# Sokoine University of Agriculture


## PhD Thesis

# Rodent Borne Pathogens Infecting *Mastomys Natalensis* in Selected Areas of Morogoro and Iringa, Tanzania

**Claus Augustino Thomas**

**February, 2024**

# Rodent Borne Pathogens Infecting *Mastomys Natalensis* in Selected Areas of Morogoro and Iringa, Tanzania

***A Thesis submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy of Sokoine University of Agriculture. Morogoro, Tanzania***

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## EXTENDED ABSTRACT

The study on the rodent borne pathogens infecting *Mastomys natalensis* in selected sites of Morogoro and Iringa was conducted between January, 2021 and July, 2022. Various studies have shown that, human and animal diseases caused by viruses, bacteria, and parasites carried by rodents are on the increase, however, relatively, little is known of the prevalence and dynamics of infections by *Trichuris* spp. *Leptospira* spp. *Bartonella* spp. and other helminths in rodents and their ectoparasites. Similarly, little is known on the effectiveness of broad spectrum anthelminthics on gastrointestinal parasites of rodents.

In this study, the prevalence and seasonal variation of *Trichuris* worm infection in rodents was investigated. In addition, molecular detection of *Leptospira* spp. and *Bartonella* spp. in *M. natalensis* and its ectoparasites was studied. Furthermore, the effect of Ivermectin® against gastrointestinal helminths was determined. These studies were carried out between January 2021 and July 2022.

The study aimed at addressing three key specific objectives; i) Determining prevalence and seasonal variation of *Trichuri*s worms infection in *M. natalensis* in Morogoro and Iringa regions ii) Molecular detection of *Leptospira* spp. and *Bartonella* spp. in *M. natalensis* and its ectoparasites in Morogoro iii) Determining the effect of Ivermectin® on Intestinal helminths in the multimmate mouse *(M. natalensis).*

The study sites were in Morogoro and Iringa regions. In Morogoro region, the studies were carried out in Choza, Kiroka and in an enclosed area (Fence) close to Sokoine University of Agriculture main campus. For Iringa region, Isimani and Idodi villages were selected.

*Mastomys natalensis* was used throughout the study as the model rodent species because it is known to carry a number of

pathogens. Also, it is the most abundant, dominant and most studied species of the small mammals in Tanzania.

To address the first specific objective, the study was conducted between January and November, 2021 in Morogoro and Iringa regions. These regions differ significantly in their eco-climatic conditions. Removal trapping was conducted using Sherman® live traps in rainy and dry seasons. Gastrointestinal tracts of captured rodents were screened for the presence of *Trichuris* worms and identified using morphological keys. The effect of geographical region, season and sex of the rodents on *Trichuris* worm infections were tested using a generalized linear model with binomial function. Data were analyzed using R Statistical Software 4.1.3 at a p- value of 0.05. For the second objective, *M. natalensis* were captured live in fallow habitats using Sherman® traps and anesthetized using halothane. Blood samples were obtained from the retroorbital sinus and ectoparasites were removed from the fur using a hard brush and preserved in 70% ethanol. Real Time – qPCR followed by Sanger sequencing was used to detect *Leptospira* spp. and *Bartonella* spp. from the blood and ectoparasites respectively. Confidence intervals (95% CI) for the prevalence of *Bartonella* spp. and *Leptospira* spp. in *M. natalensis* was determined at the level of alpha of 0.05. The statistical difference in *Bartonella* spp. infection in mites from males and females *M. natalensis* was determined using a two-tailed student's t-test.

For the third objective, rodents were live captured from the open field as well as free ranging rodents from "Fence". The animals were marked, caged individually and supplied with feed and water *ad libitum*. A total of 45 animals were released into each of two enclosed plots (treatment and control groups).

The treatment group received a single dose of an aqueous suspension of Ivermectin® (0.0007ml/l), while the control group was left untreated. The animals from both plots were captured biweekly and screened for helminths eggs over a period of eight

weeks. Analysis of variance (ANOVA) was used to determine the mean variation of gastrointestinal helminths burden before and after treatment with Ivermectin®.

The following were the results of these studies. i) A total of 200 *M. natalensis* were studied from each of the two regions, consisting of 100 animals per season. For Morogoro, the overall prevalence of *Trichuris* worms in *M. natalensis* was 36% (n=72), of which 42/200 and 30/200 were for the rainy and dry seasons respectively. For Iringa, the overall prevalence was 65% (n=130), of which 80/200 and 50/200 were for the rainy and dry seasons respectively. *Trichuris* worm infection was significantly higher during the rainy season in Iringa than in Morogoro and no significant difference in infection was observed between males and females in either of the two regions or seasons. Other forms of helminths detected were *Strongyloides* spp., *Capillaria* spp., *Hymenolepis* spp. and eggs of a yet to be confirmed helminths, possibly an *Anoplocephalid* sp.

ii) For the molecular studies, *Leptospira* spp. was demonstrated in one out of 100 *M. natalensis* while, for *Bartonella* spp., the prevalence of (14%) was recorded in mites with a higher proportion in adult males than in females. Upon Sanger sequencing, four positive samples showed a complete sequence of the *ITS* gene. Indicating that all samples belonged to *Uncultured Bartonella*.

iii) With regard to the effect of Ivermectin® on intestinal helminths, there was no significant difference in *Strongiloides* spp.*, H. nana* and *Physaloptera* spp. infection before and after treatment.

However, a significant reduction of *H. diminuta* and *Trichuris* worms was observed in the treated animals. No significant increase in the number of worms recorded in the control group. The overall prevalence of helminths in *M. natalensis* was significantly higher in male than female rodents.

To address the gaps identified from these studies, there is a need for improved surveillance of rodents borne diseases in the studied regions and elsewhere and to establish strategic control programs to reduce their adverse impact on health. This is important, considering that rodents and specifically *M. natalensis* is the most abundant rodent pest species in sub-Saharan Africa and maintenance host and carrier of diverse zoonotic pathogens. Further interventions to raise awareness of the role of commensal rodents and their ecto/endoparasites in disease transmission are recommended. Also, Ivermectin® is recommended for use in treatment against helminths. However, it is recommended to investigate the scope of its effectiveness in diverse helminths.

**Keywords**: Rodents, *Mastomys natalensis*, Trichuriosis, *Leptospira spp., Bartonella spp.*

## IKISIRI KUU

Utafiti wa vimelea vya panya wanaoambukiza Mastomys natalensis katika maeneo teule ya Morogoro na Iringa ulifanyika kati ya Januari, 2021 na Julai, 2022. Tafiti mbalimbali zimeonyesha kuwa, magonjwa ya binadamu na wanyama yanayosababishwa na virusi, bakteria na vimelea vinavyobebwa na panya ni juu ya ongezeko, hata hivyo, kwa kiasi, hakuna taarifa ya upana wake juu ya kuenea kwa hivi vimelea na mienendo ya maambukizi hasa vimelea aina ya Trichuris spp. Leptospira spp. Bartonella spp. na minyoo katika panya na vimelea vya wadudu zao. Vile vile, kuna upungufu wa taarifa juu ya ufanisi wa anthelminthics ya wigo mpana juu ya vimelea vya utumbo wa panya.

Katika utafiti huu, kuenea na tofauti za msimu za maambukizi ya minyoo ya Trichuris katika panya ilichunguzwa. Kwa kuongeza, kugundua molekuli ya Leptospira spp. na Bartonella spp. katika M. natalensis na vimelea vya wadudu yake ilichunguzwa. Zaidi ya hayo, athari ya Ivermectin® dhidi ya minyoo ya utumbo imedhamiriwa. Masomo haya yalifanywa kati ya Januari 2021 na Julai 2022.

Utafiti ulilenga kushughulikia malengo mahususi matatu muhimu; i) Kubainisha kiwango cha maambukizi na mabadiliko ya msimu wa maambukizi ya minyoo aina ya Trichuris katika M. natalensis katika mikoa ya Morogoro na Iringa ii) Utambuzi wa molekuli ya Leptospira spp. na Bartonella spp. katika M. natalensis na vimelea vya wadudu zake huko Morogoro iii) Kuamua athari za Ivermectin® kwenye minyoo ya matumbo kwenye panya ya multimmate (*M. natalensis*).

Maeneo ya utafiti yalikuwa katika mikoa ya Morogoro na Iringa. Mkoani Morogoro, tafiti hizo zilifanyika Choza, Kiroka na katika eneo lililofungwa (Uzio) karibu na Chuo Kikuu cha Sokoine cha Kilimo kampasi kuu. Kwa mkoa wa Iringa, vijiji vya Isimani na Idodi vilichaguliwa. Panya aina ya Mastomys natalensis ilitumika katika kipindi chote cha utafiti kama spishi ya panya kwa sababu inajulikana kubeba idadi ya vimelea vya magonjwa. Pia, ni spishi nyingi zaidi, zinazotawala na zilizochunguzwa zaidi za nchini Tanzania.

Ili kukabiliana na lengo mahususi la kwanza, utafiti ulifanyika kati ya Januari na Novemba, 2021 katika mikoa ya Morogoro na Iringa. Mikoa hii inatofautiana sana katika mazingira yao ya hali ya hewa. Utegaji wa ulifanywa kwa kutumia mitego ya moja kwa moja ya Sherman® katika misimu ya mvua na kiangazi. Njia za utumbo za panya waliokamatwa zilichunguzwa kwa uwepo wa minyoo ya Trichuris na kutambuliwa kwa kutumia funguo za kimofolojia. Athari za eneo la kijiografia, msimu na jinsia ya panya kwenye maambukizo ya minyoo ya Trichuris yalijaribiwa kwa kutumia modeli ya jumla ya mstari yenye utendaji wa binomial. Data ilichanganuliwa kwa kutumia Programu ya Takwimu ya R 4.1.3 kwa thamani ya p-0.05. Kwa lengo la pili, M. natalensis walinaswa moja kwa moja katika maeneo ya mashambani kwa kutumia mitego ya Sherman® na kutiwa ganzi kwa kutumia kemikali ya halothane. Sampuli za damu zilipatikana kutoka kkatika vein ya retroorbital.

Vimelea vya wadudu ziliondolewa kwenye manyoya kwa kutumia brashi ngumu na kuhifadhiwa katika ethanol 70%. Wakati Halisi - qPCR ikifuatiwa na mpangilio wa Sanger ilitumiwa kugundua Leptospira spp. na Bartonella spp. kutoka kwa damu na vimelea vya wadudu kwa mtiririko huo. Vipindi vya kujiamini (95% CI) kwa kuenea kwa Bartonella spp. na Leptospira spp. katika *M. natalensis* iliamuliwa kwa kiwango cha alpha ya 0.05.

Tofauti ya takwimu katika Bartonella spp. maambukizi katika utitiri kutoka kwa wanaume na wanawake M. natalensis ilibainishwa kwa kutumia mtihani wa  wa mwanafunzi mwenye mikia miwili.

Kwa lengo la tatu, panya walinaswa moja kwa moja kutoka kwa uwanja wazi na vile vile panya kutoka kwa "Uzio". Wanyama waliwekwa alama, wamefungwa kila mmoja na walipewa malisho na maji. Jumla ya wanyama 45 walitolewa katika kila moja ya viwanja viwili vilivyofungwa (makundi ya matibabu na udhibiti). Kikundi cha matibabu kilipokea dozi moja ya kusimamishwa kwa maji ya Ivermectin® (0.0007ml / l), wakati kikundi cha udhibiti kiliachwa bila kutibiwa. Wanyama hao kutoka sehemu zote mbili walikamatwa kila wiki mbili na kuchunguzwa kwa mayai ya minyoo katika kipindi cha wiki nane. Uchambuzi wa tofauti (ANOVA) ulitumiwa kuamua tofauti ya wastani wa kiwango cha minyoo ya utumbo kabla na baada ya matibabu na Ivermectin®.

