperature of the Komati River recorded during a similar time frame in the following year. River temperatures were significantly higher ($p = 0.002$, average 27.0˚C ± 1.6˚C).

5.4.2 Trial 2

T_2G_1 (n = 5) was euthanized at day 28 p.i. No larvae or adult parasites were recuperated from any of the fish. T_2G_2 (n = 6) was euthanized on day 35 p.i. and all individuals were similarly negative upon analysis.

Average water temperature for T_2G_1 was recorded at 25.1˚C ± 1.3˚C (Table 5.1) during the 28 day period compared to significantly higher average recorded in the Komati river ($p = 0.0002$) of 26.4˚C ± 1.3˚C during the same period. Average water temperature for T_2G_2 was

24.9˚C ± 1.2˚C (Table 5.1) compared to the significantly higher average of 26.2˚C ± 1.3˚C recorded during the same period in the Komati river ($p = 0.002$).

5.4.3 Trial 3

T_3G_1 (n =5) was euthanized on day 7 p.i., group 2 (n = 5) on day 21 p.i. and group 3 (n = 5) at day 33 p.i. No adult or larvae of *T. zimbabwensis* were recuperated from fish in these groups.

Average water temperature for T_3G_1 was artificially maintained at 26.9˚C ± 0.4˚C for the duration of the 7 day period (Table 5.1). Average water temperature for T_3G_2 and T_3G_3 was artificially maintained at 26.4˚C ± 0.9˚C and 26.4˚C ± 1.6˚C respectively (Table 5.1). The average water temperature in the Komati river during the first 7 days (T_3G_1) was 27.7˚C ± 1.0˚C, 27.3˚C ± 1.7˚C during the 28 day period (T_3G_2) and 28.7˚C ± 1.4˚C during the 33 day period for T_3G_3. Temperatures in the Komati river were significantly higher than those reported for T_3G_2 ($p = 0.04$) and T_3G_3 ($p < 0.0001$) but not so for T_3G_1 ($p = 0.07$).

5.5 Discussion

Tomašovičová, (1981) assessed the infectivity of European ruffe (*Gymnocephalus cernuus*), European perch (*Perca fluviatilis*), the common bleak (*Alburnus alburnus*) and the common carp (*Cyprinus carpio*) to *Trichinella pseudospiralis* and *T. spiralis*. In the study, larvae were delivered per os at a rate of 500 larvae/ fish. The size of fish used were not reported and hence the the larvae per gram of fish is not known. *Trichinella pseudospiralis* larvae reportedly migrated without moulting from the gastrointestinal tract to the body cavity, organs and muscles of fish while *T. spiralis* larvae only migrated to the body cavity of fish.

In a study by Moretti *et al*., (1997), common carp, tench (*Tinca tinca*), perch (*P. fluviatilis*), cat fish (*Ictalerus melas*) and eel (*Anguilla anguilla*) were infected using an orogastric tube and larvae administered at dosages varying between 100, 200 and 100 larvae per fish. The size of fish and dosage per gram of fish were not reported. *Trichinella britovi* larvae were isolated from the gastrointestinal tract and body cavity of common carp and from the gastrointestinal tract of catfish after experimental infection.

In a study by Pozio and La Rosa (2005) involving piranha (*Serrasalmus rhombeus* and *S. nattereri*), *T. zimbabwensis* and *T. papuae* larvae were administered per os at a dosage of 1000 larvae per fish. However, the exact method of infection, size of fish and inoculum per gram of fish was not reported. In this study, the mode of infection was through natural feeding of infected chicken hearts to tigerfish.

The use of natural feeding is probably the preferred method for experimental infection since it eliminates the stress associated with physical handling of the animals and any

subsequent undesirable consequences, such as regurgitation, that may occur. There is lack of data in respect of the size of fish and larvae per gram of fish administered in previous studies.

This precludes a comparison to assess the minimum larvae dosage required for *T. zimbabwensis* infection to establish in fish. It is thus not known whether the infective dosages as presented in Table 5.1 were sufficient to establish the infection in tigerfish.

Previous studies with other poikilothermic hosts experimentally infected with different *Trichinella* taxa suggest temperature to play an integral part in larval survival and/or development (Cristea and Perian, 1999; Asatrian *et al*., 2000; Pozio *et al*., 2004). Overall, temperatures recorded during this study was much higher than those reported in the studies conducted by Tomašovičová (1981) and Moretti *et al*. (1997) and compared favourably with the lower end of temperatures reported by Pozio and La Rosa (2005). In this study, the average temperature measured was 25.6˚C ± 1.6˚C during the first trial that yielded positive results.

Despite a higher average temperature recorded during Trial 3 (26.4˚C ± 1.3˚C), no positive results were obtained. Overall temperature during this study was well within the natural range encountered in the fish's natural habitat but fringed on the lower range of temperatures required for *T. zimbabwensis* development. This precludes any definitive conclusion in respect of the influence of temperature on larval development and survival in tigerfish.

