Parasites of *Rattus norvegicus* trapped in Durban, eThekwini Municipality, South Africa

By

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ABSTRACT

Synanthropic rodents of the genus *Rattus* are cosmopolitan, as are many of the parasites that they have acquired as they spread across the globe. This work narrows the gap in our knowledge of endoparasites carried by *Rattus norvegicus* in the port city of Durban, South Africa. The study was conducted over a one year period to include the wet and dry seasons, and rodents were trapped at 56 sites across four locations: central business district (CBD), harbour (HBR), informal settlements (IS) and urban/peri-urban (UPU) areas. The city's Vector Control Division conducted the trapping using custom-made live traps. Three hundred and seventy nine *R. norvegicus* were caught, plus by-catches of 10 *R. rattus* and 11 *Mastomys natalensis*. Rodents were humanely euthanased, blood samples drawn, all ectoparasites collected for a parallel study, various body measurements and mass recorded, then they were dissected, their organs removed and faeces collected. Organs were individually processed, parasites removed and preserved in 70% ethanol prior to identification. Faeces were collected in 10% formal saline for parasite egg and cyst identification. Parasites of public health importance recovered from *R. norvegicus* were: *Trypanosoma lewisi* (22.8%) from blood; *Moniliformis moniliformis* (9.5%), *Hymenolepis diminuta* (17.2%), *H. nana* (0.8%) and *Gongylonema* sp. (25.3%) from the small intestine; *Calodium hepaticum* (2.6%) from the liver and *Angiostrongylus cantonensis* (15.3%) from the heart and lungs. Serological testing for *Toxoplasma gondii* yielded a prevalence of 11.2%. Parasite ova mechanically transmitted in the rodents' faeces, and a potential infection risk for humans, were *Ascaris* sp. (4.8%), *Taenia* sp. (0.3%), *Schistosoma mansoni* (0.3%), *Calodium hepaticum* (0.8%), *Ascaridia galli* (0.5%) and *Toxocara* sp. (0.3%). *Xenopsylla cheopis*, *Polyplax spinulosa*, *Laelaps lamborni* and *L. echidnina* were investigated as drivers of *T. lewisi* infection. Rats infected with *T. lewisi* and *X. cheopis* were more prevalent at CBD and HBR, and juveniles were most frequently affected. Trypanosome infections were positively associated with fleas, negatively associated with lice, and not associated with mites. Extrinsic and intrinsic interactions between helminths of the gut were examined and location and rat age were found to be the most significant drivers. The helminths were: *Gongylonema neoplasticum*, *Protospirura muricola*, *Moniliformis moniliformis*, *Hymenolepis diminuta*, *H. nana*, *Nippostrongylus brasiliensis*, *Strongyloides* spp., *Heterakis spumosa*, and *Syphacia muris*. *Taenia taeniaformis* was most prevalent and abundant at IS, in males, and in rats as they aged. *Trichosomoides crassicauda* was most prevalent and abundant at CBD, HBR and UPU, in males and in rats as they aged (no pups were infected). Common gut protozoans were identified and reported, as were the eggs voided by rats unrelated to their helminth infections. The city centre offers harbourage and abundant food for rats, and suitable habitats for the successful breeding of arthropod vectors of some of these parasites, making it an area of high transmission and a potential public health risk.

PREFACE

The experimental work described in this thesis was carried out in the School of Life Sciences, University of KwaZulu-Natal Durban, from January 2009 to December 2016, under the supervision of Professors C. C. Appleton and S. Mukaratirwa.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

All the procedures used on the rodents collected for this study were approved by the Animal Ethics Sub-Committee of the University of KwaZulu-Natal, clearance certificate reference number: 032/09/Animal.

DECLARATION 1 – PLAGIARISM

I, Colleen Edith Archer, declare that

1. The research reported in this thesis, except where otherwise indicated is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. 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.
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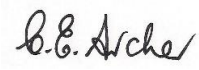
Signed: 

DECLARATION 2 – PUBLICATIONS

DETAILS OF CONTRIBUTIONS TO PUBLICATIONS

Paper I (Chapter 1):

Archer CE, Appleton CC, Mukaratirwa S, Lamb J, Schoeman MC. (2017) Endo-parasites of public-health importance recovered from *Rattus norvegicus* (Berkenhout, 1769) in Durban, South Africa. *Southern African Journal of Infectious Diseases* 32(2):57–66

Author contributions: CEA, CCA and SM conceived and designed the study; CEA collected and identified the parasites; CEA and MCS analysed the data and led the writing; CCA and SM assisted in the writing and editing. JL's laboratory did the genetics work and GenBlast searches. The manuscript was submitted in October 2016 and published in January 2017.

Paper II (Chapter 2):

Archer CE, Schoeman MC, Appleton CC, Mukaratirwa S, Hope KJ, Matthews GB. (2018) Predictors of *Trypanosoma lewisi* in *Rattus norvegicus* from Durban, South Africa. *Journal of Parasitology* 104 (3): 187-195

Author contributions: CEA, CCA and SM conceived and designed the study; CEA collected and identified the endo-parasites; KJH collected and identified the ecto-parasites; CEA, GBM and MCS analysed the data; CEA and MCS led the writing, CCA, SM and GBM assisted in the writing and editing. The manuscript was submitted in June 2017, corrected, re-submitted in January 2018, accepted in March 2018 and published in June 2018.

Paper III (Chapter 3):

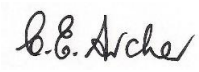
Archer CE, Appleton CC, Mukaratirwa S. Intestinal parasites of *Rattus norvegicus* in Durban, South Africa. Not yet submitted to a journal.

Author contributions: CEA, CCA and SM conceived and designed the study; CEA collected and identified the parasites, analysed the data and led the writing; CCA and SM assisted in writing and editing.

Appendix C:

Archer CE, Appleton CC, Mukaratirwa S, Hope KJ. (2011) The rat lung-worm *Angiostrongylus cantonensis*: A first report in South Africa. *South African Medical Journal* 101(3):174-175

Author contributions: CEA, CCA and SM conceived and designed the project; CEA and KJH collected the parasites; CEA analysed the data and led the writing; CCA and SM assisted in the writing. The manuscript was submitted in June 2010 and published in March 2011.

Signed: 

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- Last, and most importantly, thank you to my parents and my maternal grandmother, all deceased, for raising me to be a determined, hard-working and ethical person – I trust you are proud of me!

FOREWORD

Excerpt from the poem *The Pied Piper of Hamelin*
By: Robert Browning

Hamelin Town's in Brunswick,
By famous Hanover city;
The river Weser, deep and wide,
Washes its wall on the southern side;
A pleasanter spot you never spied;
But, when begins my ditty,
Almost five hundred years ago,
To see the townsfolk suffer so
From vermin, was a pity.
Rats!
They fought the dogs and killed the cats,
And bit the babies in the cradles,
And ate the cheeses out of the vats,
And licked the soup from the cooks' own ladles,
Split open the kegs of salted sprats,
Made nests inside men's Sunday hats,
And even spoiled the women's chats,
By drowning their speaking
With shrieking and squeaking
In fifty different sharps and flats.
At last the people in a body
To the Town Hall came flocking:
``Tis clear,'' cried they, ``our Mayor's a noddy;
And as for our Corporation -- shocking
To think we buy gowns lined with ermine
For dolts that can't or won't determine
What's best to rid us of our vermin!
You hope, because you're old and obese,
To find in the furry civic robe ease?
Rouse up, sirs! Give your brains a racking
To find the remedy we're lacking,
Or, sure as fate, we'll send you packing!''
At this the Mayor and Corporation
Quaked with a mighty consternation……

GENERAL INTRODUCTION

Rattus norvegicus originated in northern China, but by 1800, it was present throughout Europe and had also spread to the New World (Nowak and Paradiso, 1983). With increased shipping trade between ports, invasive rats spread to all continents across the globe, except for Antarctica (Pascal, 2011). Previously natives of forests and other terrestrial biomes, when rats became anthropophilic, they found nesting areas anywhere where food was plentiful, e.g. city garbage dumps, sewers, zoos, badly constructed homes, and old buildings (Myers and Armitage, 2004). Home ranges can be very small (50m in diameter) (Nowak and Paradiso, 1983) if their needs are met, but if food is scarce, are reported to extend up to 5.8ha in extent (Innes, 2001). *Rattus norvegicus* is polygynandrous, thus breeding is prolific and young are born and raised in communal groups of nesting females, and suckled until weaning occurs at 3 – 4 weeks. They become independent at 4-5 weeks and attain sexual maturity at 3 – 4 months (Myers and Armitage, 2004). This served as the rationale for aging the rats according to Hirata and Nass (1974). Many parasites have a pre-patent period of 4 – 6 weeks. Thus, by separating pups from juveniles instead of including pups and juveniles in one weight group (< 100gm or < 140gm) as researchers have previously done (Abu-Madi et al., 2001; Kataranovski et al., 2010), allowed us to get a better idea of when the infection occurred and thus a more accurate host/parasite age relationship.

Rattus norvegicus is a known reservoir of at least 60 zoonotic diseases, among them, plague, rat-borne typhus, leptospirosis and toxoplasmosis (Taylor et al., 2008). In fact, rat-borne diseases have reportedly been responsible for more deaths than all the wars in history (Tufty, 1966). Rats also destroy much of the food crops stored by farmers (Tufty, 1966) and bite people, especially children, while they sleep (Papayya, 2008). As invasive rats spread, so too did their parasites. Durban is the busiest port in Africa (Hutson, 2011) and these rats, along with their cosmopolitan parasites, are thus able to enter South Africa, presenting a very real health risk to people and may spread parasitic diseases to indigenous rodents (Smith and Carpenter, 2006; Julius et al., 2017). Our laboratory previously collaborated on the 'Ratzooman (Rodent Zoonosis Management) Project', an international study funded by the European Commission that monitored 14 sites in four African countries between 2003 and 2006. The only urban site was Durban (now known as eThekwini) and the diseases monitored were bubonic plague, leptospirosis and toxoplasmosis in rodents, humans and domestic livestock (Taylor, 2008). The gastrointestinal tracts of the trapped rats (not the other organs) were examined in our parasitology laboratory at University of KwaZulu-Natal. We found three nematodes and one cestode, identified for us by the Faculty of Veterinary Science, University of Pretoria, South Africa: *Nippostrongylus brasiliensis*, *Strongyloides ratti*, *Heterakis spumosa*, and *Hymenolepis diminuta*. The gut contents and faeces were examined and eggs of the aforementioned worms, *Balantidium coli* cysts (not previously reported from urban rats), plus tiny coiled larvae (that neither the Veterinary Faculty, Pretoria

University, nor our lab could identify at this stage) were found (Appleton and Archer, 2006). During the present study, we discovered that these larvae were those of *Angiostrongylus cantonensis*, and this demonstrated to us the importance of investigating not only the helminths of the gastrointestinal tract but rather all endoparasites as we were not the first to report the larvae as 'unidentified' (Sumangali et al., 2012).

The Vector Control Unit, eThekwini Department of Health, continues to trap rats as part of their pest control and plague surveillance programmes and the Durban Natural Science Museum euthanases them as part of their 'EcoRat' Project, an international collaboration involving the Natural Resources Institute, United Kingdom (UK), the European Union and the Southern African Development Community. When dissecting rats, the Museum staff notified us that they had noticed lesions in the liver strongly resembling those caused by *Capillaria hepatica* (renamed *Calodium hepaticum*). This proved to be an opportune time to thoroughly examine the captured rodents for parasites.

The main objective of this study was thus to identify all endoparasites, particularly those of public health importance, found in rodents trapped daily on week days, over a one year period, to include both the wet and dry seasons, at 56 sites within four locality types (locations) of the eThekwini metropolitan area. Locations were: harbour (HBR), central business district (CBD), informal settlements (IS) internationally known as 'slums', and urban/peri-urban (U/PU or UPU) areas. Age and gender of the rodents were also considered as predictors of parasite infections. A parallel study by Hope (2011), focused on the ectoparasites of these same rodents.

Much has been written on parasite zoonoses carried by rats (Bonfante et al., 1961; Easterbrook et al., 2007; Paramasvaran et al., 2009), although information in South Africa is scant. Chapter 1, Paper I, focused on the eight parasitic infections of human health importance found in *Rattus norvegicus*, as well as other human parasites mechanically spread by these rats due to coprophagy, and aimed to raise awareness of the public health risks. Some of these infections were also found in other rodent species trapped as by-catches, viz. *R. rattus* and *Mastomys natalensis*. As soon as *Angiostrongylus cantonensis* was discovered from the heart and lungs of these rats, an article was written, in the form of a short communication to the *South African Medical Journal*, to inform clinicians of the presence of angiostrongyliasis as a possible differential diagnosis for eosinophilic meningitis (Archer et al., 2011; Appendix C).

Chapter 2, Paper II, examined extrinsic and intrinsic drivers of *T. lewisi* in *R. norvegicus*. Ectoparasites found by Hope (2011) at prevalences >20%, were included in this paper as possible drivers of rodent trypanosomiasis. These were: the flea, *Xenopsylla cheopis* (42.2%), the louse, *Polyplax spinulosa* (21.6%), and the two mites, *Laelaps lamborni* (79.9%) and *L. echidnina* (23.7%). As common murid

fleas (e.g. those of the genera, *Nosopsylla* and *Xenopsylla*, *Ceratophyllus* and *Parapulex*) are known cyclical vectors of *T. lewisi*, it is surprising that many researchers have neglected to examine the associations that may not only drive this infection in rats, but also in other at-risk animals (e.g. chimpanzees in rescue facilities, animals in zoos) or humans. When examining the blood smears for haemoparasites, we expected to find both *Trypanosoma lewisi* (rodent trypanosome) as well as the haemogregarine, *Hepatozoon muris*. Despite *Laelaps echidnina*, the definitive host of *H. muris*, being present on 23.7% of *R. norvegicus*, and the fact that at least one thin and two thick blood smears were thoroughly examined, no *H. muris* were found in the leucocytes of any of the rodents. There are few reports of this genus in wild rats (Eyles, 1952) and mice (Bajer et al., 2006).

The crowding effect of cestodes has been examined in laboratory rats under controlled conditions and associations between size of cestodes and numbers present have been shown to be inversely proportional (Read, 1951). Testing for helminth species interactions in wild-caught rodents has not been particularly successful (Behnke et al., 2001), as age of parasites, date of acquisition of the infection and when challenge infections occurred are unknown. Chapter 3, Paper III, thus examined the extrinsic (location and season) and intrinsic (rat age and gender) drivers of helminth infections and looked at co-occurrences of helminths in relation to life cycles and transmission modes that may influence co-existence.

Chapter 4 deals with parasites found in *R. norvegicus*, but not included in any of the papers, as well as endoparasites of the by-catch rodents, *R. rattus* and *M. natalensis*. Included in Chapter 4 are: (1) *Cysticercus fasciolaris* - the intermediate host liver stage (or metacestode of the cosmopolitan cat tapeworm, *Taenia taeniaformis*); (2) *Trichosomoides crassicauda*, the rat urinary tract worm; (3) protozoans of the gut (*Entamoeba muris* cysts, *E. hartmanni*-like cysts, *Giardia muris* cysts and trophozoites, *Chilomastix bettencourti* cysts, amoebic trophozoites and flagellate trophozoites, *Eimeria nieschulzi* and *Eimeria parastieda*); (4) tissue cysts (*Taenia parva*), and (5) two haemoparasites, *Plasmodium* sp. (possibly *P. berghei*) and microfilariae, possibly *Dirofilaria* sp., found in *M. natalensis*. Helminth eggs found in the faeces of rats that did not have adult infections were reported as infection risks for the mechanical spread of rodent parasites amongst not only other rodents, but also to other indigenous fauna (this infection parameter has been largely neglected in the literature).

Unfortunately, due to safety and logistic issues, sampling was carried out by the eThekwini Vector Control Division of the Department of Health. It was consequently opportunistic and impossible to trap consistently across all locations. However, this study is the first comprehensive work on endoparasites of synanthropic rodents in an African city, and it thus aimed to not only fill the gap in our knowledge of the subject, but also to be a foundation for further studies in this field.

Background information for non-Parasitologists

The rodent endoparasites found in this study are broadly classified in the two tables below and infective stages and risks are briefly described (Table 1 and 2). The classification follows the 5-kingdom format, with the single-celled, nucleated organisms falling into the kingdom, Protoctista (Table 1), and the multicellular parasites into the kingdom, Animalia (Table 2) (NCBI website, accessed 22 August 2019). The nomenclature is accurate at this time but is subject to change as geneticists discover new associations between organisms.

The phylum, Apicomplexa, consists of protozoa that generally have no flagella, cilia or pseudopods. These include the coccidians (*Eimeria* spp.) and the sporozoan, *Toxoplasma gondii*. The phylum, Amoebozoa, describes the amoebae (that use pseudopodia for locomotion). The phyla, Sarcomastigophora, Metamonada and Euglenozoa, encompass the protozoans that have flagella for locomotion (hence the common name, flagellates) (Table 1).

Parasites in the phylum Acanthocephala, are usually termed acanthocephalans or thorny-headed worms; those in the phylum Platyhelminthes, are either flukes or tapeworms; those in the phylum Nematoda, are roundworms. As there are numerous roundworms, a species of roundworm may also be referred to broadly by its order or class, e.g. *Heterakis spumosa* may be termed 'the ascarid' (from its class, Ascaridida) or 'the heterakid' (from its family, Heterakidae) (Table 2).

Some important terminology and biological information will also aid in understanding this work:

(1) A definitive (or final) host is the one in which sexual reproduction of the parasite occurs; asexual reproduction occurs in the intermediate host. Not all parasites have intermediate hosts.

(2) The infective stage of a parasite is either directly transmitted to another host through anus-paw-mouth-infection, through auto/allo-grooming, suckling pups, coprophagy, geophagy, when foraging, indirectly through the bite of an intermediate host, or through ingestion of an arthropod vector and/or its faeces.

(3) The term 'pre-patent period' is the time taken from initial infection of the definitive host by a parasite up until the first visible proof of infection, usually through microscopic detection of the diagnostic stage.

(4) Mean intensity of infection is used by medical scientists to calculate the severity of a disease in a group of individuals, thus negative individuals are not included in the calculation.

(5) Mean abundance is more commonly used in epidemiological or ecological studies as it gives a better overall indication of infection risk at the community level. Mean abundance is thus calculated as the total number of parasites found in all the subjects included in a group divided by the total number of subjects in that group (both those that are positive as well as those that are negative) for that particular infection, and this methodology is followed throughout my thesis.

(6) 'Mechanical transmission' means that the 'host' did not have a true infection whereby adult stages were present in the gastrointestinal tract or in the blood or other tissues of the body, but rather that eggs or larvae had been ingested while foraging or grooming and these life-stages were then excreted in the faeces.

Table 1: **Protoctista** are classified and the infective stage, intermediate host (if required), and human health risks are given.

Phylum	Class	Order	Family	Genus	Species	Infective stage for rodents (Intermediate host)	Human health risk
Apicomplexa	Coccidia	Eucoccidiorida	Eimeriidae	*Eimeria*	*E. nieschulzi*	Oocysts passed in faeces. (None)	No, rodent *Eimeria* spp. are host specific
					E. parasteida		
			Sarcocystidae	*Toxoplasma*	*T. gondii*	Ingestion of flesh containing tissue cysts. (Felines are the definitive host. All warm-blooded animals are potential intermediate hosts like the rodents. Felines excrete oocysts in their faeces that are infective for intermediate hosts.)	Yes for pregnant women (foetal pathology), and for immunocompromised people. Also a risk for all mammals and birds.
	Aconoidasida	Haemosporida	Plasmodiidae	*Plasmodium*	*P. berghei*	Trophozoites present in red blood cells of peripheral blood. (Female Anophelene mosquitoes)	No, host specific for certain rodents
Amoebozoa	Lobosa	Amoebida	Entamoebidae	*Entamoeba*	*E. muris*	Cysts and trophozoites in faeces. (None)	No, host specific
					E.hartmanni-like	Cysts and trophozoites in faeces. (None)	Unknown
Sarcomastigophora	Zoomastigophora	Diplomonadida	Hexamitidae	*Giardia*	*G. muris*	Cysts and trophozoites in faeces. (None)	No
Metamonada	Retortamonadea	Retortamonadida	Retortamonadidae	*Chilomastix*	*C. bettencourti*	Cysts and trophozoites in faeces. (None)	No
Euglenozoa	Kinetoplastea	Trypanosomatida	Trypanosomatidae	*Trypanosoma*	*T. lewisi*	Flagellates in peripheral blood. (Male and female fleas)	Yes (see Chapter 1)

Table 2: **Animalia** are classified and the infective stage, intermediate host (if required), and human health risks are given.

Phylum	Class	Order	Family	Genus	Species	Infective stage for rodents (Intermediate host)	Human health risk
Acanthocephala	Archi-acanthocephala	Moniliformida	Moniliformidae	*Moniliformis*	*M. moniliformis*	Ingestion of infected arthropod. (Cockroaches and flour beetles)	Yes (see Chapter 1)
Platyhelminthes	Trematoda	Plagiorchiida	Dicrocoeliidae	*Dicrocoelium*	*D. dendriticum*	Ingestion of ants containing the metacercaria. (Both snails & ants)	Yes, uncommon
	Cestoda	Cyclophyllidea	Hymenolepididae	*Hymenolepis*	*H. diminuta*	Ingestion of infected arthropods. (Fleas, flour beetles)	Yes (see Chapter 1)
					H. nana	Ingestion of infected arthropods. (Fleas, flour beetles; but also direct transmission)	Yes (see Chapter 1)
			Taeniidae	*Taenia*	*T. taeniaformis*	Eggs in feline faeces. (Rodents)	No
					T. parva	Eggs in canine faeces. (Rodents)	No
Nematoda	Chromadorea	Spirurida	Onchocercidae	*Dirofilaria*	*Dirofilaria* sp.	L3 larvae in mosquito saliva when it takes blood meal. Dog normal definitive host. (Mosquitoes)	Yes, for a variety of mammals, not common
			Gongylonematidae	*Gongylonema*	*G. neoplasticum*	Cockroaches containing infective larvae. (Cockroaches)	Yes for *G. pulchrum* (see Chapter 1)
			Spiruridae	*Protospirura*	*P. muricola*	Arthopods containing infective larvae. (Cockroaches, beetles, fleas)	No
		Oxyurida	Oxyuridae	*Syphacia*	*S. muris*	Embryonated eggs. (None)	No
	Secernentea	Ascaridida	Heterakidae	*Heterakis*	*H. spumosa*	Embryonated eggs. (None)	No
		Strongylida	Metastrongylidae	*Angiostrongylus*	*A. cantonensis*	Ingestion of infective larvae left in snail slime trails, in snails, in paratenic hosts, e.g. crabs, lizards. (Land snails)	Yes (see Chapter 1)
	Chromadoridea	Rhabditida	Strongyloididae	*Strongyloides*	*S. ratti*	Skin penetration by filariform larvae. (None)	No
					S. venezuelensis		No
			Heligmonellidae	*Nippostrongylus*	*N. brasiliensis*	Skin penetration by filariform larvae. (None)	No
	Enoplea	Trichinellida	Trichuridae	*Trichuris*	*Trichuris* sp.	Embryonated eggs. (None)	No
	Adenophorea	Trichocephalida	Trichinellidae	*Trichosomoides*	*T. crassicauda*	Embryonated eggs. (None)	No
			Capillariidae	*Calodium*	*C. hepaticum*	Embryonated eggs. (None)	Yes (see Chapter 1)

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CHAPTER 1

PUBLICATION I

ENDO-PARASITES OF PUBLIC HEALTH IMPORTANCE RECOVERED FROM RODENTS IN THE DURBAN METROPOLITAN AREA, SOUTH AFRICA

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RESEARCH

Endo-parasites of public-health importance recovered from rodents in the Durban metropolitan area, South Africa

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Background: Parasite infections of public health importance carried by *Rattus* spp. on the African continent (excluding toxoplasmosis) have not been adequately researched. The aim of this study was to investigate endoparasites of public health importance, particularly those associated with *R. norvegicus*, at different locations and seasons within the port-city, Durban.
Methods: Four hundred rodents (379 *R. norvegicus*, 10 *R. rattus* and 11 *Mastomys natalensis*) were live-trapped at 60 sites in four locations, during wet and dry seasons in 2009. Rats were humanely euthanased, cardiac blood drawn (for blood smears and serology), ectoparasites removed and dissected. Each organ was separately processed to collect parasites. Binary logistic regression and four-way ANOVAs were used to test for the effects of location, season, rodent age and gender on parasite prevalence, richness and abundance.
Results: Eight parasites of public health importance were detected: *Gongylonema* sp. (25.3%), *Trypanosoma lewisi* (22.8%), *Hymenolepis diminuta* (17.2%), *Angiostrongylus cantonensis* (15.3%), *Toxoplasma gondii* (11.2%), *Moniliformis moniliformis* (9.5%), *Calodium hepaticum* (2.6%) and *H. nana* (0.8%). *Ascaris* spp. (probably *A. lumbricoides*) ova, assumed to have been acquired from consuming infected human faeces were found in rat faeces (4.8%). Parasite species richness was positively associated with location, season and rodent age. Location, season, rat age and gender differentially affected prevalence and worm abundance of parasite species.
Conclusions: These occurrence data of parasites of public health importance provide valuable information to local and provincial organisations and medical practitioners for diagnoses of possible zoonoses, and a reference point for further studies in metropolitan areas of Africa.

Keywords: helminths, parasites, protozoa, public health, *Rattus norvegicus*, rodents, zoonoses

Background

Maritime trade dates back at least two millennia and linked Asia to Europe and West Africa.[1] *Rattus rattus* and *R. norvegicus*, both natives of Asia, probably began their global spread along these trade routes and were also likely the cause of the three major pandemics of plague since the 6th century. The factors responsible for rodents co-habiting with humans have remained the same for many centuries, viz. harbourage,[2] lack of a sanitary environment[3] and availability of food.[4] Since the 15th century, trade routes have expanded globally. For example, >4 500 commercial vessels dock in Durban in eThekwini metropolitan area, South Africa (the 10th largest in the world) every year.[3] Shipping has consequently facilitated the spread of rats and their cosmopolitan parasites and diseases to people across the globe.

The world's urban population is expected to rise by 2.1 billion by 2030.[3] Durban, along with most cities in developing countries, has seen a considerable shift from rural to city dwellers. This influx of people into cities has resulted in the proliferation of informal housing, illegal occupation of dwellings and degradation of formal housing due to low socioeconomic conditions which, in turn, has favoured the breeding of rats.[5] For example, in Johannesburg, South Africa, more rats were reported from: informal dwellings than from apartments; homes where occupants did not pay rent than houses where rent was paid or homes were owned; houses with cracks, leaks, dampness, mould and crowding and where waste collection by the municipality was "never" than well-maintained brick homes with daily or weekly waste collection; and households with no income compared to those with an income of > ZAR 5 000 (±US$ 320) per month.[5] Because rodents scavenge for food and are coprophagous, they are at risk of ingesting infective agents of disease, e.g. parasite eggs and insects (or their faeces). Furthermore, many intermediate hosts of diseases that rats host (such as fleas and ticks) and their endoparasites contaminate human food stores.[6] Hence, zoonotic infections in urban-dwelling humans are probably increasing. Furthermore, mechanical spread of parasites by rats may pose a particularly high risk for disease transmission in slums and informal settlements in urban and peri-urban areas where humans practice open defaecation and foraging animals live and wander freely.[6]

Parasites of *Rattus* spp. from across the globe have been described in the literature for more than a century,[7–10] yet relatively few studies investigated the influence of location, season, rodent age and gender on endoparasite prevalence and diversity.[11] Moreover, comprehensive studies on parasites of *Rattus* spp. from Africa are scant, and limited to reports of select gastrointestinal tract (GIT) parasites[12] or zoonoses.[13] Specifically, a decade ago, two zoonotic parasites, *Hymenolepis diminuta* and *H. nana* were found in *R. norvegicus* in Durban, and serological testing revealed that 4.1% of *R. norvegicus* and 35% of human inhabitants of an informal settlement had antibodies to *Toxoplasma gondii*.[14]

The aim of this study was to therefore investigate the occurrence and distribution of endoparasites of public health importance, particularly those associated with rodents, within the eThekwini metropolitan area, South Africa. Other parasites found will be dealt with elsewhere, and ectoparasites from these rodents were reported by Hope (2011).[15] We gauged the influence of location, season, age and gender of rodents on parasite prevalence,

abundance and richness. Finally, we assessed risk factors for rat to human disease transmission in the urban landscape.

Methods

Study locations and seasons

To capture rats across the eThekwini metropolitan area, the study area was divided into four locality types, namely: central business district (CBD), harbour (HBR), informal settlements/slums (IS) and urban/peri-urban (U/PU) (Figure 1). There were 60 collection sites across these four locations. The CBD and HBR were characterised by many closely juxtaposed buildings, heavy human traffic and widespread food trade, whereas IS was characterised by slums, informal settlements and low-cost housing developments, and U/PU by formal setllements (i.e. houses and apartments), de-centralised shopping areas, recreational and small-wildlife parks, small poultry farms, and wastewater treatment works. The study spanned one year to allow for seasonal variations. There were two distinct periods of rainfall: five wet months (January, February, October, November and December) and seven dry months (March to September). Climate data were provided by weather-station number 461, Mount Edgecombe, 29°42′0″ S, 31°2′0″ E, 96 m above sea level.

Sampling of rodents

Ethical approval for this study was granted by the Animal Ethics Committee of the University of KwaZulu-Natal (Ref. 031/09/Animal) with the proviso that euthanasia was performed by trained mammalogists in accordance with international ethical guidelines.[16] For logistical and safety reasons, rodents were trapped by eThekwini Health Department's Vector Control Division. Trapping was largely opportunistic and often done in response to complaints from the public. Custom-made traps resembling the Monarch Rat Trap were used, with bread, vegetables and meat as bait.

Chloroform was used to euthanase each animal before a cardiac puncture was performed to obtain blood. Thin and thick blood smears were made and serum was harvested and frozen for serological tests [plague and leptospirosis by the National Health Laboratory Services (NHLS), Johannesburg, South Africa]. Any remaining sera were returned to our laboratory by NHLS to test for toxoplasmosis antibodies. Thin blood smears were fixed in 100% methanol and thick smears were air-dried for one hour. The former were stained with May-Grünewald/Giemsa and the latter with Giemsa,[17] allowed to dry and then stored in wooden slide boxes until examination.