Yafuatayo yalikuwa matokeo ya tafiti hizi. i) Jumla ya M. natalensis 200 zilifanyiwa utafiti kutoka kila kanda mbili, zikiwa na wanyama 100 kwa msimu. Kwa Morogoro, jumla ya maambukizi ya minyoo aina ya Trichuris katika M. natalensis ilikuwa 36% (n=72), ambapo 42/200 na 30/200 walikuwa wa misimu ya mvua na kiangazi mtawalia. Kwa Iringa, kiwango cha maambukizi kilikuwa 65% (n=130), ambapo 80/200 na 50/200 walikuwa wa msimu wa mvua na kiangazi mtawalia. Maambukizi ya minyoo aina ya Trichuris yalikuwa makubwa zaidi wakati wa msimu wa mvua huko Iringa kuliko Morogoro na hakuna tofauti kubwa ya maambukizi iliyoonekana kati ya panya wa kiume au wa kike katika mojawapo ya mikoa hiyo miwili au misimu. Aina zingine za minyoo zilizogunduliwa ni *Strongyloides spp., Capillaria spp., Hymenolepis spp.* na mayai ya minyoo ambayo bado hayajathibitishwa, labda *Anoplocephalid* sp.

ii) Kwa masomo ya molekuli, Leptospira spp. ilionyeshwa katika moja kati ya 100 M. natalensis wakati, kwa Bartonella spp., maambukizi ya (14%) yalirekodiwa katika sarafu na idadi kubwa zaidi kwa panya wa kiume wa wazima kuliko wakike. Baada ya mpangilio wa Sanger, sampuli nne chanya zilionyesha mlolongo

kamili wa vinasaba vya ITS. Ikionyesha kuwa sampuli zote ni za Uncultured Bartonella Asiyekuwa na utamadunisho.

iii) Kuhusiana na athari za Ivermectin® kwenye minyoo ya matumbo, hakukuwa na tofauti kubwa katika Strongiloides spp., H. nana na Physaloptera spp. maambukizi kabla na baada ya matibabu. Hata hivyo, upungufu mkubwa wa minyoo ya H. diminuta na Trichuris ulionekana katika wanyama waliotibiwa. Hakuna ongezeko kubwa la idadi ya minyoo iliyorekodiwa katika kikundi cha kudhibiti. Kiwango cha jumla cha maambukizi ya minyoo katika M. natalensis kilikuwa kikubwa zaidi kwa panya wa kiume kuliko wa kike.

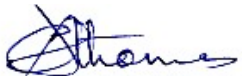
Ili kukabiliana na maeneo ambayo hajafanyiwa tafiti yaliyoainishwa na tafiti hizi, kuna haja ya kuboreshwa kwa ufuatiliaji wa magonjwa yanayoenezwa na panya katika mikoa iliyofanyiwa utafiti na kwingineko na kuanzisha programu za udhibiti wa kimkakati ili kupunguza athari zao kwa afya. Hili ni muhimu, kwa kuzingatia kwamba panya na hasa M. natalensis ndio aina ya wadudu waharibifu walio wengi zaidi katika Afrika Kusini mwa Jangwa la Sahara na ni mwenyeji na mbeba vimelea mbalimbali vya magonjwa ya zoonotiki. Hatua zaidi za kuongeza ufahamu wa jukumu la panya wa katika uambukizaji wa magonjwa zinapendekezwa. Pia, Ivermectin® inapendekezwa kwa matumizi katika matibabu dhidi ya minyoo. Hata hivyo, inashauriwa kuchunguza upeo wa ufanisi wake katika minyoo mbalimbali.

**Maneno muhimu:** Panya, *Mastomys natalensis*, Trichuriosis, *Leptospira* spp., *Bartonella* spp.

## DECLARATION

I, Claus Augustino Thomas, do hereby declare to the Senate of Sokoine University of Agriculture that, this thesis is my own

original work done within the period of registration and that it has neither been submitted for a degree award in any other institution.



_____ **19/01/2024**

Claus Augustino Thomas Date

(PhD Candidate)

The above declaration is confirmed by;

_____ **9/01/2024**

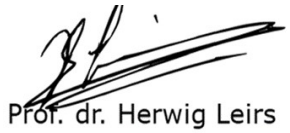
Dr. Isaac Makundi Date

(Supervisor)

_____ **12/01/2024**

Prof. Robert S. Machang'u Date

(Supervisor)

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(Supervisor) Date

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## DEDICATION

This work is dedicated to my family and friends. A special gratitude to my beloved parents, Augustino Thomas and Adellah Thomas for consistent prayers and encouragement to complete this work. To my very special and loving siblings who have never left me alone.

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## LIST OF ABBREVIATIONS/ACRONYM

| | |
|---|---|
| ACE-IRPM& BTD | African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development |
| EPG | Egg Per Gram |
| EVECO | Evolutionary Ecology Group at the |

| | |
|---|---|
| | University of Antwerp |
| IHI | Ifakara Health Institute |
| IPM | Institute of Pest Management |
| qPCR | Quantitative Polymerase Chain Reaction |
| SFUCHAS | St. Francis University College of Health and Allied Sciences |
| SUA | Sokoine University of Agriculture |
| WHO | World Health Organization |

# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION

### 1.1 Background Information

Rodents represent 42% of mammalian species (Wilson & Reeder, 2005) and they are reservoirs of pathogens of diseases such as plague, Lassa fever, murine typhus and many others diseases in humans (Hardin *et al.,* 2019). They are mammals with broad and complex ecological range; they are cosmopolitan except in Antarctica (Sharma, 2013). Because of their high reproductive potential, rodents have successfully exploited a wide variety of habitats and environments throughout the world (Makundi *et al*., 2003). Also, being in proximity to humans they constitute a potential threats for human health by being reservoir of many pathogenic diseases, inluding, leptospirosis, bartonellosis, rickettsioses, lyme borreliosis, toxoplasmosis, babesiosis, trypanosomiasis, trichuriasis as well as viral (hantaviral, arenaviral and coronaviral diseases (Griffiths *et al.,* 2011; Schmidt *et al.,* 2014 and Gryseels *et al.*, 2015).

*Mastomys natalensis* is a species of rodents in the family Muridae, also known as the Natal multimammate mouse is the most commonly found rodent species in Sub Saharan Africa. Its population dynamics is reported to have regular seasonal fluctuations, occasionally resulting to population outbreaks (Leirs *et al.,*2014). The species are hosts of various pathogens and are involved in the transmission cycles of bacterial, viral and parasitic diseases. They closely associate with humans and are commonly found in and around human settlements (Leirs *et al.,* 1997; Leirs *et al.,*2014 and Gryseels *et al.,* 2015).

### 1.2 Literature Review

#### 1.2.1 Rodents as hosts of zoonoses

Rodent-borne diseases can be spread to humans via three major transmission pathways. The first pathway is the direct route in

which there is contact between the rodents and people, e.g. by eating rodents or touching them (Thomas *et al.,* 2020). Indirect transmission occurs following exposure to rat-infected feces, urine, or in contact with objects that have been contaminated with rodent excreta (e.g. leptospirosis) or air (e.g., hantaviruses) (Meerburg and Kijlstra, 2009), and also by indirect transmission via arthropods (Figure 1).

### 1.2.2 *Mastomys natalensis*

*Mastomys natalensis* is among the most abundant and dominant rodent species in Sub-Saharan Africa, (Makundi *et al.,* 2003, Mlyashimbi *et al.,* 2020). The species is highly prolific and has successfully colonized a wide range of habitats and environments (Mariën *et al.,* 2022; Mlyashimbi *et al.,* 2020). In addition, their populations is markedly seasonal, depending on the availability of rainfall and food abundance (Mlyashimbi, 2018).

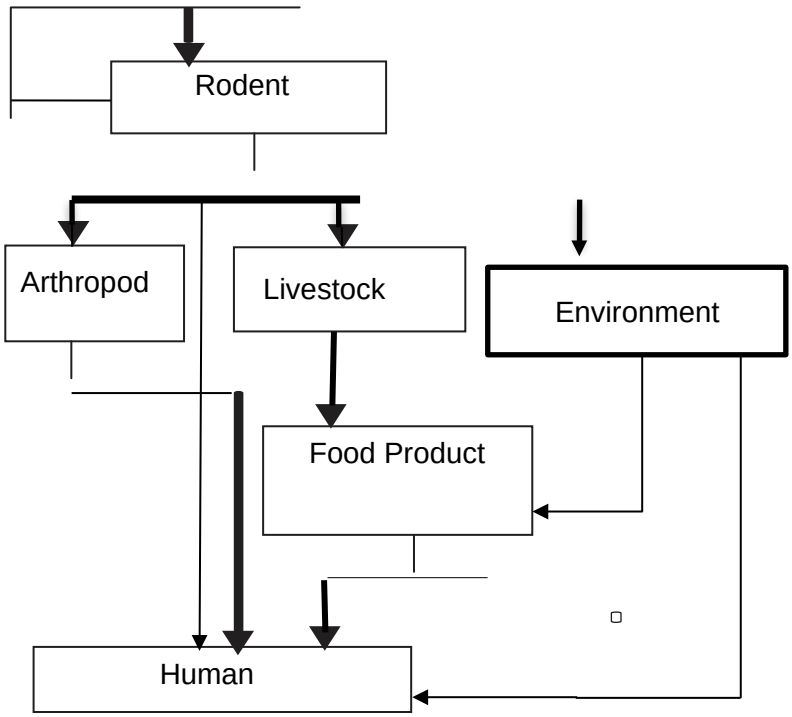


**Figure 1: Pathogen transmission pathways from rodents to humans**
Adapted and modified from Meerburg *et al.* (2009)

### 1.2.3 Common rodent borne pathogens

#### 1.2.3.1 Bacteria

Rodent is one of the group of small mammals known to be a reservoir of different pathogenic bacteria for example, *Yersinia pestis*, *Leptospira* spp. and *Bartonela* spp. (Kilonzo *et al*., 2005, Machang'u *et al*., 2004). Following are few example of bacteria pathogens:

***Leptospira* spp**

Leptospirosis is a rodent borne zoonosis of cosmopolitan in distribution (Machang'u *et al.,* 2004). It is shaped like a corkscrew with hooked and spiral ends. Infect humans, dogs, rodents and many other wild and domesticated animals. The disease is caused by a spirochete of the genus *Leptospira,* which consists of 22 species (Boey *et al.,* 2019a). According to WHO, at least 1.03 million cases of leptospirosis occur annually worldwide with 58 900 deaths reported. In the East and Central African region, leptospirosis is known to be prevalent for more than three decades (Machang'u *et al.,* 2004) and Tanzania is among the countries of Sub-Saharan Africa where leptospirosis is endemic (Mgode *et al.*, 2021). Any mammal can be infected with one or more *Leptospira* serovars, however, rodents are the most commonly affected animals which are also the major natural reservoirs of this microorganism (Dehio, 2001a; Mgode *et al.,* 2021b). In animals, including humans, Leptospirosis present with varieties of clinical manifestations, ranging from mild, flue-like symptoms to severe, febrile conditions associated with in appetence, stiffness, abdominal pain and occasionally fatal septicaemic complications (Desai *et al.,* 2009).

***Bartonella* spp**

Bacteria of the genus *Bartonella* are small, fastidious, slow-growing gram-negative aerobic rods, they are Facultative intracellular parasites (Dehio, 2001a). To date they are 45

described species and subspecies of *Bartonella* of which 20 are found in rodents (Gutiérrez *et al.,* 2015; Malania *et al.,* 2016; Theonest *et al.,* 2019). Global prevalence of 6.4 cases per 100,000 people in adults and 9.4 cases per 100,000 people in children aged 5 to 9 years (Theonest *et al.,* 2019).

It is reported that, rodent associated *Bartonella* are the cause of human infections particularly in areas where humans have close contact with rodents. Recently, *Bartonella* spp. have been reported to cause febrile illnesses in humans in northern Tanzania (Theonest *et al.,* 2019). The *Bartonella* spp. cause a disease with a wide range of clinical manifestations in humans and animals, for example: Cat-scratch fever, trench fever, Carrion's disease, bacteremia with fever, bacillary angiomatosis, peliosis, endocarditis and neuroretinitis (Broecke, 2021; Malania *et al.,* 2016). *Bartonella* spp. causes damage of the endothelial cells and erythrocytes of their mammalian hosts (Malania *et al.,* 2016).

#### 1.2.3.2 Rodent Helminthiasis

Helminthiasis are considered to be neglected diseases with low public health importance because they cause diseases that are not severe. Nevertheless, in immunocompromised individuals (e.g human immunodeficiency virus [HIV]) or other disorders of the immune system, helminths may cause chronic infection and hyper infection syndrome (Rinderknecht & Blanco, 2008, Herbreteau *et al.,* 2012, Ribas *et al*., 2013 and Chaisiri *et al.,* 2015). These include: Echinococcosis, taeniasis, trichinosis, schistosomiasis, filariasis and opisthorchiasis (Meerburg *et al.,* 2009). Word Health Organisation (WHO) estimates that, two billion people harbor parasitic worm infections while for the sub-Saharan Africa, more than one billion people are infected with soil transmitted helminths (Malsawmtluangi & Tandon, 2009, Mulungu *et al.,* 2011, Pakdel *et al.,* 2013, Broecke, 2021). Several studies on gastro-intestinal parasites of rodents have revealed various zoonotic helminths in

more than 50% of the rodent population, such as: *Strongyloides* Spp., *Hymenelopis diminuta, Hymenelopis nana, Physoloptera* spp*., Syphacia* spp. and *Trichuris* spp. (Herbreteau *et al.,* 2012; Chaisiri *et al.,* 2015; Ranjbar *et al.,* 2017).