Previous studies have suggested several reasons for the failure of establishment of *Trichinella* infections in fish which include: 1) lack of villi in the small intestine which prevents larvae from anchoring themselves to the intestinal wall and resist peristaltic movements, 2) the copious amounts of bile and mucous excreted in the intestine of fish, 3) low body temperature of the host, 4) quick passage of larvae through the intestine and 5) the possible destruction of larvae by digestive processes of fish (Moretti *et al*., 1997).

Both Tomašovičová (1981) and Moretti *et al*. (1997) reported that *Trichinella* larvae migrated through the intestinal wall of the host and did not undergo any further development.

Results from this study remain inconclusive on this aspect since no positive results could be obtained from the fish during the earlier stages after infection (Day 7 p.i.) to assess larval development. Also, the low number of larvae recuperated at days 26 p.i. and 28 p.i. of T_1G_1 and the fact that most of the larvae were dead, prevented attempts to assess the infectivity of the larvae to secondary hosts.

In this study, *T. zimbabwensis* larvae were observed in muscle tissue of two fish on day 26 and day 28 p.i., respectively. However, the number of larvae recovered were very low (0.02 lpg and 0.1 lpg). This is in agreement with findings reported in previous studies involving *T.*

britovi (Moretti *et al*., 1997) and *T. spiralis* and *T. pseudospiralis* (Tomašovičová, 1981) where very low numbers of larvae were isolated from some fish species.

In assessing the epidemiological role of fish in the maintenance and transmission of *T. zimbabwensis*, it is sensible to align experimental infection methods with the natural route of transmission which, in this case, involves natural feeding behaviour. The successful migration of *Trichinella* spp. through the gastrointestinal tract of some fish species (Tomašovičová, 1981; Moretti *et al*., 1997) could possibly be attributed to the unnatural methods of infection used, allowing for a larger infective inoculum to reach the stomach of the fish than would be expected through natural feeding.

However, this should also consider the infective dose of the parasite required to infect specific hosts in nature. In an experimental study involving *T. zimbabwensis* and *T. papuae* in caiman (*Caiman sclerops*), savannah monitors (*Varanus exanthematicus*), African helmeted turtles (*Pelomedusa subrufa*) and Burmese pythons (*Python malurus bivittatus*), all of the animals were successfully infected with an inoculum of 3000 larvae per animal (Pozio *et al*., 2004). This equates to an average inoculum of 5.5–8.6 larvae per gram of caiman, 6.7–10 larvae per gram of monitor, 30–60 larvae per gram of turtle and 3.5–8.5 larvae per gram of python.

These inocula were much greater than the 0.6–2.9 larvae per gram of fish used for tigerfish in this study.

The positive result from this study is interesting from an epidemiological perspective although it is not certain whether tigerfish could be considered a reservoir host for *T. zimbabwensis*.

5.6 Conclusion

Tigerfish, in general, appear not to be suitable hosts for *T. zimbabwensis.* However, results from this study suggest that some individuals could, under very specific circumstances, maintain the larvae for *T. zimbabwensis* and it is not known whether the parasite can fully develop and reproduce in this host.

In the case of infectivity of *T. zimbabwensis* to fish, the minimum infective dose is not known and requires further investigation. Results from this study show that the initial infective inoculum used to infect tigerfish was far less compared to inocula given to other poikilothermic hosts in a previous study (Pozio *et al*., 2004). This may have had a negative impact on the establishment of *T. zimbabwensis* larvae in the tigerfish from this study.

The influence of temperature on *T. zimbabwensis* larval development in fish remains inconclusive and it appears as if development occurs when temperatures are closer to the upper limit of the hosts' preferred temperature range (Pozio *et al*., 2004). This may suggest that tigerfish could only become infected during warmer seasons and in warmer climates.

Future research should focus on the influence of temperature on larval development and survival in tigerfish and should consider the average temperatures prevailing in the natural environments where these fish occur to establish their actual epidemiological role in different geographic regions. Similar studies should also consider other potential fish host species that cohabit with Nile crocodiles and Nile monitor lizards.

5.7 References

Asatrian A, Movsessian S, Gevorkian A. 2000. Experimental infection of some reptiles with *T. spiralis* and *T. pseudospiralis*. Abstract book, p. 154. The Tenth International Conference on Trichinellosis, 20-24 August, Fontaineblue, France.

Bell-Cross G. 1965. The distribution of fishes in Central Africa. Fisheries Research Bulletin of Zambia, 4, 3-20.Cristea G, Perian A. 1999. Experimental infestation with *Trichinella* in vipers. Revista Romana de Parsitologia, 9, 45.

Gagiano CL. 1997. An ecological study on the tigerfish (*Hydrocynus vittatus*) in the Olifants and Letaba rivers with special reference to artificial reproduction. Thesis (MSc). Rand Afrikaanse Universiteit, 94 pages.

Goodier SAM, Cotterill FPD, O'Ryan C, Skelton PH, de Wit MJ. 2011. Cryptic diversity of African tigerfish (Genus *Hydrocynus*) reveals palaeogeographic signatures of linked neogene geotectonic events. PloS One, 6, e28775.