Rodents were weighed with a Pasola scale (to the nearest 0.5 g), gender and breeding status recorded, and selected body measurements taken (to the nearest 0.1 mm): total body length (body + tail length), tail length, length of right ear and right hind foot (excluding and including claw). Rodents were then dissected: the diaphragm was removed first, followed by the heart and lungs, the liver, the GIT, the kidneys and bladder and lastly the tongue. Organs (except GIT) were placed into separate jars, covered with digestive medium for tissue (5 g pepsin + 7 ml hydrochloric acid, in 1 l distilled water) and incubated at 37 °C for 12 to 18 h to free parasites for collection, preservation and identification. The GIT was divided into five sections: oesophagus, stomach, small intestine, caecum and large intestine. Each of these sections was carefully slit open, placed in a jar and covered

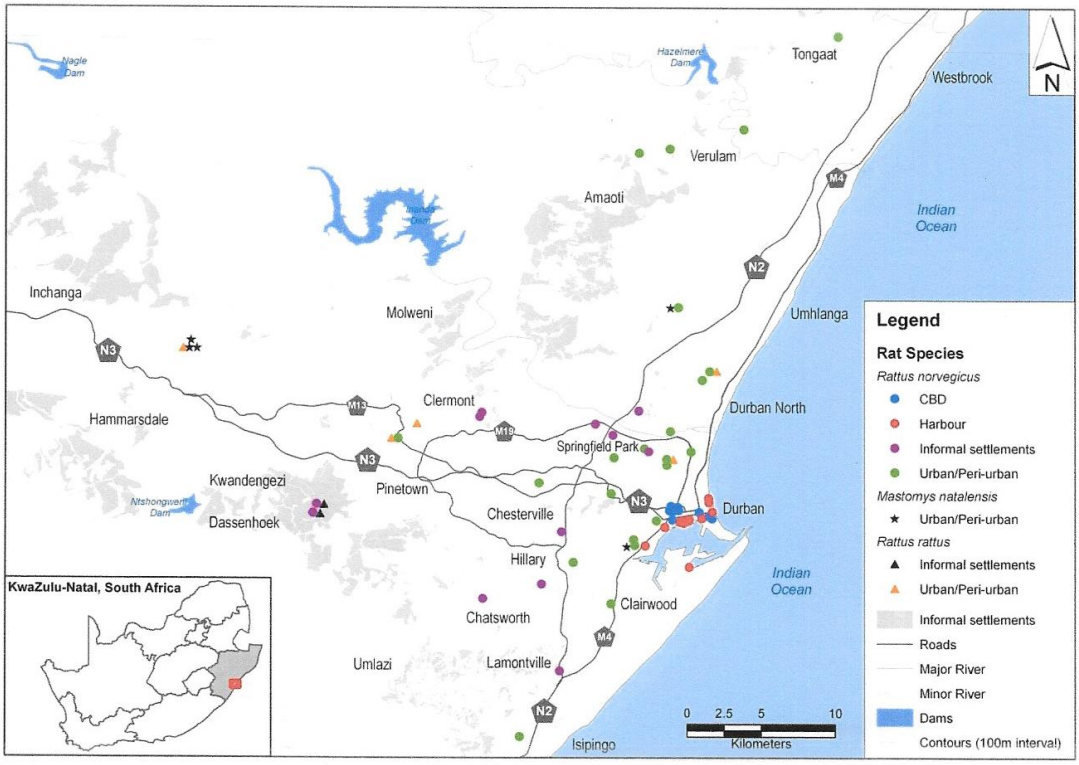

Figure 1: Map of Durban, eThekwini Metro, South Africa, showing study locations and rodents captured.

with 70% ethanol to preserve all parasites in that section. All macroscopically visible acanthocephalans, cestodes and nematodes were removed from the small intestine and preserved in 70% ethanol. Smaller worms of these three helminth taxa and those buried in the mucosa of oesophagus and stomach were removed from each section using a dissecting microscope, identified, counted and placed in 70% ethanol in separate containers for each host animal.

Faeces excreted during euthanasia or found in the rectum at dissection, were placed in 10% formal-saline. For rats that had no faecal pellets, a caecal sample was taken and processed as for the faecal pellets. Samples were processed using the modified formal-ether concentration method[18] to check for helminth eggs and protozoan cysts/oocysts. Adult worms from the caecal sub-samples were stored for future work on parasites of no public health importance.

Aging of rodents

Because the prepatent periods of most parasites are four to six weeks, prevalence of parasites in very young rats (unweaned pups) are likely to differ from weaned juveniles. Thus, *R. norvegicus* were categorised into three age-groups: pups, juveniles and adults. *Rattus rattus* by-catches were classified into age groups according to Hirata and Nass.[19] Pups included unweaned rats up to approximately four weeks; juveniles included rats of approximately five to 10 weeks, probably weaned and some sexually mature; and adults were all sexually mature and > 10 weeks old. *Mastomys natalensis* are difficult to age yet those ≤ 30 g typically are juveniles.[20] In this study, all *M. natalensis* by-catches were adults (>30 g), hence those > 50 g were categorised as old adults and those < 50 g as young adults.

Identification of parasites of zoonotic importance

Detection of Toxoplasma gondii

T. gondii infection was determined serologically using the Bio-Rad Pastorex™ Toxo kit (USA). All weak positives and inconclusive results on initial testing were re-run and confirmed using a second observer. Results were reported as either positive or negative.

Zoonotic protozoans and helminths

Trypanosoma lewisi, was identified morphologically according to Hoare.[21] To identify the acanthocephalan, the taxonomic key by Van Cleave, which uses the armament of the proboscis, was followed.[22] Cestodes were identified based on the morphology of their scolices and eggs. Nematodes in the liver were identified by broken pieces of gravid female worms and characteristic eggs. Nematodes in oesophagus and stomach mucosa were identified to genus by characteristic scutes at anterior end of the body.[23] Adult nematodes in the heart and its associated vessels and eggs, and L1 larvae in the lungs, were identified using morphological features as described by Macherras and Sandars.[24]

Genetic confirmation of A. cantonensis

DNA was isolated from tissue samples using a DNeasy® DNA isolation kit (QIAGEN Inc., USA). Analyses were based on the mitochondrial cytochrome oxidase 1 (CO1) gene (primers: LCO (forward) 5'GGTCAACAAATCATAAAGATATTGG and HCO (reverse) 5'-TAAACTTCAGGGTGACCAAAAAATCA) and nuclear ribosomal RNA ITS2 region (primers: NC1 (forward) 5'-ACGTCTGGTTCAGGGTTGTT and NC2 (reverse) 5'-TTAGTTTCTTTTCCTCCGCT). PCR amplifications were performed in 25 µl volumes. Each reaction contained 0.8 µl sterile water, 2.5 µl 10 X reaction buffer (SuperTherm, UK), 4 µl 25 mM $MgCl_2$ (SuperTherm, UK), 0.5 µl 10 mM deoxynucleoside-triphosphate mixture (dNTPs) (Roche Diagnostics, Switzerland), 0.2 µl *Taq* polymerase (5 U/µl) (SuperTherm, UK) and 4 µl of each primer (6 µM) (forward and reverse) per reaction. The thermal cycling parameters used were as follows: CO1 — 94 °C for 4 min, followed by 40 cycles of (95 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min) and followed by 72 °C for 10 min; ITS2 — 95 °C for 5 min, followed by 40 cycles of (95 °C for 1 min, 58 °C for 1 min and 72 °C for 90 s) and followed by 72 °C for 10 min.

Target fragments were purified from excised gel bands using the QIAquick® Gel Extraction Kit (QIAGEN Inc., USA) and sequenced at InqabaBiotec, South Africa. All fragments were sequenced in both directions to allow reconciliation of ambiguous positions. They were aligned using the CLUSTAL W option[25] of the BioEdit program (ver. 5.0.9 for Windows 95/98/NT) and by visual inspection. Similar sequences were identified by BLAST searches of the NCBI GenBank and downloaded for inclusion in the analyses. For the ITS analysis, the following GenBank sequences were included: *Angiostrongylus catonensis* EU636007, GQ18112, HQ540543, HQ540544, HQ540547; *Angiostrongylus daskalovi* KX242346; *Angiostrongylus vasorum* EU627592, EU627593 - EU627596; outgroups *Aelurostrongylus abstrusus* DQ372965 and JX948745. Sequences used in the CO1 analysis were: *Angiostrongylus catonensis* GQ398121, KT947978; *Angiostrongylus malayensis* KT947979; *Angiostrongylus vasorum* GQ982872, JX268542; *Angiostrongylus costaricensis* KR827449, GQ398122; outgroups *Caenorhabditis briggsiae* EU407785 and *Dictyocaulus viviparous* JX519460.

Trees were constructed using the neighbour-joining and parsimony methods in PAUP 4.0b10.[26] The software jModelTest 0.1.1[27] was used, applying the AKAIKE information criterion, to determine the most appropriate evolutionary model (GTR+G) to use in neighbour-joining analyses. For parsimony analysis, the addition sequence was random, with one tree held at each step and with ten replicates. A total of 1000 bootstrap replicates were carried out for both parsimony and neighbour-joining analyses.

Examination of faecal pellets for parasite ova

Helminth eggs and larvae of parasites normally parasitising rats were reported as absent or present on a plus-scale of 1–4. Only eggs from parasites not normally infecting rodents (i.e. those mechanically transmitted via ingestion and excretion) were counted.

Statistical analyses

Two-way ANOVAs were used to test differences in numbers of *R. norvegicus* captured between location and season. Binary logistic regression was used to identify the most significant predictors - location, season, age and gender - of parasite infection (prevalence). Four-way ANOVAs on the ranked data were used to examine differences in (i) mean worm abundance of zoonotic helminths identified, (ii) mean intensity of trypanosome infections and (iii) species richness, in *R. norvegicus* among location, season, age and gender. All statistical analyses were performed using SPSS (version 23, College Station, Texas, USA).

Results

Rodents trapped per location and season

Rattus norvegicus comprised 94.8% (n = 379) of the 400 rodents sampled. Additionally, 10 *R. rattus* and 11 *M. natalensis* were trapped. Numbers of *R. norvegicus* trapped per location were: 101 from CBD, 93 from HBR, 97 from U/PU and 88 from IS. There was a significant difference in number of *R. norvegicus* sampled

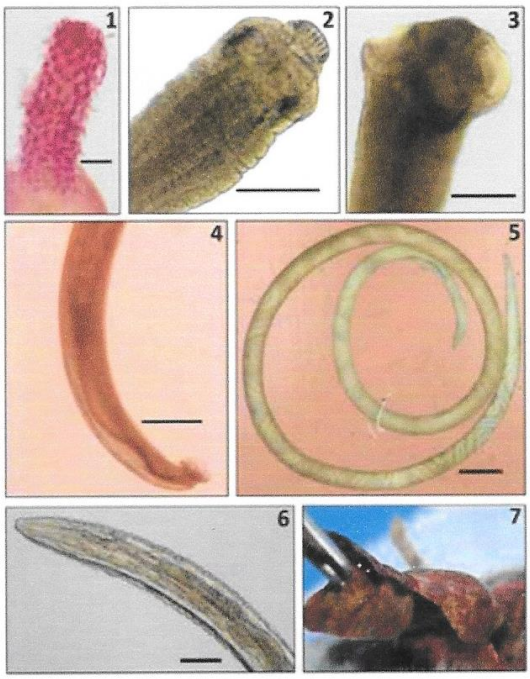

Figure 2: Adult worms and tissue pathology (scale bars = 100 µm) 1: Proboscis of *M. moniliformis* showing characteristic rows of recurved hooks; 2: *H. nana* scolex with 4 suckers and raised rostellum armed with hooklets; 3: *H. diminuta* scolex showing 4 cup-shaped suckers and unarmed rostellum; 4: Rear end of *A. cantonensis* male worm showing copulatory bursa and long spicules; 5: *A. cantonensis* female worm with the characteristic "barber's pole" appearance; 6: *Gongylonema* head end showing characteristic scutes; 7. Liver of male *M. natalensis* at dissection showing the extent of his *C. hepaticum* infection (yellow lesions in the surface parenchyma).

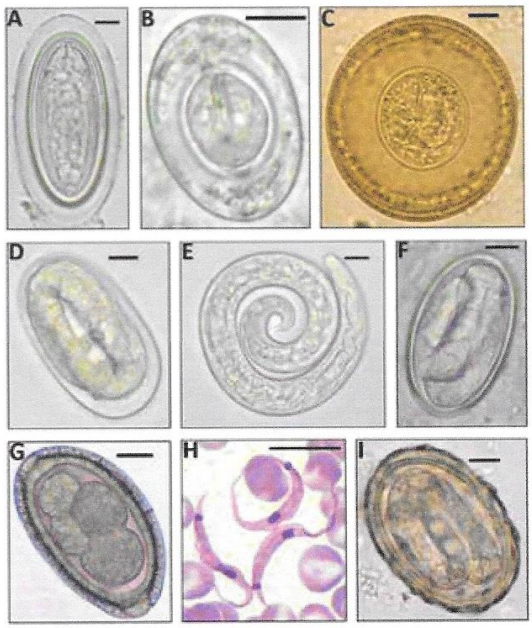

Figure 3: Helminth eggs and larva & the protozoan blood parasite found (scale bars = 10 µm). A: *M. moniliformis* egg; B: *H. nana* egg; C: *H. diminuta* egg; D: *A. cantonensis* egg; E: *A. cantonensis* L1 larva; F: *Gongylonema* sp. egg; G: *C. hepaticum* egg; H: *Trypanosoma lewisi* trypomastigotes; I: *Ascaris* sp. egg.

among locations and between seasons (2-way ANOVA: $F_{(7, 378)} = 22.136$; $p < 0.001$). Post-hoc Tukey tests showed that significantly more *R. norvegicus* were trapped at CBD than IS ($p < 0.001$) and U/PU ($p = 0.001$). Further, significantly fewer rats were captured during the wet months ($n = 137$) than dry months ($n = 242$) ($p < 0.001$). Abundance of *R. norvegicus* was highest in September ($n = 75$) and lowest in December ($n = 2$). *Rattus rattus* and *M. natalensis* were captured only at U/PU ($n = 8$ and 11, respectively; in both seasons) and IS [2 *R. rattus* (in both seasons) and 1 *M. natalensis* (in dry season only)].

Parasites of public health importance

Patent infections of *Trypanosoma lewisi* (Protista), *M. moniliformis* (Acanthocephala), *H. diminuta* and *H. nana* (Cestoda); *Gongylonema* sp., *A. cantonensis* and *C. hepaticum* (Nematoda), were found in *R. norvegicus* (Figures 2 and 3), while *T. gondii* (Protista) was confirmed serologically. *Rattus rattus* harboured only *T. gondii*, *H. diminuta* and *A. cantonensis*, and *M. natalensis* was infected with *H. diminuta*, *C. hepaticum* (Figure 2) and *Angiostrongylus cantonensis*.

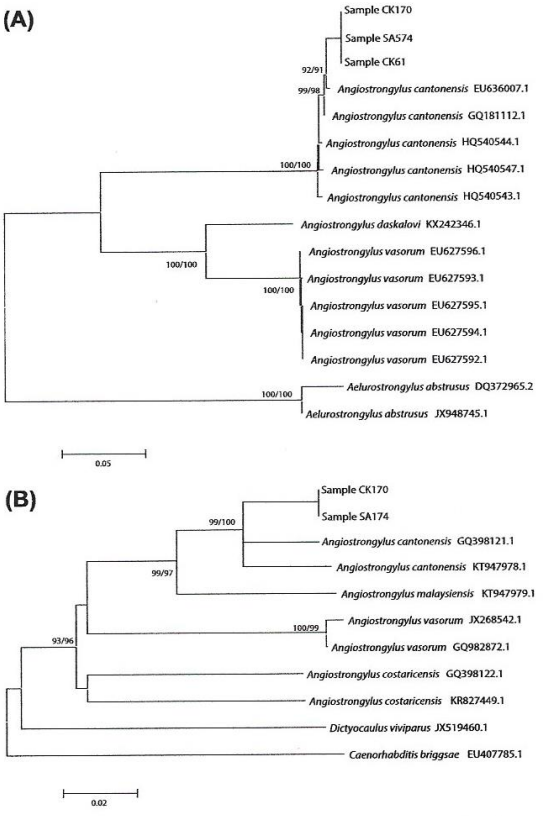

Figure 4: (A) Neighbour-joining tree based on 367 nucleotides of the nuclear ribosomal ITS2 DNA region illustrating relationships between experimental samples CK61, CK170 and SA574, *Angiostrongylus* sequences downloaded from the GenBank, and the outgroups *Aleurostrongylus abstrusus*. Bootstrap support for nodes is given as [nj%/p%] for neighbour-joining (nj) and congruent parsimony(p) analyses and (B) Neighbour-joining tree based on 611 nucleotides of the mitochondrial cytochrome oxidase 1 gene illustrating relationships between experimental samples CK170 and SA574, *Angiostrongylus* sequences downloaded from the GenBank, and the outgroups *Dictyocaulus viviparous* and *Caenorhabditis briggsae*. Bootstrap support for nodes is given as [nj%/p%] for neighbour-joining (nj) and congruent parsimony(p) analyses.

The CO1 and ITS2 alignments were trimmed to 611 and 367 nucleotides, respectively, and used in further analyses. Analyses based on both the CO1 and ITS2 regions (Figures 4(A) and (B)) were congruent and indicated that the experimental samples were previously unreported haplotypes of *Angiostrongylus cantonensis*. This is based on the inclusion of the experimental samples in a very stongly supported clade (99–100% bootstrap support) which included GenBank samples of *A. cantonensis*. This enabled the experimental samples to be referred to *A. cantonensis* according to the phylogenetic species concept.[28]

No adult worms were found in some *R. norvegicus* although eggs of *M. moniliformis*, *H. diminuta* and *Gongylonema* sp. and L1 larvae of *A. cantonensis* were detected in the faeces. Other eggs found were: *Calodium hepaticum* (ingested via environmental contamination or through necrophagy), *Taenia* sp., *Schistosoma mansoni* and *Ascaris* sp. (for which rats are not natural hosts). *Toxocara* sp. ova were found in the faeces of one *R. rattus* individual and *C. hepaticum* ova were found in the faeces of the same *M. natalensis* that harboured a patent *C. hepaticum* infection. This was considered as mechanical transmission (meaning: 'the transmitter is not infected in that tissues are not invaded and the agent does not multiply').[29] Ova of *Moniliformis moniliformis*, *H. diminuta*, and *Gongylonema* sp., and L1 larvae of *A. cantonensis* were mechanically transported by seven, 51, 11 and seven *R. norvegicus*, respectively.

Most of the helminth eggs of public health importance that did not infect rodents, but were mechanically transported by them, were the roundworm *Ascaris* sp., probably *A. lumbricoides* (Figure 3). These eggs were present in the gut contents of 20 rats: three rats in U/PU (range of 1–6 eggs/rat); four rats in CBD (1 egg each); and 13 in IS (range of 2 - 287 eggs/rat).

Influence of location, season, rodent age and gender on parasite prevalence, richness and abundance

Because of low sample sizes, endoparasite infections in *Rattus rattus* and *M. natalensis* were not statistically compared with those in *R. norvegicus*.

Hosmer and Lemeshow's Goodness of Fit Test was satisfied for logistic models of *T. gondii*, *T. lewisi*, *M. moniliformis*, *Gongylonema* sp. and *A. cantonensis*, but not for *H. diminuta*.

Sero-prevalence of T. gondii

Prevalence of *T. gondii* among *R. norvegicus* was 11.3% (n = 14/124) and among *R. rattus* it was 12.5% (n = 1/8). In terms of age groups, 16% (n = 4/25) were pups, 6.1% (n = 3/49) were juveniles and 14.0% (n = 7/50) were adults. No *M. natalensis* (n = 5) tested positive. Logistic regression showed that location, season, rat age and gender, were not significant predictors for *T. gondii* prevalence (all $p > 0.05$).

Patent helminth infections

Gongylonema sp. was found in 25.3% of *R. norvegicus* (n = 96/379), but was absent from the other two rodent species. This nematode was more prevalent in HBR and CBD than in IS and U/PU (Table 1, Figure 5). Prevalence increased with the age of rats (Table 1). Mean worm abundance was highest in HBR and CBD and lowest in IS, and it increased with the age of rats, particularly in the wet season (Table 2).

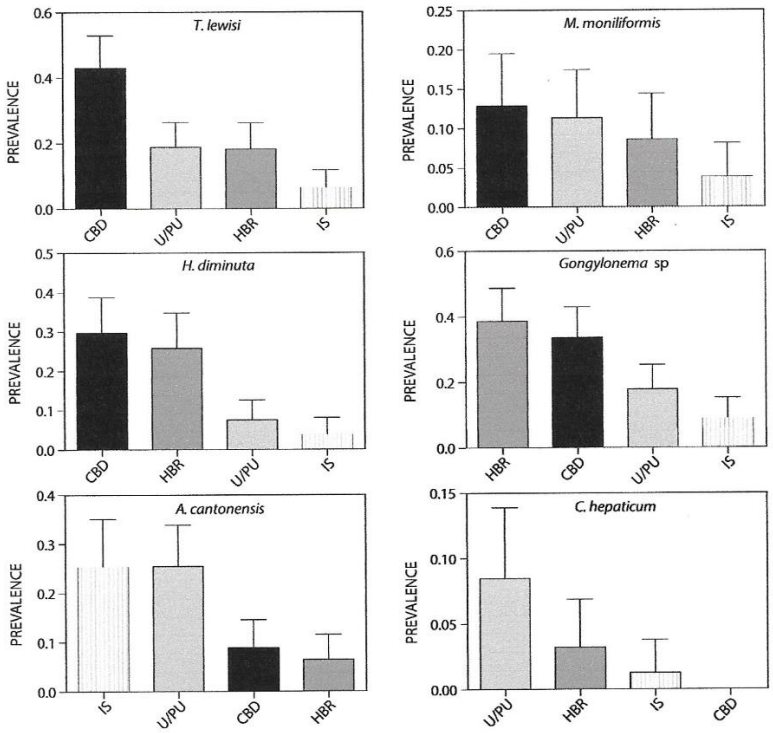

Figure 5: Prevalence (Mean ± 95% CI) of *T. lewisi*, *M. moniliformis*, *H. diminuta*, *A. cantonensis*, *Gongylonema* sp., and *C. hepaticum* in *R. norvegicus* across 4 locations (CBD = central business district; HBR = harbour; IS = informal settlements; U/PU = urban/peri-urban areas) in Durban.

Table 1: Significant statistical results for binary logistic regression testing the influence of age and gender of *Rattus norvegicus*, location and season on prevalence of endoparasites

Parasite	χ^2	*df*	*p*-value	Variable	Significant variables	*p*-value
T. lewisi	41.764	7	<0.001			
				Location	[1]CBD > IS by 6.45x	<0.001
					CBD > HBR by 3.40x	0.001
					CBD > U/PU by 3.56x	<0.001
				Age	J > A by 2.85x	0.003
M. moniliformis	15.556	7	0.029			
				Age	A > P by 3.85x	0.024
					A > J by 2.93x	0.020
H. diminuta	74.685	7	<0.001			
				Location	CBD > HBR by 2.20x	0.033
					CBD > IS by 13.00x	<0.001
					CBD > U/PU by 5.86x	<0.001
					HBR > IS by 5.89x	0.007
					HBR > U/PU by 2.65x	0.036
				Season	Wet > Dry by 3.35x	<0.001
				Age	A > P by 6.51x	0.002
					J > P by 8.25x	<0.001
Gongylonema sp.	68.553	7	<0.001			
				Location	CBD > IS by 5.67x	<0.001
					HBR > IS by 6.82x	<0.001
					CBD > U/PU by 2.70x	0.008
					HBR > U/PU by 3.25x	0.002
				Age	A > P by 10.89x	<0.001
					A > J by 3.11x	<0.001
					J > P by 3.50x	0.006
A. cantonensis	93.376	7	<0.001			
				Location	IS > HBR by 9.93x	<0.001
					U/PU > HBR by 6.50x	<0.001
				Season	Wet > Dry by 2.29x	0.026
				Age	A > J by 5.55 x	<0.001
					J > P by 19.09 x	0.006
					A > P by 105.85 x	<0.001

Notes: A = adults; J = juveniles; P = pups; CBD = central business district; HBR = harbour; IS = informal settlements; U/PU = urban/peri-urban areas.
[1]Example of how to read 'Significant variables' column: 'CBD > IS by 6.4x' means rats in CBD are 6.4x more likely to be infected than rats in IS.

Prevalence of *Trypanosoma lewisi* was 22.8% (n = 86/378), and was highest in CBD and juveniles (Table 1). Mean intensity of infection was affected by location, age and gender (Table 2).

Prevalence of *H. diminuta* in *R. norvegicus* was 17.2% (n = 65/379; Figure 5), in *R. rattus*, 30% (n = 3/10) and in *M. natalensis*, 36.4% (n = 4/11). *R. rattus* positives were from U/PU. One juvenile (dry season) was infected with six *H. diminuta* and two adults (wet season) with two *H. diminuta* each. Positive *M. natalensis* were adults and from U/PU, three that were caught in the dry season harboured three, eight and two *H. diminuta*, respectively, and one caught in the wet season harboured six worms. Location, season and rat age were significant predictors of *H. diminuta* prevalence and abundance in *R. norvegicus* (Tables 1 and 2). Prevalence was significantly higher at CBD and HBR than at IS and U/PU (Figure 5); in the wet than in the dry season; and in adults and juveniles than in pups. Highest abundances were found in juveniles at HBR and U/PU and in adults at HBR. Juveniles had high mean worm burdens in both seasons, yet adults had high mean worm burdens principally in the wet season. The few infected pups had very low worm burdens.

Prevalence of *A. cantonensis* was 15.0% (n = 57/379) in *R. norvegicus* and 10% (n = 1/10) in *R. rattus*. The two *M. natalensis* (18.2%; n = 2/11) infections were possibly *A. sandarsae* given that this species was previously recorded from *Mastomys natalensis* in Kenya[30] and Mozambique.[31] Location, season and age were significant predictors for *A. cantonensis* prevalence in *R. norvegicus* (Table 1) – prevalence was significantly higher at IS and U/PU than HBR (Figure 5), in the wet season than dry season, in adults than juveniles and pups, and in juveniles than pups.

Mean worm abundance of *A. cantonensis* was significantly influenced by location, season and age as well as interactions between location and season, location and age, and season, age and gender (Table 2). Highest worm abundance was at IS and U/

Table 2: Significant statistical results for 4-way ANOVAs testing differences in trypanosome intensity of infection, worm abundance and species richness between locations and seasons and among age groups and gender of *R. norvegicus*

Parasite/Spp. Richness	$F_{(df)}$	*p*-value	Significant variables	*p*-value
T. lewisi	$F_{(46, 363)} = 2.166$	<0.001		
			Location	<0.001
			Age	0.016
			Location*Age	0.022
			Location*Gender	0.046
M. moniliformis	$F_{(46, 364)} = 1.411$	0.048		
			Location*Season	0.046
H. diminuta	$F_{(46, 364)} = 3.848$	<0.001		
			Location	<0.001
			Age	0.001
			Location*Age	0.001
			Season*Age	0.021
Gongylonema sp.	$F_{(46, 364)} = 2.085$	<0.001		
			Location	<0.001
			Age*Season	0.018
A. cantonensis	$F_{(46, 364)} = 4.939$	<0.001		
			Location	<0.001
			Season	0.007
			Age	<0.001
			Location*Season	0.001
			Location*Age	<0.001
			Season*Age* Gender	0.010
Species richness	$F_{(46, 364)} = 2.895$	<0.001		
			Location	<0.001
			Age	<0.001

PU in the wet season (alone and combined). Juveniles had the highest worm abundance in U/PU and juveniles and adults had the highest worm abundance in the wet season. Male adult rats in the wet season had the highest mean worm abundance.

Prevalence of *M. moniliformis* in *R. norvegicus* was 9.5% (n = 36/379; Figure 5). Neither *R. rattus* nor *M. natalensis* were infected. The only significant predictor for *M. moniliformis* prevalence was age; prevalence was significantly higher in adults than in juveniles and pups (Table 1). Mean worm abundance was significantly influenced by the interaction of location and season (Table 2) where the highest mean worm abundance was during the wet season at HBR and IS.

Calodium hepaticum prevalence in *R. norvegicus* was 2.6% (n = 10), *viz.*:

(1) HBR: one juvenile male trapped in the wet season had only *C. hepaticum* eggs in its liver. Fragments of a single worm were recovered from the liver of one juvenile male rat (dry season). One adult female (dry season) harboured a damaged female worm with eggs and her liver had characteristic creamy-yellow tracks visible on the surface.

(2) U/PU (dry season only): fragments of worms and eggs were recovered from the livers of one juvenile male rat, two adult females and four adult males.

No *R. rattus* were infected and only one adult male *M. natalensis* (prevalence 9.1%), trapped in the dry season at U/PU was positive. This mouse was the most heavily infected animal trapped and its liver parenchyma showed many yellow lesions (Figure 2), fragments of male and female worms and thousands of eggs. He also passed *C. hepaticum* eggs in his faeces (unrelated to his own infection) (Figure 3).

Hymenolepis nana was recovered from three *R. norvegicus* only at HBR in the wet season. One pup had 49 worms (prevalence 0.9%), one juvenile had eight worms (co-infected with *H. diminuta*; prevalence 0.7%), and one adult had a single worm (prevalence 0.8%). No *H. nana* infections were found in the other two rodent species.

Species richness

The model for species richness was significant for location, season and age (as independent effects). Multiple infections were significantly low at IS and in the dry season and increased with age of rats (Table 2).

Discussion

This study is the first detailed account of endoparasites of public health importance from urban rats, principally *Rattus norvegicus,* in Africa. Similar to previous studies in eThekwini,[3] *R. norvegicus* was the most common rodent sampled (94.8% of the total catch) and it harboured eight zoonoses. Globally, this rodent species often occurs with other *Rattus* species and is not always the dominant species, especially in Asia.[9] The eight parasite species of public health importance are cosmopolitan.[32] Location, season and age were significant predictors of parasite species richness – significantly low at informal settlements and in the dry season and increasing with age of hosts. By contrast, neither site nor host age influenced parasite richness in *R. norvegicus* in Kuala Lumpur, Malaysia, yet parasite species richness in *R. rattus*, was higher at wet markets and in older rats.[11]

Prevalence of *Gongylonema* sp. in rodents was relatively high (25.3%). A study in Kuala Lumpur found only 0.4% of *R. rattus* was infected (no *R norvegicus*)[11]; one study in India found 17.5% *Rattus* spp. infected[9]; and, one study in San Juan found 35% *Rattus* spp. infected.[33] Specimens will be identified to species level based on the gongylonemids in future work. Among 47 recognised species of *Gongylonema*, 15 are found in rodents, including *G. neoplasticum* and *G. pulchrum*.[34] By 2013 there were 57 reported cases worldwide of human infections with *Gongylonema* spp. (and mostly assigned *G. pulchrum*), yet the first genetically confirmed case of *G. pulchrum* was in 2013.[23] Infection is acquired through ingestion of infected intermediate hosts such as beetles or cockroaches, which in human cases, usually occurs unintentionally.[34] Human gongylonemiasis creates the sensation of something moving in the buccal mucosa and some patients removed worms from their mouths.[35] Infected patients complained of reflux (which indicates possible involvement of the oesophagus[35]) and high fever with digestive disturbances and vomiting.[36] Future work should combine genetic identification with morphology to identify the source of human infections and clarify the role that rats play in the transmission of this parasite.