***Trichuris* worms**

*Trichuris* spp. is a nematodes worm causing a trichuarisis diseases in mammals, (Ribas *et al.,* 2013; Wang *et al.,* 2013). Trichuriasis is distributed worldwide, being the most abundant disease in the tropical regions (Jones, 2021). Its Infections affect about one billion people around the globe and more than a quarter of the world's population is predicted to be at risk. In 2020, WHO considered sub-Saharan Africa as one of the regions heavily affected by *Trichuris* worms. *Trichuris* worms and other intestinal parasites have received much attention in recent years, worldwide, due to the exponential increase in their infestation rates in humans and animals (Gul *et al.,* 2016). About 80 species are currently identified in the genus *Trichuris* and most seem to have specific mammalian hosts (Xie *et al.,* 2018). For instance, *Trichuris muris* is a rodent intestinal parasitic nematode that inhabits the large intestine of its host and induces a strong immune response (Xie *et al.,* 2018).

### 1.2.4 Control of helminths

Ivermectin® is a well-characterized drug that is used to treat parasitic infections in humans and animals (Bosco *et al.*, 2020). This anthelminthic targets a glutamate-gated chloride channel in some invertebrates as the chloride channel of gamma-aminobutyric acid type A (GABA-A) receptors hence promoting cellular hyperpolarization and worm paralysis (Foletto *et al.,* 2015a; Cullin *et al.*, 2017). Ivermectin is known to reduce gastrointestinal helminths burden in diverse animals due to its safety, availability, ease of administration and mechanism of action as compared to other anthelminths.

### 1.2.5 Mechanism of action

Initial research into IVM's antiparasitic activity against gut-dwelling nematodes led to the proposal that IVM mimics the action of the

nematode inhibitory neurotransmitter, GABA. This conclusion was based on the indirect evidence that IVM interacts with mammalian and other invertebrate GABA receptors and on the direct evidence that synaptic communication between ventral inhibitory neurons and dorsal excitatory neurons of Ascaris was blocked by micromolar amounts of IVM ~3-1 s. (Cullin *et al.*, 2017).

### 1.2.6 Side effect

Drug-related side-effects include rash, and tenderness and enlargement of lymph nodes. These effects appear to peak about three days (Foletto *et al.,* 2015a).

### 1.2.7 Climatic conditions of Morogoro and Iringa

According to the world climate database, Morogoro has an average temperature of 21.9 ◦C during the coldest month of July, and 27.3 ◦C during the warmest month of January; the precipitation amounts to 890 mm per year (Van Aelst *et al*., 2018). In Iringa, the average temperature of the coldest month (July) is 19.0 ◦C, and that of warmest month (November) is 23.1$^{0}$C. The precipitation amounts to 740 mm per year (Mbululo *et al.,* 2012).

## 1.3 Statement of the problem and Justification of the study

Various studies have shown that human and animal diseases caused by rodent borne viruses, bacteria and parasites are on the increase and are considered to be a serious concern (Griffiths *et al.,* 2011; Kumar *et al.,* 2018). Numerous studies have focused mostly on incidence and prevalence of single, rather than multiple infections and their effects on the host immune systems (Gunther *et al.,* 2009; Jittapalapong *et al.,* 2009; Ziwa *et al.,* 2013; Giles *et al.*, 2016 and Peterson *et al.,* 2017). Relatively few studies have focused on infection involving helminths (worms) and bacteria for example (*Leptospira* spp. and *Bartonella* spp.) in rodent

populations and their ectoparasites in Morogoro. (Sugahara *et al.,* 2004; Lecompte *et al.,* 2006; Griffiths *et al.,* 2011, Margaletic, 2012 Villette *et al.,* 2017; Antonio *et al.,* 2019) and even less information is available on the molecular epidemiology, prevalence and modes of transmission of the diseases caused by these pathogens. Effective treatments against gastrointestinal helminths and seasonal variations of infection by these pathogens in climatically different regions of Morogoro and Iringa have also not been fully understood. Morogoro and Iringa regions were selected due to the ongoing cohort studies on rodents for more than 15 years.

### 1.4 Study Rationale

The findings of this study will contribute to the following;

i) Designing of effective control strategies of these diseases and their carriers understanding the influence of seasons on the dynamics of the infections iii) developing appropriate research model for studying coinfection involving rodent transmitted helminths and other parasites in small mammals.

#### 1.4.1 Research questions

i. How do variation of season influence the rate of *Trichuris* worms infection in *M. natalensis* in climatically different regions of Morogoro and Iringa?
ii. What are the molecular characteristics of *Leptospira* spp. and *Bartonella* spp.in *M. natalensis* and its ectoparasites in Morogoro?
iii. What is the effect of Ivermectin® on intestinal helminths in *M. natalensis*?

#### 1.4.2 Objectives of the study

##### 1.4.2.1 General objective

To investigate rodent borne pathogens infecting *M. natalensis* in selected areas of Morogoro and Iringa, Tanzania.

### 1.4.3 Specific objectives

i. To determine the prevalence and seasonal variation of *Trichuri*s worms infection in *M. natalensis* in Morogoro and Iringa regions, Tanzania.
ii. To carry out molecular detection of *Leptospira* spp. and *Bartonella* spp. in *M. natalensis* and its ectoparasites in Morogoro, Tanzania.
iiii. To determine the effect of Ivermectin® on intestinal helminths in *M. natalensis*

### 1.4.4 Limitations of the study

i. Limited time and resources to carrying out phylogenetic studies to determine species of *a Trichuris, Leptospira*, mites and fleas infecting/infesting *M. natalensis.*
ii. Scarcity of *M. natalensis* in the study areas during the study period.

### 1.4.5 Organisation of the thesis

This thesis presents three publishable manuscripts organised into chapters. The thesis is organised into six chapters and starts with an introduction in chapter one. This sets the general background information for the research problem. Chapter two presents manuscript number one, which focuses on the prevalence and seasonal variation of *Trichuris* worms Infection in *M. natalensis* in Morogoro and Iringa Regions (derived from the first specific objective) which has been accepted for publication in the journal of Parasitologia. This is followed by Chapter three, which deals with Molecular detection of *Leptospira* and *Bartonella* in *M. natalensis* and its ectoparasites in Morogoro (derived from the second specific objective) which has been published in the journal of Mammalia. Chapter four presents manuscript number three which concentrates on the effect of ivermectin® on intestinal helminths in *M. natalensis* derived from the third specific objective which has been submitted for publication in the journal of

Mammalia. This was then followed by Chapter five, which presents a general discussion and provides an overall synthesis and interpretation of thesis findings. Finally, in Chapter six, the thesis draws a clear conclusions and recommendations of the entire study.

**REFERENCES**

Blanco, R. E. (2008). “The Largest Fossil Rodent.” pp. 923–928.

Boey, K., Shiokawa, K., & Rajeev, S. (2019a). *Leptospira* infection in rats: A literature review of global prevalence and distribution. *PLoS Negl Trop Dis* 13, e0007499.

Bosco, A., Kiebler, J., Amadesi, A., Varady, M., Hinney, B., Ianniello, D., Maurelli, M. P., Cringoli, G., & Rinaldi, L. (2020). The threat of reduced efficacy of anthelmintics against gastrointestinal nematodes in sheep from an area considered anthelmintic resistance-free. *Parasites & Vectors,* 13(1), 457.

Broecke, B.V. (2021). Linking Behavior, Co-infection Patterns, and Viral Infection Risk With the Whole Gastrointestinal Helminth Community Structure in *Mastomys natalensis*. *Frontiers in Veterinary Science,* 8, 15.

Chaisiri, K., Siribat, P., Ribas, A., & Morand, S. (2015). Potentially Zoonotic Helminthiasis of Murid Rodents from the Indo-Chinese Peninsula: Impact of Habitat and the Risk of Human Infection. *Vector-Borne and Zoonotic Diseases*, 15(1), 73–85.

Chen, H., Mozzicafreddo, M., Pierella, E., Carletti, V., Piersanti, A., & Ali, S.M. (2021). Dissection of the gut microbiota in mothers and children with chronic *Trichuris trichiura* infection in Pemba Island, Tanzania. *Parasites Vectors,* 14, 62.

Cullin, C. O., Sellers, M. S., Rogers, E. R., Scott, K. E., Lee, D. N., Ophir, A. G., & Jackson, T. A. (2017). Intestinal Parasites

and Anthelmintic Treatments in a Laboratory Colony of Wild-caught African Pouched Rats (*Cricetomys ansorgei*). *Comparative Medicine* 67(5), Article 5.

Dehio, C. (2001a). *Bartonella* interactions with endothelial cells and erythrocytes. *Trends in Microbiology* 9, 279–285.

Foletto, V., Vanz, F., Gazarini, L., Stern, C., & Tonussi, C. (2015a). Efficacy and security of Ivermectin® given orally to rats naturally infected with *Syphacia* spp., *Giardia* spp. and *Hymenolepis nana*. *Laboratory Animals,* 49(3), Article 3.

Griffiths, E. C. (2011). "The Nature and Consequences of Coinfection in Humans." *Journal of Infection,* 63(3), 200–206.

Gryseels, S. (2015). Gairo Virus, a Novel Arenavirus of the Widespread *Mastomys Natalensis* : Genetically Divergent, but Ecologically Similar to *Lassa* and *Morogoro Viruses*." *Virology,* 476, 249–56.

Gul, N., & Tak, H. (2016). Prevalence of *Trichuris* spp. in small ruminants slaughtered in Srinagar District (J&K). *Journal of Parasitic Diseases,* 40(3), 741–744.

Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I.S., & Harrus, S., (2015). *Bartonella* Infection in Rodents and Their Flea Ectoparasites: An Overview. *Vector-Borne and Zoonotic Diseases,* 15, 27–39.

Hardin, A. (2019). "Comparative Genomic Characterization of the Multimammate Mouse. *Mastomys Coucha,* 1–8.

Herbreteau, V., Bordes, F., Jittapalapong, S., Supputamongkol, Y., & Morand, S. (2012). Rodent-borne diseases in Thailand: Targeting rodent carriers and risky habitats. *Infection Ecology & Epidemiology, 2*(1), 18637.

Jones, K.R. (2021). *Trichuris* spp. in Animals, with Specific Reference to Neo-Tropical Rodents, *Veterinary Sciences,* 8(2),15.

Kilonzo, B. S., Mhina, J., Sabuni, C., & Mgode, G. (2005). The role of rodents and small carnivores in plague endemicity in Tanzania. *Belgian Journal of Zoology*, 135 (Suppl.), 119-125.

Leirs, H. (1994). “Population Ecology of *Mastomys Natalensis* (Smith, 1834). Implications for Rodent Control in Africa. pp. 286.

Leirs, H., Ron, V., & Walter, V. (2014).“Productivity of Different in a Population of *Mastomys Natalensis* Rats in Tanzania. *Veterinary Sciences,* 68: 53–60.

Machang’u, R.S., Mgode, G.F., Assenga, J., Mhamphi, G., Weetjens, B., Cox, C., Verhagen, R., Sondij, S., Goris, M.G., & Hartskeerl, R.A. (2004). Serological and molecular characterization of *leptospira* serovar Kenya from captive African giant pouched rats (*Cricetomys gambianus*) from Morogoro Tanzania. *FEMS Immunology & Medical Microbiology,* 41,117–121.

Makundi, R.H., Kilonzo, B.S., & Massawe, A.W. (2003). Interaction between rodent species in agro-forestry habitats in the western Usambara Mountains, north-eastern Tanzania and its potential for plague transmission to humans. pp. 5.

Makundi, R.H., Apia, W. M., & Loth, S. M. (2007). “ Reproduction and Population Dynamics of *Mastomys Natalensis* Smith, 1834 in an Agricultural Landscape in the Western Usambara Mountains, Tanzania .” *Integrative Zoology,* 2(4), 233–38.

Malania, L., Bai, Y., Osikowicz, L.M., Tsertsvadze, N., Katsitadze, G., Imnadze, P., & Kosoy, M., 2016. Prevalence and Diversity of Bartonella Species in Rodents from Georgia (*Caucasus*). *The American Journal of Tropical Medicine and Hygiene,* 95, 466–471.