Huchzermeyer KDA. 2018. Fish Disease Course for Veterinarians: Notes. Rhodes University. 119 pages.

Jubb RA. 1967. Freshwater fishes of Southern Africa. Cape Town, Balkema.

La Grange LJ, Mukaratirwa S. 2020. Epidemiology and hypothetical transmission cycles of *Trichinella* infections in the Greater Kruger National Park of South Africa: an example of host-parasite interactions in an environment with minimal human interference. Parasite, 27, 1-12.

La Grange LJ, Govender D, Mukaratirwa S. 2013. The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. Journal of Helminthology, 87, 91-96.

McCafferty JR, Ellender BR, Weyl OLF, Britz PJ. 2012. The use of water resources for inland fisheries in South Africa. Water SA, 38, 327-344.

Moretti A, Piergili Fioretti D, Pasquali P, Mechelli L, Rossodivita ME, Polidori GA. 1997. Experimental infection of fish with *Trichinella britovi*: biological evaluations. In: Ortega-Pierres G, Gamble HR, van Knapen F, Wakelin D. (Eds.) Trichinellosis. *Centro de* Investigacion y Estudios Avanzados IPN, Mexico City, 135-142.

Mukaratirwa S, La Grange LJ, Malatji MP, Reininghaus B, Lamb J. 2019. Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga provinces of South Africa. Journal of Helminthology, 93, 50-56.

Nöckler K, Kapel CMO. 2007. Detection and surveillance for *Trichinella*: Meat inspection hygiene, and legislation. In: Dupouy-Camet J. & Murrell KD. (Eds.), FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis. Paris, World Organisation for Animal Health Press, 69–97.

O'Brien GC, Jacobs F, Evans SW, Smit NJ. 2014. First observation of African tiger fish (*Hydrocynus vittatus*) predating on barn swallows (*Hirundo rustica*) in flight. Journal of Fish Biology, 84, 263-266.

Pozio E, La Rosa G. 2005. Evaluation of the infectivity of *Trichinella papuae* and *Trichinella zimbabwensis* for equatorial freshwater fishes. Veterinary Parasitology, 132, 113-114.

Pozio E, Marucci, G, Casulli A, Sacchi L, Mukaratirwa S, Foggin CM, La Rosa G. 2004. *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles. Parasitology, 128, 333-342.

Roux F. 2013. A study on the behaviour of tiger fish (*Hydrocynus vittatus*) using biotelemetry, to determine habitat utilisation and survival strategies in the Lower Incomati River system. Thesis (P.hD.). University of Johannesburg, 258 pages.

Roux F, Steyn G, Hay C, Wagenaar I. 2018. Movement patterns and home range size of tiger fish (*Hydrocynus vittatus*) in the Incomati River system, South Africa. Koedoe, 60, 1-13.

Skelton P. 2001. A complete guide to the freshwater fishes of Southern Africa. 2nd Edition, Struik Publishers, Cape Town.

Somaweera R, Brien M, Shine R. 2013. The role of predation in shaping crocodilian natural history. Herpetological monographs, 27, 23-51.

Tomašovičová O. 1981. The role of fish in transfer and maintenance of Trichinellae under natural conditions. Biologia, 36, 115-125.

Webb GJW. 1979. Survey of a pristine population of freshwater crocodiles in the Liverpool River, Arnhem Land, Australia. National Geographic Society Research Reports, 1979, 841–852.

Woodborne S, Huchzermeyer KDA, Govender D, Pienaar DJ, Hall G, Myburgh JG, Deacon AR, Venter J, Lübcker N. 2012. Ecosystem change and the Olifants River crocodile mass mortality events. Ecosphere, 3, Article #87, 1-17.

CHAPTER 6: DISCUSSION SYNTHESIS, RESEARCH GAPS AND SUGGESTIONS FOR FUTURE STUDIES

6.1 Introduction

This study aimed to contribute to the knowledge and understanding of the epidemiology of *Trichinella* spp. in the GKNP. Particular emphasis was placed on the infectivity of *Trichinella zimbabwensis* to selected fish species known to cohabit with two known poikilothermic hosts, Nile crocodiles (*Crocodylus niloticus*) (Pozio *et al*., 2007; La Grange *et al*., 2009) and Nile monitor lizards (*Varanus niloticus*) (Pozio *et al*., 2007).

The background provided in the first chapter imparts a historic overview of the genus *Trichinella* and its 13 species currently known to exist globally (Pozio and Zarlenga, 2013; Sharma *et al*., 2019). *Trichinella* spp. infections naturally occur in a wide range of sylvatic mammals, birds and reptiles that exhibit scavenger, omnivorous and carnivorous behaviour.

Since its first description in a human cadaver in 1835 (Campbell, 1979), the zoonotic potential and ability of these parasites to spill over into domestic cycles have driven a myriad of studies aimed at prevention and control. Trichinellosis, however, remains a notable food-borne parasitic zoonosis with often severe although less frequent, fatal consequences (Murrell and Pozio 2011; Mukaratirwa *et al*., 2013). Facilitating the design and implementation of control measures depends in part on knowledge of potential sylvatic reservoirs, their epidemiological contribution to transmission cycles and potential risk to public health.