Trypanosoma lewisi had the second highest prevalence (22.8%) among parasites reported in this study. Prevalence and intensity of infection were influenced by location, age, and gender with significantly more rats infected and with highest parasite burdens in the central business district in juveniles and in females. By contrast, prevalences ranged from 1.5% in Malaysia[37] to 21.7% in Brazil.[38] In the latter study, prevalence was highest in the wet season, in males and in young rats. *Xenopsylla cheopis*, the plague flea, is the common vector in warm climates[39] (also the most prevalent on the rats in this study[15]), and infection is by ingestion of flea faeces or fleas.[40] Two African studies, one in Nigeria and another in selected sites in three African countries (Tanzania, Swaziland and Namibia), found 75.7%[41] and 45.2%[42] *R. rattus* respectively, infected with *T. lewisi*. This haemoflagellate can prove fatal in humans[43] and in young rats.[44] Human cases (often involving infants), have been reported from India, Thailand, Malaysia and Gambia.[43] Common symptoms are fever, coughing, anorexia, depression and lassitude.[43]

Hymenolepis diminuta, a common cosmopolitan parasite of rats and mice, was the only parasite found in all three rodent species. This tapeworm has been found across the globe at prevalences of up to 66.7% in *Rattus* spp.[45] Often, human cases in developing countries are incidentally reported when mass faecal parasite surveys in school children are carried out. Clinical case reports by 2004 were < 500, with most cases in Southeast Asia probably due to the cultural practice of entomophagy.[46] We found that location, season and age group were significant predictors of both *H. diminuta* prevalence and worm abundance. Although *H. diminuta* has a relatively short prepatent period of 18–20 days, *R. norvegicus* pups were the least affected (prevalence = 3.8%). Potential intermediate hosts for *H. diminuta* include fleas (*Xenopsylla cheopis*), and flour beetles (*Tenebrio molitor*) that are often found in grain storage facilities.[6] The latter may explain the high prevalence of *H. diminuta* at the harbour and central business district. Significantly high infections after weaning in the wet season may occur because rats become independent and more likely to encounter abundant intermediate hosts while exploring and foraging.

Prevalence of *Angiostrongylus cantonensis* in *R. norvegicus* is the first from Africa.[47] Rats are the definitive host, *A. cantonensis* does not occur naturally in other rodents. This nematode was first discovered in *R. norvegicus* and *R. rattus* in Canton (now Guangzhou), China in 1933,[48] and has since been found globally. Location, season and age had significant effects on prevalence and worm abundance of *A. cantonensis*, where highest prevalence and abundance were in adults at informal settlements and urban/peri-urban areas during the wet season. These factors were probably mediated by the intermediate hosts – terrestrial snails and slugs – of *A. cantonensis*. In support, species of the snail families Achatinidae and Helicidae and slugs of the families Urocyclidae and Veronicellidae are more commonly found in sub-urban and rural areas than built-up city and harbour areas, especially in the wet season.[49] Achatinids are eaten by humans in West Africa,[50] yet information on snail-eating in southern Africa is lacking. Slime trails containing infective L1 larvae, left by molluscs on vegetables, are also a source of infection.[51] By 2010, there were more than 2 877 human case reports worldwide, the most common symptom being eosinophilic meningitis, but pathology varied from mild to severe, sometimes resulting in death.[51] It is frequently encountered in humans in China and the Far East due to their penchant for strange and exotic culinary delicacies.[52]

Seroprevalence of the most ubiquitous parasite, *T. gondii*, was 11.3%. One reason for the lack of statistical differences in the number of *T. gondii*-positive rats among location, season, or age groups could be because infections become established within seven days in the rodent host. Although cats, the definitive hosts of *T. gondii*, are common in informal settlements, the status of toxoplasmosis in these cats is not known, nor how inhabitants interact with them. Seroprevalence reports from Mozambique, Zimbabwe, Tanzania and South Africa ranged between 1–21.3% in rodents and 4.1–51.2% in humans.[14] A comprehensive review listed seroprevalences of 4–100% for women of child-bearing age in countries across the globe between 1990 and 2000.[53] Humans can contract *T. gondii* by eating raw or undercooked flesh of various intermediate hosts, therefore human infections of *T. gondii* are not always related to rat infections.[53]

Age of rodents was the only significant predictor for *M. moniliformis* prevalence (9.5%), as pups are much less likely to be infected than adults due to the five-week prepatent period of the parasite. This "thorny-headed worm" was described from *R. rattus* and *R. norvegicus* in Egypt[54] yet is rare in southern Africa. Prevalence of *M. moniliformis* in the gut of *Rattus* spp. from across the globe range between 0% and 59.3%.[8] There have been clinical case reports of *M. moniliformis* infections in people from Australia, Asia, Europe, America and Africa (Sudan, Nigeria, Egypt, Madagascar and Zimbabwe).[55,56] In Ghana, West Africa, larval stages of *M. moniliformis* were found in the intermediate host, *Periplaneta americana*.[57] There is evidence that worm abundance is positively correlated with abundance of common arthropod intermediate hosts, particularly in the hot, humid rainy season.[58] Although we found no evidence for the influence of season on prevalence, worm abundance was significantly high during the wet season at harbour and informal settlement sites.

Calodium hepaticum was present in male and female *R. norvegicus* in both seasons at both the harbour ($n = 3$) and urban/peri-urban ($n = 7$), and one *M. natalensis* (U/PU, dry season) had a severe liver infection. Transmission of parasite eggs is facilitated mainly by cannibalism and necrophagy, particularly inside burrows; and, once eggs mature to the infective stage, allogrooming can cause re-infection.[59,60] We found no evidence that prevalence and intensity were related to season or gender of the host. Low prevalence of *C. hepaticum* may be due to the relatively mild winters and abundant food resources throughout the year in Durban, which, in turn, may result in low levels of cannibalism and necrophagy. Human hepatic capillariasis is a serious infection that is usually diagnosed at autopsy, yet there were only two clinical cases and one autopsy reported from South Africa between 1957 and 1973.[61–63]

Hymenolepis nana was found only in three rats at the harbour in the wet season. This tapeworm needs only one host to complete its lifecycle.[64] Eggs are infective when they leave the gravid segments and can infect the same host by anus-hand-mouth contamination or via oncospheres hatching from eggs laid in the gut, or from another host via eggs in contaminated food. Previous studies have estimated *H. nana* prevalence in *R. norvegicus* between 0% and 42.4%[32,65]; reports have been varied in humans. For example, low prevalences in school-based surveys where infected children were symptomless,[66,67] and individual symptomatic cases in children from disadvantaged backgrounds as a concomitant infection with *Giardia intestinalis*,[68] or in immunocompromised patients together with *Crytosporidium parvum*.[69]

The most common mechanically transmitted eggs carried by *R. norvegicus* in this study belonged to *Ascaris* sp. (probably *A. lumbricoides*). Infected rats were caught, mainly at urban/peri-

urban sites; almost half of the carrier rats were caught under a wooden hut that was used as a crèche. Previously, children in informal settlements of eThekwini exhibited high prevalence of *A. lumbricoides* (81.7–96.3%).[70] It is perhaps notable that 39% of boys and 53% of girls in northern KwaZulu-Natal regularly ate soil.[71]

Conclusions

All eight parasites identified in this study are of public health importance and capable of causing pathology, with *C. hepaticum* the most serious and *H. diminuta* the least. Although prevalences of parasites were relatively low, the mere presence of these parasites merits further investigation in eThekwini as well as other metropolitan areas in Africa. We found evidence that location, season, rat age and gender differentially affected the prevalence and mean worm abundance of parasite species. However, one caveat of this study is that it was not possible to design the sampling protocol ourselves, especially in the slums, partly because of the high crime rates. Thus sampling effort was not consistent and equal across locations and seasons, which may have affected our statistical analyses of parasite prevalence and abundance. Future work should standardise the sampling effort and replicate sampling in other urban landscapes, to better understand the patterns and drivers of parasite loads in rats in African urban landscapes.

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Conflict of Interest – The authors declare that they do not have a commercial or any other association which might pose a conflict of interest.

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CHAPTER 2

PUBLICATION II

PREDICTORS OF *TRYPANOSOMA LEWISI* IN *RATTUS NORVEGICUS* FROM DURBAN, SOUTH AFRICA

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PREDICTORS OF *TRYPANOSOMA LEWISI* IN *RATTUS NORVEGICUS* FROM DURBAN, SOUTH AFRICA

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ABSTRACT: This study investigated associations between *Trypanosoma lewisi* and *Xenopsylla cheopis*, a common cyclical vector of *T. lewisi*; *Polyplax spinulosa*, a reported mechanical vector; and *Laelaps echidnina* and *Laelaps lamborni*, 2 rodent mites of *Rattus norvegicus* in Durban, South Africa. In total, 379 *R. norvegicus* individuals were live-trapped at 48 sites in 4 locality types around Durban during a 1-yr period. Rats were euthanized, cardiac blood was taken to check for hemoparasites, and ectoparasites were removed for identification. Parasite species richness was higher in pups (2.11) and juveniles (1.02) than adults (0.87). Most rats in the study harbored 1 or 2 of the 5 parasites surveyed. Rats with trypanosomes and fleas were more prevalent in the city center and harbor, where juveniles were most affected. Rats with lice were more prevalent in informal settlements and urban/peri-urban areas, where pups had the highest infestations. There was a significant positive association between rats with fleas and trypanosomes and a negative association between rats with lice and trypanosomes. Location and rat age were significant predictors of *T. lewisi*, *X. cheopis*, and *P. spinulosa*. Mites showed no strong association with trypanosomes. Ectoparasite associations are possibly habitat and life-cycle related. We conclude that Durban's city center, which offers rats harborage, an unsanitary environment, and availability of food, is a high-transmission area for fleas and trypanosomes, and consequently a potential public health risk.

Rodents are reservoirs of a number of zoonoses (e.g., plague, murine typhus, leptospirosis, angiostrongyliasis, and toxoplasmosis) that can be transmitted to humans directly or indirectly via vectors such as ectoparasites (Begon, 2003; Meerburg et al., 2009). Common ectoparasites of rats belong to the following orders: Siphonaptera (fleas), Phthiraptera (lice), Mesostigmata (mites), and Acarina (ticks) (Paramasvaran et al., 2009). Ixodid ticks, mites, and fleas are temporary obligate parasites, whereas lice (both adults and nymphs) are permanent parasites (Askew, 1971; Service, 1980). Life stages of ticks, some mites, and lice, as well as adult fleas, are hematophagous (Noble and Noble, 1976). Although these arthropods can transmit bacterial and viral diseases, only fleas are vectors of the helminths *Hymenolepis diminuta* and *Hymenolepis nana*, and the protozoan *Trypanosoma lewisi* (Beaver et al., 1994). Lice (*Polyplax spinulosa*) have been implicated (in laboratory studies) as mechanical vectors of rodent trypanosomiasis (Mac Neal, 1904; Nuttall, 1908); however, other experiments with lice, ticks, mites, and bugs did not produce infection with *T. lewisi* (Strickland and Swellengrebel, 1910).

Trypanosoma lewisi is a blood flagellate of the sub-genus, *Herpetosoma* (stercoraria section) that parasitizes *Rattus* spp. Fleas are cyclical vectors of *T. lewisi*, and the most common species are *Xenopsylla cheopis* in tropical and sub-tropical areas and *Nosopsyllus fasciatus* in temperate regions (Hoare, 1972). Transmission to the mammalian host is via ingestion of the vector's moist feces or the vector itself (Minchin and Thomson, 1915). In 1845, Chaussat found trypanosomes in the blood of *Rattus rattus*, and it was Lewis' work in 1878 on *T. lewisi* in wild rats in India that highlighted trypanosomiasis in mammals (Laveran and Mesnil, 1907). Plummer (1913) reported *T. lewisi* as a highly host-specific parasite exclusively found in *Rattus* spp. However, this has since been disproved, as the trypanosome has been isolated and genetically confirmed from humans (Howie et al., 2006; Shah et al., 2011; Verma et al., 2011), captive monkeys (da Silva et al., 2010), and *Bandicota* rodent species in Thailand (Jittapalapong et al., 2008). More recently, in Southeast Asia, mice, shrews, and rats of the genera *Bandicota*, *Berylmys*, *Niviventer*, *Moxomys*, and *Rattus* were found to be infected with *T. lewisi* and *Trypanosoma evansi* (Pumhom et al., 2015).

Prevalence of *T. lewisi* in *Rattus* spp. on most continents ranges from 1.5% (Siti Shafiyyah et al., 2012) to 82.3% (Laha et al., 1997). Data on *T. lewisi* prevalence on the African continent are relatively scant; however, a prevalence of 75.7% was recorded in *R. rattus* in Nigeria (Akinboade et al., 1981). Few studies have statistically examined the influence of extrinsic (location, season) or intrinsic (age and gender) factors, or the prevalence of ectoparasite infestations, on *T. lewisi* prevalence. One example found no significant effect of rodent habitat (described as rice fields, upland fields, secondary forests, and domestic habitats) on *T. lewisi* infection in 3 of the 12 rodent species trapped, namely, *Rattus exulans*, *Bandicota savilei*, and *Bandicota indica*, in Thailand (Jittapalapong et al., 2008).

There are also few studies on other *Trypanosoma* species of indigenous rodents and their corresponding flea vectors. One comprehensive study on the interactive effects of extrinsic and intrinsic factors on hemoparasite and ectoparasite infections in indigenous spiny mice (*Acomys dimidiatus*) in Egypt found an overall prevalence of 17.9% for fleas (*Parapulex chephrensis* and *Xenopsylla dipodilli*), 17.5% for trypanosomes (*Trypanosoma acomys*), and 32.1% for 2 *Polyplax* species of lice (Bajer et al., 2006). Fleas and trypanosomes were aggregated in 2 of the 4 wadis (rivers or valleys), and abundance of both these parasites on

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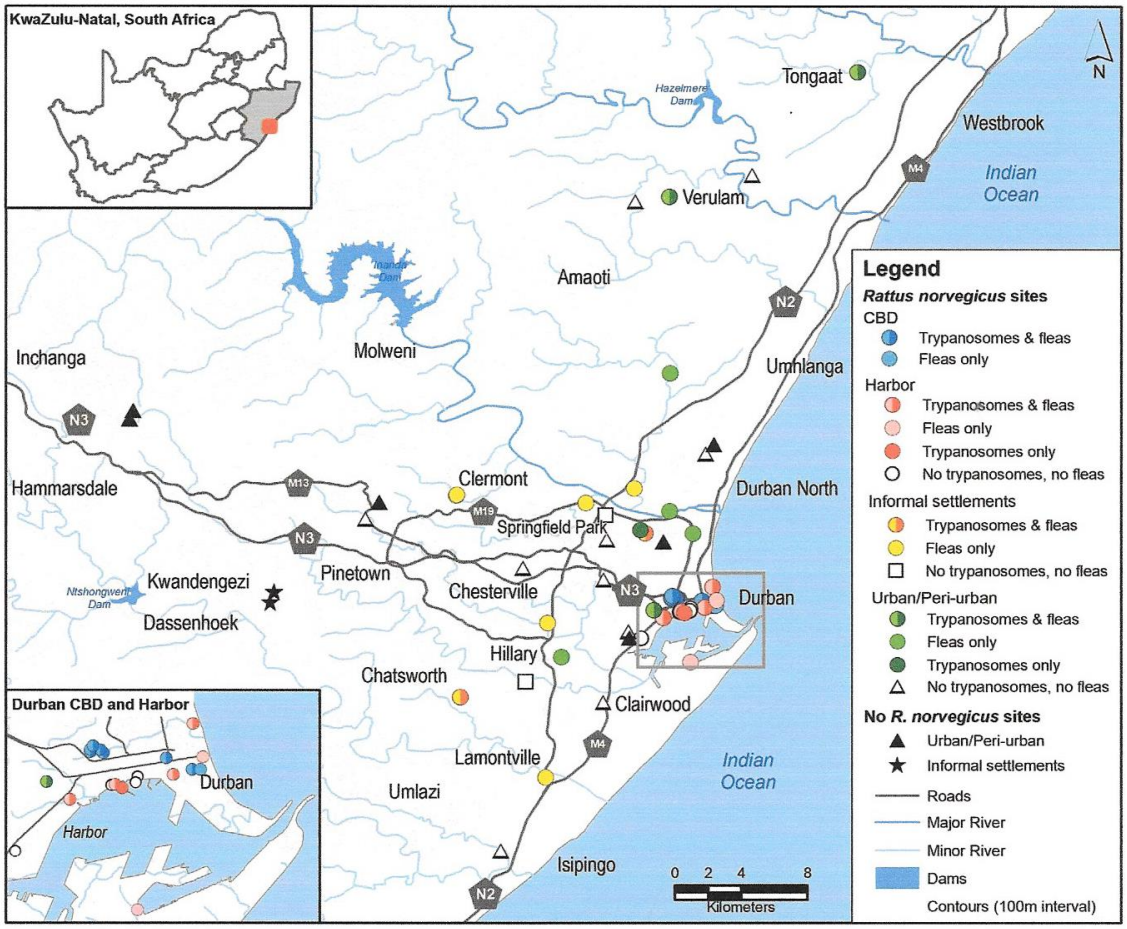

FIGURE 1. Map of eThekwini municipality (Durban) showing the 56 trapping sites, 48 where *Rattus norvegicus* were trapped, and 8 where only by-catches of *R. rattus* and *Mastomys natalensis* were trapped. Inset in top-left corner shows Durban (black or red square) in relation to KwaZulu-Natal province (gray), within South Africa. Inset at bottom-left corner is an expanded view of the harbor and central business district (CBD), to show the separate sites. Color version available online.

spiny mice was significantly affected by site (Bajer et al., 2006). However, studies on factors driving *T. lewisi* infection in *Rattus* spp. within cities in developing countries are lacking.

Building on our comprehensive database of endoparasites of wild rats sampled in 2009 in the port city of Durban, South Africa (Archer et al., 2017), we report here on *T. lewisi*, one of its common cyclical vectors, *X. cheopis*, and its potential arthropod mechanical vectors, including *P. spinulosa*, *Laelaps lamborni*, and *Laelaps echidnina* in *Rattus norvegicus*, the most common rodent captured in Durban. The aim of our study was to investigate the effects of abiotic (location and season) and biotic (rat age, gender, and abundance of 4 ectoparasites) factors on the prevalence and intensity of *T. lewisi* infection in *R. norvegicus*. We predicted that the prevalence and abundance of *T. lewisi* and the arthropod vector(s) in the rats would be interdependent.

MATERIALS AND METHODS

Study locations and seasons

The study area, located in the eThekwini metropolitan area commonly known as Durban, South Africa, was divided into 4 locations: central business district (CBD), harbor (HBR), informal settlements/slums (IS), and urban/peri-urban (U/PU) (Fig. 1). The CBD and HBR form a typical metropolis of high-rise buildings that is densely populated, with an abundant food trade and consequent litter. The IS consist of densely populated shacks and low-cost houses, and U/PU includes formal residences, food shops, markets, a wildlife facility, parks, and waste-water treatment stations. The study spanned 1 yr (2009) and included both wet and dry seasons, with mean temperature/rainfall of 21.8 C/121 mm and 19.1 C/30.9 mm, respectively. For 75% of 2009, humidity was ≥70%. Climate data were provided by weather-

station number 461, Mount Edgecombe, 29°42′0″S, 31°2′0″E, at 96 m above sea level (South African Weather Services).

Sampling of rodents

The Animal Ethics Committee of the University of KwaZulu-Natal (Ref. 031/09/Animal) approved this study, providing that euthanasia was performed according to international ethical guidelines (Gannon et al., 2007). Custom-made Monarch-like live-traps were baited with food scraps, including meat, cereal-based foods, and vegetables, and were set up and collected by eThekwini Health Department's Vector Control Division. This was for safety and logistic reasons, because traps needed to be placed where they would not be stolen, and high crime rates make entering settlements dangerous for researchers. Trapping of rodents was carried out at 56 sites in total; however, *R. norvegicus* individuals were trapped at only 48 of these, with by-catches of *R. rattus* and *Mastomys natalensis* captured at the other 8 sites (Fig. 1). The Durban Natural Science Museum, as part of their own research, identified the rodents from our study using detailed morphometric measurements and genetic confirmation where necessary.

Euthanasia was performed using chloroform, followed by cardiac puncture to obtain blood samples. To prevent carry-over of ectoparasites from one rat to the next, a different brush and comb were used for each animal processed on one day. Afterwards, all implements were thoroughly washed and dried before the next day's cohort of rats was processed.

Each rat was thoroughly dry-combed and brushed to dislodge and remove ectoparasites from the fur. This was done over a tray lined with a white paper towel to easily see ectoparasites. The surface of each body region was systematically and meticulously inspected so as to avoid missing any ectoparasites, and those still clinging to the skin or fur were removed with forceps or by further brushing. Skin lesions were excised using a scalpel. All containers and surfaces where the euthanized rat had been placed were also checked for ectoparasites that may have left the dying host. Brushings from the paper towel were inspected using a stereomicroscope. All ectoparasites collected were placed directly into appropriately labelled 1.5-ml Eppendorf tubes containing 70% ethanol for preservation. Fleas, mites, and lice were separated and stored in appropriately labelled Eppendorf tubes and then prepared and mounted on microscope slides using a modification of the Canada balsam technique (Palma, 1978). This involved cleaning specimens in 10% sodium hydroxide without damaging the exoskeletons, gradual dehydration in increasing strengths of ethanol for 1 hr per concentration, clearing in clove oil, mounting in Canada balsam, and allowing time to dry and set. Sample slides of each parasite were sent to various South African experts for confirmation of identifications.

Thin and thick blood smears were made immediately from blood drawn by cardiac puncture, and serum was harvested and frozen for use by the National Institute of Communicable Diseases (NICD) in Johannesburg, South Africa. Thin blood smears were fixed in 100% methanol, and thick smears were air-dried for 1 hr. The former were stained with May-Grünewald/Giemsa, and the latter were stained with Giemsa (Lynch et al., 1969); samples were then allowed to dry, stored in wooden slide boxes, and examined later using a compound light microscope and 100× oil-immersion objective.

Rodents were weighed, gender and breeding status were recorded, and selected body parameters were measured (Archer et al., 2017), and this information was used to age the rodents. The rats were then dissected; all internal organs were removed, and parasites were harvested for further studies. Feces were collected from the rectum and preserved in 10% formal saline for further examination.

Aging of rodents

Given that the patency period of most of the helminth fauna of rodents is 4–6 wk, we decided to use the age tables of Hirata and Nass (1974) to differentiate among un-weaned pups, weaned juveniles, and fully mature rats. Pups included those <5 wk (females <70 g, males <77 g); juveniles were approximately 5–10 wk, probably weaned and some sexually mature (females <142 g, males <222 g); and adults were all sexually mature and >10 wk old (females >113 g, males >164 g). There was an overlap in mass ranges between each week of age. Thus, at the age of 5 wk, where we separated pups from juveniles, and at 10–11 wk (separation of juveniles from adults), total body length and sexual and breeding status were also used to allocate them to age groups.

Morphological identification of parasites

Trypanosoma lewisi was morphologically identified according to Hoare (1972). Thick and thin smears were examined, and if positive, the degree of infection was recorded based on a scale of 1–4: 1 = light, 2 = moderate, 3 = heavy, and 4 = severe. (However, to simplify reporting, this infection intensity of *T. lewisi* will be termed 'abundance,' as for the ectoparasites.) All thin and thick smears were meticulously examined to ensure no light *T. lewisi* infections were missed and to check for other hemoparasites.

Mounted ectoparasites were identified to species level, counted, and differentiated by gender. Fleas, mites, and lice were identified according to Haeselbarth et al. (1966), Matthee and Ueckermann (2009), and Ledger (1980), respectively. The prevalences of other ectoparasites were ≤6.9% and were thus excluded from this study, because they would not have had any statistical significance.

Statistical analyses

Statistical tests were run in IBM SPSS Statistics for Windows (version 24.0; IBM Corp., Armonk, New York) and R (v. 3.4.2). First, differences in the number of rats caught between locations, seasons, and in age classes and genders were assessed using 2-way ANOVAs and Tukey's post-hoc tests. To assess the associations between parasites and trapping locations, cross-tabulations were run on the prevalence of *T. lewisi* and each of the 4 ectoparasites among the 4 locations, and a Pearson's chi-square test was included to test the null hypothesis that the parasite infections were not dependent on location.

Binary logistic regression (BLR) was used to test the prevalence data. BLR1 consisted of 5 models (a–e) to examine the abiotic (location and season) and biotic (rodent age and gender) factors as predictors of the prevalence of each of the following parasites: (a) *T. lewisi*, (b) *X. cheopis*, (c) *P. spinulosa*, (d) *L. lamborni*, and (e) *L. echidnina*. BLR2 consisted of 2 models, (a) a full model that tested all the abiotic and biotic (including the 4 ectoparasites)

predictor variables on the prevalence of *T. lewisi* as the dependent variable, and (b) the best sub-model that was identified based on the Akaike information criterion (AIC) using the package glmulti (Calcagno, 2013) in R.

Due to the large number of absolute zeros in our database, the count outcome variables were over-dispersed, and so negative binomial regression (NBR) was used to test the abundance data. Instead of using the default dispersion parameter of 1 in SPSS, we chose the estimate option, which allows SPSS to estimate this value. AIC values indicated that the latter option produced better models than the former. NBR1(a) tested the effects of location, season, and rat age and gender, and the abundance of each of the 4 ectoparasites on the abundance of *T. lewisi*, and NBR1(b) included only the significant variables from NBR1(a). NBR2 examined the effects of location, season, and rat age and gender on parasite species richness.

RESULTS

Rodents trapped per location and season

The number of rodents trapped at each location (CBD = 101, HBR = 93, IS = 88, U/PU = 97) and season (wet = 137, dry = 242) were significantly different (2-way ANOVA: $F_{7,378} = 22.136$; $P < 0.001$). A post-hoc Tukey's test showed that significantly more *R. norvegicus* individuals were trapped at CBD than IS ($P < 0.001$) and U/PU ($P = 0.001$), and significantly more rats were trapped during the dry months (n = 242 in 7 mo) than wet months (n = 137 in 5 mo) ($P < 0.001$). One rat (pup) was excluded from the analyses because we were unable to draw blood from it.

Examination of the data

The cross-tabulations showed that 50.6% of *T. lewisi*–positive rats were from CBD, 20% were from HBR, 20% were from U/PU, and 9.4% were from IS. The chi-square test showed that there were significant differences in *T. lewisi* prevalence among locations ($\chi^2 = 35.515$, df = 3, $P < 0.001$). We found that 51.3% of the rats infested with *X. cheopis* were from CBD, 21.9% were from HBR, 16.9% were from IS, and 10% were from U/PU. There were significant differences in the prevalence of fleas among locations ($\chi^2 = 94.791$, df = 3, $P < 0.001$). The percentages of rats with trypanosomiasis and fleas were both predominant in the CBD.

A different picture emerged with the prevalence of *P. spinulosa*. There were significant differences in lice prevalence among locations (chi-square test: $\chi^2 = 42.995$, df = 3, $P < 0.001$), and most were found from IS (46.3%), with much lower prevalence at HBR (25.6%), U/PU (23.2%), and CBD (4.9%).

There were significant differences among locations for *L. lamborni* and *L. echidnina* (chi-square test: $\chi^2 = 25.167$, df = 3, $P < 0.001$ and $\chi^2 = 14.76$, df = 3, $P = 0.002$, respectively). The prevalence of *L. lamborni* was 29.4% at CBD, 26.4% at IS, 23.4% at HBR, and 20.8% at U/PU, whereas *L. echidnina* prevalence was 32.2% at HBR, 32.2% at IS, 22.2% at CBD, and 13.3% at U/PU.

Chi-square results confirmed a strong association between the parasites and location and supported our decision not to run location as a random factor, but rather as an independent, categorical variable in the BLRs.

Prevalence and mean intensity/abundance of parasites

Table I shows the prevalence for each parasite, mean intensity of *T. lewisi*, mean abundance of each ectoparasite, and mean species richness, overall, and for each location, season, rat age, and rat gender. For both *T. lewisi* and its cyclical vector, *X. cheopis*, the highest prevalence had correspondingly higher mean abundance per location, season, age, and gender, except for fleas on pups and juveniles. This pattern was not found for the remaining ectoparasites (Table I).

An assessment of trypanosome- and flea-infected and -uninfected rats showed the following: 51.6% (n = 195/378) of rats had no fleas and no trypanosomes; 25.9% (n = 98/378) of rats had fleas but no trypanosomes; 15.6% of rats (n = 59/378) had both fleas and trypanosomes; and 6.9% (n = 26/378) of rats had trypanosomes but no fleas.

Statistical analyses of prevalence and abundance data

The 5 individual BLR1 models that examined location, season, rat age, and rat gender as predictors for (a) *T. lewisi*, (b) *X. cheopis*, (c) *P. spinulosa*, (d) *L. lamborni*, and (e) *L. echidnina* were all significant. The Hosmer-Lemeshow test showed that the models for *T. lewisi*, *L. lamborni* and *L. echidnina* were a good fit ($P > 0.05$), while that for *X. cheopis* was acceptable, and that for *P. spinulosa* was not a good fit (Tables II, III).

Location had a significant effect on the prevalence of all parasites, except *L. echidnina* ($P = 0.072$; Table II). Odds of CBD rats having trypanosomiasis as opposed to HBR rats were 3.4 (1/0.294), as opposed to IS rats were 6.5 (1/0.155) and as opposed to U/PU rats were 3.6 (1/0.281). Odds of CBD rats harboring fleas were 7.9 times that of HBR rats, 11.9 times that of IS rats, and 23.3 times that of U/PU rats. Odds of HBR rats harboring lice were 8.3 times that of CBD rats, and odds of U/PU rats having lice were 8.9 times that of CBD rats. The prevalence of lice was highest at IS (46.3%), and the odds of these rats harboring lice were 20.8 times that of CBD rats, 2.5 times that of HBR rats, and 2.3 times that of U/PU rats. Odds of rats with *L. lamborni* mites were greater at CBD and IS than HBR and U/PU. Age was significant for *T. lewisi*, *X. cheopis*, and *L. echidnina* models, where odds of having trypanosomiasis, fleas, and *L. echidnina* mites were between 2 to 3 times greater for both pups and juveniles than for adults. Odds of *P. spinulosa* and *L. echidnina* on rats in the wet rather than the dry season were 2.2 and 1.8 times, respectively. These were the only parasites significantly affected by season, and none of the parasites displayed any prevalence associations with gender (Table II).