Malsawmtluangi, C., & Tandon, V. (2009). Helminth parasite spectrum in rodent hosts from bamboo growing areas of Mizoram, North-east India. *Journal of Parasitic Diseases, 33*(1–2), 28–35.

Mariën, J., Vanden, B. B., Tafompa, P.J.J., Bernaerts, L., Ribas, A., & Mnyone, L.L. (2022). Host related factors determine co-occurrence patterns between pathogenic bacteria, protozoa and helminths in populations of the multimammate mouse, *Mastomys natalensis. Ecology,* 10, 111 – 163.

Meerburg, B.G., Grant, R. S., & Aize, K. (2009). 35 Critical Reviews in Microbiology Rodent-Borne Diseases and Their Risks for Public Health Rodent-Borne Diseases and Their Risks for Public Health. *Critical Reviews in Microbiology*, *35*(3), 221–270.

Mehraj, V., Hatcher, J., Akhtar, S., Rafique, G., & Beg, M.A. (2008). Prevalence and Factors Associated with Intestinal Parasitic Infection among Children in an Urban Slum of Karachi. Sutherland CJ, editor. *PLoS ONE,* 3, e3680.

Mgode, G.F., Mhamphi, G.G., Massawe, A.W., & Machang'u, R.S. (2021b). *Leptospira* Seropositivity in Humans, Livestock and Wild Animals in a Semi-Arid Area of Tanzania. *Pathogens,* 10, 696.

Mlyashimbi, E.C.M., Mariën, J., Kimaro, D.N., Tarimo, A.J.P., Machang'u, R.S., & Makundi, R.H. (2020). Home ranges, sex ratio and recruitment of the multimammate rat (*Mastomys natalensis*) in semi-arid areas in Tanzania. *Mammalia,* 84(4), 336–343.

Mlyashimbi, E.C.M. (2018). Relationships between seasonal changes in diet of Multimammate rat (*Mastomys natalensis*) and its breeding patterns in semi-arid areas in Tanzania. The Doctorial at Sokoine University of Agriculture, Morogoro - Tanzania. 149pp.

Ribas, A., López, S., Makundi, R.H., Leirs, H., & de Bellocq, J.G. (2013). *Trichuris* spp. (Nematoda: Trichuridae) from Two Rodents, *Mastomys natalensis* and *Gerbilliscus vicinus* in Tanzania. *Journal of Parasitology,* 99(5), 868.

Rinderknecht, A., & Blanco, R.E. (2008). The largest fossil rodent. *Procedure Research Sociology,* 275(1637), 923–928.

Schmidt, S. (2014). "Multiple Infections of Rodents with Zoonotic Pathogens in Austria." *Vector-Borne and Zoonotic Diseases,* 14(7), 467–75.

Theonest, N.O., Carter, R.W., Amani, N., Doherty, S.L., Hugho, E., Keyyu, J.D., Mable, B.K., Shirima, G.M., Tarimo, R., Thomas, K.M., Haydon, D.T., Buza, J.J., Allan, K.J., & Halliday, J.E.B. (2019). Molecular detection and genetic

characterization of *Bartonella* species from rodents and their associated ectoparasites from northern Tanzania. *PLoS ONE* 14: e0223667.

Vanden, B.B., Bernaerts, L., Ribas, A., Sluydts, V., Mnyone, L., & Matthysen, E. (2021). Linking Behavior, Co-infection Patterns and Viral Infection Risk with the Whole Gastrointestinal Helminth Community Structure in *Mastomys natalensis*. *Front Veterinary Sciences,* 13(8): 669058.

Wang, D., Wang, X., Li, W., & Wang, S. (2013). Trichuriasis diagnosed by colonoscopy: case report and review of the literature spanning 22 years in mainland China. *International Journal of Infectious Diseases* Nov, 17(11), e1073–1075.

Xie, Y., Zhao, B., Hoberg, E.P., Li, M., Zhou, X., & Gu, X. (2018). Genetic characterization and phylogenetic status of whipworms (*Trichuris* spp.) from captive non-human primates in China, determined by nuclear and mitochondrial sequencing. *Parasites Vectors,* 11(1), 516.

# CHAPTER TWO
# PAPER I

*parasitologia*

MDPI

*Article*

## Prevalence and Seasonal Variation of *Trichuris* Worms Infection in *Mastomys natalensis* in Morogoro and Iringa Regions, Tanzania

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**Abstract:** Trichuriosis is a disease in mammals caused by the whipworms of the genus *Trichuris*. These worms are known for the high disease burden they cause in humans and domestic animals, especially in sub-Saharan Africa. In this study, we investigated the seasonal variations of *Trichuris* worms in multimammate rats (*Mastomys natalensis*). The study was conducted between January and November 2021 in Tanzania, in two regions (Morogoro and Iringa) that differ in their eco-climatic conditions. Removal trapping was conducted using Sherman® live traps during the rainy and dry seasons. The gastrointestinal tracts of captured rodents were screened for the presence of *Trichuris* worms, which were identified using morphological keys. A total of 200 *M. natalensis* rats were collected from each of the regions, with 100 animals in each season. For Morogoro, the overall prevalence of *Trichuris* worms in *M. natalensis* was 36% ($n$ = 72), of which 42% ($n$ = 42) and 30% ($n$ = 30) were for the rainy and dry seasons, respectively. For Iringa, the overall prevalence was 65% ($n$ = 130), of which there were 80% ($n$ = 80) and 50% ($n$ = 50) for the rainy and dry seasons, respectively. *Trichuris* worm infections were significantly higher during the rainy season in Iringa than in Morogoro; however, no significant difference in infections between males and females was noted in either region or season. Other helminths detected were *Strongyloides* spp., *Capillaria* spp., *Hymenolepis* spp. and eggs of a helminth that has yet to be confirmed, possibly an Anoplocephalid species. Since *M. natalensis* is the most important pest species in sub-Saharan Africa, and is a carrier of several zoonotic helminths, there is a need for improved surveillance of helminths infections in the studied regions, in order to establish strategic control programs to reduce their adverse impacts on health.

**Keywords:** rodents; Trichuriosis; whipworm; zoonosis



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### 1. Introduction

Trichuriosis is a disease of mammals caused by nematodes belonging to the genus *Trichuris* [1–3]. The disease has spread worldwide, and is most abundant in tropical regions of the world. Infections affect about one billion people around the globe, and more than a quarter of the world's population is predicted to be at risk. In 2020, the World Health Organization (WHO) considered sub-Saharan Africa as one of the regions most heavily affected by *Trichuris* worms infections. *Trichuris* worms and other intestinal parasites have received much worldwide attention in recent years due to the exponential increase in their infection rates in humans and animals [4,5].

Approximately 80 species of the worms are currently identified in the genus *Trichuris*, and most seem to have specific mammalian hosts. For instance, *Trichuris muris* is a rodent intestinal parasitic nematode that inhabits the large intestine of its host, and induces a strong immune response [5]. Indeed, rodents are reservoirs of many zoonotic pathogens, including *Trichuris* worms [1,6]. *Mastomys natalensis* is among the most abundant and dominant rodent species in sub-Saharan Africa, including Tanzania [7–9]. This rodent species is highly prolific, and has successfully exploited a wide variety of habitats and environments [7–9]. In addition, their populations are strongly seasonal, depending on the availability of rainfall and feed abundance [10].

Several studies suggested that differences in environmental conditions, such as temperature and rainfall, lack of access to potable water, poor hygiene and poverty, are major risks for trichuriosis infections [5–9]. It is also hypothesized that infections occur more often during the rainy season [11–15]. Consequently, little is known about the prevalence of *Trichuris* worms in climatically different environments in Tanzania. Therefore, this comparative study aimed to determine the prevalence and seasonality of *Trichuris* infections in two climatically different regions of Tanzania. The findings will help to determine the influence of season on infection, which can help organize worm control programs, including deworming of vulnerable communities.

## 2. Materials and Methods

### *2.1. Study Sites and Design*

A cross sectional study was carried out in two climatically different regions of Tanzania, namely, Morogoro and Iringa, from January to November 2021. In Morogoro, the study was carried out in Choza village close to the Sokoine University of Agriculture main campus, and in Kiroka village (6°50′34.9794″ S; 37°38′8.232″ E). The villages experience a bimodal rainfall pattern that is characterized by short rains from November to January, and long rains from March to May. The drier season lasts for six months in Morogoro, from May to November [16] (Figure 1).

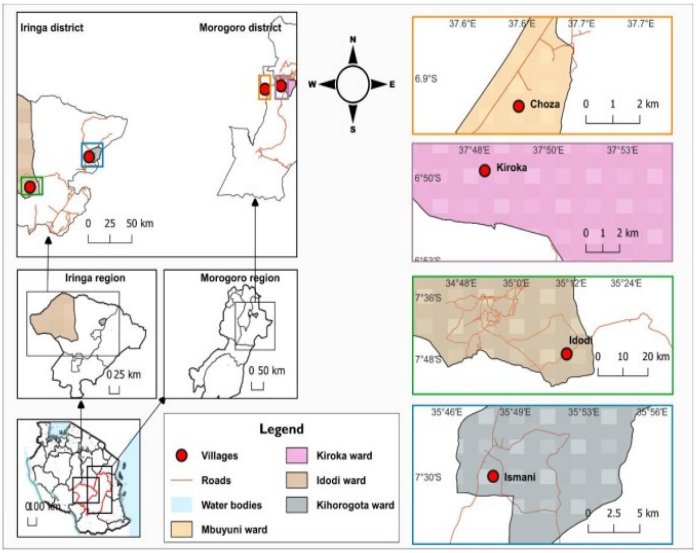


**Figure 1.** Map of selected study areas of Morogoro and Iringa regions, Tanzania.

**3. Results**

A total of 200 *M. natalensis* were collected from each region and screened for *Trichuris* infections. For each season, an equal number of 100 animals were screened. For the Morogoro region, 97 (49%) and 103 (51%) of the animals were males and females respectively. Overall, the prevalence of *Trichuris* worms infection for both seasons in Morogoro was 36% ($n$ = 72), of which males accounted for 33% ($n$ = 32) while females accounted for 39% ($n$ = 40); moreover, incidences of 22% ($n$ = 22) and 30% ($n$ = 30) were recorded during the rainy and dry seasons, respectively.

For the rainy season, the prevalence were 12% ($n$ = 12) in males and 10% ($n$ = 10) in females, while for the dry season, the prevalence were 16% ($n$ = 16) and 14% ($n$ = 14) for males and females respectively.

For the Iringa region, 105 (53%) were males and 95 (47%) were females. The overall prevalence of infection was 65% ($n$ = 130), of which 61% ($n$ = 64) and 70% ($n$ = 66) were in males and females for both seasons respectively. For the rainy season, the total number of males was 57% (57), while females was 43 (43%). The overall prevalence of infection was 80% ($n$ = 80), of which males and females accounted for 44% ($n$ = 44) and 36% ($n$ = 36), respectively. For the dry season, the overall prevalence was 50% ($n$ = 50), of which 20% ($n$ = 20) were males and 30% ($n$ = 30) were females (Figure 2). During the study, the prevalence of *Trichuris* worm infections varied significantly between the two regions and seasons; with the rainy season showing a higher prevalence than the dry season in both regions (Figure 2) (df = 1, $\chi^2$ = 31.443, $p < 0.05$), with infection being significantly higher in the Iringa region than in the Morogoro region (df = 1, $\chi^2$ = 16.438, $p$ = 0.0001). The generalized linear model showed no significant difference between males and females in infection with *Trichuris* worms in either region or season (Figure 2) (df = 1, $\chi^2$ = 1.3, $p$ = 0.431). During the study, eggs and adult worms other than *Trichuris* spp. were observed via stereomicroscopy [23,25–28] (Tables 1 and 2).

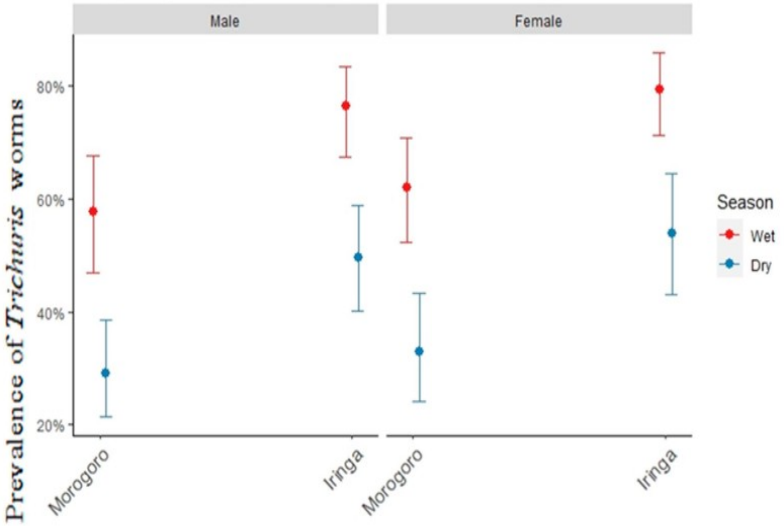


**Figure 2.** Plot showing the effect of season, location and sex of *Mastomys natalensis* on *Trichuris* worm infections in Morogoro and Iringa regions, Tanzania.