Trichinella zimbabwensis was first described in wild Nile crocodiles (*C. niloticus*) from the Kruger National Park (KNP) by La Grange *et al*. (2009). Subsequent studies revealed not only a high prevalence (83.3%) among crocodiles (La Grange *et al*., 2013) but also led to the first description of this specie in a naturally infected mammal (La Grange *et al*., 2010). These reports as well as reports of a mixed infection of *T. nelsoni* and *Trichinella* T8 in a lion (*Panthera leo*) from the Greater Kruger National Park (GKNP) (Marucci *et al*., 2009) prompted a review of *Trichinella* infections in sub-Saharan Africa (Mukaratirwa *et al*., 2013) in which hypothetical transmission cycles for *T. nelsoni* and *Trichinella* T8 and a separate hypothethetical transmission cycle for *T. zimbabwensis* was proposed. This was followed by the first description of a mixed infection of *T. nelsoni* and *Trichinella* T8 in a leopard (*P. pardus*) from the GKNP (La Grange *et al*., 2014). Between 2012 and 2016 several unidentified isolates of *Trichinella* spp. were collected from several hosts in the GKNP and surrounding areas and there was a need to identify these isolates to species level.

6.2 Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga provinces of South Africa

Chapter 2 reports on the molecular identification and prevalence of *Trichinella* species collected from Limpopo and Mpumalanga provinces between 2012 and 2016. Earlier studies conducted between 1964 and 2011 reported 14/85 *Trichinella* spp. positive lions (prevalence 16.5%). Of these, one was *T. zimbabwensis*, three were *T. nelsoni*, four were *Trichinella* T8 and six were unidentified to species level (Young and Kruger, 1967; Pozio *et al.*, 1994; La Rosa and Pozio, 2000; La Grange *et al*., 2010). During the same period *Trichinella* spp. isolates were identified from 12/18 hyaenas (prevalence 66.7%). Only a single isolate of *Trichinella* T8 was identified with the remaining 11 unidentified (Young and Kruger 1967; Marucci *et al*., 2009). In addition, unidentified isolates were obtained from 1/3 side-striped jackal (*Canis adustis*), 1/1 African civet (*Civettictis civetta*) and 1/44 multimammate mouse (*Praomys natalensis*) (Young and Kruger, 1967; Young and Whyte, 1975; Marucci *et al*., 2009).

A total of ninety samples from 15 mammalian, two bird- and three reptile species were initially screened for *Trichinella* infection between 2012 and 2016 and twenty found to be positive. Subsequent PCR analysis in the current study revealed that 3/8 lions, 2/5 hyaenas, 1/2 leopards, 1/1 Nile monitor lizards and 1/1 small spotted genet harboured *T. zimbabwensis*. The remaining isolates from 5 lions and 2 hyaenas could not be identified to species level due to degradation of DNA and one isolate from a hyaena could only be confirmed to fall under the encapsulated clade. Additionally, isolates from 1/1 black-backed jackal (*Canis mesomelas*), 1/1 African wild cat (*Felis sylvestris lybica*), 1/6 chacma baboons (*Papio ursinus*) and 1/1 marsh owl (*Asio capensis*) could not be identified to species level due to DNA degradation.

My results represent the second report of *T. zimbabwensis* natural infection in a lion and this confirms the previous report by La Grange *et al,* (2010) that the lion is an exceptional host for all the three *Trichinella* taxa (*Trichinella* T8, *T. nelsoni* and *T. zimbabwensis*) circulating in South Africa.

Results from this study significantly contribute to the existing body of knowledge on the prevalence and natural host species of *Trichinella* spp. in the GKNP. These results suggests a maintenance cycle for *T. zimbabwensis* between lions and hyaenas.

Another significant finding is that of *T. zimbabwensis* infection in the small spotted genet. This opportunist omnivore preys on small mammals, amphibians, reptiles, fruits and birds (Lariviere and Calzada, 2001) suggesting that it may have acquired infection from infected small mammals such as rodents or reptiles. This study also reports the first known infection of *T. zimbabwensis* in a leopard. For the first time *Trichinella* spp. larvae were

reported in a baboon, and a *Trichinella* spp.-like infection in a bird from South Africa. Only a single taxon (*T. pseudospiralis)* is known to infect birds (Pozio and Murrell, 2006).

6.3 Epidemiology and hypothetical transmision cycles of *Trichinella* infections in the Greater Kruger National Park of South Africa: An example of host-parasite interactions in an environment with minimal human interactions.

Chapter 3 provides an update on the epidemiology and hypothetical transmission cycles of *Trichinella* spp. in the GKNP. At least four species of *Trichinella* have been confirmed in sub-Saharan Africa, including *T. nelsoni*, *Trichinella* T8, *T. britovi* and *T. zimbabwensis* (Mukaratirwa *et al*., 2013). Of these all except *T. britovi,* have been reported in the GKNP (Mukaratirwa *et al*., 2013; 2019).