The best sub-model, BLR2(b), had a lower AIC value than the full model BLR2(a), and in both models, only rat age, *X. cheopis*, and *P. spinulosa* were significant predictors. The goodness of fit statistics for BLR2(b) were: Hosmer-Lemeshow test $\chi^2(8) = 4.852$, $P = 0.773$, AIC = 334.9; cases correctly predicted = 77.5%. The odds of CBD rats having trypanosomes compared to HBR and IS rats were 2.1 times ($P = 0.057$) and 2.4 times ($P = 0.075$), respectively (Table IV).

NBR models showed that *P. spinulosa* abundance had no effect on *T. lewisi* abundance, and there were no significant associations between the abundance of mites and trypanosomiasis. However, location was a significant predictor of trypanosome abundance. The significant results for NBR1(a), NBR1(b), and NBR2 are presented in Table V. Coefficients (B) for each of the predictor

TABLE I. Prevalence (Prev.) and mean infection intensity/abundance (Mean) data for *Trypanosoma lewisi*, *Xenopsylla cheopis*, *Polyplax spinulosa*, *Laelaps lamborni*, and *Laelaps echidnina*; and mean species richness at 4 locations: central business district (CBD), harbor (HBR), informal settlements (IS), and urban/peri-urban (U/PU). These data are also given for season, rodent age, and rodent gender.

	T. lewisi		*X. cheopis*		*P. spinulosa*		*L. lamborni*		*L. echidnina*		
Variables	Prev. (%)	Mean ± SD	Prev. (%)	Mean ± SD	Prev. (%)	Mean ± SD	Prev. (%)	Mean ± SD	Prev. (%)	Mean ± SD	Parasite species richness, mean ± SD
Overall	22.5	0.60 ± 1.25	42.2	3.26 ± 7.83	21.6	1.83 ± 6.99	79.9	13.25 ± 21.22	23.7	0.69 ± 2.07	1.89 ± 1.05
Location											
CBD	43.0	1.20 ± 1.59	81.2	7.19 ± 10.68	4.0	0.06 ± 0.34	88.1	19.15 ± 29.13	19.8	0.32 ± 0.71	2.35 ± 0.85
HBR	18.3	0.56 ± 1.28	37.6	4.06 ± 9.51	22.6	0.94 ± 2.47	76.3	12.08 ± 17.59	31.2	1.49 ± 3.61	1.86 ± 1.01
IS	9.1	0.24 ± 0.83	30.7	1.05 ± 2.27	43.2	4.22 ± 8.31	90.9	16.28 ± 20.76	33.0	0.81 ± 1.65	2.07 ± 1.16
U/PU	17.5	0.36 ± 0.89	16.5	0.41 ± 1.23	19.6	2.35 ± 10.68	64.9	5.47 ± 9.68	12.4	0.20 ± 0.62	1.29 ± 0.88
Season											
Wet	16.8	0.44 ± 1.08	42.3	3.37 ± 8.41	32.1	3.58 ± 10.31	83.9	11.99 ± 16.80	33.6	1.24 ± 3.01	2.07 ± 1.09
Dry	25.7	0.70 ± 1.33	42.1	3.20 ± 7.50	15.7	0.83 ± 3.72	77.7	13.96 ± 23.35	18.2	0.38 ± 1.16	1.79 ± 1.02
Age											
Pups	18.1	0.53 ± 1.26	50.0	3.42 ± 6.48	31.1	2.90 ± 7.44	81.1	13.83 ± 17.50	31.1	0.70 ± 1.56	2.11 ± 1.17
Juveniles	30.9	0.88 ± 1.45	47.1	4.70 ± 9.82	16.9	1.90 ± 9.17	81.6	11.85 ± 21.82	25.7	1.05 ± 3.02	2.01 ± 1.02
Adults	14.6	0.30 ± 0.83	28.5	1.73 ± 6.45	20.3	1.02 ± 2.93	75.6	11.98 ± 21.09	16.3	0.31 ± 0.84	1.54 ± 0.87
Gender											
Females	22.5	0.61 ± 1.25	41.0	3.15 ± 7.92	20.2	1.26 ± 3.69	78.2	10.15 ± 17.29	25.0	0.68 ± 1.98	1.86 ± 1.04
Males	20.9	0.55 ± 1.22	42.4	3.51 ± 8.02	24.3	2.56 ± 9.45	80.8	14.92 ± 22.99	23.2	0.72 ± 2.23	1.90 ± 1.06

variables (including dummy variables) were all positive, except for *P. spinulosa*. Results for predictors with dummy variables, e.g., location, are interpreted as follows. NBR1(a): Compared to HBR, the expected log count of CBD increased by 1.136 (B-value); compared to adults, the expected log count of juveniles increased by 1.025; and for the continuous scale result for the covariate *X. cheopis*, for each 1-unit increase in *X. cheopis* mean abundance, the expected log count of the abundance of *T. lewisi* increased by 0.045. NBR2 produced a negative B-value for *P. spinulosa*; i.e., for each 1-unit increase in the abundance of *P. spinulosa*, the expected log count of *T. lewisi* abundance decreased by 0.079.

The incident rate ratios (IRRs) for the same examples showed that the incident rate of a higher abundance of *T. lewisi* at CBD was 3.1 times that for the reference group (HBR), holding all other variables constant; the incident rate of a higher abundance of *T. lewisi* for juveniles was 2.8 times that for the reference group (adults). Each positive variable's contribution to the model can be read in the same way, and for *P. spinulosa*, the IRR is interpreted as follows: The percent change in abundance of *T. lewisi* is a 0.9% decrease for every 1-unit increase in *P. spinulosa* abundance. Rats at CBD, HBR, and IS had higher incidence rates of the greatest number of parasites (parasite species richness), as did pups and juveniles compared to adult rats. All the significant parameters are shown in Table V and can be interpreted as described above.

DISCUSSION

We found that in the port city of Durban, *T. lewisi* prevalence in *R. norvegicus* had a significant positive association with *X. cheopis*, a negative association with *P. spinulosa*, as well as a significant association with rat age, with younger rats more likely to be infected with *T. lewisi* (and *X. cheopis*). Further, *X. cheopis* abundance, rat age, and location were significant predictors of trypanosome abundance. A similar situation was reported in Egyptian spiny mice (*A. dimidiatus*), where trypanosome infections were 3–4 times higher in flea-infested than non-infested mice. Prevalence of fleas peaked in mice from age class 2 (which corresponds to our juveniles) and then decreased in the oldest age group (Bajer et al., 2006). To the best of our knowledge, there are no comprehensive studies on *T. lewisi* and its flea vectors in *Rattus* spp. across varying habitat types within large cities. However, there are studies that incorporated season, rodent age, and rodent gender. Perhaps the most comparative study in this regard was in Brazil, where *R. norvegicus* individuals were trapped at dumps in the Belo Horizonte municipality (Linardi and Botelho, 2002). Consistent with our results, overall *T. lewisi* prevalence was 21.7% (n = 93/429; cf. 22.5% in Durban), and the highest prevalence of *T. lewisi* coincided with the greatest *X. cheopis* infestations. Further, there were significantly more infected young (29.3%) and immature (27.1%) rats than adults (8.8%), similar to our study (pups 18.1%, juveniles 30.9%, and adults 14.6%). Significantly more rats were infected in the cooler, dry months than the rainy, wet months, and significantly more males than females were infected (Linardi and Botelho, 2002), whereas we found no significant differences in seasons and genders. Conversely, *T. lewisi* prevalence in *R. norvegicus* in Memphis, Tennessee, was higher in winter than summer, yet, similar to Durban rats, prevalence was significantly higher in rats <200 g, with very few rats >300 g infected (Eyles, 1952). A recent Egyptian study on associations between fleas and trypanosomes in *Rattus* spp. on farms and houses in rural areas reported an overall prevalence of 24.7%, no significant difference in infection between genders, and also a significant inverse correlation between parasite load and host age (Danesh and Mikhail, 2016). This common trend, where *T. lewisi* declines as the rat ages, is due to the development of immunity when the host

TABLE II. Significant results of binary logistic regressions (BLRs) for each parasite as dependent variable (BLR1a–BLR1e), with location, season, and rat age as predictor variables. Odds ratios (OR), 95% confidence intervals (CI), and *P*-values are given. Abbreviations: central business district (CBD), harbor (HBR), informal settlements (IS), and urban/peri-urban (U/PU), reference category (ref.).

			95% CI for OR		
BLR; Parasite	Significant variable	OR	Lower	Upper	*P* value
1(a) *Trypanosoma lewisi*	Location CBD (ref.)	1			
	HBR	0.294	0.143	0.602	0.001
	IS	0.155	0.066	0.367	<0.001
	U/PU	0.281	0.140	0.564	<0.001
	Rat age Juveniles (ref.)	1			
	Adults	0.352	0.178	0.694	0.003
1(b) *Xenopsylla cheopis*	Location CBD (ref.)	1			
	HBR	0.126	0.062	0.257	<0.001
	IS	0.084	0.040	0.177	<0.001
	U/PU	0.043	0.020	0.094	<0.001
	HBR (ref.)	1			
	U/PU	0.341	0.166	0.702	0.004
	Rat age Adults (ref.)	1			
	Pups	2.774	1.410	5.458	0.003
	Juveniles	2.625	1.398	4.929	0.003
1(c) *Polyplax spinulosa*	Location CBD (ref.)	1			
	HBR	8.293	2.326	29.564	0.001
	IS	20.749	6.010	71.637	<0.001
	U/PU	8.914	2.493	8.914	0.001
	IS (ref.)	1			
	HBR	0.400	0.199	0.803	0.010
	U/PU	0.430	0.212	0.872	0.019
	Season Wet (ref.)	1			
	Dry	0.456	0.257	0.809	0.007
1(d) *Laelaps lamborni*	Location CBD (ref.)	1			
	HBR	0.398	0.176	0.898	0.026
	U/PU	0.265	0.123	0.574	0.001
	IS (ref.)	1			
	HBR	0.285	0.116	0.699	0.006
	U/PU	0.190	0.079	0.457	<0.001
1(e) *Laelaps echidnina*	Season Wet (ref.)	1			
	Dry	0.556	0.331	0.934	0.027
	Rat age Adults (ref.)	1			
	Pups	2.260	1.143	4.470	0.019
	Juveniles	1.921	1.004	3.676	0.049

TABLE III. Goodness-of-fit statistics for binary logistic regressions (BLR1a–BLR1e) in Table II. Table also shows Akaike information criterion (AIC).

		Hosmer-Lemeshow test			
BLR1; Parasite	AIC	χ^2 (8)	*P*	Cases correctly classified (%)	*P* value of model
(a) *Trypanosoma lewisi*	353.07	6.204	0.624	78.6	<0.001
(b) *Xenopsylla cheopis*	400.59	13.468	0.097	74.5	<0.001
(c) *Polyplax spinulosa*	340.43	27.382	0.001	83.0	<0.001
(d) *Laelaps lamborni*	358.64	2.335	0.969	79.2	<0.001
(e) *Laelaps echidnina*	390.97	8.709	0.367	75.9	<0.001

TABLE IV. Binary logistic regressions BLR2(b), with location, rat age, *Xenopsylla cheopis*, and *Polyplax spinulosa* as predictors of *Trypanosoma lewisi* in rats. Abbreviations: central business district (CBD), harbor (HBR), informal settlements (IS), odds ratio (OR), confidence intervals (CI), reference category (ref.).

			95% CI for OR		
BLR2(b)	Significant variable	OR	Lower	Upper	*P* value
T. lewisi	Location CBD (ref.)	1			
	HBR	0.480	0.225	1.023	0.057
	IS	0.421	0.162	1.092	0.075
	Rat Age Juveniles (ref.)	1			
	Pups	0.488	0.244	0.976	0.043
	Adults	0.446	0.228	0.871	0.018
	Absence of fleas (ref.)	1			
	Xenopsylla cheopis	3.022	1.566	5.830	0.001
	Presence of lice (ref.)	1			
	Polyplax spinulosa	4.719	1.580	14.096	0.005

produces IgM antibodies to surface antigens on the trypanosomes, resulting in lysis of the flagellates in the blood of the mature host, with consequent immunity to challenge infections (Linardi and Botelho, 2002).

This study is the first to investigate whether lice or mites act as mechanical vectors in wild *Rattus* spp. We found no statistical associations between mites and the prevalence or abundance of *T. lewisi* in rats. Conversely, there was a significant negative association between rats with trypanosomes and lice (the odds of louse-free rats having *T. lewisi* were 4.7 times that of louse-infected rats), and this association was strongly related to location, as the odds of rats at IS with this ectoparasite were 20.8 times that of rats at CBD (where fleas and trypanosomes were most prevalent), and it was also strongly related to age, with lice more abundant on pups.

Taken together, the ecologies of *R. norvegicus* and its ectoparasites may explain the findings of our study. *Rattus norvegicus* is known to be synanthropic (Tufty, 1966) and more common in areas where poor communities reside, buildings are poorly constructed or in disrepair, and the environment is unsanitary (Donaldson, 1925; Jassat et al., 2013). In support, large numbers of rats were frequently trapped where these conditions existed, particularly at the CBD (Archer et al., 2017). *Xenopsylla cheopis* is also more common in commercial than residential areas, especially where foods like cereals are handled or stored (Cole and Koepke, 1946; Pollitzer, 1954; this study). Only adult fleas feed on blood, and they leave the host to breed, which they often do in rats' burrows (Briscoe, 1956), and in suitable feeding sites like cereal and grain stores, where cereal debris provides an ideal substrate for the development of their young (Pollitzer, 1954). This could explain why juvenile rats, which are likely the most active age group, may be at higher risk to become infested with fleas while foraging. Further, *T. lewisi* infection is often relatively common in rodents inhabiting highly built-up human settlements such as the CBD (Pumhom et al., 2013; this study). Given that the home ranges of synanthropic rats are relatively small (Davis et al., 1948), and trapping sites at the CBD and HBR were more closely set together than trapping sites at U/PU and IS (Fig. 1), CBD rats were probably more likely to interact and infect other CBD and HBR rats with *T. lewisi* and *X. cheopis* than rats at U/PU and IS. By contrast, lice are obligatory ectoparasites that live, breed, and feed on their hosts, and they spread by direct contact (Ledger, 1980). We propose that this probably explains why pups, which have close contact with their own mothers, as well as other nursing females in the nest, had the highest abundance of lice in this study. However, the inverse relationship between lice and *T. lewisi* has not been previously reported, and it remains a question that requires further research.

We concede that the standard approach to identify parasite and vector inter-relationships, would be to dissect each arthropod and

TABLE V. Significant results from negative binomial regression models (NBRs), with *Trypanosoma lewisi* abundance (NBR1) and parasite species richness (NBR2) as dependent variables, and the following as predictors: NBR1(a)—location, season, rat age, and rat gender, abundance of 4 ectoparasites; NBR1(b)—location, rat age, abundance of *Xenopsylla cheopis*; and NBR2—location, season, rat age, and rat gender. Abbreviations: Akaike information criterion (AIC); the coefficient estimate of the model (B); incidence rate ratio (IRR); lower to upper confidence intervals (CI [l–u]); juveniles (Juv.); central business district (CBD); harbor (HBR); informal settlements (IS); urban/peri-urban areas (U/PU). Significant variables: first dummy variable is reference category, e.g., within location, CBD is compared with HBR, and written 'Location HBR (ref.)/CBD.'

Model no. and parasite (AIC)	Significant variables	B	IRR	95% CI for IRR (l–u)	*P* value
1(a). *T. lewisi* (666.20)	Location HBR (ref.)/CBD	1.136	3.113	1.436–6.749	0.004
	Location IS (ref.)/CBD	1.323	3.755	1.634–8.627	0.002
	Location U/PU (ref.)/CBD	0.884	2.421	1.079–5.432	0.032
	Rat age Adults (ref.)/Juv.	1.025	2.788	1.398–5.556	0.004
	X. cheopis abundance	0.045	1.047	1.002–1.093	0.038
1(b). *T. lewisi* (662.95)	Location HBR (ref.)/CBD	1.031	2.804	1.353–5.812	0.006
	Location IS (ref.)/CBD	1.488	4.430	2.018–9.726	<0.001
	Location U/PU (ref.)/CBD	0.947	2.577	1.217–5.461	0.013
	Rat age Pups (ref.)/Juv.	0.661	1.937	0.993–3.778	0.052
	Rat age Adults (ref.)/Juv.	1.051	2.860	1.480–5.525	0.002
2. Parasite species	Location U/PU (ref.)/CBD	0.554	1.740	1.393–2.174	<0.001
richness (1083.22)	Location U/PU (ref.)/HBR	0.290	1.337	1.052–1.698	0.017
	Location U/PU (ref.)/IS	0.401	1.493	1.176–1.897	0.001
	Location HBR (ref.)/CBD	0.264	1.302	1.058–1.602	0.013
	Rat age Adults (ref.)/Pups.	0.248	1.281	1.043–1.573	0.018
	Rat age Adults (ref.)/Juv.	0.272	1.313	1.083–1.591	0.006

examine for the presence of *T. lewisi* life stages. Unfortunately, we could not investigate this because the ectoparasites were used for a separate study by Hope (2011). Moreover, the unambiguous identification of endo- and ectoparasites of rats has significant implications for parasitologists and vector biologists. Thus, identification based on both morphology and genetic markers should be an integral part of future studies investigating the pathogens and their vectors carried by urban rats.

To conclude, this study highlights the inter-dependence of the well-established trypanosome-flea vector cycle parasitizing *R. norvegicus*, particularly at the CBD of Durban. There are no reports of *T. lewisi* from humans in South Africa. However, there is 1 report from Gambia, North Africa (Howie et al., 2006), as well as a number of documented cases in India and Asia (Truc et al., 2013). It is important to note that the flea vector *X. cheopis* is also the vector of plague (Bitam et al., 2010) and intermediate host for the tapeworm *H. diminuta* (Smit, 1973). Durban and other densely populated African cities all experience the same problems of harborage, unsanitary environments, and plentiful rodent food, conditions that are highly favorable for synanthropic rats (Taylor et al., 2008). If vigorous steps are not taken to clean up the city and rid it of invasive *Rattus* spp., the result could pose a threat to public health (Jassat et al., 2013).

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CHAPTER 3

PUBLICATION III

DRIVERS OF GASTROINTESTINAL HELMINTH INFECTIONS IN *RATTUS NORVEGICUS*, DURBAN, SOUTH AFRICA

DRIVERS OF GASTROINTESTINAL HELMINTH INFECTIONS IN *RATTUS NORVEGICUS*, DURBAN, SOUTH AFRICA

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ABSTRACT

Gastrointestinal helminths of indigenous and synanthropic rodents are well documented. The interactions and effects of one helminth species on another have been examined mostly in laboratory settings in order to control primary and challenge infections and draw conclusions. However, in the wild, this is not possible, so we examined the interspecific relationships between helminths found in the gastrointestinal tract of *Rattus norvegicus* and attempt to explain our findings. In total, 379 *R. norvegicus* were live-trapped at 48 sites in four locality types around Durban during a one-year period. Rats were euthanased and the following nine gastrointestinal helminth species were recovered and identified: *Gongylonema neoplasticum* (25.3%), *Protospirura muricola* (3.2%), *Moniliformis moniliformis* (10.0%), *Hymenolepis diminuta* (18.2%), *H. nana* (0.8%), *Nippostrongylus brasiliensis* (82.1%), *Strongyloides ratti* and *S. venezuelensis*, grouped as *Strongyloides* spp (24.5%), *Heterakis spumosa* (28.0%) and *Syphacia muris* (2.6%). Univariate crosstabulations and multivariate regression analyses were used to analyse the data. Location and rat age were significant predictors of both prevalence and abundance of helminth species. Both arthropod-borne helminths and those directly transmitted were most prevalent in the city centre and harbour, with adults and juveniles mostly affected. Informal settlements (IS) had the lowest prevalence and abundance of helminth species compared with the central business district (CBD), harbour (HBR) and urban/peri-urban (UPU) areas. Parasite species richness was significantly higher in CBD than in UPU (incident rate ratio [IRR] 1.45; $P < 0.001$) and IS (IRR 1.83; $P < 0.001$); and in HBR than UPU (IRR 1.25; $P = 0.041$) and IS (IRR 1.57; $P < 0.001$). Parasite species richness was also higher in the wet rather than dry season (IRR 1.28; $P = 0.002$), in adults than juveniles (IRR 1.19; $P = 0.050$) in adults than pups (IRR 2.03; $P < 0.001$) and in juveniles than pups (IRR 1.71; $P < 0.001$). We used binary logistic regression together with the crosstabulation univariate analyses of prevalence and means data to determine which associations were not confounded by location. We conclude that Durban's CBD and harbour that provide rats with an unsanitary environment, harbourage in badly maintained buildings, easy access to food, and abundant arthropod vectors, are high-transmission areas for gastrointestinal helminths.

Key words: *Rattus norvegicus*, gastrointestinal helminths, location, helminth associations, Durban, South Africa

INTRODUCTION

Gastrointestinal helminths of indigenous and synanthropic rodents are well documented, although there is a paucity of studies that have examined various extrinsic (e.g. location, season, climate change, etc.) and intrinsic (e.g. age and gender) effects on their prevalence and/or abundance (Harkema, 1936; Behnke et al., 2004; Behnke et al., 2008a,b; Froeschke et al., 2010). Most studies on parasites of synanthropic rodents of the genus *Rattus*, simply reported on prevalence, with some giving basic species richness and age/gender related differences and others only examining location (Luttermoser, 1936; Calero et al., 1950; Stojcevic et al., 2004; Rafique et al., 2009; Chaisiri et al., 2010; Milazzo et al., 2010). Some reported on risks that helminths pose for human health (Paramasvaran, 2009; McGarry et al., 2013, Archer et al., 2017), yet others statistically tested for drivers of infection (Waugh et al., 2006; Mohd Zain et al., 2012).

The gastrointestinal helminths of synanthropic rodents, including *Rattus norvegicus* and *R. rattus*, are cosmopolitan. These include the acanthocephalan, *Moniliformis moniliformis*, the cestodes, *Hymenolepis diminuta* and *H. nana*, and a number of nematodes: the stomach spirurids, *Mastophorus muris*, *Protospirura muricola*, and *Gongylonema neoplasticum*; the small intestine strongylid, *Nippostrongylus brasiliensis*, and rhabditids, *Strongyloides ratti* and *S. venezuelensis*; the large intestine/caecum ascarid, *Heterakis spumosa* and oxyurid, *Syphacia muris*. However, some originated from indigenous rodents, e.g. *Protospirura muricola*, an African spirurid of murid rodents. *Rattus* spp., possibly together with the Madeiran cockroach intermediate host, *Leucophaea madera*, have spread this nematode across the globe (Smales, et al., 2009).

Some of these helminths are directly transmitted, either through ingesting eggs that have rapidly developed to the infective stage, e.g. *Syphacia muris*, whereby embryonated eggs laid on the perianal surface become infective within about 30 minutes of deposition (Stahl, 1961). Others, like *Nippostrongylus brasiliensis*, and *Strongyloides* spp., live in the mucosa of the small intestine and lay eggs that are voided in the faeces and subsequently mature in the environment to infective filariform larvae that infect new hosts via penetration of the skin (Yokogawa, 1922; Viney and Lok, 2007). *Heterakis spumosa*, lays eggs that are voided in the faeces and require time in optimal environmental conditions to mature to infective larvae (L_2-stage) before becoming infective for the rat host (Smith, 1953).

The helminths that are indirectly transmitted (via an arthropod intermediate host) are: *Mastophorus muris*, vectored by a number of different insect hosts e.g. cockroaches, flour beetles, dipterans and fleas (Smith and Kinsella, 2011); *Gongylonema neoplasticum*, vectored mainly by a variety of cockroaches and dung beetles (Sato et al., 2005); *Moniliformis moniliformis*, transmitted by the cockroach,

Periplaneta americana, and tenebrionid beetles e.g. *Blaps mucronata*, *Tenebrio molitor* and *Tribolium confusum* (Moore, 1946); *Hymenolepis diminuta* and *H. nana*, transmitted via ingestion of the same beetle intermediate hosts as *M. moniliformis*, as well as by fleas (Riley and Shannon, 1922). However, *H. nana* is also known to be directly transmitted (unusual for tapeworms). Here, the villi in the upper part of the small intestine become the location for development of a similar cysticercoid intermediate host stage normally found in flour beetles or fleas. Once the cysticercoid larvae emerge from the villi they attach to the mucosa slightly lower down the small intestine and mature to adulthood (Sadaf et al., 2013).

The pre-patent period of the common rat gastrointestinal tract (GIT) helminths documented to date are: *G. neoplasticum* ≥ 60 days (Sato et al., 2005); *P. muricola* > 60 days (Cram, 1926); *M. muris* ≥ 28 days (Smith and Kinsella, 2011); *M. moniliformis* 5 – 6 weeks (Moore, 1946); *H. diminuta* 2 – 3 weeks (Riley and Shannon, 1922); *H. nana* ± 14 days (Beaver et al., 1984); *N. brasiliensis* 6 - 9 days (Yokogawa, 1922); *Strongyloides* spp. ± 4 days (Abadie, 1963); *H. spumosa* ± 30 days (Smith, 1953); and *S. muris* 7 - 10 days (Stahl, 1961).

Here, we focus on the gastrointestinal helminths of *R. norvegicus*, the most common, widespread, synanthropic rodent in the Durban municipality, South Africa (Archer et al., 2017). We concede that it is extremely difficult to determine one helminth species' presence or absence with respect to other co-infecting helminths in the wild as opposed to in controlled, laboratory environments (Behnke et al., 2001). Rather, we attempted to examine interactions between environmental (abiotic) factors and rodent age and helminth life-cycles (biotic factors) to investigate associations between these parasites found in the gut ecosystem with the objective of identifying drivers or determinants of their presence and abundance in *R. norvegicus* from different habitats of the Durban metropolitan area.

MATERIALS AND METHODS

Study locations and seasons

This study was conducted in the eThekwini metropolitan area (city of Durban) and was divided into four locations: central business district (CBD), harbour (HBR), informal settlements/slums (IS) and urban/peri-urban areas (UPU) (Figure 1). The densely populated CBD and HBR areas consist of high-rise buildings with an abundant food trade and consequent litter. The IS consist of numerous shacks and low-cost houses that are densely populated, whereas the UPU includes formal residences, food shops, markets, a wildlife facility, parks and waste-water treatment stations. Durban does not have four distinct seasons, but rather a wet and a dry season, with mean temperature/rainfall of 21.8°C/121 mm and 19.1°C/30.9 mm respectively. The study period, 2009, had five wet and seven dry months. Climate data

were provided by weather-station number 461, Mount Edgecombe, 29°42'0"S, 31°2'0"E; 96 m above sea level (South African Weather Services).

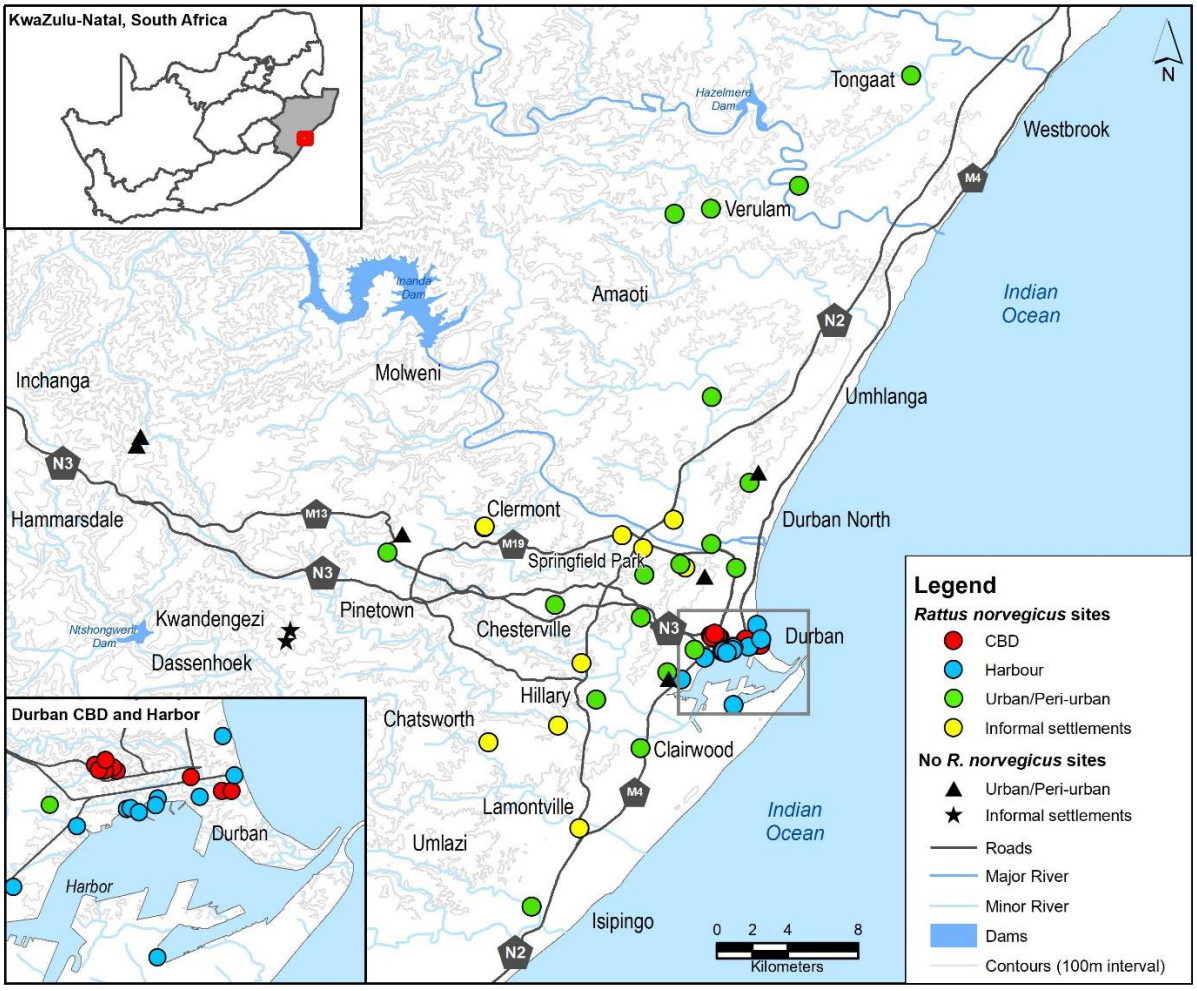

Figure 1: Map of Durban (eThekwini Municipality) showing sites where *Rattus norvegicus* were trapped.