**Table 1.** Prevalences of helminth eggs detected during the screening of *Trichuris* worms in the fecal samples of *Mastomys natalensis* in the dry and rainy seasons in Morogoro region, Tanzania.

| | Rainy Season | | | Dry Season | | |
|---|---|---|---|---|---|---|
| **Helminths Detected** | **Total Prevalence ($n$ = 100)** | **Male** | **Female** | **Total Prevalence ($n$ = 100)** | **Male** | **Female** |
| *Trichuris* spp. | 22% | 12% | 10% | 30% | 16% | 14% |
| Anoplocephalid species. * | 10% | 4% | 6% | 22% | 15% | 7% |
| *Strongyloides* spp. | 0% | 0% | 0% | 50% | 22% | 28% |
| *Capillaria* spp. | 0% | 0% | 0% | 14% | 6% | 8% |
| *Hymenolepis* spp. | 0% | 0% | 0% | 55% | 27% | 28% |

* Yet to be confirmed.

**Table 2.** Prevalences of helminth eggs detected during the screening of *Trichuris* worms in the fecal samples of *Mastomys natalensis* in the dry and rainy seasons in Iringa region, Tanzania.

| | Rainy Season | | | Dry Season | | |
|---|---|---|---|---|---|---|
| **Helminths Detected** | **Total Prevalence ($n$ = 100)** | **Male** | **Female** | **Total Prevalence ($n$ = 100)** | **Male** | **Female** |
| *Trichuris* spp. | 80% | 44% | 36% | 50% | 20% | 30% |
| Anoplocephalid species. * | 20% | 12% | 8% | 28% | 15% | 13% |
| *Strongyloides* spp. | 90% | 56% | 34% | 50% | 39% | 21% |
| *Capillaria* spp. | 0% | 0% | 0% | 16% | 6% | 10% |
| *Hymenolepis* spp. | 0% | 0% | 0% | 6% | 2% | 4% |

* Yet to be confirmed.

## 4. Discussion

The current study aimed to determine the seasonal variations of *Trichuris* worm infections in *M. natalensis* in two regions of Tanzania (Morogoro and Iringa) that are climatically different. The study showed different infection levels according to the regions and seasons (Table 1, Figure 2). The prevalence of *Trichuris* worms and other helminths in both regions was higher during the rainy than the dry seasons. Moreover, the prevalence of *Trichuris* worm infections was higher in Iringa than in Morogoro.

The relatively high prevalence of *Trichuris* worms in *M. natalensis* has also been reported in other studies [1,9], with higher abundances during the rainy season compared to the dry season [7]. Our results also suggest that male and female rodents are equally infected. This result is in contrast to other studies that showed females to be more susceptible [27–29]. This finding calls for more studies to explain this disparity.

During the screening, ova and adult worms belonging to other species were also detected. During the rainy season, the eggs of an unconfirmed helminth, possibly Anoplocephalid species were also found in Morogoro and Iringa, while *Strongyloides* spp. were detected in Iringa. For the dry season, *Strongyloides* spp., *Capillaria* spp., Anoplocephalid species and *Hyemenolepis* spp. were found in rodents from both regions. This suggests that coinfection with worms of different species is common in rodents. These findings were similar to those of other studies conducted elsewhere, where helminthic infections in mice were screened [28,30].

Furthermore, during this study, the highest prevalence was noted during the rainy season in Iringa. This result agrees with those of previous studies on seasonal variations of *Trichuris* spp. and other intestinal helminth infections [7,29]. However, a study conducted in India by [15] conflicted with this study by showing that *Trichuris* worm infections were higher during the dry season than the rainy season.

Other studies have shown that *Trichuris* worm ova can adapt easily under different environmental conditions, thus enabling them to survive well even during dry seasons [29]. Various studies have shown that helminth infections in *M. natalensis* can adversely impair digestive function in a host, and consequently affect its efficiency in absorbing nutrients from the gut. Other reports showed that parasitic infections in *M. natalensis* can compromise an animal's health, adversely impacting its intestinal microbiota and digestive and immunoregulation [3].

Generally, the examined rodents were more infected with *Trichuris* worms, *Strongyloides* spp. and presumptive an Anoplocephalid species with a higher prevalence found in Iringa than in Morogoro. The differences in rainfall pattern, temperature and humidity could cause this variation in favor of the Iringa region. Iringa is located at a higher altitude (1564 masl) compared to Morogoro (254 masl), and has an overall higher rainfall abundance and lower average temperature than Morogoro. These factors may probably be favorable for the survival of the *Trichuris* ova deposited on the soils in Iringa, and hence a higher chance of infection than in Morogoro.

Based on the findings of this study and other studies [3,31], *M. natalensis* is shown to be involved in transmitting a series of helminths known to be potential pathogens to humans and animals. Therefore, human, animal and environmental health professionals (One Health) need strategic awareness on control programs to reduce their adverse impact. These programs shall include periodic screening of rodents for helminths, and management of the small mammal populations where there is imminent danger of them causing disease. Moreover, further studies to characterize the different species of *Trichuris* from different animal species at the molecular level are highly recommended.

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## References

1. Ribas, A.; López, S.; Makundi, R.H.; Leirs, H.; de Bellocq, J.G. *Trichuris* spp.(Nematoda: Trichuridae) from two rodents, *Mastomys natalensis* and *Gerbilliscus vicinus* in Tanzania. *J. Parasitol.* **2013**, *99*, 868–875. [CrossRef] [PubMed]
2. Wang, D.D.; Wang, X.L.; Wang, X.L.; Wang, S.; An, C.L. Trichuriasis diagnosed by colonoscopy: Case report and review of the literature spanning 22 years in mainland China. *Int. J. Infect. Dis.* **2013**, *17*, e1073–e1075. [CrossRef] [PubMed]
3. Jones, K.R. *Trichuris* spp. in animals, with specific reference to neo-tropical rodents. *Vet. Sci.* **2021**, *8*, 15. [CrossRef] [PubMed]
4. Gul, N.; Tak, H. Prevalence of *Trichuris* spp. in small ruminants slaughtered in Srinagar District (J&K). *J. Parasit. Dis.* **2016**, *40*, 741–744. [PubMed]
5. Xie, Y.; Zhao, B.; Hoberg, E.P.; Li, M.; Zhou, X.; Gu, X.; Lai, W.; Peng, X.; Yang, G. Genetic characterisation and phylogenetic status of whipworms (*Trichuris* spp.) from captive non-human primates in China, determined by nuclear and mitochondrial sequencing. *Parasit. Vectors* **2018**, *11*, 516. [CrossRef]
6. Rinderknecht, A.; Blanco, R.E. The largest fossil rodent. *Proc. R. Soc. B Biol. Sci.* **2008**, *275*, 923–928. [CrossRef]
7. Mlyashimbi, E.C.; Mariën, J.; Kimaro, D.N.; Tarimo, A.J.; Machang'u, R.S.; Makundi, R.H.; Isabirye, M.; Massawe, A.W.; Leirs, H.; Mdangi, M.E.; et al. Home ranges, sex ratio and recruitment of the multimammate rat (*Mastomys natalensis*) in semi-arid areas in Tanzania. *Mammalia* **2020**, *84*, 336–343. [CrossRef]

8. Makundi, R.H.; Massawe, A.W.; Mulungu, L.S. Reproduction and population dynamics of *Mastomys natalensis* Smith, 1834 in an agricultural landscape in the Western Usambara Mountains, Tanzania. *Integr. Zool.* **2007**, *2*, 233–238. [CrossRef]
9. Mariën, J.; Vanden Broecke, B.; Tafompa, P.J.; Bernaerts, L.; Ribas, A.; Mnyone, L.L.; Mulugu, L.S.; Leirs, H. Host related factors determine co-occurrence patterns between pathogenic bacteria, protozoa, and helminths in populations of the multimammate mouse, *Mastomys natalensis*. *bioRxiv* **2022**. [CrossRef]
10. Mlyashimbi, E.C.; Mariën, J.; Kimaro, D.N.; Tarimo, A.J.; Isabirye, M.; Makundi, R.H.; Massawe, A.W.; Mdangi, M.E.; Kifumba, D.; Nakiyemba, A.; et al. Relationships between seasonal changes in diet of Multimammate rat (*Mastomys natalensis*) and its breeding patterns in semi-arid areas in Tanzania. *Cogent Food Agric.* **2018**, *4*, 1507509. [CrossRef]
11. Mehraj, V.; Hatcher, J.; Akhtar, S.; Rafique, G.; Beg, M.A. Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. *PLoS ONE* **2008**, *3*, e3680. [CrossRef] [PubMed]
12. Riaz, M.; Aslam, N.; Zainab, R.; Aziz-Ur-Rehman; Rasool, G.; Ullah, M.I.; Daniyal, M.; Akram, M. Prevalence, risk factors, challenges, and the currently available diagnostic tools for the determination of helminths infections in human. *Eur. J. Inflamm.* **2020**, *18*. [CrossRef]
13. Chen, H.; Mozzicafreddo, M.; Pierella, E.; Carletti, V.; Piersanti, A.; Ali, S.M.; Ame, S.M.; Wang, C.; Miceli, C. Dissection of the gut microbiota in mothers and children with chronic *Trichuris trichiura* infection in Pemba Island, Tanzania. *Parasit. Vectors* **2021**, *14*, 62. [CrossRef]
14. Vanden Broecke, B.; Bernaerts, L.; Ribas, A.; Sluydts, V.; Mnyone, L.; Matthysen, E.; Leirs, H. Linking behavior, co-infection patterns, and viral infection risk with the whole gastrointestinal helminth community structure in *Mastomys natalensis*. *Front. Vet. Sci.* **2021**, *8*, 669058. [CrossRef] [PubMed]
15. Kanojiya, D.; Shanker, D.; Sudan, V.; Jaiswal, A.K. Prevalence and seasonal variation of *trichurid* worm infections of small ruminants of Mathura, India. *J. Parasit. Dis.* **2016**, *40*, 199–202. [CrossRef]
16. Van Aelst, K.; Holvoet, N. Climate change adaptation in the Morogoro Region of Tanzania: Women's decision-making participation in small-scale farm households. *Clim. Dev.* **2018**, *10*, 495–508. [CrossRef]
17. Mbululo, Y.; Nyihirani, F. Climate Characteristics over Southern Highlands Tanzania. *Atmos. Clim. Sci.* **2012**, *2*, 454–463. [CrossRef]
18. Kassian, L.M.; Tenywa, M.; Liwenga, E.T.; Dyer, K.W.; Bamutaze, Y. Implication of climate change and variability on stream flow in Iringa region, Tanzania. *J. Water Clim. Change* **2017**, *8*, 336–347. [CrossRef]
19. Claus, A.T.; Abdul, A.S.; Rhodes, H.M.; Robert, S.; Stella, T.K. Prevalence of *Rickettsia typhi* in rodent fleas from areas with and without previous history of plague in Mbulu district, Tanzania. *Afr. J. Microbiol. Res.* **2020**, *14*, 65–70. [CrossRef]
20. Aplin, K.P.; Brown, P.R.; Jacob, J.; Krebs, C.J.; Singleton, G.R. *Field Methods for Rodent Studies in Asia and the Indo-Pacific*; Australian Centre for International Agricultural: Canberra, Australia, 2003.
21. Tun, S.; Ithoi, I.; Mahmud, R.; Samsudin, N.I.; Kek Heng, C.; Ling, L.Y. Detection of helminth eggs and identification of hookworm species in stray cats, dogs and soil from Klang Valley, Malaysia. *PLoS ONE* **2015**, *10*, e0142231. [CrossRef]
22. Moravec, F. Review of Keys to the Nematode Parasites of Vertebrates. *Parasit. Vectors* **2009**, *2*, 42. [CrossRef]
23. Hansen, J.; Perry, B.D. *The Epidemiology, Diagnosis and Control of Gastro-Intestinal Parasites of Ruminants in Africa: A Handbook*; ILRI (aka ILCA and ILRAD): Nairobi, Kenya, 1990.
24. Ghai, R.R.; Simons, N.D.; Chapman, C.A.; Omeja, P.A.; Davies, T.J.; Ting, N.; Goldberg, T.L. Hidden population structure and cross-species transmission of whipworms (*Trichuris* sp.) in humans and non-human primates in Uganda. *PLoS Neglected Trop. Dis.* **2014**, *8*, e3256. [CrossRef]
25. Haydée Sardella, N.; Horacio Fugassa, M. Parasites in rodent coprolites from the historical archaeological site Alero Mazquiarán, Chubut Province, Argentina. *Memórias Inst. Oswaldo Cruz* **2009**, *104*, 37–42. [CrossRef]
26. Jaran, A.S. Prevalence and seasonal variation of human intestinal parasites in patients attending hospital with abdominal symptoms in northern Jordan. *EMHJ-East. Mediterr. Health J.* **2016**, *22*, 756–760. [CrossRef] [PubMed]
27. Kataranovski, M.; Mirkov, I.; Belij, S.; Popov, A.; Petrović, Z.; Gačić, Z.; Kataranovski, D. Intestinal helminths infection of rats (*Ratus norvegicus*) in the Belgrade area (Serbia): The effect of sex, age and habitat. *Parasite J. Société Française Parasitol.* **2011**, *18*, 189. [CrossRef]
28. Malsawmtluangi, C.; Tandon, V. Helminth parasite spectrum in rodent hosts from bamboo growing areas of Mizoram, North-east India. *J. Parasit. Dis.* **2009**, *33*, 28–35. [CrossRef] [PubMed]
29. Yevstafieva, V.A.; Kravchenko, S.O.; Gutyj, B.V.; Melnychuk, V.V.; Kovalenko, P.N.; Volovyk, L.B. Morphobiological analysis of *Trichuris vulpis* (Nematoda, Trichuridae), obtained from domestic dogs. *Regul. Mech. Biosyst.* **2019**, *10*, 165–176. [CrossRef]
30. Pakdel, N.; Naem, S.; Rezaei, F.; Chalehchaleh, A.A. A survey on helminthic infection in mice (*Mus musculus*) and rats (*Rattus norvegicus* and *Rattus rattus*) in Kermanshah, Iran. In *Veterinary Research Forum*; Faculty of Veterinary Medicine, Urmia University: Urmia, Iran, 2013; Volume 4, p. 105.
31. Mafiana, C.F.; Osho, M.B.; Sam-Wobo, S. Gastrointestinal helminth parasites of the black rat (*Rattus rattus)* in Abeokuta, southwest Nigeria. *J. Helminthol.* **1997**, *71*, 217–220. [CrossRef]