Current prevalence data confirms lions and hyaenas (*Crocuta crocuta*) to be the major reservoirs for *Trichinella* infections in GKNP. (Mukaratirwa *et al*., 2013; 2019). Additionally, *Trichinella* spp. infections were confirmed in at least six mammalian and two reptilian species from the GKNP (Pozio *et al*., 1994; La Rosa and Pozio, 2000; Marucci *et al*., 2009; La Grange *et al*., 2014; Mukaratirwa *et al*., 2019). *Trichinella*-like infections have also been reported in six additional mammalian hosts (Young and Kruger, 1967; Young and Whyte, 1975; Marucci *et al*., 2009; Mukaratirwa *et al*., 2019).

In this study, published information on *Trichinella* infection in wildlife in the GKNP of South Africa from 1964–2019 was reviewed. Subsequent results from the review enabled the construction of hypothetical transmission cycles for the three taxa known to circulate in the GKNP. Factors that may influence the establishment of these cycles, potential spillage into domestic environments and risk for human infections are discussed as further justification of the hypotheses.

To assess the probability of *T. nelsoni*, *Trichinella* T8 and *T. zimbabwensis* parasites being transmitted between potential hosts in the GKNP, published literature on dietary habits of specific host species were reviewed together with published reports from other sub-Saharan countries involving similar host species. The additional data obtained from these reviews supplemented the information portrayed in the hypothetical transmission cycles

The previous hypothetical cycle by Mukaratirwa *et al*. (2013) was updated with recent findings by Mukaratirwa *et al*. (2019) and, based on the sympatric existence of *T. nelsoni* and *Trichinella* T8, the single hypothetical transmission cycle for these two taxa was retained.

The separate hypothetical cycle for *T. zimbabwensis* (Mukaratirwa *et al*., 2013) was similarly updated to include recent findings by Mukaratirwa *et al*. (2019). Two additional apex predators (hyaena and leopard) and a mesopredator, the small spotted genet (*Genetta genetta*)

were included in the hypothetical transmission cycle. In addition to these recently confirmed hosts, the multimammate mouse (*P. natalensis*) (Young and Kruger, 1967), African civet (*C. civetta*) (Young and Whyte, 1975), black-backed jackal (*C. mesomelas*) (Young and Kruger, 1967) and African wild cat (*F. sylvestris lybica*) (Mukaratirwa *et al*., 2019) were added as probable host species in both hypothetical cycles. These hosts were previously found to be infected by unidentified species of *Trichinella* which we assume could have been any of the three *Trichinella* taxa known to circulate in the GKNP.

Results from this study also highlights unresolved gaps in the epidemiology of *Trichinella* spp. and call for maintenance and improvement of collaboration with other stakeholders. In order to provide assistance with surveillance and increase capacity, existing knowledge and expertise should be employed to establish a *Trichinella* Reference Centre for Africa.

Furthermore, the results from this study must be considered preliminary and on-going in nature rather than complete and, undoubtedly, will require revision in consideration of future evidence that may be presented.

6.4 Experimental infection of African sharp tooth catfish with *Trichinella zimbabwensis*.

Chapter 4 reports on the results from an experimental study conducted on African sharp tooth catfish (*Clarias gariepinus*) to assess the infectivity of *Trichinella zimbabwensis* to this fish species. Previous chapters in this thesis showed that *T. zimbabwensis* has been reported to naturally infect at least two poikilothermic hosts, Nile crocodiles (Mukaratirwa and Foggin, 1999; Pozio *et al*., 2004; La Grange *et al*., 2009; 2013) and monitor lizards (Pozio *et al*., 2004; Mukaratirwa *et al*., 2019). La Grange *et al*. (2013) and La Grange and Mukaratirwa (2020) affirmed the need to elucidate the role of probable host species as potential sources of infection to crocodiles.

Experimental studies to assess the infectivity of *Trichinella* spp. to fish have been limited. Tomašovičová (1981) reported the migration of *T. pseudospiralis* larvae in unaltered form from the gastrointestinal tract to the body cavity, organs and muscles of fish. *Trichinella spiralis* larvae migrated in a similar fashion but were confined to the body cavity of fish. Moretti *et al*. (1997) isolated *T. britovi* larvae from the gastrointestinal tract and body cavity of experimentally infected common carp (*Cyprinus carpio*) and from the gastrointestinal tract of experimentally infected catfish (*Ictalurus melas*). Pozio and La Rosa, (2005) reported that *T. zimbabwensis* larvae failed to develop or survive in the gastrointestinal tract and cavities of experimentally infected piranha (*Serrasalmus nattereri* and *S. rhombeus*).