Sampling of rodents

The Animal Ethics Committee of the University of KwaZulu-Natal approved the study (Ref. 031/09/Animal) providing that trained mammalogists euthanased the animals according to international ethical guidelines [Gannon and Sikes, 2007].

The Vector Control Division of eThekwini Health Department used custom-made traps (similar to Monarch Rat Traps) baited with vegetables, bread and meat scraps, to trap the rats. *Rattus norvegicus* were captured across 56 sites within the four locations (CBD n = 101, HBR n = 93, IS n = 88, and UPU n = 97 rats). Of these 242 rats were trapped in the dry season and 137 in the wet season. Other rodents, *R. rattus* (n = 10) and *Mastomys natalen*sis (n = 11) were 'by-catches', but due to the low numbers, they were excluded here.

After euthanasia of the rats with chloroform, they were weighed, sexed, breeding status noted, and various measurements taken (total length - body + tail; lengths of tail, right ear, and right hind foot – excluding and including claw). Each rodent was then dissected and the gastrointestinal tract (GIT) removed to recover all helminth parasites that were identified, sexed and counted. Faeces were either collected when passed by the rodents during euthanasia or from the rectum at dissection. Faeces were processed by the modified formal-ether concentration method of Allen and Ridley (1970) and microscopically examined for the presence of helminth eggs, larvae, and protozoans. (Note: All parasites, not only those included here, were collected, sampled by various means, and analysed [Hope, 2011; Archer et al., 2011; Archer et al., 2017; Archer et al., 2018]).

Aging of rodents

The average pre-patent period of many GIT helminths averages four weeks, so we decided to use the age tables of Hirata and Nass (1974) to age the rats rather than place them into weight classes. Thus, un-weaned pups included those ≤ 5 weeks (females < 70 g, males < 77g); juveniles were approximately 5 – 10 weeks, probably weaned and some sexually mature (females < 142 g, males < 222 g); and adults were all sexually mature and > 10 weeks old (females > 113 g, males > 164 g). There was a slight overlap in mass ranges between each week of age. Thus at the age of five weeks where we separated pups from juveniles, and 10 – 11 weeks (separation of juveniles from adults), total body length and sexual and breeding status were also used to allocate the rats to age groups.

Collection and identification of GIT parasites

At dissection of each rat, the GIT was divided into oesophagus, stomach, small intestine, caecum, and large intestine (including rectum). All helminth parasites were carefully removed to try and keep them intact, then preserved in 70% ethanol. They were later cleared in lacto-phenol, identified to species level, sexed and counted.

Helminths were identified according to relevant keys and descriptions: stomach nematodes according to Kruidenier and Peebles (1958), and Smales et al., (2009); small intestine nematodes according to Yokogawa (1920), and Little (1966); the small intestine acanthocephalan and cestodes according to Van Cleave (1923) and Hughes (1941) respectively; large intestine ascarid according to Robles et al. (2008), and the oxyurid found in the caecum, according to Hussey (1957).

Statistical Methods

All statistical analyses were performed using IBM SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, N.Y., USA). Crosstabulations (univariate Chi-square statistics) were used to compute prevalence of rats positive for each helminth species across location, season, age and gender, and between other helminth species. Pearson's Chi-square test was included to test the null hypothesis that

each helminth species was not dependent on location, season, age and gender, nor on the prevalence of other helminth species. Mean abundance of each helminth, as well as mean species richness, across location, season, rodent age and gender were also computed. Mean abundance was calculated as the mean number of parasites found in the total number of rats, whether infected or not.

Binary logistic regression (BLR) was used to determine the driver/s of prevalence of each helminth species (as the dependent variable). Eight full models were run for each helminth species in turn (as dependent variable) against abiotic independent variables (location and season) and biotic independent variables (rodent age and gender), plus the other helminth species as covariates.

The helminth interactions using BLR, when examined in relation to the crosstabulations, showed that when the BLR produced significant associations, (i.e. where the odds of rats that were positive for the predictor helminth were significantly more likely to have the dependent variable helminth as opposed to negative predictor helminth rats) these were actually caused by 2-way interactions, of which only one of the ways was significant. These cases had to be examined in the crosstabulations to establish which helminth was actually the significant predictor and are marked with an asterisk (*) in Table II. On a case-by-case basis, we examined each interaction, compared the BLR and crosstabulation results, and marked the ones that had a very low ratio of predictor helminth positive to negative rats for each DV helminth – these are marked with a hash (#) in Table II. Finally, the predictor variables marked with an arrow (→) all had a > 50% positive association with the DV helminth, and for this reason we considered these as being worthy of further examination as interspecific drivers (Table II).

Our dataset contained a large number of absolute zeros, hence negative binomial regression (NBR) was used to examine associations between mean worm abundance of each helminth species, as well as mean species richness, and location, season, rat age and gender. Instead of using the default dispersion parameter of ‘1’ in SPSS, we chose the estimate option that allows SPSS to estimate this value, and Akaike information criterion (AIC) values indicated that this option produced better models.

RESULTS

Identification of helminths recovered from the GIT

Nine different helminth species were isolated from sections of the GIT and identified as: *Gongylonema neoplasticum* (oesophagus and stomach), *Protospirura muricola* (stomach), *Moniliformis moniliformis*, *Hymenolepis diminuta*, *Hymenolepis nana, Nippostrongylus brasiliensis,* and *Strongyloides* spp. - both single infections with each of *Strongyloides venezuelensis* and *S. ratti*, and co-infections with these two species - (small intestine); *Heterakis spumosa* (large intestine), and *Syphacia muris* (caecum).

Prevalence and abundance of helminth species

The total number of *R. norvegicus* trapped was 379, and the most prevalent helminth species was *N. brasiliensis* (82.1%, n = 311), followed by *H. spumosa* (28.0%, n = 106), *G. neoplasticum* (25.3%, n = 96), *Strongyloides* spp. (24.5%, n = 93), *H. diminuta* (18.2%, n = 69), *M. moniliformis* (10.0%, n = 38), *P. muricola* (3.2%, n = 12), *S. muris* (2.6%, n = 10) and *H. nana* (0.8%, n = 3).

Prevalence, mean abundance of each helminth species and mean species richness, overall, and for each location, season, rat age and gender are shown in Table I. Although *H. nana* results are included (and show that location and season are significant), it should be noted that only three rats were infected (one male pup, one male juvenile and one female adult), all trapped in the wet season and at the HBR (Table I). This cestode species was thus excluded from all further statistical analyses of both prevalence and abundance data.

Pearson's chi-square test showed that there were significant differences in the prevalence of helminth species amongst locations for *G. neoplasticum* ($x^2 = 28.249$, *df* 3, $P < 0.001$), *P. muricola* ($x^2 = 9.379$, *df* 3, $P = 0.005$), *H. diminuta* ($x^2 = 32.146$, *df* 3, $P < 0.001$), *Strongyloides* spp. ($x^2 = 28.120$, *df* 3, $P < 0.001$), *H. spumosa* ($x^2 = 35.875$, *df* 3, $P < 0.001$), and *S. muris* ($x^2 = 17.361$, *df* 3, $P = 0.001$); but not for *M. moniliformis* and *N. brasiliensis.*

There were also significant differences in prevalence among age groups for *G. neoplasticum* ($x^2 = 37.390$, *df* 2, $P < 0.001$), *M. moniliformis* ($x^2 = 12.342$, *df* 2, $P = 0.002$), *H. diminuta* ($x^2 = 20.865$, *df* 2, $P < 0.001$), *N. brasiliensis* ($x^2 = 20.120$, *df* 2, $P < 0.001$), and *H. spumosa* ($x^2 = 40.897$, *df* 2, $P < 0.001$); but not for *P. muricola*, *Strongyloides* spp. and *S. muris*. Season was significant for prevalence of *P. muricola* ($x^2 = 8.557$, *df* 1, $P = 0.003$), *H. diminuta* ($x^2 = 17.406$, df 1, $P < 0.001$), and *S. muris* ($x^2 = 8.557$, *df* 1, $P = 0.003$); however, gender was only significant for prevalence of *H. spumosa* ($x^2 = 5.359$, *df* 1, $P = 0.021$).

Pearson's chi-square and Fisher's exact test were both considered for the helminth species interactions in order to remove border-line significances as these interactions need to be highly significant in order to be of any value (preferably, $P < 0.04$). The following helminths had significant interactions with each other: (1) *G. neoplasticum* and *M. moniliformis* ($x^2 = 20.008$, df 1, $P < 0.001$), (2) *G. neoplasticum* and *H. diminuta* ($x^2 = 32.140$, df 1, $P < 0.001$), (3) *G. neoplasticum* and *H. spumosa* ($x^2 = 25{,}395$, df 1, $P < 0.001$), (4) *P. muricola* and *H. diminuta* ($x^2 = 26.843$, df 1, $P < 0.001$), (5) *P. muricola* and *H. spumosa* ($x^2 = 13{,}606$, df 1, $P < 0.001$); (6) *M. moniliformis* and *H. diminuta* ($x^2 = 9.850$, df 1, $P = 0.002$), (7) *M. moniliformis* and *H. spumosa* ($x^2 = 12{,}752$, df 1, $P < 0.001$), (8) *H. diminuta* and *Strongyloides* spp. ($x^2 = 34{,}793$, df 1, $P < 0.001$), (9) *H. diminuta* and *H. spumosa* ($x^2 = 14{,}190$, df 1, $P < 0.001$) (10) *N.*

brasiliensis and *H. spumosa* (x^2 = 10,800, df 1, *P* = 0.001), (11) *Strongyloides* spp. and *H. spumosa* (x^2 = 5,716, df 1, *P* = 0.017), (12) *H. spumosa* and *S. muris* (x^2 = 5,231, df 1, *P* = 0.022).

Gongylonema neoplasticum was more prevalent and abundant in HBR (37.5%, 1.92 ±5.04) and CBD (35.4%, 1.74 ±4.36) and *P. muricola* was more prevalent and abundant at CBD (58.4%, 0.57 ±0.27) and was absent from IS. *Moniliformis moniliformis* had the highest prevalence and abundance at CBD (36.8%, 0.66 ±3.94) and UPU (34.2%, 0.59 ±1.84), whereas *H. diminuta* had the highest prevalence, but second lowest abundance at CBD (46.4%, 0.82 ±1.99), the second highest prevalence and abundance at HBR (34.8%, 2.32 ±8.72), and the second lowest prevalence but highest abundance at UPU (13.0%, 2.94 ±21.00). *Nippostrongylus brasiliensis* had a very similar prevalence across all four locations, however, mean abundance was highest at HBR (89.42 ±234.24) and lowest at IS (31.44 ±52.99). *Strongyloides* spp. had the highest prevalence and abundance at CBD (45.2%, 5.58 ±13.03) and UPU (23.6%, 5.35 ±28.37). *Heterakis spumosa* had the highest prevalence and abundance at HBR (40.6%, 3.65 ±9.51); and *S. muris* had the highest prevalence and abundance at HBR (80.0%, 9.28 ±42.13), and was absent from CBD (Table I).

For all helminth species, prevalence and abundance were lowest, or absent in IS. Age showed a general pattern of increasing prevalence and abundance in rats as they aged, with some helminths being similarly present and abundant in both juveniles and adults while still being lowest in pups. Gender and season did not show any patterns between prevalence and abundance. (Table I).

Table I. Prevalence (Prev), mean abundance (Mean) and standard deviation (±SD) data for *Gongylonema neoplasticum* (*Gn*), *Protospirura muricola* (*Pm*), *Moniliformis moniliformis* (*Mm*), *Hymenolepis diminuta* (*Hd*), *Hymenolepis nana* (*Hn*), *Nippostrongylus brasiliensis* (*Nb*), *Strongyloides* spp. (*S*spp), *Heterakis spumosa* (*Hs*), and *Syphacia muris* (*Sm*); and mean helminth species richness (HSR) at 4 locations (LOC): central business district (CBD), harbour (HBR), informal settlements (IS) and urban/per-urban (UPU). These data are also given for season (SEAS), rodent age (AGE) and gender (GEN); M = males, F = females.

Variable	***Gn***	***Pm***	***Mm***	***Hd***	***Hn***	***Nb***	***S*spp**	***Hs***	***Sm***	**HSR**
	Prev Mean (±SD)	Prev Mean (±SD)	Prev Mean (±SD)	Prev Mean (±SD)	Prev Mean (±SD)	Prev Mean (±SD)	Prev Mean (±SD)	Prev Mean (±SD)	Prev Mean (±SD)	Mean (±SD)
Overall	25.3% 1.27 (±4.03)	3.2% 0.17 (±1.67)	10.0% 0.45 (±2.55)	18.2% 1.72 (±11.74)	0.8% 0.15 (±2.55)	82.1% 52.07 (±142.83)	24.5% 3.36 (±16.15)	28.0% 2.54 (±7.48)	2.6% 2.30 (±21.17)	1.94 (±1.33)
LOC: CBD	35.4% 1.74 (±4.36)	58.4% 0.57 (±3.20)	36.8% 0.66 (±3.94)	46.4% 0.82 (±1.99)	0.0% 0.00 (±0.00)	26.0% 47.66 (±131.37)	45.2% 5.58 (±13.03)	32.1% 2.54 (±6.82)	0.0% 0.00 (±0.00)	2.42 (±1.47)
HBR	37.5% 1.92 (±5.04)	33.3% 0.05 (±0.27)	21.1% 0.45 (±2.47)	34.8% 2.32 (±8.72)	100% 0.62 (±5.14)	23.8% 89.42 (±234.24)	16.1% 0.76 (±2.56)	40.6% 3.65 (±9.51)	80.0% 9.28 (±42.13)	2.31 (±1.33)
IS	7.3% 0.30 (±1.23)	0.0% 0.00 (±0.00)	7.9% 0.03 (±0.18)	5.8% 0.77 (±4.79)	0.0% 0.00 (±0.00)	23.8% 31.44 (±52.99)	15.1% 1.35 (±4.72)	6.6% 0.95 (±4.17)	10.0%0.01 (±0.107)	1.25 (±0.82)
UPU	19.8% 1.05 (±4.10)	8.3% 0.01 (±0.10)	34.2% 0.59 (±1.84)	13.0% 2.94 (±21.00)	0.0% 0.00 (±0.00)	26.4% 39.56 (±72.84)	23.6% 5.35 (±28.37)	20.7% 2.92 (±8.13)	10.0% 0.06 (±0.61)	1.71 (±1.26)
SEAS: Wet	38.5% 1.33 (±4.38)	75.0% 0.44 (±2.75)	36.8% 0.61 (±3.81)	58.0% 2.46 (±8.20)	100% 0.42 (±4.24)	36.3% 74.55 (±201.90)	44.1% 6.66 (±25.35)	37.7% 2.32 (±8.08)	80.0% 5.53 (±33.74)	2.22 (±1.48)
Dry	61.5% 1.24 (±3.83)	25.0% 0.02 (±0.13)	63.2% 0.35 (±1.42)	42.0% 1.30 (±13.33)	0.0% 0.00 (±0.00)	63.7% 39.34 (±92.44)	55.9% 1.49 (±6.09)	62.3% 2.67 (±7.14)	20.0% 0.56 (±7.14)	1.78 (±1.21)
AGE: Pups	7.7% 0.18 (±0.81)	0.0% 0.00 (±0.00)	12.1% 0.24 (±2.05)	6.1% 0.04 (±0.19)	33.3% 0.46 (±4.76)	24.7% 14.86 (±30.94)	25.6% 1.27 (±4.20)	4.9% 0.06 (±0.27)	30.0% 2.71 (±23.85)	1.14 (±0.81)
Juvniles	35.6% 0.99 (±3.08)	40.0% 0.04 (±0.23)	27.3% 0.32 (±1.43)	51.5% 2.85 (±17.85)	33.3% 0.06 (±0.69)	37.1% 27.01 (±41.05)	41.1% 3.24 (±9.27)	48.5% 3.04 (±6.77)	50.0% 4.07 (±28.26)	2.08 (±1.36)
Adults	56.7% 2.50 (±5.97)	60.0% 0.46 (±2.90)	60.6% 0.66 (±3.63)	42.4% 2.09 (±8.36)	33.3% 0.01 (±0.09)	38.2% 104.46 (±219.56)	33.3% 5.58 (±26.24)	46.6% 4.20 (±10.59)	20.0% 0.24 (±2.53)	2.41 (±1.38)
GEN: F	58.9% 1.23 (±4.29)	60.0% 0.03 (±0.20)	60.6% 0.32 (±1.47)	60.6% 1.79 (±15.36)	66.7% 0.32 (±3.73)	53.5% 40.54 (±86.60)	50.0% 4.40 (±21.69)	61.2% 1.72 (±5.04)	40.0% 3.21 (±23.59)	1.76 (±1.26)
M	41.1% 1.29 (±3.86)	40.0% 0.30 (±2.35)	39.4% 0.50 (±3.23)	39.4% 1.77 (±7.50)	33.3% 0.01 (±0.07)	46.5% 58.09 (±170.38)	50.0% 2.56 (±9.08)	38.8% 3.36 (±9.31)	60.0% 1.60 (±19.50)	2.07 (±1.39)

Statistical analyses of prevalence data - BLR

Eight helminths were tested in individual BLR models with each helminth in turn as the dependent variable, location, season, rat age and gender as predictors, and the other helminths (*G. neoplasticum, P. muricola, M. moniliformis, H. diminuta, N. brasiliensis, Strongyloides* spp., *H. spumosa* and *S. muris*) as covariates. All models were significant. The Hosmer and Lemeshow test showed that all models were a good fit ($P > 0.05$) except for *H. diminuta* ($P = 0.050$). Only significant interactions were tabulated (Table II).

Location had a significant effect on the prevalence of *G. neoplasticum, H. diminuta, Strongyloides* spp., and *H. spumosa* ($P < 0.05$). Even though location was not significant for *M. moniliformis*, the dummy variables of HBR and UPU had a significant interaction - odds of UPU rats compared with HBR rats having this helminth were 3.7 ($P = 0.025$) (Table II).

Age was significant for *G. neoplasticum, H. diminuta, N. brasiliensis and H. spumosa* models. Generally, odds of rats having these helminths increased with age (Table II).

Season was significant for *H. diminuta* and *S. muris*, where odds of rats trapped in the wet season as opposed to the dry season having each of these helminths were 3.2 ($P = 0.002$), and 39.7 ($P = 0.011$) respectively. Season approached significance for *P. muricola* ($P = 0.059$) and for *Strongyloides* spp. ($P = 0.058$), where odds of rats trapped in the wet season as compared with the dry season having the spirurid and the rhabditid nematode were 9.0 and 1.8 respectively (excluded from table). None of the parasites displayed any prevalence associations with gender (Table II).

Table II: Binary logistic regression (BLR) models for each parasite as dependent variable: *Gongylonema neoplasticum* (*Gn*), *Protospirura muricola* (*Pm*), *Moniliformis moniliformis* (*Mm*), *Hymenolepis diminuta* (*Hd*), *Nippostongylus brasiliensis* (*Nb*), *Strongyloides* spp. (*S*spp), *Heterakis spumosa* (*Hs*) and *Syphacia muris* (*Sm*), and location, season, rat age and gender as predictors, plus the other helminths as covariates. Akaike information criterion (AIC), odds ratios (OR), 95% confidence intervals (CI), and *P*-values are given. Abbreviations: central business district (CBD), harbour (HBR), informal settlements (IS), urban/per-urban areas (UPU), and reference category (ref). Significant variables: first dummy variable is reference category, e.g. within location, CBD is compared with IS and is written 'Location IS (ref)/CBD'; → = significant driver; * = inverse relationship causing significance; # = not a very good predictor.

Dependent variable: helminth	Significant variable	OR	95% CI for OR		*P*- value
			Lower	Upper	
Gn	**Location** IS (ref)/CBD	3.873	1.382	10.854	0.010
(AIC = 341.85)	**Location** IS (ref)/HBR	5.409	1.992	14.689	0.001
	Location UPU (ref)/CBD	2.428	1.070	5.509	0.034
	Location UPU (ref)/HBR	3.391	1.497	7.677	0.003
	Rat age Pups (ref)/Juveniles	2.668	1.000	7.118	0.050
	Rat age Pups (ref)/Adults	8.385	3.032	23.187	< 0.001
	Rat age Juveniles (ref)/Adults	3.143	1.647	5.997	0.001
	→*Mm* neg (ref)/*Mm* pos	3.927	1.651	9.344	0.002
	→*Hd* neg (ref)/*Hd* pos	2.740	1.325	5.665	0.007
Pm	**Hd* neg (ref)/*Hd* pos	11.152	1.061	117.209	0.044
(AIC = 75.17)	**Hs* neg (ref) *Hs* pos	16.107	1.580	164.167	0.019
Mm	**Location** HBR (ref)/UPU	3.736	1.180	11.829	0.025
(AIC = 208.32)	**Gn* neg (ref)/*Gn* pos	3.552	1.477	8.541	0.005
	**Hs* neg (ref)/*Hs* pos	3.226	1.305	7.978	0.011
Hd	**Location** IS (ref)/ CBD	4.688	1.364	16.113	0.014
(AIC = 252.87)	**Location** UPU (ref)/CBD	4.069	1.498	11.053	0.006
	Season Dry (ref)/Wet	3.182	1.524	6.643	0.002
	Age Pups (ref)/Juveniles	7.867	2.339	26.459	0.001
	Age Pups (ref)/Adults	4.765	1.294	17.554	0.019
	**Gn* neg (ref)/*Gn* pos	2.680	1.271	5.648	0.010
	**Mm* neg (ref)/*Mm* pos	2.998	1.075	8.359	0.036
	*Sspp neg (ref)/*S*spp pos	4.167	1.994	8.706	< 0.001
Nb	**Rat age** Pups (ref)/Juveniles	2.142	1.066	4.305	0.032
(AIC = 331.52)	**Rat age** Pups (ref)/Adults	5.997	2.384	15.087	< 0.001
	Rat age Juveniles (ref)/Adults	2.800	1.168	6.710	0.021
	#*Hd* pos (ref)/*Hd* neg	2.435	1.004	5.903	0.049
	#*Hs* neg (ref)/*Hs* pos	4.326	1.630	11.484	0.003
***S*spp**	**Location** HBR (ref)/CBD	5.127	2.291	11.476	< 0.001
(AIC = 373.05)	**Location** HBR (ref)/UPU	3.034	1.235	7.456	0.016
	Location IS (ref)/CBD	3.068	1.360	6.920	0.007
	→*Hd* neg (ref)/*Hd* pos	4.771	2.312	9.843	< 0.001
	**Hs* neg (ref)/*Hs* pos	2.075	1.076	4.004	0.029

Hs	**Location** IS (ref)/CBD	4.461	1.601	12.427	0.004
(AIC = 341.82)	**Location** IS (ref)/HBR	6.493	2.398	17.584	< 0.001
	Location UPU (ref)/CBD	2.357	1.061	5.237	0.035
	Location UPU (ref)/HBR	3.431	1.574	7.481	0.002
	Age Pups (ref)/Juveniles	7.776	2.663	22.703	< 0.001
	Age Pups (ref)/Adults	7.183	2.319	22.250	0.001
	→*Pm* neg (ref)/*Pm* pos	24.604	2.521	240.096	0.006
	→*Mm* neg (ref)/*Mm* pos	3.238	1.366	7.676	0.008
	#*Nb* neg (ref)/*Nb* pos	3.619	1.362	9.618	0.010
	#*S*spp neg (ref)/*S*spp pos	2.011	1.021	3.959	0.043
	→*Sm* neg (ref)/*Sm* pos	6.299	1.290	30.759	0.023
Sm	**Season** Dry (ref)/ Wet	39.744	2.296	687.839	0.011
(AIC = 74.11)	**Hs* neg (ref)/*Hs* pos	67.364	3.356	1352.200	0.006

Statistical analyses of abundance data - NBR

Unfortunately, due to 'missing data' from entire dummy variable predictors (i.e. no rats in IS plus no pups infected with *P. muricola*, and no rats infected with *S. muris* in CBD), Hessian matrix singularity caused errors in computing full models (that included the other helminths as covariates), consequently the abundance data for each helminth could only be examined against location, season, rat age and gender.

The Wald Chi-square statistics are given here for each NBR helminth model: location for *M. moniliformis* abundance (x^2 = 13.668, *df* 3, P = 0.003); location and age were significant for abundance of *G. neoplasticum* (x^2 = 15.535, *df* 3, P = 0.001 and x^2 = 36.647, *df* 2, P < 0.001); location and season for *P. muricola* (x^2 = 7.539, *df* 2, P = 0.023 and x^2 = 8.470, *df* 1, P = 0.004), and *Strongyloides* spp. (x^2 = 18.128, *df* 3, P < 0.001 and x^2 = 15.854, *df* 1, P < 0.001), location, season and rat age for *H. diminuta* (x^2 = 14.263, *df* 3, P = 0.003; x^2 = 12.696, *df* 1, P < 0.001 and x^2 = 30.650, *df* 2, P < 0.001); season, rat age and gender for *N. brasiliensis* (x^2 = 18.904, *df* 1, P < 0.001; x^2 = 81.242, *df* 2, P < 0.001 and x^2 = 6.736, *df* 1, P = 0.009); and age for *H. spumosa* (x^2 = 60.307, *df* 2, P < 0.001). There were no significant predictors of *S. muris* abundance, however there was a significant interaction between the dummy variables, HBR and IS, within location. Although location was not significant for the *N. brasiliensis* model, rats in the harbour were prone to higher mean abundance than rats in CBD; the same situation occurred for *H. spumosa*, where CBD and HBR rats were prone to higher mean abundance of this helminth than IS rats. Rat age was not significant for *Strongyloides* spp., however juveniles were prone to higher mean abundance of this nematode than pups. Males were more prone than female rats to higher infections with *N. brasiliensis*.

Using *G. neoplasticum* as an example, we interpret the statistics produced for predictors with dummy variables, here location is compared with IS and UPU, thus: the expected log counts of CBD increased by 1.568 and 1.286 (B-value) and the expected log counts of HBR increased by 1.214 and 0.932. The incident rate ratios (IRRs) for these locations showed that the incident rate of a higher abundance of *G. neoplasticum* at CBD were 4.8 ($P = 0.001$) and 3.6 ($P = 0.004$) times that of the two reference groups, IS and UPU, holding all other variables constant. Likewise, the IRRs for a higher abundance of this spirurid at HBR were 3.4 ($P = 0.014$) and 2.5 ($P = 0.037$) times that of the two reference groups, holding all other variables constant. All the significant abundance results are presented in Table III below.

The NBR model for mean species richness was significant for location (x^2=30.677, *df* 3; P < 0.001); season (x^2=9.630, *df* 1; P = 0.002); and rat age (x^2=39.100, *df* 2; P < 0.001). Rats from CBD and HBR had higher incidence rate ratios of the greatest number of parasites (i.e. parasite species richness) as compared to IS and UPU; had the highest IRRs in the wet rather than the dry season and in juveniles and adult rats compared with pups (Table III). When the means were computed, the maximum number of helminths for each predictor were given: CBD = 7; UPU = 6; HBR = 5; IS = 4; wet season = 7; dry season = 6; pups = 4; juveniles = 6; adults = 7; females = 7, and males = 6.

Table III: Negative binomial regression models with the abundance of each helminth, and helminth species richness as dependent variables, and the following as predictors: location, season, rat age and gender. Abbreviations: AIC = Akaike information criterion; B = the coefficient estimate of the model; IRR = incidence rate ratio; CI (l – u) = lower to upper confidence intervals for IRR; *Gongylonema neoplasticum* (*Gn*), *Protospirura muricola* (*Pm*), *Moniliformis moniliformis* (*Mm*), *Hymenolepis diminuta* (*Hd*), *Hymenolepis nana* (*Hn*), *Nippostrongylus brasiliensis* (*Nb*), *Strongyloides* spp. (*Sspp*), *Heterakis spumosa* (*Hs*), and *Syphacia muris* (*Sm*); Juv = juveniles; CBD = central business district, HBR = harbour, IS = informal settlements, UPU = urban/peri-urban areas. Significant variables: first dummy variable is reference category, e.g. within location, CBD is compared with IS, and written ‘Location IS (ref)/CBD’.