# CHAPTER THREE

**PAPER II**

DE GRUYTER Mammalia 2023; aop

**Original Study**

Claus Thomas*, Venance Msoffe, Natalie Van Houtte, Ginethon Mhamphi, Joachim Mariën, Christopher Sabuni, Isaac Makundi, Jahashi Nzalawahe, Robert Machang'u and Herwig Leirs

# Molecular detection of *Leptospira* and *Bartonella* in *Mastomys natalensis* and its ectoparasites in Morogoro, Tanzania

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**Abstract:** Rodents play an important role in the transmission of zoonotic diseases. This study investigated the prevalence of *Leptospira* spp. and *Bartonella* spp. in *Mastomys natalensis* and its ectoparasites (fleas and mites) in selected villages of Morogoro, Tanzania. *Mastomys natalensis* were captured live in fallow habitats using Sherman® traps and anesthetized using Halothane. Blood samples were obtained from the retroorbital sinus Ectoparasites were removed from the fur using a hard brush and preserved in 70 % ethanol. Real time–qPCR was used to detect Leptospira spp. and *Bartonella* spp. from *Mastomys natalensis* blood and ectoparasites respectively. The study revealed a relatively larger number of males than females captures. *Leptospira* spp. was demonstrated in one out of 100 *Mastomys natalensis*. For *Bartonella* spp., prevalence of (14 %) was recorded in mites with a higher proportion in mites from adult male *Mastomys natalensis* than females. Upon Sanger sequencing, four positive samples showed a complete sequence of the *ITS* gene. Indicating that all samples belonged to *Uncultured Bartonella*. Low prevalence of *Leptospira* spp. and a high prevalence of *Bartonella* spp. was observed in *Mastomys natalensis*. Further exploration of rodent pathogens is recommended to raise awareness of the role of commensal rodents in disease transmission via their ectoparasites.

**Keywords:** *Bartonella* spp.; ectoparasites; *Leptospira* spp.; *Mastomys natalensis*; mites

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## 1 Introduction

Rodents are mammals with broad and complex ecological range; they are cosmopolitan except in Antarctica (Makundi et al. 2003). Given their high prolificacy, rodents have successfully colonized a wide variety of habitats and environments throughout the world (Cortez et al. 2018; Dahmana et al. 2020; Holt et al. 2006; Meerburg et al. 2009). *Mastomys natalensis* is an abundant and dominant species within the small mammal community of Morogoro, Tanzania, and it is known to carry a number of infectious agents of diverse diseases, including: Bartonellosis, leptospirosis, rickettsioses, lyme borreliosis (Katakweba et al. 2012; Machang'u et al. 2004; Mariën et al. 2022).

Rodent borne pathogens may enter a human host through diverse routes including ectoparasite bites, direct contact with rodent excreta or consumption of food and water contaminated with fecal materials of the small mammals (Boey et al. 2019; Machang'u et al. 2004).

Leptospirosis is a rodent borne zoonosis of worldwide distribution (Machang'u et al. 2004). The disease is caused by a spirochete of the genus *Leptospira*, which consists of 22 species (Boey et al. 2019). According to WHO at least

1.03 million cases of leptospirosis occurred annually, worldwide, with 58,900 deaths in the past decade. In the East and Central African region, leptospirosis is known to be prevalent for more than three decades (Machang'u et al. 2004). Tanzania is among the tropical countries of Sub-Saharan Africa where leptospirosis is endemic (Mgode et al. 2021).

Any mammal can be infected with one or more *Leptospira* serovars, however, rodents are the most commonly affected animals which are also the major natural reservoirs of this microorganism (Dehio 2001; Mgode et al. 2021). In animals, including humans, leptospirosis present with varieties of clinical manifestations, from mild, flue-like symptoms to severe, febrile conditions associated with, inappetance, stiffness, abdominal pain and occasionally may cause fatal septicaemic complications (Desai et al. 2009).

Bacteria of the genus *Bartonella* are small, fastidious, slow-growing gram-negative aerobic rods (Dehio 2001). To date they are 45 described species and subspecies of *Bartonella* of which 20 are found in rodents (Gutiérrez et al. 2015; Malania et al. 2016; Theonest et al. 2019).

It is reported that, rodent associated *Bartonella* are the cause of human infections particularly in areas where humans have close contact with rodents. Relatively recently, *Bartonella* spp. have been reported to cause febrile illnesses in humans in northern Tanzania (Theonest et al. 2019).

The *Bartonella* spp. cause a disease with a wide range of clinical manifestations in humans and animals, for example: Cat-scratch fever, trench fever, Carrion's disease, bacteremia with fever, bacillary angiomatosis, peliosis, endocarditis and neuroretinitis (Broecke 2021; Malania et al. 2016). *Bartonella* spp. cause damage of the endothelial cells and erythrocytes of their mammalian hosts (Malania et al. 2016).

Studies on *Leptospira* spp. and *Bartonella* spp. transmission through rodents and their ectoparasites have shown that rodent urine and rodent ectoparasites are major drivers in the transmission of leptospirosis and bartonellosis respectively (Boey et al. 2019; Dehio 2001; Mariën et al. 2022). However, studies on the genotypes of these pathogens, prevalence of the diseases caused and their modes of transmission in rodents, specifically *M. natalensis* and their ectoparasites in Morogoro needs further exploration. This study, therefore, aimed at determining the prevalence, genotypes and modes of transmission of *Leptospira* spp. and *Bartonella* spp. pathogens in Morogoro. The findings of this study will contribute to the design of effective control strategies of these diseases and their carriers.

# 2 Materials and methods

## 2.1 Study sites

This study was carried out in fallow lands at Choza and Kiroka villages, within Morogoro municipality (6°50'34.9794"S; 37°38'8.232"E) between January and March 2021. This period coincides with the breeding season of *M. natalensis* in the study areas (Broecke 2021). The study area experience a bimodal rainfall pattern characterized by short rains from November to January and long rains from March to May each year. During the period of January to March, the land is commonly dry, covered with short grasses and scattered bushes. The dry season lasts for six (6) months from May to November (Rija et al. 2014; Van Aelst and Holvoet 2018) (Figure 1).

## 2.2 Rodent trapping and sample collection

During the study, *M. natalensis* ($n$ = 50) were live captured from each of the villages, using Sherman® traps (standard medium size LFA: 7.6 × 8.9 × 23 cm). Sample size was determined by considering time of the study, budget and animal ethics as described by Aplin (2003). The trapping was conducted for three consecutive nights and inspected every morning for captures over the period of 3 months (Table 2). In each study village, five transect lines were set in fallow land, each laid with 30 Sherman® traps. A 10 m space was set from line to line and from trap to trap. A mixture of peanut butter and maize bran was used as the bait. Captured *M. natalensis* were anaesthetized using halothane and blood samples were drawn from the retro-orbital sinus using capillary tubes. Collected blood was preserved in EDTA vacutainer tubes and a drop of the blood from each rodent was absorbed on a filter paper (Claus et al. 2020).

Fleas and mites were removed from the fur of the anaesthetized animals using a hard brush then collected in a clean dish and covered with a white paper, as described by Claus et al. (2020). After sample collection, the rodents were humanely killed using an overdose of halothane (Mariën et al. 2022).

Mites ($n$ = 250) and fleas ($n$ = 80) obtained were preserved in Eppendorf tubes containing 70 % ethanol before being transported to the Institute of Pest Management (IPMC) in Morogoro. The ectoparasites were morphologically identified using a bright–field digital microscope (Zeiss Primor Star Axiocam ERaC5C) at 0.5 objective lens and a pictorial identification guide as described in Angelakis and Raoult (2014), Desai et al. (2009), and Kim et al. (2005). The fleas and mites, identified as *Xenopsylla cheopis and Laelaptine* spp. respectively were sorted and pooled separately according to their host and then crushed in a sterile mortar. Before DNA extraction (Van Houtte et al. 2014), the *X. cheopis* pulp were divided into 20 aliquots, while *Laelaptine*s spp. were aliquoted into 80 pools.

## 2.3 DNA extraction

Genomic DNA of the *M. natalensis* blood, *X. cheopis* and *Laelaptine*s spp. were extracted using the NucleoSpin® Tissue (MACHEREY-NAGEL GmbH & Co. KG, Germany), following the manufacturer's instructions. The DNA extracts were then stored at −20 °C.

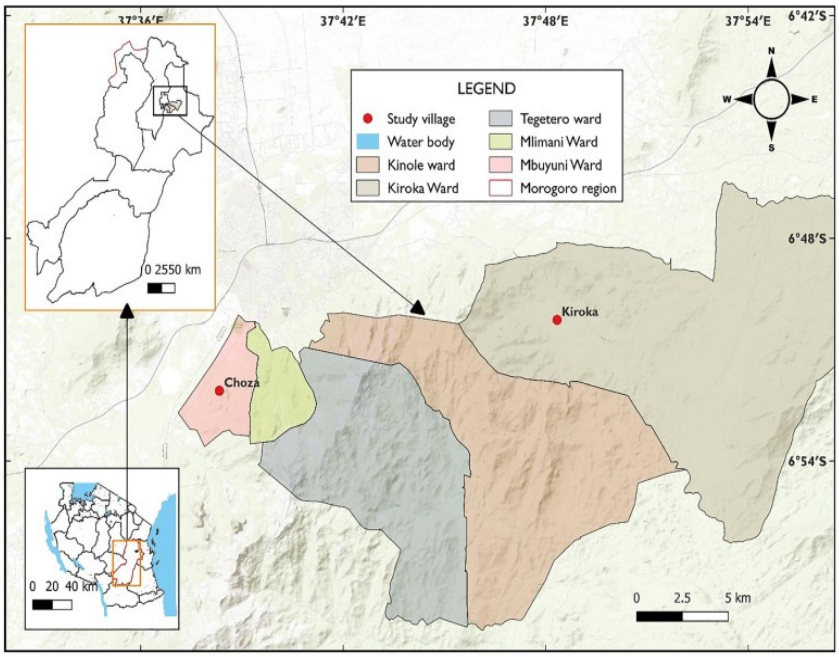


**Figure 1:** Map of selected study villages of Morogoro.

### 2.4 Molecular screening of *Bartonella* spp.

DNA extracts were screened for *Bartonella* spp. by real time-qPCR using the following pairs of primers and probes (Meerburg et al. 2009; Van Houtte et al. 2014). Barto_ITS3 F(5′GATGCCGGGGAAGGTTTTC3′), Barto_ITS3_R(5′-GCCTGGGAGGACTTGAACCT-3′) and Barto_ITS3 _Probe (6FAM-5′GCGCGCGCTTGATAAGCGTG-3′) (Invitrogen, Thermo Fisher Scientific, and Belgium).