All of the previous studies involved either *Trichinella* spp. not known to be infective to poikilothermic hosts (Tomašovičová, 1981; Moretti *et al*., 1997) or host species that do not cohabit with known poikilothermic hosts of *T. zimbabwensis* (Pozio and La Rosa, 2005). African sharp tooth catfish are omnivorous scavengers and predators and consume a variety of foods from plankton to small mammals (Bruton, 1979; Skelton, 2001). Catfish also cohabit with Nile crocodiles and Nile monitor lizards in the KNP and are predated upon by both these aquatic predators (Pienaar. 1968; Skelton, 2001).

In this study 24 African sharp tooth catfish were randomly divided into five groups and experimentally infected with *T. zimbabwensis* larvae using an orogastric tube (Groups 1 and 2), blank gelatin capsules (Groups 3 and 4) and natural feeding (Group 5). No larvae or adults of *T. zimbabwensis* were observed in the stomach, intestinal tract, body cavity or muscle tissue of any of the fish on day 7 p.i (Groups 1 and 2), day 2 p.i. (Groups 3 and 5) and day 1 p.i (Group 5).

Results from this study are in agreement with previous experimental studies on fish with *T. spiralis* and *T. pseudospiralis* (Tomašovičová, 1981); *T. britovi* (Moretti *et al*., 1997) and *T. zimbabwensis* and *T. papuae* (Pozio and La Rosa, 2005) where infection failed to establish. These results suggest that *T. zimbabwensis* is not able to develop and establish in the African sharp tooth catfish.

The non-encapsulated species of *T. zimbabwensis* and *T. papuae* requires a temperature range of 26˚C to 40˚C in order to develop in homeothermic and poikilothermic hosts (Pozio *et al*., 2004). In this study the influence of temperature on the survival and development of *T. zimbabwensis* was not assessed. The average temperature recorded in this study was well within the natural range of catfish but not aligned with the range required for *T. zimbabwensis* development. Water temperatures in the KNP and other neo-tropical habitats where catfish occur may often overlap with those required for *T. zimbabwensis* development, especially during summer periods. This precludes a definitive conclusion in respect of the fish's potential role in the epidemiology of *T. zimbabwensis.*

Our results suggest that the entozoic environment in the gastrointestinal tract of catfish might not be suitable for the survival and development of *T. zimbabwensis*. The importance of host characteristics (Soule *et al*., 1989; Kapel, 1995; Reina *et al*., 1996; La Grange *et al*., 2013) and parasite characteristics (Hurnikova *et al*., 2004; Kapel *et al*., 2005) on the development and infectivity of *T. zimbabwensis* should be considered. Additional research is required on other tropical fish such as large scale yellowfish (*L. marequensis*), Madagascar mottled eel (*A. marmorata*) and common long fin eel (*A. mossambicus*) that share aquatic habitats with other

known hosts of *T. zimbabwensis* in the GKNP to elucidate the role of any specific fish species in the epidemiology of *T. zimbabwensis*.

6.5 Experimental infection of tigerfish with *Trichinella zimbabwensis*.

Previous experimental studies of fishes have shown the ability of some *Trichinella* species to survive for short periods in some fish species (Tomašovičová, 1981; Moretti *et al*., 1997). This suggests that some tropical fish could serve as paratenic hosts for the parasite.

However, a study involving piranha (*Serrasalmus nattereri* and *S. rhombeus)* experimentally infected with *T. zimbabwensis* and *T. papuae* suggested that fish are not likely to play a role in the epidemiology of *T. zimbabwensis* (Pozio and La Rosa, 2005). Results from Chapter 4 of this thesis showed African sharp tooth catfish to be unlikely hosts for *T. zimbabwensis*; however, the results precluded a general hypothesis that fish do not play any role in the epidemiology of the parasite. Tigerfish are apex predators that inhabit fresh water systems in large parts of southern Africa (Roux, 2013; Skelton, 2001). These water systems are often cohabited by Nile crocodiles and Nile monitor lizards (Roux *et al*., 2018).

In this study 41 tigerfish were used. Three separate trials were conducted and 34 experimentally infected fish analyzed. An additional 7 fish were assessed for the presence of natural infection as a control experiment.

Only two fish from T_1G_1 yielded *T. zimbabwensis* larvae in muscle tissues on day 26 p.i. (0.1 lpg) and day 28 p.i. (0.02 lpg), respectively. No adults or larvae of *T. zimbabwensis* were detected in body cavities, stomachs, intestinal tracts or muscle tissue of any of the other experimentally infected fish on days 7, 21, 28, 33 or 35 post infection (p.i.) or from the control group.

Results from this study are in agreement with those from previous studies (Tomašovičová, 1981; Moretti *et al*., 1997; Pozio and La Rosa, 2005) as well as the results presented in Chapter 4 of this thesis and suggest fish to be unsuitable hosts for *T. zimbabwensis.*

However, the positive results obtained from two fish in this study suggest that some tigerfish could, under very specific and as yet to be elucidated circumstances, maintain the larvae for *T. zimbabwensis.* During this study it could not be confirmed whether the larvae of *T. zimbabwensis* undergo any further development or, could successfully reproduce in tigerfish.