Helminth (AIC)	Significant variables	B	IRR	95% CI (l – u)	*P*-value
Gn	Location IS (ref)/ CBD	1.568	4.795	1.908 – 12.049	0.001
(AIC = 812.65)	Location UPU (ref)/ CBD	1.286	3.617	1.513 – 8.649	0.004
	Location IS (ref)/ HBR	1.214	3.367	1.276 – 8.886	0.014
	Location UPU (ref)/ HBR	0.932	2.540	1.060 – 6.088	0.037
	Rat age Pups (ref)/Juv	1.509	4.523	1.943 – 10.528	< 0.001
	Rat age Pups (ref)/Adults	2.818	16.742	6.661 – 42.082	< 0.001
	Rat age Juv (ref)/Adults	1.309	3.701	1.836 – 7.460	< 0.001
Pm	Location IS (ref)/CBD	2.617	13.701	1.128 – 166.356	0.040
(AIC = 120.03)	Location UPU (ref)/CBD	3.611	36.991	2.056 – 665.465	0.014
	Season Dry (ref)/Wet	4.249	70.034	4.005 – 1224.673	0.004
Mm	Location IS (ref)/CBD	2.285	9.829	1.563 – 61.792	0.015
(AIC = 364.53)	Location IS (ref)/HBR	2.192	8.949	1.349 – 59.390	0.023
	Location IS (ref)/UPU	3.954	52.159	6.364 – 427.525	< 0.001
Hd	Location CBD (ref)/UPU	1.794	6.013	1.830 – 19.752	0.003
(AIC = 676.07)	Location HBR (ref)/UPU	1.706	5.506	1.321 – 22.949	0.019
	Location IS (ref)/UPU	2.481	11.955	2.941 – 48.592	0.001
	Season Dry (ref)/Wet	2.056	7.815	2.522 – 24.214	< 0.001
	Rat age Pups (ref)/Juv	3.996	54.382	13.113 – 225.527	< 0.001
	Rat age Pups (ref)/Adults	3.539	34.424	7.581 – 156.318	< 0.001
Nb	Season Dry (ref)/Wet	0.799	2.224	1.551 – 3.189	< 0.001
(AIC = 3097.21)	Rat age Pups (ref)/Juv	0.718	2.050	1.322 – 3.179	0.001
	Rat age Pups (ref)/Adults	2.169	8.752	5.351 – 14.317	< 0.001
	Rat age Juv (ref)/Adults	1.452	4.270	2.819 – 6.467	< 0.001
	Gender Female (ref)/Male	0.497	1.643	1.129 – 2.391	0.009
Sspp	Location HBR (ref)/CBD	2.291	9.889	3.291 – 29.714	< 0.001
(AIC = 982.96)	Location HBR (ref)/IS	1.486	4.418	1.361 – 14.347	0.013
	Location HBR (ref)/UPU	2.017	7.516	2.382 – 23.720	0.001
	Season Dry (ref)/Wet	1.589	4.898	2.240 – 10.706	< 0.001
Hs	Rat age Pups (ref)/Juv	3.772	43.481	14.914 – 126.770	< 0.001
(AIC = 1020.03)	Rat age Pups (ref)/Adults	4.279	72.184	23.967 – 217.409	< 0.001
HSR	Location UPU (ref)/CBD	0.374	1.454	1.183 – 1.787	<0.001
(AIC = 1114.69)	Location UPU (ref)/HBR	0.221	1.248	1.009 – 1.543	0.041
	Location IS (ref)/CBD	0.607	1.834	1.451 – 2.318	< 0.001
	Location IS (ref)/HBR	0.454	1.574	1.239 – 1.999	< 0.001
	Season Dry (ref)/Wet	0.247	1.280	1.095 – 1.495	0.002
	Rat age Pups (ref)/Juv	0.536	1.709	1.372 – 2.129	< 0.001
	Rat age Pups (ref)/Adults	0.708	2.029	1.624 – 2.535	< 0.001
	Rat age Juv (ref)/Adults	0.171	1.187	1.000 – 1.409	0.050

DISCUSSION

Gastrointestinal helminths of *Rattus* spp. are cosmopolitan, although overall prevalence rates may vary in different studies from across the globe. Some studies found similar results to our study, while others varied considerably. References cited in the introduction provide these data. Behnke et al. (2001) cite studies that have examined component community structures influenced by extrinsic (location, season, year) and intrinsic factors (rodent age and gender, presence of other infections), albeit on indigenous rodents. This type of thorough research is scant for synanthropic rats.

The one extrinsic factor commonly used as a predictor for parasite prevalence and abundance, is location. Studies on *Rattus* spp. from Asia mostly looked at wet markets and sometimes residential suburbs (Singla et al., 2008; Paramasvaran et al., 2009; Mohd Zain et al., 2012) and a study in the Netherlands compared different farm types, and suburban and rural areas (Franssen et al., 2016) and found significance. However, no publication that we have found has divided a city up in a similar manner as for this study. Here, we examined rats trapped at sites within two closely located areas (CBD and HBR) where rats' home ranges may lie very close to one another, and other sites that were within different IS and UPU areas where the likelihood of rats meeting others within these two widespread locations was not likely as home ranges, when food and harbourage suffice, are known to be limited (Davis et al., 1948) (Figure 1).

The second extrinsic factor, season, was significant for the prevalence and abundance of *H. diminuta*, prevalence only of *S. muris* and abundance only of *P. muricola*, *N. brasiliensis*, *Strongyloides* spp., as well as helminth species richness, where the wet season produced higher results than the dry. Helminths that rely on arthropods, especially tenebrionid beetles, to vector them would be expected to be more common in the wet than dry season due to these vectors increased activity in hot humid conditions (Howe, 1965) and trichostrongylid and rhabditid nematodes that infect via larval penetration of the skin, are better suited to transmission in moist environments (Haley, 1962).

To understand rat age in relation to parasitoses, we recap on the helminth biology: *G. neoplasticum, P. muricola, M. moniliformis, H. diminuta* and *H. nana* require an arthropod intermediate host in order to be transmitted to the final host (rat) and complete the life-cycle. Pre-patent periods for these five helminths are ≥ 60 days, > 60days, 35 – 42 days, 14 – 21 days and ±14 days, respectively. *Nippostrongylus brasiliensis*, *Strongyloides* spp., and *H. spumosa* are transmitted directly from the larvae- or egg-contaminated environment to the rat via the skin or by ingestion (pre-patent periods are 6 – 9 days, ±4 days and ±30 days respectively). *Syphacia*

muris eggs are deposited on the perianal skin of the rat host and are infective within ±30 minutes, while the pre-patent period is 7 – 10 days. With this in mind, rat pups (i.e. those up to ± 35 days of age) should not be infected with *G. neoplasticum* and *P. muricola*, but pups around 30 days could be infected with *M. moniliformis* and *H. spumosa*. On the other hand, most pups (if exposed) could easily become infected with *H. diminuta* and *H. nana*, and frequently with *N. brasiliensis*, *Strongyloides* spp. and *S. muris*. Juvenile rats ≥ 60 days of age and adults would be more likely to acquire infections with all the helminths as infections are known to accumulate with time and exposure (Behnke et al., 2001).

Ageing rats in retrospect is obviously not ideal, as with most animals, there will always be those that are abnormally small and underweight for their age. This must have been the case with the seven 'pups' we found infected with *G. neoplasticum*, however no pups were infected with *P. muricola*. *Moniliformis moniliformis* prevalence increased significantly with age (univariate analysis) and a similar trend (though not significant) was seen with abundance. Both prevalence and abundance were significant for rats with *H. diminuta* as they aged. Although there were only three rats infected with *H. nana*, all age groups were equally at risk of infection and this makes sense as this cestode can be directly transmitted between hosts and the pre-patent period is short. Prevalence and abundance were significant for *N. brasiliensis* as rats aged, though prevalence between juveniles and adults was not very different, but abundance increased markedly between age groups. Prevalence and abundance were also significant for *H. spumosa* as rats aged with the difference between pups and juveniles and pups and adults being almost 10-fold for prevalence and even higher for abundance. Age was not significant for rats infected with *Strongyloides* spp. and *Syphacia muris* due to both helminths' extremely rapid time to infectivity and their short pre-patent periods. Ageing our rats according to Hirata and Nass (1974) has shown clearly how important it is to separate un-weaned pups from juveniles so as to clearly see the patterns described above, which would not have been possible if pups were included with older, weaned rats simply lumped together as "< 100gm" which is common place (Abu-Madi et al., 2001; Stojcevic et al., 2004).

Regression analyses are multivariate (computed using interactions between all the variables entered into the equation), so results varied slightly from the univariate prevalence and means data given in Table I. While the predictors: location, season, rat age and gender gave fairly comparable results, it should also be noted that presence-absence data for the helminth covariates (occurrence of ones and zeros) were very 'unbalanced'. *Hymenolepis nana*, *S. muris*, *P. muricola*, *M. moniliformis* and *H. diminuta* had more than 76 occurrences of '0' (< 20% presence of each helminth), and *N. brasiliensis* had more than 303 occurrences of '1' (> 80% presence of the

helminth). Results from the BLR statistics thus need to be conservatively interpreted for helminths that have a large imbalance of zeros and ones (Babyak, 2004; Salas-Eljatib et al., 2018). For this very reason, we considered it necessary to examine the results of the helminth interactions from the crosstabulations and means data, the BLR results, and the NBR results to determine which covariates had real biological meaning. The independent variable that was most likely to prove a confounder for helminth interactions was location as this has proven a driving predictor for other parasite interactions studied in this group of rats (Archer et al., 2017; Archer et al., 2018).

The two predictor-helminths for *G. neoplasticum* were *M. moniliformis* and *H. diminuta*. The extrinsic factor that was significant for both prevalence and abundance of *G. neoplasticum* was indeed location, where this helminth was most frequently found in highest abundance at both CBD and HBR as compared with IS and UPU. The intrinsic variable, age, showed an increase in prevalence and abundance of this helminth in rats as they aged. Next, we examined the predictors for similar location and age associations. Indeed, although *M. moniliformis* was more prevalent in UPU as compared with HBR, abundance was highest at CBD, HBR and UPU as compared with IS. Age was not significant in the BLR, but in the univariate analyses there was a distinct increase in prevalence (but not so distinct for abundance) as rats aged. *Hymenolepis diminuta* was more prevalent in CBD than IS and UPU, however, abundance was highest at UPU, and for both prevalence and abundance, this cestode increased in numbers as rats aged. Now, while 55% of *M. moniliformis*-positive and 52% of *H. diminuta*-positive rats as opposed to only 22% of *M. moniliformis*-negative and 19% of *H. diminuta*-negative rats were infected with *G. neoplasticum*, the overwhelming evidence is that these associations were likely driven by location and rat age.

Next, the BLR did not produce significant results for *P. muricola* as a predictor for *H. diminuta*, probably due to low prevalence of this spirurid and its absence from the IS. However, the univariate statistics showed that 75% (n=9) of *P. muricola*-positive rats as opposed to only 16% (n = 3) of *P. muricola*-negative rats were infected with *H. diminuta*. While the numbers were low and there were no other significant associations for prevalence, *P. muricola* abundance was highest in CBD as opposed to IS and UPU and higher in the wet than the dry season. Both these helminths were associated with the CBD.

Hymenolepis diminuta was a significant driver of *Strongyloides* spp. – 52% of *H. diminuta*-positive rats as compared with 18% of *H. diminuta*-negative rats had *Strongyloides* spp. Rats in the CBD and UPU compared with HBR, and CBD compared with IS, had a higher prevalence of *Strongyloides* spp., whereas rats in CBD, IS and UPU had a higher abundance of this helminth than HBR rats. *Strongyloides* spp. was more abundant in CBD, IS and HBR than in UPU, in the

wet rather than the dry season and in juveniles rather than pups. Here too, we see that location was a driver of this association.

Protospirura muricola, *M. moniliformis* and *S. muris* were all significant covariates of *H. spumosa*, where 75% *P. muricola*-positive, 53% *M. moniliformis*-positive and 60% *S. muris*-positive rats, as opposed to 26% *P. muricola*-negative, 25% *M. moniliformis*-negative and 27% *S. muris*-negative rats had *H. spumosa*. *Syphacia muris* had one significant extrinsic independent variable, season, where prevalence of rats with this pinworm were higher in the wet rather than the dry season. Although the BLR did not find location significant for this helminth (probably due to its absence from the CBD), whereas the NBR did, the univariate analysis showed that prevalence/mean abundance was 80% / 9.28 in HBR, 10% / 0.01 in IS and 10% / 0.06 in UPU. *Protospirura muricola*, *M. moniliformis*, *S. muris* and *H. spumosa* were all strongly associated with location. Mean species richness was highest in CBD, in the wet season and in adults.

There was another driver that we did not account for, the presence of vectors of the two spirurids, the acanthocephalan and the two cestodes. These helminth species are all vectored by insects – predominantly cockroaches and tenebrionid beetles, and cestodes also by fleas - and any Durbanite will attest to their plentiful presence in our city. Our previous publication reported on the predominance of the flea ectoparasite, *Xenopsylla cheopis*, on CBD rats (Archer et al., 2018). Temperatures and high humidity in the wet season create a perfect haven for these insects (Howe, 1965; Robinson, 2005). Ideal breeding grounds and food sources for flour beetles abound in the numerous grocery stores, fast-food outlets, street food-vendors and formal restaurants found in the CBD and HBR areas which have stores of milled grain products for food preparation (Howe, 1965). There are also old buildings that are dark and damp and in disrepair in these two locations, and these conditions are well suited to cockroach infestations (Robinson, 2005). Kitchens are moist and steamy and there are numerous bins outside food outlets that provide littered, moist environments, frequented by both cockroaches and rats. Some sites within the UPU offered similar attractions for insect vectors, thus the significance for *M. moniliformis* (vectored by both cockroaches and flour beetles) associated with this location.

The most striking results were those for IS. As these areas are densely populated, polluted and covered with litter, one would not have expected to find that these areas would have both the lowest prevalence and abundance of rodent GIT helminths. The highest prevalence of all the helminths was *N. brasiliensis* (23.6%), followed by *Strongyloides* spp. at 15.1%. *Protospirura muricola* and *H. nana* were absent from IS and all other helminths were found at $\leq$ 10%. A possibility for the low numbers of insect-vectored helminths could be a result of the poverty and

high crime rates in these areas, where living from hand to mouth is the norm rather than purchasing large bags of milled grain products and storing them (which is both unsafe and unaffordable).

It may be pertinent to mention here that there are many subtle drivers that are possibly immuno-regulators of disease, or have synergistic or antagonistic interactions with other helminths. There is also the consideration of niches occupied by adult helminths that may vary due to interspecific cohabitation (Behnke et al., 2001). Laboratory based research has raised many questions that need answers from natural populations, but this type of research is difficult and needs careful planning.

We thus conclude that while there is no single, obvious helminth-driven interaction between any of the helminths in the GIT of the rats in this study, location and age were significant drivers of infection, with rats in CBD and HBR, and adult rats as opposed to pups and juveniles having the highest overall prevalence and abundance of infections. While some of the helminths are not zoonoses, *M. moniliformis*, *H. diminuta* and *H. nana* are, and while the vectors and hosts abound, the risk for transmission will not dissipate.

It may be of value for the Vector Control Unit of the Health Department of eThekwini to inform people who complain of the rat problem that trapping and removing rats from the area is a one-sided and ineffective measure for both the control of the rodent population and of disease. The complainants, especially business owners in the CBD and HBR areas, also need to clean up their environment and consider storing dry foods in sealed packaging in a deep freeze to reduce the arthropod vectors and the number of rats that forage for food on their premises.

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CHAPTER 4

OTHER PARASITES OF *RATTUS NORVEGICUS* AND BY-CATCH RODENTS

INTRODUCTION

The parasites discussed in this chapter comprise two organ parasites of *Rattus norvegicus*, viz. *Taenia taeniaformis*, the hepatic metacestode, intermediate host stage of the cat tapeworm, and *Trichosomoides crassicauda* (the rodent urinary tract worm). It also includes all parasites found in *R. rattus* and *Mastomys natalensis*, protozoans of the intestinal tract of all three rodent species, and helminth eggs mechanically transmitted by the rodents in the absence of an actual infection with adult worms. As adult stages were recovered, it was simple to differentiate between the rodents that actually had patent infections and those that had ingested eggs or larvae and were simply excreting these life-cycle stages in their faeces.

Taenia taeniaformis liver cysts have been found in *Rattus* spp., mice (*Mus musculus*), bandicoot rats (*Bandicota* spp.) gerbils (*Tatera indica*), and the grey squirrel (*Sciurus carolinensis*) (Harkema, 1936; Smales, 1997; Singla et al., 2008; Sumangali et al., 2012) and are cosmopolitan in *Rattus* spp. (Forbes, 1942; Calero et al., 1950; Seong et al., 1995; Milazzo et al., 2010). Singla et al. (2008) also reported *Taenia taeniaformis* cysts attached to the mesentery and abdominal wall in a Bandicoot rat, while Sumangali et al. (2012) called their find "an unidentified strobilocercus larva of *Taenia* sp. in the body cavity". The period from ingestion of the eggs (passed in the faeces of cats infected with adult tapeworms) to development of liver cysts of this cestode is approximately 4 weeks (Greenfield, 1942). This parasite is usually found in small numbers in wild rodents, but occasional heavy infections have been reported (Miller and Dawley, 1928). Greenfield (1942) found that nurseling pups appear to be like old rats, 'resistant' to infection, whereas younger animals past the weaning stage appear to be more susceptible to heavier infections. Her study found that when pups < 15 days old were infected, either no cysts or dead cysts were found four weeks later at autopsy. The same was found for adults ≥ 6 months, but 25-day-old rats were most susceptible.

Trichosomoides crassicauda is a rat species-specific nematode of the urinary bladder (McGarry et al., 2015), but although it is also cosmopolitan (Harkema, 1936; De Leon, 1964) it is not as frequently reported as *T. taeniaformis* due to the fact that many studies only focus on the helminths of the gastrointestinal tract and liver. This delicate worm, oddly and very cleverly,

keeps her mate close – the male is parasitic inside her uterus (Thomas, 1924). Young un-weaned pups are possibly infected from exposure to their mothers' urine, however the pre-patent period is fairly long (8 - 12 weeks), thus the infection only becomes patent when they are juveniles or young adults (Zubaidy and Majeed, 1981). Al-Humaid et al. (1999) were the first to report on the presence of this nematode in Saudi Arabia. They described a fatal pneumonia attributed to *T. crassicauda* in a colony of laboratory rats and were also the first to record the presence of juvenile worms in the seminiferous tubules of rat testes.

Trichuris spp., nematodes of the caecum and large intestine of rodents, have been reported far less frequently from *R. norvegicus* than other intestinal nematodes like *Nippostrongylus brasiliensis* and *Heterakis spumosa* (Calero et al., 1950; Kataranovski et al., 2010). Ribas et al. (2013) state that the main host of *Trichuris muris* is *R. rattus*. Their study in Tanzania looked at indigenous rodents and they found only two of the five genera infected with *Trichuris* spp.: 23.4% of 321 *M. natalensis* were infected with a species of *Trichuris* that was genetically identified as *T. mastomysi*, and 30.8% of 26 *Gerbilliscus vicinus* were infected with another genetically identified species, *T carlieri*. It appears that the reports of invasive rodents infecting indigenous fauna may actually be erroneous with regards to this nematode genus and it is recommended that both morphological and genetic analyses are needed for accurate identification. (Ribas et al., 2013).

Trematode infections are sometimes reported in wild rodents: *Plagiorchis elegans* and *Notocotylus* sp. were found in *Crethrionomys*, *Microtus*, *Arvicola* and *Apodemus* spp. in Finnland (Tenora et al., 1983); *Caballerolecythus* n. gen. (a new dicrocoeliid) was found in *Liomys irroratus* and *Peromys difficilis* in Mexico (Lamothe-Argumendo et al., 2005); and natural infections of *Schistosoma mansoni* in indigenous rodents, mainly *M. natalensis*, in the old Eastern Transvaal province, now known as Mpumulanga, in South Africa (Pitchford and Visser, 1962). Laboratory rodents, e.g. *Mastomys coucha* and BALB/c mice, have also been used together with the appropriate snail intermediate hosts to maintain the life cycle of *Schistosoma mansoni* (Higgins-Opitz et al., 1990).

Of the 400 rodents trapped in this study, 379 were *Rattus norvegicus*, 10 were *R. rattus* and 11 were *Mastomys natalensis*. Due to the small numbers of the two by-catch species, their parasites could not be included in any statistical analyses. The aim of this chapter is thus to examine the effects of location and season, and rodent age and gender on the prevalence and abundance of *T. taeniaformis* and *T crassicauda*. Further, the basic prevalence and intensity data for coccidian

oocysts, amoebae and flagellates is presented and mechanical transmission of parasite infections, as well as parasites found in the two by-catch rodent species are discussed.

MATERIALS AND METHODS

Rodent Trapping

Durban Natural Science Museum's authorisation by Ezemvelo KZN Wildlife (permit number 4827/2007; Appendix B) to collect rodents and other small mammals was taken into consideration when the Animal Ethics Sub-Committee of the University of KwaZulu-Natal approved this study (clearance certificate reference number: 032/09/Animal; Appendix A). Rodents were captured by the Vector Control Division of eThekwini Health Department. Unfortunately, this meant that trapping was largely opportunistic due to traps often being placed in response to complaints about rats, and in areas where they were less likely to be stolen or constantly moved. Custom-made live traps, resembling the Monarch Rat Traps, were baited with the same kinds of foods that attracted rats to the trapping sites, e.g. bread, vegetables, meat. Rodents were euthanased in accordance with the international ethical guidelines of the American Society of Mammalogists (Gannon and Sikes, 2007).

Sampling sites and seasons

For our study purpose, we divided the eThekwini Metro (commonly known as Durban) into four locality-types: central business district (CBD), harbour (HBR), informal settlements (IS) and urban/peri-urban areas (UPU). These demarcations allowed for differentiation between business areas in the city centre and harbour (CBD and HBR) where there is heavy human traffic, extensive food trade and movement of goods, and residential areas (IS and UPU) where there are slums, low-cost housing settlements, local shopping malls, formal residences, small poultry farms and small game and bird parks (Figure 1). The map clearly shows how close together the CBD and HBR sites are compared with the IS and UPU sites, as well as the extent of the eThekwini Metro (Figure 1).

Durban does not have well-defined seasons with extremes of temperature, but rather a wet and dry season. In 2009, there were five wet months - January, February, October, November and December; and seven dry months - March to September (Figure 2). Over the one year period, 400 rodents were trapped: 379 *Rattus norvegicus*, 10 *Rattus rattus* and 11 *Mastomys natalensis*.

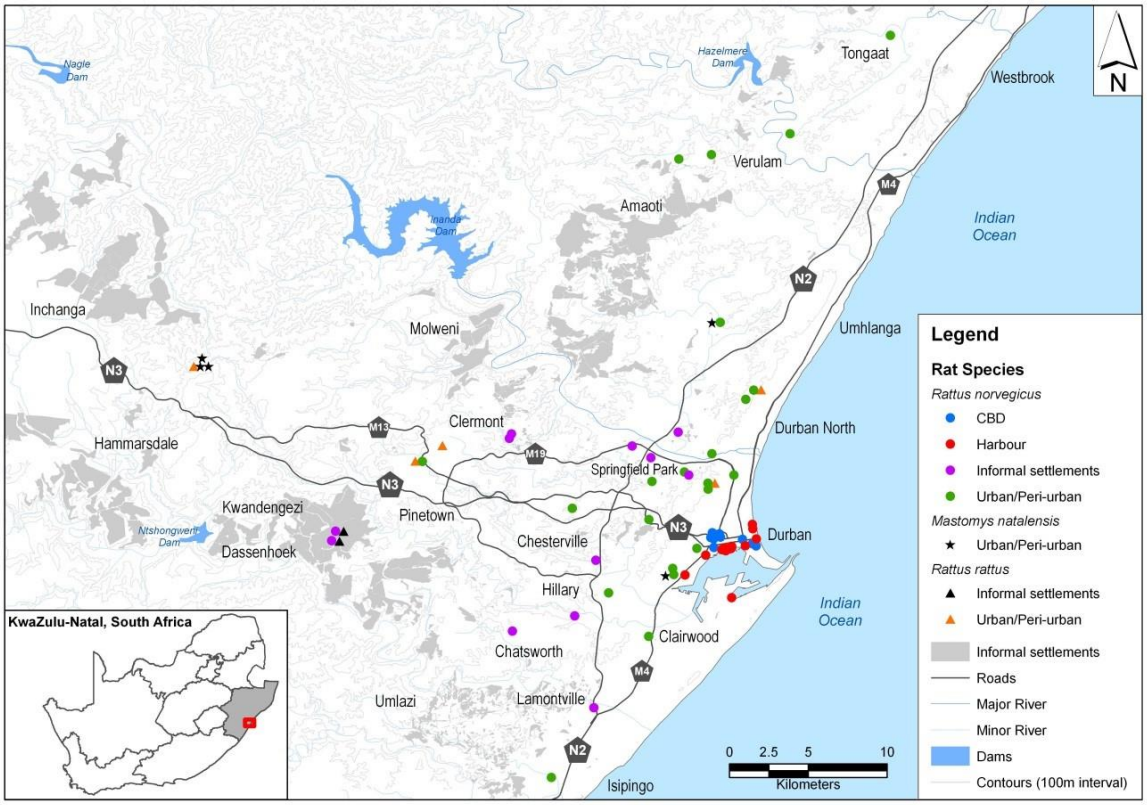

Figure 1: Map showing trapping sites of rodents within the eThekwini municipality (Durban).

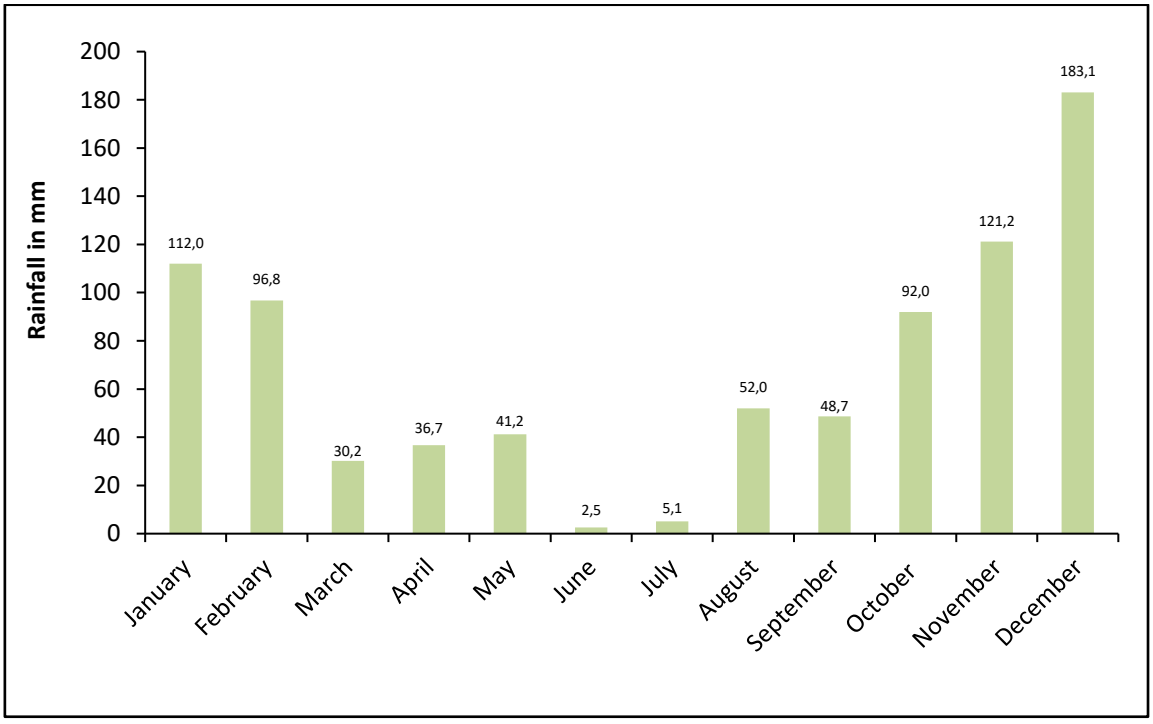

Figure 2: Mean rainfall (mm) per month for study period (January to December 2009). Data provided by SASRI, Mount Edgecombe (Weather station no. 461; 29°42'0" S, 31°2'0" E, 96m elevation above sea level).

Processing of rodents and collection of samples

Euthanasia was performed using chloroform, followed by cardiac puncture to obtain blood for making blood smears for haemoparasite detection and harvesting serum for serological tests. Each animal was thoroughly brushed and combed to collect ectoparasites which were also collected from all surfaces that the rat had been placed upon during processing. These were used by Hope (2011) for a separate study. All animals were then weighed, selected body measurements taken: overall length (body + tail), tail length only, length of right ear and right hind foot excluding as well as including claw, and gender and breeding status noted. Lastly, rodents were dissected and the complete gastrointestinal tract (GIT), faecal pellets, heart + lungs, kidneys + bladder, liver, tongue and diaphragm collected. Each set of organs (excluding GIT) were covered with digestive fluid (consisting of 5gm pepsin powder and 7ml hydrochloric acid in 1,000ml of distilled water) and incubated at 37°C for 12 to 18 hours to break down organ tissue and release parasites. The GIT (placed into a Petri dish at dissection) was divided into sections: oesophagus, stomach, small intestine (SI), caecum, and large intestine (LI). Each was carefully slit open to avoid damaging helminths and all macroscopically visible helminths were removed and preserved in 70% ethanol (ETOH). Later all remaining helminths were removed from each section of the alcohol preserved-GIT using a dissecting microscope, sexed, counted and stored in 70% ETOH. Faecal pellets passed during euthanasia or found in the rectum at dissection, were collected, preserved in 10% formal-saline, and later processed by the formal-ether concentration method (Allen and Ridley, 1970) to check for helminth eggs and protozoan cysts.

The carcass remains of a random number of *R. norvegicus* and all by-catch rodents, plus tissue samples of liver and kidney (in 90% ETOH) were deposited with the Durban Natural Science Museum for skull morphology (to differentiate between *Rattus* species, specifically *R. rattus* and *R. tanezumi*) and the tissue for genetic studies. All remaining carcasses were incinerated at the University of KwaZulu-Natal's Biomedical Resource Centre.

Aging of rodents

During collection and examination of the rodents, differences were noted in the presence/absence of many of the parasites in different sized rodents. It was thus decided to attempt to 'age' the animals (in retrospect), using body mass, gender and relevant literature. Kataranovski et al. (1994), used biochemical (dry lens weight and tyrosine content of the insoluble fraction of the lens) and morphometric parameters (body weight and ratio of body length to weight) to age rodents. This method was not an option as aging was done retrospectively in the present study and we did not keep the eyes. Conlogue et al. (1979) used three divisions of weight to divide the age groups of *R. norvegicus*, namely, < 100gm = juvenile; 100-200gm = adolescent and > 200gm

= adult. Mohd Zain et al. (2012) also used three divisions but rather different weight groups for *R. norvegicus* (Class 1: < 140gm; Class 2: 140-240gm and Class 3: > 240gm) and *R. rattus* (Class 1: < 90gm; Class 2: 90-150gm and Class 3: > 150gm).

Hirata and Nass (1974), gave a range for mass in grams per week of age for male and female *R. norvegicus* and *R. rattus*, and this method made the most sense to me as a parasitologist, as it meant that separating very young pups from juveniles that are independent, and juveniles from mature adult rats, would in turn, better relate to infection risk for rats at different age-periods in their lives than the weight-classes used by the aforementioned authors. This aging method has shown significant differences between our age categories and parasite infections. Hirata and Nass' weight categories per week of age do overlap, so the weight and total body length of each rat in the present study was carefully assessed and allocated as closely as possible to the appropriate age in weeks. The categories were: pups (< 5 weeks, un-weaned), juveniles (5-10 weeks, weaned, many sexually mature) and adults (> 10 weeks, all sexually mature). The 10 *R. rattus* were also classified using Hirata and Nass (1974). *Mastomys natalensis* are extremely difficult to age as their growth and sexual maturity is highly dependent on seasonal factors (Leirs et al., 1990). Sall-Dramé et al. (2010) categorized all *M. natalensis* ≥ 50gm as old and those ≤ 30gm as juveniles. As all 11 of the mice caught in our traps were adults, these authors were broadly followed, with mice >50gm being categorised as old adults and those >30 but <50gm as young adults.