Amplification of Internal Transcribed Spacer (ITS) gene was conducted in a final volume of 20 µL containing 10 µL of 2 × Eurogentec TakyonTM Mix (Eurogentec, Liège, Belgium), 1 µL of each primer (0.5 µM), 0.12 µL of probe, 2.5 µL of DNase-free water, and 5 µL of DNA template. The real time–qPCR was performed on the StepOneTM Real-Time PCR system (by Thermo Fisher Scientific) using the following thermal profile: an incubation step at 50 °C for 2 min for eliminating PCR amplicons, then an activation step at 95 °C for 3 min followed by 40cycles of denaturation at 95 °C for 15 s and an annealing-extension at 60 °C for 30 s. Samples were confirmed as *Bartonella* spp. if they tested positive for the first and second runs on real time–qPCR (Mariën et al. 2022) Further amplification of the ITS region (453–780 bp) (Sokhna et al. 2013)-(Böge et al. 2021) was done using conventional PCR system before sequencing (Sokhna et al. 2013). The amplification reactions were conducted in a final volume of 15 µL, containing 7.5 µL of Hot Goldstar master mix, 0.3 µL of each primer, 5.4 µL of DNA free water and 2.5 µL of DNA template. Reactions were conducted in a thermal cycler (TProfessional Basic Thermocycler by Biometra) under the following amplification conditions; 40 cycles for 30 s at 94 °C, for 30 s at 66 °C, for 50 s at 72 °C. PCR products were prepared with DNA Gel Loading Dye (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) for gel electrophoresis in 1 % agarose. Visualization was done using UV light. Amplicons of positive samples were purified and then Sanger sequenced with forward and revers primers at Neuromics Support Facility- Vlaams Instituut voor Biotechnologie (Antwerp-Belgium). The DNA extracts from the ectoparasites were also screened for other potential rodent pathogens including; *Borrelia* spp., *Babesia* spp. and *Anaplasma* spp. by real time-qPCR.

### 2.5 Molecular screening of *Leptospira* spp.

The blood DNA extracts were screened for *Leptospira* spp. by real time-qPCR using the following pairs of primers and probes (Dahmana et al. 2020):

Primer F lip32B (AGCTCTTTTGTTCTGAGCGA), Primer R lip32BR (TACGAACTCCCATTTCAGCGATTA), Probe (FAM-AAAGCCAGGACAAGCG CCG-NFQ-MGB) (Invitrogen, Thermo Fisher Scientific, Belgium). Amplification of 16S-RNA gene was conducted in a final volume of 10 µL containing 5 µL of 2 × master mix, 0.5 µL of each primer forward and reverse, 0.2 µL of probe, 1.0 µL of DNase-free water, and 1 µL of DNA template. The real time–qPCR was performed on the StepOneTM

Real-Time qPCR system by (Thermo Fisher Scientific) using the following thermal profile: pre incubation step at 95 °C for 3 min by one cycle then an activation step at 95 °C for 15 s for 40 cycles, followed by 40 cycles of denaturation at 60 °C for 30 s and an annealing-extension at 60 °C for 30 s (Cortez et al. 2018).

### 2.6 Statistical analysis

Confidence intervals (95 % CI) for the prevalence of *Bartonella* spp. and *Leptospira* spp. in *M. natalensis* was determined at the level of alpha of 0.05 (Böge et al. 2021).

The statistical difference in *Bartonella* spp. infection of mites from males and females of *M. natalensis* was determined using a two-tailed student's *t*-test.

Prevalence (%) was calculated as number of individuals infected divided by the number of individuals examined. Statistical analysis and molecular analyses were done using *R* Statistical Software 4.1.3 and Genious software respectively.

## 3 Results

### 3.1 Molecular detection of *Leptospira and Bartonella* spp. in *M. natalensis* and their ectoparasites

One out of 100 *M. natalensis* screened for *Leptospira* spp by real time–qPCR, was positive 1(1 %), (95 %, CI: 4.7–7.9 %), indicating a relatively low prevalence of leptospirosis in the multimammate rats in the study areas.

Following real time-qPCR, *Bartonella* spp. were detected in mites, but not in fleas at a prevalence of 14 (14 %), (95 % CI: 7.08–20.9 %). The positive samples were further amplified by conventional PCR followed by sequencing. Of the 14 samples, four showed a complete sequence of the *ITS* gene (Table 1). The sequenced samples belonged to the same *Bartonella* strain (RN3BJ), predominantly *Uncultured Bartonella* (MW194941).Others were*Bartonella queenslandensis* (MZ570393), *Candidatus Bartonella thailandensis* (FJ411484,) and *Bartonella* sp. (EF190331). *Borrelia* spp., *Babesia* spp. and *Anaplasma* spp. were not detected. *Bartonella* spp. infection was shown to be higher in mites from adult male *M. natalensis* than females. However the difference was not considered significant ($p > 0.05$). Juvenile *M. natalensis* were relatively few in the study areas and no ectoparasites were found on them. The study revealed a larger population of males than females in the study areas (Table 2).

**Table 1:** Mites from *Mastomys natalensis* screened for *Bartonella* spp. by real time qPCR.

| Study location | *M. natalensis* mites positive for *Bartonella* spp. | *Bartonella* spp.-positive males | *Bartonella* spp.-positive females | *P*-value |
|---|---|---|---|---|
| Choza | 6/14(42 %) | 4/6 (67 %) | 2/6 (33 %) | 0.06 |
| Kiroka | 8/14 (58 %) | 5/8 (63 %) | 3/8 (37 %) | 0.07 |

**Table 2:** *Mastomys natalensis* captured in a three-month period (trap nights, sex and age).

| | Choza | | | Kiroka | | |
|---|---|---|---|---|---|---|
| | Jan | Feb | Mar | Jan | Feb | Mar |
| Trap nights | 3 | 3 | 3 | 3 | 3 | 3 |
| Number of traps | 150 | 150 | 150 | 150 | 150 | 150 |
| *M. natalensis* captured | 10 | 19 | 21 | 11 | 19 | 20 |
| Males | 7 | 10 | 10 | 7 | 10 | 10 |
| Females | 3 | 9 | 11 | 4 | 9 | 10 |
| Adults | 9 | 19 | 20 | 11 | 18 | 20 |
| Juveniles | 1 | 0 | 1 | 0 | 1 | 0 |
| Mites collected from *M. natalensis* | 17 | 40 | 54 | 29 | 42 | 50 |
| Fleas collected from *M. natalensis* | 6 | 10 | 14 | 12 | 11 | 21 |

## 4 Discussion

In this study, the prevalence of *Leptospira* spp. and *Bartonella* spp. in *M. natalensis* and their ectoparasites (*Laelaptine* spp. and *X. cheopis*) in selected areas of Morogoro were determined. There was no co-infestation found of mites and fleas in the multimmamate rats. This finding is in agreement with report by (Kaminskienė et al. 2017).

Molecular analysis of the pathogens demonstrated a relatively low prevalence of *Leptospira* in *M. natalensis*. This finding contradicts with a previous report by (Katakweba et al. 2012), which states that, a dry environment is limiting the survival of *Leptospira* spp. because leptospirosis is a water borne disease. Therefore, the prevalence would be expected to be higher during the study period which was between the end of the short rain season and beginning of long rains which would be favorable for the *Leptospir*a spp. to survive in the environment. Therefore, it is plausible to believe that, the overall prevalence of leptospirosis in *M. natalensis* in the study areas is relatively low.

Other studies by Kaminskienė et al. (2017) and Mgode et al. (2021) have also reported a low prevalence of *Leptospira* spp. in *M. natalensis*, but a comparatively higher prevalence in *Rattus rattus* and *Cricetomys* spp. This relatively small proportion of *Leptospira* spp. infected *M. natalensis* may also be due to their ecology and habitats which differ from those of *R. rattus* and *Cricetomys* spp. (Massawe et al. 2005). Low prevalence of *Leptospira* spp. in

blood samples has also been reported by Cortez et al. (2018) and Bal et al. (1994), suggesting a number of factors contributing to the low prevalence, including: the species of the rodent, habitat and development of immunity with age. However, leptospirosis has a short phase in which the infectious agent is found in the circulatory system (leptospiremia) before entering the kidney tubules where it remains for a long time with intermittent discharge with urine in relatively large numbers (Bal et al. 1994).

*Laelaptine*s spp. from 14 rodents tested positive for *Bartonella* spp. by real time–qPCR, however, upon conventional PCR and sequencing, four samples demonstrated a complete *ITS* gene sequence with preponderance of *Uncultured Bartonella.* These findings agreed with previous studies done elsewhere, showing that *Bartonella* strains found in rodent ectoparasites belonged to *Uncultured Bartonella* spp. (Angelakis and Raoult 2014; Böge et al. 2021; Dehio 2001; Gundi et al. 2009). In this study, other species of *Bartonella* spp. that were detected and known to be zoonotic pathogens includes: *Bartonella queenslandensis, Candidatus Bartonella, Thailandensis Bartonella* spp. In this study, more mites were isolated from males than females *M. natalensis*, however the difference was not significant. This is in agreement with studies by Beery (2018).

This study also showed that, the overall prevalence of *Bartonella* spp. in *M. natalensis* mites was higher in males than females. This could be explained by the fact that, males have a larger home range compared to females, thus, predisposing them to more frequent contacts with the pathogens. This is in agreement with Ferrari et al. (2004) and Kataranovski et al. (2011), who suggested that males have a bigger role in driving the dynamics of transmission of infections.

*Bartonella* spp. infection was higher in mites from adult *M. natalensis* than in juveniles, because, adults had a higher chance of exposure to the pathogens than the juveniles. The detection of *Bartonella* spp. in mites has been reported by Alsarraf et al. (2017) which proposed that, ticks and mites be added to the list of potential reservoirs and vectors of pathogens, however, further investigations are necessary to describe their potential roles in *Bartonella* infection and transmission.

## 5 Conclusion and recommendation

This study has shown a relatively low prevalence of *Leptospira* spp. and higher prevalence of *Bartonella* spp. in mites from *Mastomys natelensis* in the study areas, suggesting that mites are potential reservoirs *cum* vectors of bartonellosis. Additional studies on the role of fleas as reservoirs or vectors of bartonellosis are essential.

Since *Leptospira* spp. and *Bartonella* spp. are zoonotic pathogens transmitted by rodents and their ectoparasites respectively. Surveillance for these bacteria should be considered alongside studies of other rodent borne pathogens such as, *Borrelia* spp., *Babesia* spp. and *Anaplasma* spp.

**Research ethics:** Risk assessment was submitted to and approved by the Ethical Committee and Decision Board of Sokoine University of Agriculture (SUA), Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH), permit number 2022-401-NA-2021-084.

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**Author contributions:** CT conceptualized the research idea, was involved during the data collection and manuscript write up. VM, NH, GM, JM undertook the field survey, collected data and performed the data analysis. CS and JN reviewed and edited the manuscript. IM and RM supervised the research work, reviewed and edited the manuscript. HL supervised the research work, reviewed, edited the manuscript, funded the research work and finalized the manuscript.

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## References

Alsarraf, M., Mohallal, E.M.E., Mierzejewska, E.J., Behnke-Borowczyk, J., Welc-Falęciak, R., Bednarska, M., Dziewit, L., Zalat, S., Gilbert, F., Behnke, et al. (2017). Description of *Candidatus Bartonella fadhilae* n. sp. and *Candidatus Bartonella sanaae* n. sp. (*Bartonellaceae*) from *Dipodillus dasyurus* and *Sekeetamys calurus* (*Gerbillinae*) from the Sinai Massif (Egypt). Vector-Borne Zoonotic Dis. 17: 483–494.