The influence of temperature on *T. zimbabwensis* larval development in tigerfish could not be elucidated. As such it remains unclear whether the two positive results obtained in this study could be attributed to differences in temperature or differences in host characteristics between tigerfish and catfish. *Trichinella zimbabwensis* is known to favour temperatures in the upper limit of the hosts' preferred temperature range (Pozio *et al*., 2004). This may suggest

seasonal climatic changes to be an additional determinant for *T. zimbabwensis* infection in fish. The seasonal temperature variation between geographic regions may subsequently cause differences in infection patterns between populations of the same fish species from different geographical regions and requires investigation.

6.6 General conclusion and future research recommendations

Overall results from this work show that *T. zimbabwensis* infect a variety of ecto- and endothermic host species indiscriminately and confirm the significant epidemiological role of mammals in the epidemiology of *T. zimbabwensis*. The confirmed infections with *T. zimbabwensis* in a small spotted genet and leopard suggest a large parasite biomass in smaller rodent and/or reptile species. From a veterinary public health perspective, these findings are disconcerting. The potential transmission of the parasite from the natural sylvatic cycle to domestic animals through rodent infestations may be a considerable risk. However, determining the risk of human infection relies on a better knowledge of the epidemiology of *Trichinella* spp. in wildlife. This risk will inevitably increase as population growth drives the search for alternative food sources and causes expansion of the game industry to ensure food security for the country's inhabitants.

The vast protected area of the GKNP combined with its diverse species richness and limited human interference provides an excellent setting for the maintenance of a sylvatic cycle of *Trichinella* spp. Similarly optimal conditions may exist in other national protected areas in sub-Saharan Africa including the Serengeti (Tanzania), Kafue (Zambia), Hwange (Zimbabwe), Masaai Mara (Kenya) and Gorongoza (Mozambique) and further studies to confirm this are recommended.

Co-ordinated surveillance efforts in the GKNP and other nature reserves are hindered by minimal stakeholder involvement, limited access to a variety of samples as a result of legislative barriers and limited financial and other resources. Overcoming these barriers will rely on sensitizing of all stakeholders to the importance of surveillance; close collaboration and effective communication between researchers, regulatory authorities and other stakeholders and motivation of funding by highlighting the potential impact of *Trichinella* on human health and its threat to commercial farming industries.

The current lack of data on human infections and cases involving domestic animals causes *Trichinella* surveillance to be neglected as a public health priority. The perceived neglible threat of *Trichinella* spp. infections needs to change. Surveillance costs care marginal in comparison to the cost of remedial action associated with a human outbreak or the cost of control and eradication that would be required in the event of domestic spill-over. Effective

implementation of existing regulations [Regulation (EU) 2015/1375] is required to maintain and improve wildlife surveillance for *Trichinella* infections in GKNP. Other African countries should be encouraged to conduct surveillance and establishing a *Trichinella* Reference Centre for Africa is of great importance. This would greatly assist in surveillance efforts and capacity building in the needed expertise.

6.7 References

Bruton MN. 1979. The food and feeding behaviour of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of cichlids. Transactions of the Zoological Society of London, 35, 47-114.

Campbell WC. 1979. History of trichinosis: Paget, Owen and the discovery of *Trichinella spiralis*. Bulletin of the History of Medicine, 53, 520-552.

Hurníková Z, Dubinsky S, Mukaratirwa S, Foggin CM, Kapel CMO. 2004. Infectivity and temperature tolerance on non-encapsulating *Trichinella zimbabwensis* in experimentally infected Red foxes (*Vulpes vulpes*). Helminthologia, 41, 189–192.

Kapel C. 1995. *Trichinella* infections in Arctic foxes from Greenland: studies and reflections on predilection sites of muscle larvae. Journal of Helminthology, 69, 325-330.

Kapel C, Webster P, Gamble H. 2005. Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife. Veterinary Parasitology, 132, 101–105.

La Grange LJ, Mukaratirwa S. 2020. Epidemiology and hypothetical transmission cycles of *Trichinella* infections in the Greater Kruger National Park of South Africa: an example of host-parasite interactions in an environment with minimal human interference, Parasite, 27, 1-12.

La Grange LJ, Reininghaus B, Mukaratirwa S. 2014. First report of a mixed infection of *Trichinella nelsoni* and *Trichinella* T8 in a leopard (*Panthera pardus*) from the Greater Kruger National Park, South Africa. Onderstepoort Journal of Veterinary Research, 81, 8-10.

La Grange LJ, Govender D, Mukaratirwa S. 2013. The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. Journal of Helminthology, 87, 91-96.

La Grange LJ, Marucci G, Pozio E. 2010. *Trichinella zimbabwensis* in a naturally infected mammal. Journal of Helminthology, 84, 35-38.

La Grange LJ, Marucci G, Pozio E. 2009. *Trichinella zimbabwensis* in wild Nile crocodiles (*Crocodylus niloticus*) of South Africa. Veterinary Parasitology, 161, 88-91.

Lariviere S, Calzada J. 2001. Mammalian species, *Genetta genetta*. American Society of Mammalogists, 680, 1-6.