Identification of parasites

The liver cestode cysts were identified morphologically according to Loos-Frank (2000). The urinary bladder-worm was identified by its general morphology, unique female-male relationship, characteristic eggs, host, and site within the rat (Thomas, 1924). The abdominal cavity cysts were identified on morphology (number and size of hooks on scolices), host and site within the mouse's body (Loos-Frank, 2000). The protozoans were morphologically identified, from the author's extensive experience, to genus (and 'human' species-type); then species were taken as from those previously reported for *R. norvegicus* (Bonfante et al., 1961). Dead amoebic and flagellate trophozoites, although identifiable as such to the trained eye, were not further identifiable on wet preparation slides and were hence referred to as amoebic or flagellate trophozoites. Eggs and larvae of helminths found in the faeces were identified, from the author's experience, to genus and then to species by using the following references: Beaver et al. (1984), Hughes (1941), Hussey (1957), Kruidenier and Peebles (1958), Little (1966), Mackerras and Sandars (1955), Robles et al. (2008), Smales et al. (2009), Van Cleave (1923), and Yokogawa (1920). Patent helminth infections were then differentiated from mechanical transmission of eggs and larvae by the presence or absence of adults within the host.

Statistical analyses

Data for this study were not normally distributed and transformation thereof did not achieve normality. Basic descriptive statistics, i.e. crosstabulations (including Pearson's Chi-square test for significance) and means, were employed to assess the data. Means were calculated as the sum of the mean number of parasites divided by the total number of rats (including both the infected and uninfected individuals) in the prescribed category. Statistical tests, binary logistic regression (BLR) for prevalence, and negative binomial regression (NBR) for abundance, equipped to deal with non-normally distributed data, were used for *T. taeniaformis* and *T. crassicauda*. Crosstabulation were used for prevalence data of the protozoan parasites (where there were no abundance data, but rather an assessment of light, moderate or heavy for degree of infection). Prevalence of the positives for each parasite across location, season, rat age and gender were described from the crosstabulations when appropriate. Due to low rodent numbers of *R. rattus* (n=10) and *M. natalensis* (n=11), only descriptive statistics were used. Critical probability for all tests was set at $P = 0.05$ and statistical analyses were performed using Statistical Package for the Social Sciences (SPSS; version 25, College Station, Texas, USA).

RESULTS

Identification of parasites

The liver cestode was identified as the intermediate host stage of the adult feline cestode, *Taenia taeniaformis*. The nematode found in the rat bladders was identified as *Trichosomoides crassicauda*. The abdominal cavity cysts were identified as *Taenia parva*. The protozoans were amoebae: (1) *Entamoeba muris* cysts, (2) *Entamoeba hartmanni*-like cysts, (3) amoebic trophozoites; flagellates: (1) *Giardia muris* cysts, (2) *Chilomastix bettencourti* cysts, (3) flagellate trophozoites; and coccidia: (1) *Eimeria nieschulzi* and (2) 'bi-polar' oocysts – possibly *Eimeria parastieda*. The eggs mechanically transmitted by the rats were identified microscopically as those of *Gongylonema neoplasticum*, *Moniliformis moniliformis*, *Hymenolepis diminuta*, *Nippostrongylus brasiliensis*, *Strongyloides* spp., *Heterakis spumosa*, *Trichuris* sp. (possibly *T. muris*) and *Trichosomoides crassicauda*, and the tiny coiled larvae as the L_1 stage of *Angiostrongylus cantonensis*. The piece of trematode uterus and eggs were identified as probably *Dicrocoelium dendriticum*. The microfilaria was not clearly identifiable and the possible early ring-form malaria trophozoites could not be confirmed or identified conclusively (Figures 3, 4 and 5).

Analysis of data: *Rattus norvegicus*

Basic prevalence data

The prevalence data for parasites found in *R. norvegicus* but not discussed in the three papers included in this thesis are presented in Table I. Total number and prevalence for each parasite is given in the first column, and only numbers of infected rats are reported in the columns beneath each location and season. The second part of Table I gives the prevalence of each helminth for eggs found in the absence of adult infections and numbers of these 'carrier rats' per location and season. Some rats infected with the bladder-worm, *T. crassicauda*, passed eggs in the faeces (n=27; 7.1%), while others that were not infected also passed eggs in their droppings (n=19; 5.0%). Many rats (41.4%) not infected with *Strongyloides* spp., mechanically transmitted the highest number of eggs and those not infected with *H. diminuta* (13.2%) transmitted the second highest number. *Trichuris* sp. eggs were mainly mechanically transmitted in the IS and UPU. The rest of the eggs were transmitted by < 3% of rats (Table I).

Table I: Prevalence data for *Taenia taeniaformis*, *Trichosomoides crassicauda*, all protozoans and helminth eggs mechanically transmitted by *Rattus norvegicus*. Abbreviations: CBD = central business district; HBR = harbour; IS = informal settlements; UPU = urban/peri-urban; Wet / Dry = season.

Parasites in *Rattus norvegicus* (n/379; prevalence %)	**CBD Wet = 29**	**CBD Dry = 72**	**HBR Wet = 50**	**HBR Dry = 43**	**IS Wet 41**	**IS Dry = 47**	**UPU Wet = 17**	**UPU Dry = 80**
T. taeniaformis liver cysts (n=69; 18.2%)	7	5	12	8	7	14	3	13
T. crassicauda worms (n=85; 22.4%)	12	15	15	10	2	6	4	21
Eimeria nieschulzi oocysts (n=145; 38.3%)	8	34	23	19	22	10	2	27
? *E. parastieda* oocysts (n=2; 0.5%)	0	0	1	0	1	0	0	0
Entamoeba muris cysts (n=11; 2.9%)	0	1	1	0	2	2	1	4
Entamoeba hartmanni-like cysts (n=2; 0.5%)	0	0	2	0	0	0	0	0
Amoebic trophozoites (n=2; 0.5%)	0	2	0	0	0	0	0	0
Giardia muris cysts & trophozoites (n=18; 4.8%)	0	3	6	0	3	4	1	1
Chilomastix bettencourti cysts (n=5; 1.3%)	0	0	1	0	0	1	1	2
Flagellate trophozoites (n=60; 15.8%)	1	6	11	5	8	8	0	21

Mechanically transmitted eggs (and *Angiostrongylus cantonensis* larvae) – no helminth infection								
Helminth – n / location/season →	**CBD Wet**	**CBD Dry**	**HBR Wet**	**HBR Dry**	**IS Wet**	**IS Dry**	**UPU Wet**	**UPU Dry**
% rats carrying helminth eggs /larvae↓	**= 29**	**= 72**	**= 50**	**= 43**	**= 41**	**= 47**	**17**	**= 80**
Gongylonema neoplasticum (n=11; 2.9%)	3	3	2	1	0	1	0	1
Moniliformis moniliformis (n=7; 1.9%)	0	0	0	1	0	0	0	6
Hymenolepis diminuta (n=50; 13.2%)	4	23	1	0	2	3	2	15
Nippostrongylus brasiliensis (n=5; 1.3%)	2	1	0	0	0	0	1	1
Strongyloides spp. (n=157; 41.4%)	5	38	11	20	16	20	7	40
Heterakis spumosa (n=6; 1.6%)	1	0	1	0	0	2	0	2
Trichuris sp. (n=13; 3.4%)	0	2	0	0	4	4	1	2
Angiostrongylus cantonensis (n=8; 2.1%)	0	3	1	0	0	1	0	3
Trichosomoides crassicauda (n=19; 5%)	2	6	3	1	0	1	2	4

The bi-polar coccidian oocysts (possibly *Eimeria parastieda*) were only found in two rats, one from HBR and one from IS, both male pups trapped in the wet season. *Chilomastix bettencourti* was absent from CBD, one female pup from HBR in the wet season and one adult female from IS in the dry season were infected, and three rats from UPU (one adult male - wet season; one adult female - dry season; one male pup - dry season) were infected. *Giardia muris* cysts and trophozoites were absent from CBD rats in the wet and HBR rats in the dry season. Two rats with age and gender information missing and one juvenile female were positive in CBD in the dry season, and three male pups and three male juveniles were infected in HBR in the wet season. Slightly more rats were infected with this flagellate in IS (two female pups, one male pup - wet season; one female pup, two male pups, one adult female - dry season) and UPU (one juvenile male - wet season; one female pup - dry season) than in CBD-dry and HBR-wet season. *Entamoeba muris* was absent from CBD-wet and HBR-dry season. One juvenile female CBD-dry season and one adult female HBR-wet season, two male pups in IS-wet season, one female pup and one adult male IS-dry season, one juvenile male UPU-wet season, and two juvenile males and two juvenile females UPU-dry season, were positive for this amoeba. *Entamoeba hartmanni*-like cysts were only present in two juvenile females in HBR-wet season, and amoebic trophozoites were present in two female rats in CBD-dry season, one pup and one juvenile.

Univariate analyses

Infections with protozoans were highest for *Eimeria nieschulzi* (38.3%) and second highest for flagellate trophozoites (15.8%). Crosstabulations (univariate analyses) were run for these two parasites to see if there were any significant relationships between each helminth and location, season, age and gender separately. Prevalence of *Eimeria*-positive rats was not significantly different across location, season and gender; however Pearson's Chi-square test was significant for age ($x^2 = 53.464$; df 2; $P < 0.001$): 46.1% of the rats positive for this coccidian fell into the

age-group pups, 40.6% were juveniles, and only 13.3% were adults. Prevalence of flagellate trophozoites was significantly different across locations (x^2 = 8.965; *df* 3; *P* = 0.030): 11.7% of positive rats were from CBD, 26.7% from HBR, 26.7% from IS and 35.0% from UPU. Season, age and gender were not significant, but there was a drop in prevalence as rats aged.

Multivariate analyses

The BLR for *T. taeniaformis* as the dependent variable and location, season, rat age and gender as predictor (independent) variables, was significant (x^2 = 96.594; *df* 7; *P* < 0.001), Hosmer and Lemeshow's test showed that the model was a good fit (x^2 = 3.868; *df* 8; *P* = 0.869) and the Akaike information criterion (AIC) was 265.44. Location, age and gender were significant, but not season. Odds of rats from IS becoming the intermediate host for this cestode was 3.3 times that of CBD rats (*P* = 0.015) and 3.4 times that of UPU rats (*P* = 0.007). Odds of juvenile and adult rats having the cestode cysts was 18.6 times (*P* = 0.006) and 168.1 times (*P* < 0.001), respectively, that of pups; and odds of adults was 9.0 times that of juveniles (*P* < 0.001). Odds of males having *T. taeniaformis* was 3.0 times that of females (*P* = 0.002).

The NBR for *T. taeniaformis* abundance showed a higher abundance in IS than in CBD and HBR, not UPU as for the BLR, but age followed the same abundance increase with age as it did for the prevalence data. When compared to CBD and HBR, the expected log count of IS increased by 1.18 and 1.22 (B value) respectively, compared to pups and juveniles the expected log count for adults increased by 4.66 and 2.08 respectively, compared to pups the expected log count for juveniles increased by 2.58, and compared to females the log count for males increased by 0.91. The NBR also showed that the incident rate ratio (IRR) of a higher abundance of *T. taeniaformis* at IS was 3.3 (*P* = 0.012) times and 3.4 times (*P* = 0.007) that for the reference groups CBD and HBR respectively, holding all other variables constant. The IRR for a higher abundance of the liver cysts in juveniles and adults was 13.2 (*P* = 0.001) and 105.3 (*P* < 0.001), respectively, times that of pups, and for a higher abundance in adults compared to juveniles, the IRR was 8.0 times (*P* < 0.001). The IRR for male rats having a higher abundance of this cestode was 2.5 (*P* = 0.008) times that of females.

The BLR testing the independent variables of location, season, rat age and gender as predictors for the dependent variable, *Trichosomoides crassicauda*, was significant (x^2 = 132.625; *df* 7; *P* < 0.001), Hosmer and Lemeshow's test showed that the model was a good fit (x^2 = 4.937; *df* 8; *P* = 0.764) and the AIC was 272.74. Location, rat age and gender were significant, however, season was not. The odds of rats at CBD being infected with *T. crassicauda* were 2.6 times those at UPU (*P* = 0.027). Odds of rats at CBD, HBR and UPU harbouring this nematode were 6.9 (*P* < 0.001),

3.8 (P = 0.011) and 2.7 (P = 0.046), respectively, times that of IS rats. No pups were infected with the urinary tract worm and the odds of adults being infected was 11.8 times that of juveniles (P < 0.001). The odds of males, as compared with females, harbouring this nematode was 2.4 (P = 0.012).

Due to 'missing data' in one of the groups, i.e. no pups infected with *T. crassicauda*, the Hessian matrix was singular and validity of the NBR model was uncertain. Nevertheless the results were as follows: When compared to IS the expected log count of CBD, HBR and UPU, increased by 0.96, 1.25 and 1.19 (B value), respectively, compared to juveniles the expected log count for adults increased by 1.87 (P < 0.001) and compared to females the log count for males increased by 1.00 (P = 0.001). The IRR of a higher abundance of *T. crassicauda* at CBD, HBR and UPU was 2.6 (P = 0.035), 3.5 (P = 0.006) and 3.3 (P = 0.006) respectively, times that for the reference group, IS, holding all other variables constant. The IRR for a higher abundance of urinary tract worms in adults was 6.5 (P = 0.001) times that of juveniles and for male rats it was 2.7 times that of females (P = 0.001).

Examination of raw data: *Rattus rattus* and *Mastomys natalensis*

Parasites found in the two by-catch rodents, *R. rattus* and *M. natalensis* are given in Table 2 and these two rodents were trapped only in two of the four locations, IS and UPU. Excluded from the table are the results for the *Toxoplasma gondii* testing that was used for Chapter 1: 1/7 *R. rattus* and 0/5 *M. natalensis* tested seropositive. Also excluded from the table were the mechanically vectored eggs passed by the by-catch rodents that did not have an infection: two *R. rattus*, one adult female caught in the IS-wet season and one juvenile male caught in UPU-wet season passed one *Strongyloides* spp. egg + one *Trichuris* sp. egg, and one *Angiostrongylus* sp. L_1 larva, respectively, and one *M. natalensis*, an old male caught at UPU-Dry season, passed one *Strongyloides* spp. egg (Figure 5).

One juvenile male rat caught in UPU in the dry season was infected with seven *A. cantonensis* adults and three old adult multimammate mice were infected with *Angiostrongylus* sp., possibly *A. sandarsae*, which has been reported from this species in Mozambique and Kenya. Unfortunately we could not genetically confirm their identity, as after extracting DNA and storing the samples over a December period, the DNA denatured due to electrical problems and repeated thawing and freezing of the samples. Three rats were infected with one *T. taeniaformis* cyst each (see Table II for location, etc.), but no mice. One *M. natalensis*, an old male, had an extremely severe infection with *Calodium hepaticum* in his liver, but no *R. rattus* were infected with this nematode.

The GIT helminths were mostly those regularly found in these rodents as well as *R. norvegicus*, however, the oesophagus/stomach spirurid, *Gongylonema neoplasticum*, was notably absent and *Trichuris muris*, while absent from *R. norvegicus*, was present in one *R. rattus* and in four *M. natalensis*. Coccidian oocysts were more commonly found in *M. natalensis* than in *R. rattus*, otherwise there was nothing else especially noted with regard to protozoan infections. An interesting find was a piece of a trematode uterus containing operculate eggs in the gut washings of one old male *M. natalensis* trapped in the dry season in UPU, identified as possibly *Dicrocoelium dendriticum* from size and egg morphology (Figure 5).

When blood smears were examined, 2/10 *R. rattus* were infected with *Trypanosoma lewisi* (there were 10 rats, but one pup was poisoned thus no blood could be drawn). One of the two had a moderate infection and the other a heavy infection. No *M. natalensis* were infected with *T. lewisi*. However, the same old male *M. natalensis* that had *Angiostrongylus* sp. juveniles and an extremely severe *C. hepaticum* infection, was also infected with unidentified microfilariae. There was only one damaged and one intact microfilaria in the thin blood film. The intact microfilaria measured ± 390 µm in length and varied in width from 2 µm at the tail to 6 µm at the widest part (Figure 3). It was not clear if there was a sheath, but there may have been as the 'dots' in the tail region did not extend further than 45 µm from the end of what was either an extremely narrow tail or a sheath. There also appeared to be ring-form malaria trophozoites in his blood, possibly *P. berghei* (Figure 3).

Two *Mastomys natalensis*, one young adult male and one old adult male, both caught in the dry season, at UPU, but at different sites within this location, had one *T. parva* cestode cyst each in the body cavity. The former's cyst was attached to the muscle wall behind the kidneys and the latter's was in the connective tissue surrounding the GIT. The scolices within the cysts numbered 15 and 10, and hooks 42 and 36 respectively, though a few hooks could have been lost during preparation for microscopy. Large hooks ranged in size from 363 – 398 µm and small hooks from 199 - 211 µm (Figure 4).

Table II: All parasites found in organs, gastrointestinal tract (GIT), and faeces of *Rattus rattus* and *Mastomys natalensis* during the two seasons (Dry, Wet) in informal settlements (IS) and urban/peri-urban areas (UPU). There were male (M) and female (F) pups, juveniles (Juv) and adults (Ad). Parasites found: *Ac = Angiostrongylus cantonensis*; *A* sp. = *Angiostrongylus* sp.; *Ch = Calodium hepaticum*; *Tt = Taenia taeniaformis*; *Pm = Protospirura muricola*; *Mm = Moniliformis moniliformis*; *Hd = Hymenolepis diminuta*; *Nb = Nippostrongylus brasiliensis*; *S*spp = *Strongyloides* spp.; *Hs = Heterakis spumosa*; *Tm = Trichuris muris*; *Sm = Syphacia muris; Tl = Trypanosoma lewisi*

Rodent: Location, Season, Age, Gender	Heart & Lungs: adults	Liver	GIT Adult worms	Blood Smears	Faeces: Eggs, larvae, cysts, trophs, oocysts
Rattus rattus:					
UPU, Dry, Ad, F	0	0	*Nb* 1	0	0
UPU, Dry, Juv, M	0	0	*Nb* 2, *Sm* 28	0	0
UPU, Dry, Pup, M	0	0	0	0	1+ flagellate trophs
IS, Dry, Pup, M	0	1 *Tt* cyst	*Tm* 1	0	0
UPU, Dry, Juv, M	*Ac* 7	0	*Mm* 1, *Hd* 6, *Hs* 3	0	0
IS, Wet, Ad, F	0	1 *Tt* cyst	*Hs* 2	0	0
UPU, Wet, Ad, M	0	0	0	*Tl* (moderate infection)	1+ oval coccidian oocysts 1+ *E. muris* cysts
UPU, Wet, Ad, M	0	1 *Tt* cyst	*Hd* 2, *Sm* 3	0	2+ flagellate trophs 1+ *E. muris* cysts
UPU, Wet, Ad, F	0	0	*Hd* 2	*Tl* (heavy infection)	0
UPU, Wet, Juv, M	0	0	*Nb* 2	0	*A*. sp. larva 1
Mastomys natalensis:					
UPU, Dry,OAd, M	*A* sp.12 Juveniles	*Ch worms, eggs*	*Pm* 5, *Hd* 3, *Nb* 134, *S*spp 10	Microfilaria, malaria	0
IS, Dry, OAd, F	*A* sp. 1	0	*Nb* 27, *Hs* 15	0	1+ flagellate trophs
UPU, Dry, OAd, F	0	0	*Nb* 14, *Tm* 2	0	1+ oval coccidian oocysts
UPU, Dry, YAd, M	0	0	*Hd* 8, *Nb* 71, *S*spp 1, *Tm* 2	0	0
UPU, Dry, YAd, F	0	0	0	0	1+ oval coccidian oocysts 1+ flagellate trophs
UP U, Dry, OAd, M	0	0	*Nb* 25, *Sm* 8	0	1+ oval coccidian oocysts
UPU, Dry, OAd, M	0	0	*Nb* 38	0	1+ oval coccidian oocysts 1+ flagellate trophs 1+ *E. muris* cysts
UPU, Dry, OAd, M	0	0	*Hd* 2, *Nb* 1	0	Piece of trematode & operculate eggs
UPU, Wet, YAd, F	0	0	*Nb* 8, *S*spp 2	0	1+ oval coccidian oocysts
U/PU, Wet, OAd, M	0	0	*Hd* 6, *Nb* 115, *Tm* 4	0	3+ oval coccidian oocysts
UPU, Wet, OAd, F	*A* sp. 2	0	*Hd* 16, *Nb* 8, *Tm* 4	0	1+ *E. muris* cysts

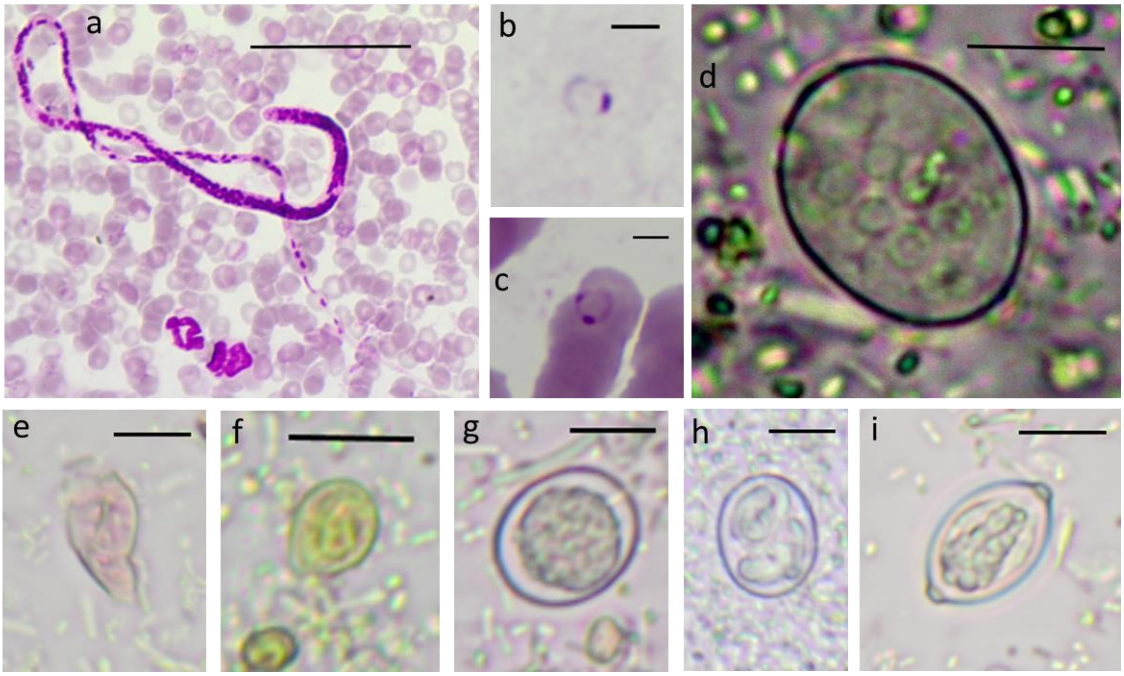

Figure 3: a. Microfilaria found in *Mastomys natalensis*; **b.** and **c.** Malaria trophozoites in same *M. natalensis*; **d.** *Entamoeba muris* cyst; **e.** Flagellate trophozoite; **f.** *Chilomastix bettencourti* cyst; **g.** *Eimeria nieschulzi* oocyst; **h.** *Eimeria nieschulzi* oocyst containing two sporocysts; **i.** *Eimeria parastieda*. Scale bars: a = 50µm, b – i = 10µm.

Figure 4: 1. Normal looking rat upon dissection. **2.** Dissected rat showing liver full of *Taenia taeniaformis* cyts. **3.** *Mastomys natalensis* at dissection showing a *Taenia parva* tissue cyst. **4.** A single scolex released from *T. parva* cyst. **5.** Enlarged view of large and small hooks from a scolex of *T. parva*. **6.** Cyst of *T. parva* opened up to show the multiple scolices.

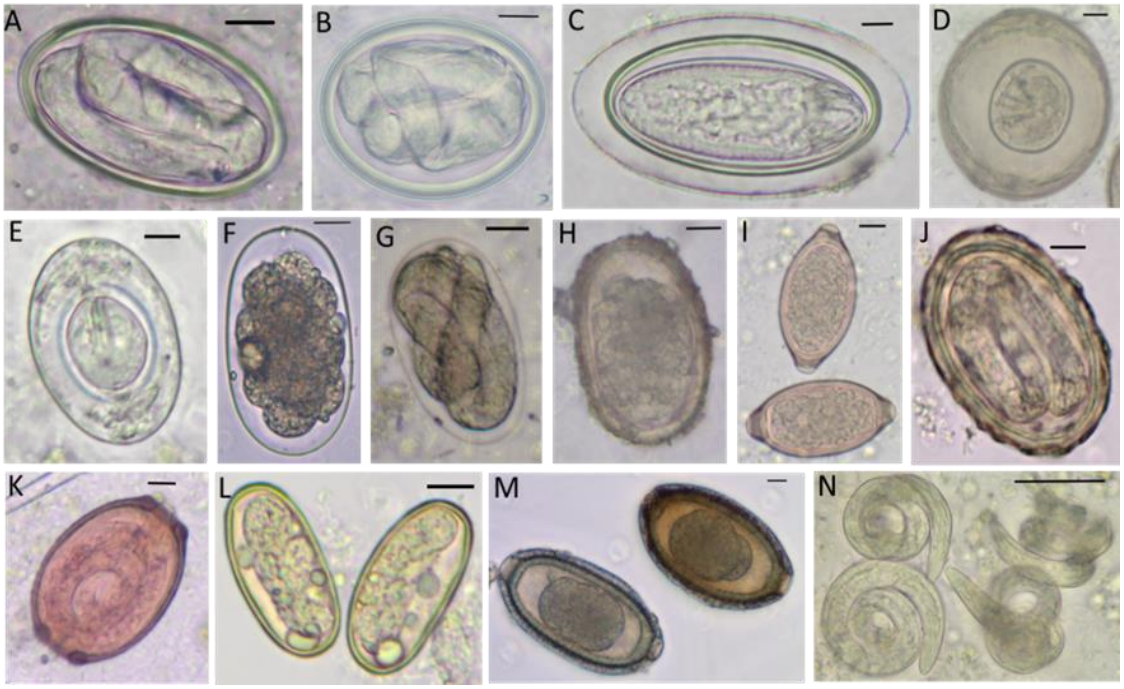

Figure 5: Eggs of: **A.** *Gongylonema neoplasticum*; **B.** *Protospirura muricola*; C. *Moniliformis moniliformis*; **D.** *Hymenolepis diminuta*; **E.** *H. nana*; **F.** *Nippostongylus brasiliensis*; **G.** *Strongyloides* spp., **H.** *Heterakis spumosa*; **I.** *Trichuris muris*; **J.** *Ascaris lumbricoides*; **K.** *Trichosomoides crassicauda*; **L.** *Dicrocoelium dendriticum*; **M.** *Calodium hepaticum*; and L_1 larvae of: **N.** *Angiostrongylus cantonensis*. All scale bars for A - M = 10µm; scale bar for N = 50µm.

DISCUSSION

None of the parasites discussed in this chapter fitted into any of the publications (Chapter 1 – 3), however, they are all important and of interest to anyone who has a passion for parasitology.

Members of all three rodent species mechanically transmitted infective stages of various parasites. The number of *R. norvegicus* mechanically transmitting *Strongyloides* spp. eggs was exceptionally high at > 40% and rats transmitting *H. diminuta* were around 13%. The *Trichuris* sp. eggs mechanically transmitted in IS and UPU could have been *T. muris* and/or the very similar *T. trichiura*. In IS especially, these eggs could have been those of *T. trichiura* as rats were found to transmit another human parasite's eggs, i.e. *Ascaris*, in sites within IS in Durban (Archer et al., 2017). With respect to the eggs of *T. crassicauda* mechanically transmitted or passed in the faeces of rats infected with this nematode, they could very well have been ingested while grooming, and both infected and non-infected rats would be equally as likely to ingest eggs from their urine-contaminated environment. Table I further demonstrates the degree of contamination of the rats' environment by the number of different helminth eggs/larvae being moved around via this oral-faecal route.

Protozoans are seldom documented in publications on the parasites of *Rattus* spp., although the amoebae and flagellates are similar to those found in humans and in primates, and belong to the same genera, they usually have the species name, 'muris'. Due to the scavenging and coprophagous nature of rats, it is difficult to know whether these are human protozoans they are mechanically transmitting or actual infections. All we can do with the results we have, is list them according to Bonfante et al. (1961) as in Table I, except for the report of possible *Eimeria parastieda*. Here, Berto et al. (2014) was consulted, and these 'bi-polar' oocysts were tentatively identified by the presence of what may well be a stiedal body at one end and parastiedal body at the opposite end (Figure 3). The only two protozoans present at a prevalence > 15% were examined using crosstabulations. *Eimeria nieschulzi* did not statistically differ across location, season and gender, however it was most prevalent in pups and decreased as rats aged. The flagellate trophozoites differed across location: they were more prevalent, though not very different, at UPU, followed by IS and HBR, but considerably lower (6.9%) at CBD. Age was not significant, but there was a gradual decrease in prevalence as rats aged.

The liver cysts of the feline cestode, *T. taeniaformis*, have been frequently reported in studies from across the world. Prevalence reports range from as low as 0.4% in Adak, Alaska (Schiller, 1952), to 65% in Chunchon, Korea (Seong et al., 1995). Mostly, numbers of cysts are low in wild caught rats, however occasionally, if there are large numbers, the liver can increase in size to the extent that it weighs one half of the mass of the rat (Miller and Dawley, 1928). Before beginning this study, I examined some rats sent to me by the Durban Natural History Museum, and one old rat had +/- 220 *T. taeniaformis* cysts in his liver (Figure 4). Examining cat faeces in localities where rats are infected with *T. taeniaformis* could give a better indication of infection risk. Ash (1962) found that 30% of rats in Hawaii were infected with the intermediate cyst stage in their livers and there was a correspondingly high prevalence in cats examined for the eggs passed by adult tapeworms in their faeces.

A Croatian study reported a prevalence difference between two villages and found that male rats had a higher prevalence of *T. taeniaformis* than females and that infection increased with age (Stojcevic et al., 2004). Locally, a study in Gauteng, South Africa reported this cestode's presence in *R. norvegicus, R. rattus* and *R. tanezumi* at an overall prevalence of 5.3% and mean intensity of 1.36 (Julius et al., 2017). As they followed Bush et.al. (1997) for parasite ecological terminology, this is the number of cysts found divided by the number of infected rats as opposed to abundance that is calculated as the number of cysts found divided by all the animals included in the sample. The overall prevalence in the present study, for both *Rattus* species was 18.5% and mean abundance was 0.44.