La Rosa G, Pozio E. 2000. Molecular investigation of African isolates for *Trichinella* reveals genetic polymorphism in *Trichinella nelsoni*. International Journal for Parasitology, 30, 663-667.

Marucci G, La Grange LJ, La Rosa G, Pozio E. 2009. *Trichinella nelsoni* and *Trichinella* T8 in a lion (*Panthera leo*) of the Kruger National Park (South Africa). Veterinary Parasitology, 159, 225-228.

Moretti A, Piergili Fioretti D, Pasquali P, Mechelli L, Rossodivita ME, Polidori GA. 1997. Experimental infection of fish with *Trichinella britovi*: biological evaluations. In: Ortega-Pierres G, Gamble HR, van Knapen F, Wakelin D. (Eds.) Trichinellosis. *Centro de* Investigacion y Estudios Avanzados IPN, Mexico City, 135-142.

Mukaratirwa S, La Grange LJ, Malatji MP, Reininghaus B, Lamb J. 2019. Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga Provinces of South Africa. Journal of Helminthology, 23, 1-7.

Mukaratirwa S, La Grange LJ, Pfukenyi D. 2013. *Trichinella* infections in animals and humans in sub-Saharan Africa: A review. Acta Tropica, 125, 82-89.

Mukaratirwa S, Foggin CM. 1999. Infectivity of *Trichinella sp*, isolated from *Crocodylus niloticus* to the indigenous Zimbabwean pig (Mukota). International Journal for Parasitology, 29, 1129–1131.

Murrell KD, Pozio E. 2011. The worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerging Infectious Diseases, 17, 2194-2202.

Pienaar U. de V. 1968. The freshwater fishes of the Kruger National Park: Pretoria, National Parks Board, 1-20.

Pozio E, Zarlenga DS. 2013. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. International Journal for Parasitology, 35, 1191- 1204.

Pozio E, Foggin CM, Gelanew T, Marucci G, Hailu A, Rossi P, Gomez-Morales MA. 2007. *Trichinella zimbabwensis* in wild reptiles of Zimbabwe and Mozambique and farmed reptiles of Ethiopia. Veterinary Parasitology, 143, 305–310.

Pozio E, Murrell KD. 2006. Systematics and epidemiology of *Trichinella*. Advances in Parasitology, 63, 371-445.

Pozio E, La Rosa G. 2005. Evaluation of the infectivity of *Trichinella papuae* and *Trichinella zimbabwensis* for equatorial freshwater fishes. Veterinary Parasitology, 132, 113-114.

Pozio E, Marucci G, Casulli A. 2004. *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles. Parasitology, 128, 333-342.

Pozio E, Verster A, Braack L, De Meneghi D, La Rosa G. 1994. Trichinellosis south of the Sahara. In: Campbell CW, Pozio E, Bruschi F. (Eds.), Trichinellosis. Istituto Superiore di Sanità Press, Rome, Italy, 527–532.

Reina D, Munoz-Ojeda M, Serrano F. 1996. Experimental trichinellosis in goats. Veterinary Parasitology, 62, 125–132.

Roux F. 2013. A study on the behaviour of Tiger fish (*Hydrocynus vittatus*) using biotelemetry, to determine habitat utilisation and survival strategies in the Lower Incomati River system. Thesis (P.hD.). University of Johannesburg, 258 pages.

Roux F, Steyn G, Hay C, Wagenaar I. 2018. Movement patterns and home range size of tiger fish (*Hydrocynus vittatus*) in the Incomati River system, South Africa. *Koedoe*, 60, 1-13.

Sharma R, Konecsni K, Scandrett B, Thompson P, Hoberg EP, Bouchard E, Buhler K, Harms NJ, Kukka PM, Jung TS, Elkin B, Mulders R, Larter NC, Branigan M, Pongracz J, Wagner B, Fenton H, Rosenthal BM, Jenkins E. 2019. Distribution and genetic diversity of *Trichinella* in Canadian wildlife: A previously undescribed species (T13) and an unexpected discovery of *T. spiralis*. Scientia Parasitologica, 20, 145-148.

Skelton P. 2001. A complete guide to the freshwater fishes of Southern Africa. 2nd Edition, Struik Publishers, Cape Town.

Soule C, Dupoy-Camet J, Georges P, Ancelle T, Gillet JP, Vaissaire J, Delvigne A, Plateau E. 1989. Experimental trichinellosis in horses: Biological and parasitological evaluation. Veterinary Parasitology, 31, 19-36.

Tomašovičová O. 1981. The role of fish in transfer and maintenance of Trichinellae under natural conditions. Biologia, 36, 115-125.

Young E, Kruger SP. 1967. *Trichinella spiralis* (Owen, 1935) Railliet 1895, infestation of wild carnivores and rodents in South Africa. Journal of the South African Veterinary Association, 38, 441–443.

Young E, Whyte IJ. 1975. Trichinosis (*Trichinella spiralis* infestations) in wild animals of the Kruger National Park. Journal of the South African Veterinary Association, 6, 233–234.