In Chapter 3, it was found that helminths were more prevalent in CBD and HBR, however *T. taeniaformis* cysts were most prevalent in IS and in males rather than females. Abundance followed a similar pattern. Age too was highly significant, with adults being 9.0 times as likely to be infected as juveniles and 168.0 times more likely than pups, and juveniles were 18.6 times more likely to be infected than pups. Cyst abundance also increased considerably with age (the IRR for a higher abundance in juveniles was 13.2 and adults was 105.3 times that of pups, and for adults compared to juveniles, the IRR was 8.0 times. The age relationship for this parasite proved interesting, as it was demonstrated that separating pups from juveniles, and juveniles from adults clearly showed that prevalence increased notably with age of the rats. This association would not have become apparent if the rough weight groups used by others had been followed, where some juveniles get put together with un-weaned pups and larger juveniles get placed in the adult group (Mohd Zain et al., 2012; Kataranovski et al., 2010), and this effect is then 'diluted'. Greenfield (1942) noted that very young rats were resistant to infection with this cestode cyst as they lacked the enzymes required to digest the tough embryo envelope of *T. taeniaformis* eggs, however, she states that the view of others that the lactating mother transfers immunity to her offspring, may also be a factor.

Trichosomoides crassicauda has, like *T. taeniaformis*, been reported from a number of countries across the globe, ranging from 5.4% in Adak rats (Schiller, 1952), to 47.1% in Taichung city, Taiwan (Tung et al., 2013). As opposed to the situation for the liver cysts, the same pattern is seen as for the GIT helminths, where the odds of CBD rats being infected with *T. crassicauda* were 2.6 times those at UPU and 6.9 times those at IS. HBR and UPU rats also had a higher odds ratio for infection than IS rats. Abundance was also higher at CBD, HBR and UPU than IS. Males were significantly more likely to be infected with the bladder-worm and to have a higher abundance than females. No pups were infected with the *T. crassicauda* as the pre-patent period is 8 – 9 weeks. The odds of adults being infected was 11.8 times that of juveniles and the IRR for a higher abundance in adults was 6.5 times that of juveniles. The age-parasite relationship is well demonstrated for this helminth as for the former. Kataranovski et al. (2010) suggest that as male rats wander further, their home ranges may overlap and this would allow them to be exposed to more parasite species and higher abundance of infection than females.

The trematode, *Brachylaemus peromysci*, has been recorded in *Peromyscus leucopus* and *Mus musculus* from Pennsylvania (Hall et al., 1955). In Mexico *Liomys irroratus* and *Peromyscus difficilis* were found infected with a new trematode species belonging to the family, Dicrocoellidae, *Caballerolecythus ibunami* n. sp. The eggs found in *M. natalensis* in Durban fell

into the size range of *Dicrocoelium dendriticum* (36-45 x 22-30 µm) and were clearly operculate (Figure 5).

The two body cavity cestode cysts, identified according to Loos-Frank (2000) as *Taenia parva*, are not unusual finds for the host genus *Mastomys*. Julius et al. (2017) recorded this intermediate cyst stage of *T. parva* in *M. coucha* in Gauteng Province, South Africa. The final (definitive) hosts are *Genetta*, *Herpestes*, *Felis* and *Ictonyx* species (Loos-Frank, 2000).

Toxoplasma gondii was included in Chapter 1 and is not discussed further here. *Rattus norvegicus* and *R. rattus* are equally at risk of becoming infected with *Trypanosoma lewisi*, it just depends on the relative number of species trapped. This blood parasite has been found across the globe and is a zoonosis of concern since it can infect people and symptoms vary from mild to severe depending on the health status of the patient. Both these parasites are discussed in Chapter 1.

The possible malaria trophozoites in the blood of the old *M. natalensis* male presents a problem as there does not appear to be any literature for South Africa on the occurrence of this haemoparasite in the blood of wild rodents. Anophelene mosquitoes vector malaria. In laboratory infections, *Anopheles stephensi* is used and in the wild in central Africa *A. dureni* is the natural vector of *P. berghei* (Killick-Kendrick, 1974).

The last parasite, the microfilaria found in *M. natalensis* is an enigma. The genus *Litomosoides* has been described from bats, rodents and opossums and is vectored by dermanyssid mites, which would make one lean towards this when looking to identify the 'find'. Unfortunately, the morphology is very different, as most of the microfilaria species in this genus are very short (< 150µm) with a rounded tail and or sheath. The possible sheath of the one found in the present study seems to fit closely to the body of the worm, only becoming visible at the end where it tapers off to a very long thin end (Figure 3). There is a strong possibility that the infection was accidental, possibly *Dirofilaria* sp.

To conclude this chapter: the two helminth infections (*T. taeniaformis* and *T. crassicauda*) endorsed the decision that was made to age the rats according to Hirata and Nass (1974) as this resulted in a clear relationship for each of these parasites with rodent age. The generally higher prevalence of protozoans in younger rats suggests a risk of infection for pups before they even leave the nest. As rats communally raise their young, this risk increases even more for all parasites that are either directly (anus-mouth) transmitted or those with an extremely short pre-patent period, e.g. ± 4 days for *Strongyloides* spp (Abadie, 1963). Although feral cats and domesticated

cats are found in all locations within Durban and could easily become the definitive host for the adult *T. taeniaformis* tapeworm, it may well be that the more common final hosts are in fact, wild cats, genets, mongooses and polecats, hence the occurrence of this parasite at UPU. The definitive hosts for this cestode, whether domestic or feral cats, could be what drives the prevalence and abundance in IS. It would really be worthwhile checking Durban's feral cats for parasites as the prevalence of not only the tapeworms would be worthwhile knowing, but also the prevalence of *Toxoplasma gondii* (see Chapter 1).

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GENERAL SUMMARY and CONCLUSIONS

The successful spread of synanthropic *Rattus* spp. across the globe has been ongoing since the advent of shipping (Karagas, 2015). Gratz (1999) estimated that by 2025, approximately 61% of the world's population will live in cities, particularly in developing countries. In South Africa, this influx of people into cities, and the low socio-economic situation, has resulted in shacks being erected, the illegal occupation of dwellings and general degradation of formal houses (Jassat et al., 2013). In Johannesburg, South Africa, rats were more commonly found in dwelling places that were informal, or in disrepair, were damp and crowded, and where refuse was never collected and income was low to non-existent, than in well-kept communities where income was > R 5000.00 per month (Jassat et al., 2013). This situation has likely facilitated an increase in numbers of synanthropic rats in most cities in developing countries, where harbourage is readily available and there are numerous food outlets, street-food vendors and restaurants to provide these 'unwelcome guests' with a smorgasbord of 'tasty' food, either stored or discarded in bins and on streets. In the hot and humid city of Durban arthropods too, e.g. grain beetles, find a good source of food and the ideal place to breed in stored grain products, while cockroaches happily live in moist dark places from where they can scurry around sourcing food left behind by humans. This all makes for an ideal situation for transmission of parasites, not only amongst rodents, but also between humans and rats.

As referenced in the preceding chapters, numerous studies on parasites of rats have been recorded from across the world, however most of the earlier ones are simply records of prevalence. More recent studies on *Rattus* spp. and indigenous rodents have examined extrinsic (e.g. location, season, year) and intrinsic (e.g. rat age and gender) factors. The present study is the first detailed account of endoparasites from urban rats, principally *R. norvegicus,* in Africa. Previous studies in eThekwini found that *R. norvegicus* was the most commonly trapped rodent (Tayor et al., 2008), however, *Rattus* spp. frequently occur together and *R. norvegicus* is not always the dominant species, especially in Asia (Paramasvaran et al., 2009).

Of the 400 rodents trapped in the present study, 94.8% were *R. norvegicus*, 2.5% were *R. rattus* and 2.7% were *Mastomys natalensis*. *Rattus norvegicus* harboured a total of 10 protozoans, one acanthocephalan, three cestodes and 10 nematodes, and mechanically transmitted six parasite species. *Rattus rattus* harboured five protozoans, one acanthocephalan, two cestodes and six nematodes, and mechanically transmitted 2 parasite species, while *M. natalensis* was infected with three protozoans, two cestodes and seven nematodes, and voided only trematode eggs while

not infected with the adult parasite. Most of the parasite species found are cosmopolitan (Luttermoser, 1936).

At the time of writing Paper I, *Gongylonema* worms had not yet been identified to species level and as rats carry both *G. neoplasticum* as well as *G. pulchrum* (the human species), this nematode was reported as *Gongylonema* sp. until later identified as *G. neoplasticum*. Also, the *Angiostrongylus* sp. worms found in *M. natalensis* were never identified to species as the extracted DNA, stored at -18°C, was unfortunately lost due to unforeseen circumstances (power outages) that resulted in repeated thawing and refreezing of the samples. It is perhaps important to note here that as the GIT protozoans were identified based on my knowledge of human protozoan infections, in the future these may change when molecular techniques are used.

To recapitulate on all parasites found and their associations (Chapters 1 – 4): Prevalences of gastrointestinal tract (GIT) protozoans were mostly low, except for *Eimeria nieschulzi* (38.3%) and flagellate trophozoites (15.8%). Unlike many multicellular parasites, these protozoans tended to be more prevalent in pups and decreased with age. Rats in CBD were far less likely than rats from the other three locations to be infected with flagellate trophozoites (species unknown). While *Toxoplasma gondii* infections were unrelated to location, season, rat age and gender, *Trypanosoma lewisi* was most prevalent and abundant in CBD rats (as was its vector, *Xenopsylla cheopis*) and juveniles were most affected.

Moniliformis moniliformis (Acanthocephala), had the lowest prevalence and abundance in IS rats (7.9%; 0.03 ±0.18) as compared with other locations. Four members of the class Cestoda were found in these rodents: *Hymenolepis nana* was found only in HBR in the wet season; *H. diminuta* was significantly more prevalent in CBD and HBR rats than in those from IS and UPU, and juvenile and adult rats were much more likely to be infected than pups. *Taenia taeniaformis* (intermediate host liver cysts/larval stage) were found in 18.2% of *R. norvegicus* and IS rats were most at risk of infection, as were adults, followed by juveniles and then pups, and males were more likely to be infected than female rats. *Taenia parva* was found in two *M. natalensis* (one metacestode/larval stage per mouse) from two unrelated sites within UPU. Season is, understandably, unlikely to be associated with larval cestodes as these infections develop over a period of time and remain with the host for the rest of its life.

The Nematoda comprised the largest number of helminths found in these rodents. The two spirurid nematodes, *Gongylonema neoplasticum* and *Protospirura muricola* were most prevalent at CBD and HBR and in rats as they aged. Prevalence of *Nippostrongylus brasiliensis* was the

same across locations, however abundance was higher in CBD and HBR than in the other two locations, and juvenile and adult rats were more prone to infection and higher abundance of this worm than pups. *Strongyloides ratti* and *S. venezuelensis*, together referred to as *Strongyloides* spp., were most prevalent in CBD and UPU and most abundant in the same two locations as well as at IS, and here season was significant in that rats in the wet season had a higher abundance of worms than rats trapped in the dry season. Prevalence, but not abundance of *Heterakis spumosa* was highest at CBD and HBR and both prevalence and abundance were higher in juveniles and adults rather than pups. Prevalence of *Syphacia muris* was higher in the wet than dry season and *Trichuris muris* was only found in one *R. rattus* species trapped at IS, and in four *M. natalensis*, one from an IS site and the other three from UPU sites. *Angiostrongylus cantonensis* was more prevalent and abundant in IS and UPU as opposed to CBD and HBR, in the wet rather than the dry season, and in rats as they aged. *Trichosomoides crassicauda*, was most prevalent at CBD and abundance was highest in CBD, HBR and UPU when compared with IS. No pups were infected, adults had a higher prevalence and abundance than juvenile rats and males were more prone to infection and a higher abundance of this nematode than females. *Calodium hepaticum* was only present in 2.6% of *R. norvegicus* and in one old male *M. natalensis*. *Trichuris muris* has mostly been reported at very low prevalence rates in *Rattus* spp. (< 6%, Kataranovski et al., 2011; 0.34%, Waugh et al., 2006), and Julius et al. (2017) reported *Trichuris* sp. only from *Mastomys coucha*, in Gauteng, South Africa. In the present study, *T. muris* was found in 1/10 *R. rattus* and in 4/11 *M. natalensis*.

All the above helminth infective stages, except for *P. muricola*, *S. muris*, and *T. muris*, were also mechanically transmitted by uninfected rats, most notably, *Strongyloides* spp. (41.4% of rats), followed by *H. diminuta* (13.2% of rats). This form of transmission, especially when as high as for *Strongyloides* spp. must surely play an important role in perpetuating the life-cycle and spreading infection within the rodent colony's home range. The role that rodents may play in mechanical transmission of infective life-stages of human parasites is seldom reported by investigators. The most common mechanically transmitted human parasite eggs found in the present study were those of *Ascaris lumbricoides*. Here, most of the rats found with *Ascaris lumbricoides* eggs in their gut contents (range 2-287 eggs/rat) were trapped at sites in IS, one of them a creche housed in a wooden hut under which numerous rat droppings were noticed. A previous study conducted in Durban informal settlements found that more than 80% of children were infected with geohelminths, most notably *Ascaris lumbricoides* (Appleton et al., 2009).

The minimal gender associations found in this study were also seen elsewhere (Milazzo et al., 2010; Mohd-Zain et al., 2012). The present study found that gender was significant for *T.*

taeniaformis and *T. crassicauda* prevalence and abundance. Chapter 1 found that for *A. cantonensis* abundance there was an interactive effect of season, age and gender and for *T. lewisi* abundance there was an interactive effect between location and gender.

Kataranovski et al. (2011) found that males were more prone to gastrointestinal helminths than females, but it should be considered that they trapped and examined 302 rats over a four year period, an average of only 75 rats per year. Although they found that the dry weight of the eye lens could be used to age rodents, there do not appear to be many parasite studies on rats where this method was employed. One would expect that these authors would then have aged their rats very specifically, however, Kataranovski et al. (2011) still categorised rats into juveniles/sub-adults (< 2.5 months old) and adults (> 2.5 months old) according to a differentiating rodent mass of 200gm. Mohd Zain et al. (2012) categorised *R. norvegicus* into rats of < 140 gm as Class 1, those of 140 – 240 gm as Class 2 and those > 240 gm as Class 3. Abu-Madi et al. (2001) used only two categories of < 100gm = juveniles and > 150 gm = adults. Franssen et al. (2016) used < 100gm for both female and male juveniles; 101 –175gm for young adult females, 101 – 200 gm for young adult males; > 175 gm for adult females and > 200 gm for adult males. Having said this, these studies found significant differences between age groups, although the results were not as marked as they may have been if pups were separated from the subsequent age group.

As mentioned in Paper III, Chapter 3, aging rats is difficult and, as with most animals, there will always be individuals that are either very large or very small for their age and in this paper we reported that seven pups were infected with *G. neoplasticum* even though the pre-patent period for this spirurid is ≥ 60 days (≥ 8.5 weeks) and pups were aged as ≤ 5 weeks. Looking back on the aging database, it was found that all seven of these 'pups' were aged between 2.5 to 4.0 weeks, with a mass rage of 38.4 – 69.3 gm and total body length + tail range of 220 – 272 mm. Due to the difficulties of accurately comparing studies where age is used as a criterion, it would be extremely helpful to have a reliable and standardised method that could be used globally for aging rodents in weeks (similarly to Hirata and Nass, 1974) but with a more fool-proof way of separating (most importantly) pups from juveniles, but also juveniles from adults, as I believe that this differentiation is especially important for understanding parasite dynamics in rodent populations.

Prior to 2006, our laboratory examined only the GIT helminths from rats euthanased by the Durban Natural Science Museum as part of the 'RatZooMan (Rodent Zoonosis Management) Project'. I was unfamiliar with all the parasites of rats and when I found numerous small tightly coiled larvae in the gut contents, the samples were sent to Onderstepoort Veterinary Institute, to aid in identification of some of the helminths found and to get clarity on the larvae. They could

also not identify them and only when I embarked on the present study, did I discover that they were L_1 larvae of *A. cantonensis* that are voided in rat faeces to be ingested by the snail intermediate host in order to perpetuate the life cycle. Other researchers may disregard them as environmental contaminants or, like Sumangali et al. (2012) report them as 'unidentifiable', thus it is recommended that to achieve comprehensive results, parasite studies on invasive rats should examine all the organs and not only the GIT and liver.

It may be prudent here to briefly discuss home range and to stress that it is different from territory in that it is the area frequented by rats and does not imply exclusive possession of, nor the need to defend the area (Davis et al., 1948). This article reported that of the rats (*R. norvegicus*) trapped, marked, and released within city blocks of Baltimore, between 75 and 88% were recaptured within 40 feet (12.2 metres) of the original capture site. Young females were the least adventurous (4.8% were recaptured in another building), adult females and young males followed with 10% of each group recaptured in a different building, and adult males were the most adventurous, with 11.6% recaptured in another building. Rats tested on farms were found to also travel very limited distances. By using a dye in corn that coloured the rats' faeces they were able to ascertain the range the rats moved around a baited food station on a farm, i.e. ±100 feet (±30.5m) in diameter (Davis et al., 1948).

We probably can safely assume that when a colony of rats has safe harbourage and a good source of food close by, then home range is limited. So, if home ranges are small, on one hand it could be expected that parasites would be hyper-transmitted within rat communities, and on the other hand, colonies with very few parasites and low abundance, may actually remain largely healthy. It should also be remembered that, with the exception of only a very few parasites (e.g. *Calodium hepaticum*), the aim of these organisms is not to kill the host as this would also result in their own death (Spratt and Singleton, 1986). Where parasite life-cycle stages are present that require the rat to be ingested by a final host in order to complete the life-cycle, host manipulation has been observed, e.g. rats with toxoplasmosis lose their fear of cats, the final host that is required to eat the rat to perpetuate the life-cycle of *Toxoplasma gondii* (Berdoy et al., 2000).

The parasites vectored by arthropods were generally most prevalent and abundant in the CBD and HBR. While we know from Chapter 2 that fleas were also highly prevalent in this location, we make the assumption based on what we know about the probability of there being plentiful stores of grains and cereals in the food shops, and Durban's notoriety for cockroaches, that the vectors too are likely plentiful here. Then, tying home range into the equation, it can be seen that location exerts a highly significant effect on these transmission cycles. So, as long as the equilibrium in

these areas is largely maintained, most rats should spend their lives in a fairly restricted area and the parasites that they carry and transmit would remain within their home range. In contrast, *A. cantonensis*, is vectored by land snails that are more commonly found at sites within IS and UPU, however, in this scenario, sites in IS and UPU are very far from one another (see the maps in any of Chapters 1 – 4), so parasite transmission patterns would be maintained at the site level within a location rather than at the location level.

Chapter 3 clearly showed that GIT helminth prevalence was lowest at IS. The interesting situation here, is that the city's Vector Control Unit knows that rats are highly prevalent in these settlements, however, they may have trapped at places where they knew that their traps would be looked after, or there is the possibility that poverty and crime preclude the inhabitants from buying large quantities of grains and cereals and they are more likely to live from hand to mouth, and this would reduce the number of intermediate hosts, which in turn would keep the numbers of infected rodents to a minimum. *Taenia taeniaformis* was significantly more prevalent and abundant in IS rats. Rats are the intermediate host and cats are required to eat the rat to contract the adult tapeworm and complete the life-cycle. Taylor et al. (2008) noted that semi-feral cats were plentiful in Cato Crest IS and that inhabitants sometimes shared the services of these cats to keep the rat population at bay. This facilitates both the perpetuation of the *T. taeniaformis* life-cycle, but also the transmission of *T. gondii* in these locations.

Unfortunately, one caveat of this study was that it was not possible to design the sampling protocol myself, especially in the slums, partly because of the high crime rates. Thus sampling effort was not consistent and equal across locations and seasons. Future work should standardise the sampling effort, the best method of aging the rodents should be very carefully applied as was attempted here, and all morphological identifications should be backed-up by DNA analyses. Notwithstanding the above, this study has considerably improved our knowledge of parasites of synanthropic rodents in Durban, as has the work of Julius et al. (2017) in Gauteng, and maybe researchers will be inspired to replicate the sampling in other cities in South Africa.

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APPENDIX A

UNIVERSITY OF
KWAZULU-NATAL

RESEARCH OFFICE
WESTVILLE CAMPUS
E-Mail: moodleyv@ukzn.ac.za
Tel.: 27-31-260 2273 Fax: 27-31-260 2384

29 January 2009

Reference: 031/09/Animal

Mrs CE Archer
Research Assistant / MSc student
School of Biological and Conservation Sciences
University of KwaZulu-Natal
WESTVILLE

Dear Mrs Archer

Ethical Approval of Research Project using Animals

I have pleasure in informing you that on recommendation of the review panel, the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2009 on the following project:

"Endoparasites of Invasive Rats in eThekwini Metro".

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar
Research Office
Head of School

APPENDIX B

DURBAN NATURAL SCIENCE MUSEUM
PO Box 4085 Durban 4000 South Africa Tel (031) 3112241/56 Fax (031) 3112242

20 Jan 2009

Prof Chris Appleton
School of Biological & Conservation Sciences
UKZN

Dear Chris

Permit to collection small mammals

This letter confirms that the Durban Natural Science Museum is authorized to collect rodents and other small mammals under a permit from Ezemvelo KZN Wildlife (Permit No. 4827/2007) which is renewed annually. This applies to any rats which be included in the MSC studies of your students, Colleen Archer and Karen Hope. Rodents will be euthenased in accordance with the international ethical guidelines of the American Society of Mammalogists (*Journal of Mammalogy* 88(3):809–823, 2007).

Regards

Peter Taylor
Curator of Mammals

APPENDIX C

SCIENTIFIC LETTERS

The rat lung-worm *Angiostrongylus cantonensis*: A first report in South Africa

C E Archer, C C Appleton, S Mukaratirwa, K J Hope

To the Editor: A study of the parasites of invasive rats in the eThekwini Municipality of KwaZulu-Natal has led to this first report of *Angiostrongylus* (*Parastrongylus*) *cantonensis*, commonly known as the rat lung worm, in South Africa. *A. cantonensis* is clearly endemic in this region and probably also in other areas of South Africa. There are a few reports of this nematode from Africa (excluding South Africa): in rats and snails in Egypt (Fouad and Abdulla, 1978), in snails in Nigeria (Sowemimo and Asaolur, 2004), and a human case from Ivory Coast (1980).[1] As humans are accidental hosts, the parasite cannot complete its life cycle, and immature worms lodge in the central nervous system where they elicit a condition known as eosinophilic meningitis.

Background

A. cantonensis is endemic in rats in Asia, the Pacific Islands, China, Australia and parts of North and South America, where human cases of eosinophilic meningitis are common.[2]

The life cycle of *A. cantonensis* involves the rat as definitive host and a mollusc (snail or slug) as intermediate host. Adult worms live in the pulmonary arteries of infected rats, where they mate and lay eggs. First-stage larvae hatch, ascend the bronchial tree, are swallowed, pass out in the rat faeces and are ingested by an intermediate host. Fish, shellfish, frogs and monitor lizards serve as paratenic hosts, while vegetables contaminated by slime-trails and faeces of molluscs are also sources of infection for the definitive host. If a rat ingests any of these, the infective third-stage larvae penetrate the gut wall and are carried via the bloodstream to the brain where they moult to fourth-stage larvae, grow rapidly to about 1 mm, re-enter the bloodstream and are carried to their final site in the pulmonary arteries, where they feed on blood and mature to adulthood. Mature females are 18.5 - 33 mm in length and 0.28 - 0.5 mm in breadth, and males measure 15.5 - 22 mm by 0.25 - 0.35 mm. One female may lay up to 15 000 eggs per day.[3]

Humans are accidentally infected when they ingest raw or undercooked, infected intermediate or paratenic hosts, or vegetables contaminated with third-stage larvae. The parasites migrate from the gastrointestinal tract via the circulatory system, to the brain where they remain and die, so not completing their life cycle. There have been reports of patent infections (i.e. adolescent worms reach the lungs, mature to adults, mate and lay eggs) where the patients have died, but these are exceptional cases.[4]

Many species of snails and slugs serve as intermediate hosts for *A. cantonensis* but no survey has been done in South Africa.

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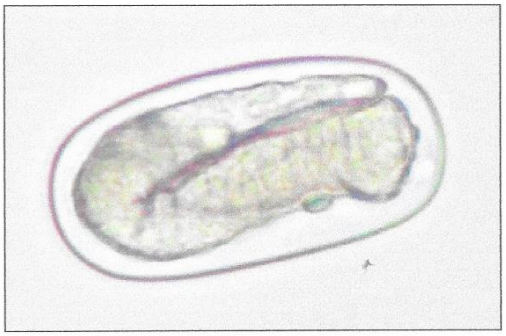

Fig. 1. A. cantonensis *egg.*

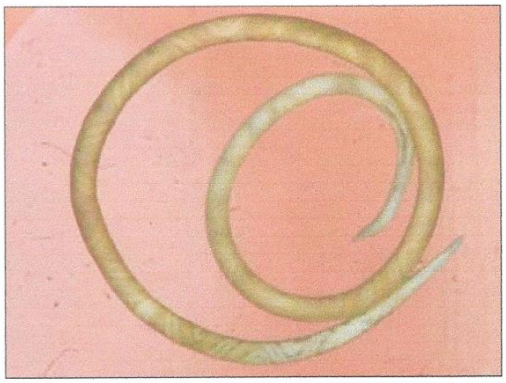

Fig. 2. A. cantonensis *female.*

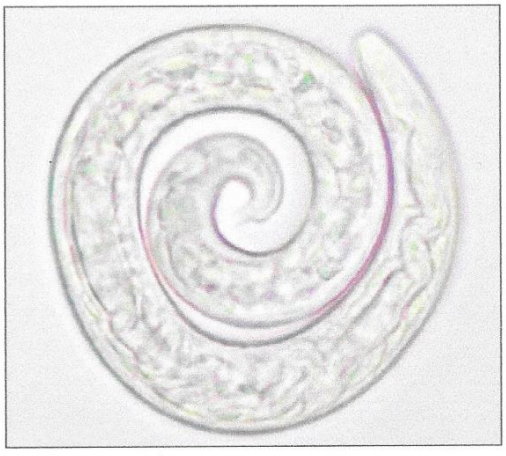

Fig. 3. A. cantonensis *larva.*

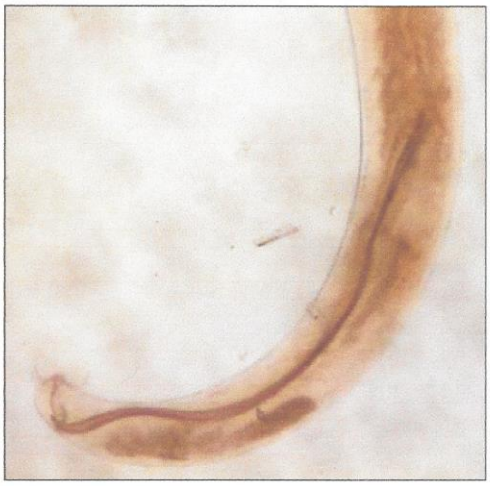

Fig. 4. A. cantonensis *male rear end.*

The study

eThekwini Health's Vector Control Programme has authorisation from the Durban Natural Science Museum (permit no. 4827/2007) to collect live specimens of *Rattus* spp. in baited traps. Our study in 2009 on parasites of invasive rats fitted in with this programme (approval by University of KwaZulu-Natal's Ethics Committee: Ref. 031/09/Animal). A total of 398 *Rattus* spp. (391 *R. norvegicus* and 7 *R. rattus*) were trapped during the year. Once killed, all ecto- and endoparasites were recovered for identification. Detailed results for these will be published in other journals. The heart and lungs were examined under a dissecting microscope.

Adult worms were isolated from the heart, pulmonary arteries and arterioles of 56 rats, (55 *R. norvegicus* and 1 *R. rattus*), giving an overall prevalence of 14.1%. Polymerase chain reaction (PCR) testing confirmed the identification of the worms as *A. cantonensis*. Further PCR testing and parasite morphology will be carried out and the results published.

Clinical information

When healthy patients become infected with *A. cantonensis*, the larvae and sub-adults remain in the CNS, commonly causing eosinophilic meningitis/meningoencephalitis. Other symptoms may result from human infections with this parasite and, although not as common, may cause severe illness and occasionally death.

After being ingested, infective larvae migrate to the CNS, resulting in eosinophilia in the CSF (30 - 45%; 16 - 72%) and to a lesser degree in the peripheral blood (20 - 32%; 0 - 19%).[5,6] (The first pair of figures in each set of parentheses (i.e. 30 - 45% and 20 - 32%) were taken from Panackel *et al.*, and the second set from Koo *et al.*) Clinical symptoms may also begin at this time or up to 4 weeks later. Patients most commonly present with severe headaches, neck stiffness, nausea, vomiting, confusion and hyperaesthesia. Other symptoms include papilloedema, hemiparesis, facial paralysis, hyperreflexia, fever and visual problems.[7]

Human angiostrongyliasis is usually mild and self-limiting, and recovery takes place within a week, but paraesthesiae and muscular weakness may persist for years and represent chronic forms of the disease. Such cases may indicate heavy infections.

A spinal tap is necessary for constructing a diagnosis of angiostrongyliasis, as eosinophilia is initially more likely to be seen in the CSF than in peripheral blood, and *A. cantonensis* larvae may occasionally be identified.[8] It is advisable to specifically request the laboratory to perform an eosinophil count on the CSF. This requires centrifuging of the sample, making a smear from the deposit and staining with a routine haematological stain, such as Wright's.

There is no specific treatment, but high doses of the corticosteroid, prednisone (40 - 60 mg daily) for a few weeks, or in chronic cases a few months, is helpful.[9] CSF aspiration is effective for the relief of intracranial pressure and consequently the persistent headaches experienced by most patients. Other therapies include analgesics, antibiotics and anthelminthics.[7]

Although angiostrongyliasis is the most common cause of eosinophilic meningitis, the differential diagnoses include other infectious diseases, allergic reactions and malignancies. Among the parasitic diseases included here is gnathostomiasis – caused by the Asian nematode *Gnathostoma spinigerum*.[5]

Conclusions

A. cantonensis is clearly endemic in the urban rat population of the eThekwini area of KwaZulu-Natal, and may be more widespread. It might also have spread to the indigenous rodent community. Since *A. cantonensis* is the most common cause of eosinophilic meningitis in people, a retrospective analysis of all cases of eosinophilic meningitis of unknown aetiology is necessary to assess the likelihood of human *A. cantonensis* infections occurring in South Africa.

Although fatalities are uncommon in eosinophilic meningitis caused by angiostrongyliasis, young children and immuno-compromised people are at risk of developing a fatal patent infection (i.e. the larvae migrate to the heart and lungs, mate and lay eggs) with severe lung pathology. This is consequently another opportunistic infection to be aware of in AIDS patients!

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