Angelakis, E. and Raoult, D. (2014). Pathogenicity and treatment of *Bartonella* infections. Int. J. Antimicrob. Agents 44: 16–25.

Aplin, K.P. (2003). *Field methods for rodent studies in Asia and the Indo-Pacific*. Australian Centre for International Agricultural Research, Canberra.

Bal, A.E., Gravekamp, C., Hartskeerl, R.A., De Meza-Brewster, J., Korver, H., and Terpstra, W.J. (1994). Detection of leptospires in urine by PCR for early diagnosis of leptospirosis. J. Clin. Microbiol. 32: 1894–1898.

Beery, A.K. (2018). Inclusion of females does not increase variability in rodent research studies. Curr. Opin. Behav. Sci. 23: 143–149.

Boey, K., Shiokawa, K., and Rajeev, S. (2019). Leptospira infection in rats: a literature review of global prevalence and distribution. PLoS Negl. Trop. Dis. 13: e0007499.

Böge, I., Pfeffer, M., Htwe, N.M., Maw, P.P., Sarathchandra, S.R., Sluydts, V., Piscitelli, A.P., Jacob, J., and Obiegala, A. (2021). First detection of *Bartonella* spp. in small mammals from rice storage and processing facilities in Myanmar and Sri Lanka. Microorganisms 9: 658.

Broecke, B.V. (2021). Linking behavior, co-infection patterns, and viral infection risk with the whole gastrointestinal helminthes community structure in Mastomys natalensis. Front. Vet. Sci. 8: 15.

Claus, A.T., Abdul, A.S., Rhodes, H.M., Robert, S., and Stella, T.K. (2020). Prevalence of Rickettsia typhi in rodent fleas from areas with and without previous history of plague in Mbulu district, Tanzania. Afr. J. Microbiol. Res. 14: 65–70.

Cortez, V., Canal, E., Dupont-Turkowsky, J.C., Quevedo, T., Albujar, C., Chang, T.-C., Salmon-Mulanovich, G., Guezala-Villavicencio, M.C., Simons, M.P., Margolis, E., et al. (2018). Identification of *Leptospira* and *Bartonella* among rodents collected across a habitat disturbance gradient along the inter-oceanic highway in the southern amazon basin of Peru. PLoS One 13: e0205068.

Dahmana, H., Granjon, L., Diagne, C., Davoust, B., Fenollar, F., and Mediannikov, O. (2020). Rodents as hosts of pathogens and related zoonotic disease risk. Pathogens 9: 202.

Dehio, C. (2001). *Bartonella* interactions with endothelial cells and erythrocytes. Trends Microbiol. 9: 279–285.

Desai, S., van Treeck, U., Lierz, M., Espelage, W., Zota, L., Sarbu, A., Czerwinski, M., Sadkowska-Todys, M., Avdicová, M., Reetz, J., et al. (2009). Resurgence of field fever in a temperate country: an epidemic of Leptospirosis among seasonal strawberry harvesters in Germany in 2007. Clin. Infect. Dis. 48: 691–697.

Ferrari, N., Cattadori, I. M., Nespereira, j., Rizzoli, A., and Hudson, P. J. (2004). The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse Apodemus flavicollis. Ecol. Lett. 7: 88–94.

Gundi, V.A.K.B., Taylor, C., Raoult, D., and La Scola, B. (2009). *Bartonella rattaustraliani* sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats. Int. J. Syst. Evol. Microbiol. 59: 2956–2961.

Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I.S., and Harrus, S. (2015). *Bartonella* spp. infection in rodents and their flea ectoparasites: an overview. Vector Borne Zoonotic Dis. 15: 27–39.

Holt, J., Davis, S., and Leirs, H. (2006). A model of Leptospirosis infection in an African rodent to determine risk to humans: seasonal fluctuations and the impact of rodent control. Acta Trop. 99: 218–225.

Kaminskienė, E., Radzijevskaja, J., Balčiauskas, L., Gedminas, V., Paulauskas, A. (2017) Laelapidae mites (Acari: Mesostigmata) infesting small rodents in the Curonian Spit, Lithuania. Biologija 63, https://doi.org/10.6001/biologija.v63i2.3528.

Katakweba, A.A.S., Mulungu, L.S., Eiseb, S.J., Mahlaba, T.A., Makundi, R.H., Massawe, A.W., Borremans, B., and Belmain, S.R. (2012). Prevalence of haemoparasites, Leptospires and Coccobacilli with potential for human infection in the blood of rodents and shrews from selected localities in Tanzania, Namibia and Swaziland. Afr. Zool. 47: 119–127.

Kataranovski, M., Mirkov, I., Belij, S., Popov, A., Petrović, Z., Gačić, Z., and Kataranovski, D. (2011). Intestinal helminths infection of rats (*Ratus* [sic] *norvegicus*) in the Belgrade area (Serbia): the effect of sex, age and habitat. Parasite 18: 189–196.

Kim, C.M., Kim, J.Y., Yi, Y.H., Lee, M.J., Cho, M., Shah, D.H., Klein, T.A., Kim, H.C., Song, J.W., Chong, S.T., et al. (2005). Detection of Bartonella species from ticks, mites and small mammals in Korea. J. Vet. Sci. 6: 327–334.

Machang'u, R.S., Mgode, G.F., Assenga, J., Mhamphi, G., Weetjens, B., Cox, C., Verhagen, R., Sondij, S., Goris, M.G., and Hartskeerl, R.A. (2004). Serological and molecular characterization of leptospira serovar Kenya from captive African giant pouched rats (*Cricetomys gambianus*) from Morogoro Tanzania. FEMS Immunol. Med. Microbiol. 41: 117–121.

Makundi, R.S., Kilonzo, B.S., and Massawe, A.W. (2003). Interaction between rodent species in agro-forestry habitats in the western Usambara Mountains, north-eastern Tanzania, and its potential for plague transmission to humans. SUA IR (2003-1).

Malania, L., Bai, Y., Osikowicz, L.M., Tsertsvadze, N., Katsitadze, G., Imnadze, P., and Kosoy, M. (2016). Prevalence and diversity of Bartonella species in rodents from Georgia (Caucasus). Am. J. Trop. Med. Hyg. 95: 466–471.

Mariën, J., Vanden Broecke, B., Tafompa, P.J.J., Bernaerts, L., Ribas, A., Mnyone, L.L., Mulugu, L.S., and Leirs, H. (2022). Host related factors determine co-occurrence patterns between pathogenic bacteria, protozoa, and helminths in populations of the multimammate mouse, *Mastomys natalensis*. bioRxiv 2022-01.

Massawe, A.W., Rwamugira, W., Leirs, H., and Makundi, R.H. (2005). Influence of land preparation methods and vegetation cover on population abundance of Mastomys natalensis in Morogoro, Tanzania. SUA IR (2005-12).

Meerburg, B.G., Singleton, G.R., and Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. Crit. Rev. Microbiol. 35: 221–270.

Mgode, G.F., Mhamphi, G.G., Massawe, A.W., and Machang'u, R.S. (2021). Leptospira seropositivity in humans, livestock and wild animals in a semi-arid area of Tanzania. Pathogens 10: 696.

Rija, A.A., Bugingo, A., Said, A., and Mwamende, K.A. (2014). Wet season bird species richness and diversity along urban-rural gradient in Morogoro municipality and surrounding areas, Tanzania. Tanzania J. Forestry Nat. Conserv. 83: 1–12.

Sokhna, C., Mediannikov, O., Fenollar, F., Bassene, H., Diatta, G., Tall, A., Trape, J.F., Drancourt, M., and Raoult, D. (2013). Point-of-care laboratory of pathogen diagnosis in rural Senegal. PLoS Negl. Trop. Dis. 7: e1999.

Theonest, N.O., Carter, R.W., Amani, N., Doherty, S.L., Hugho, E., Keyyu, J.D., Mable, B.K., Shirima, G.M., Tarimo, R., Thomas, K.M., et al. (2019). Molecular detection and genetic characterization of Bartonella species from rodents and their associated ectoparasites from northern Tanzania. PLoS One 14: e0223667.

Van Aelst, K. and Holvoet, N. (2018). Climate change adaptation in the Morogoro Region of Tanzania: women's decision-making participation in small-scale farm households. Clim. Dev. 10: 495–508.

Van Houtte, N., de Bellocq, J.G., Akaibe, D., Wilschut, L., Verheyen, E., Amundala, N., Socolovschi, C., Falay, D., Raoult, D., Laudisoit, A., et al. (2014). High prevalence of *Rickettsia typhi* and *Bartonella* species in rats and fleas, Kisangani, Democratic Republic of the Congo. Am. J. Trop. Med. Hyg. 90: 463–468.

## CHAPTER FOUR
## PAPER III

# EFFECT OF IVERMECTIN® ON INTESTINAL HELMINTHS IN MULTIMMATE MOUSE (*MASTOMYS NATALENSIS*)

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## ABSTRACT

Rodents are reservoirs of various zoonotic pathogens, including parasitic helminths. In this study, the effect of Ivermectin® against gastrointestinal helminths of the multimammate mouse (*Mastomys natalensis*) was investigated under field condition. It is hypothesized that Ivermectin® is effective against a broad spectrum of gastrointestinal helminths such as, *Physaloptera* spp., *Strongiloides* spp. and *Trichuris* spp., however, no controlled studies have been carried out to assess its effectiveness in *M. natalensis* in the field. The rodents were live captured using Sherman® traps from an open field as well as free ranging rodents from an enclosed field (Fence) near Sokoine University of Agriculture.

The animals were marked and housed individually in clean cages and supplied with feed and water *ad libitum*. After collecting faecal samples from every rodents, the animals were released into two enclosed plots in the Fence, each containing 45 animals. The animals in the first plot received a single dose of an aqueous suspension of Ivermectin®. Animals in the group were left untreated. Thereafter the rodents were captured biweekly from the plots and faecal materials were collected for screening of helminth eggs. Five different species of intestinal parasites were identified which were; *Hymenolepis diminuta* (42.6%), *Physaloptera* spp. (28%), *Strongiloides* spp. (12%), *Hymenelopsis nana* (10%) and *Trichuris* spp. (7%). There was no significant difference in mean worm infection before and after treatment in *Strongiloides* spp*., H. nana* and *Physaloptera* spp. However, there was a significant reduction in the number of *H.diminuta* and *Trichuris* worms in the treated animals*.* For the untreated group, no significant increase in the number of worms were observed across eight weeks. The overall prevalence of helminths infection in *M. natalensis* before treatment appeared to be significantly higher in male than female rodents. This study has revealed a greater diversity of helminths infection in *M. natalensis*, especially in male individuals. Also, Ivermectin® has shown to be effective against *H. diminuta* and *Trichuris* worms but not the other worms found in this study.

**Key words;** Helminths, anthelminths, Ivermectin®, *Mastomys natalensis*

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## 1.0 Introduction

Rodents are reservoirs of various parasites including zoonotic helminths (Malsawmtluangi & Tandon, 2009 and Stojcevic *et al.,* 2012). Helminthiasis are considered to be neglected diseases with low public health importance because they cause diseases that are not severe. Nevertheless, in immune compromised individuals (e.g human immunodeficiency virus (HIV) or other disorders of the immune system, helminths may cause chronic infection and hyper infection syndrome (Herbreteau *et al.,* 2012 and Chaisiri *et al.*, 2015). Moreover, some zoonotic Helminthiasis are of public health significance. These include: Echinococcosis, taeniasis, trichinosis, schistosomiasis, filariasis, and opisthorchiasis (Meerburg *et al.,* 2009). The World Health Organization estimates that, two billion people harbor parasitic worm infections (WHO, 2022), while for the sub-Saharan Africa, more than one billion people are infected with soil transmitted helminths (Malsawmtluangi & Tandon, 2009, Mulungu *et al.*, 2011, Pakdel *et al.,* 2013, Broecke, 2021).

Studies on gastro-intestinal parasites of rodents have revealed various helminths in more than 50% of the rodent population, such as: *Strongyloides* spp., *Hymenelopis diminuta, Hmenelopis nana, Physoloptera* spp.*, Syphacia* Spp. and *Trichuris* spp. (Malsawmtluangi & Tandon, 2009, Mulungu *et al.,* 2011; Herbreteau *et al.,* 2012; Chaisiri *et al.,* 2015; Ranjbar *et al.,* 2017). The challenge by the helminths can cause increased morbidity and mortality to the rodents (Paul *et al.*, 2016; Broecke, 2021).

The increasing anthelmintic resistance (AR) is a threat to animal and human health (Peña-Espinoza, 2018). Furthermore, side-effects, lack of efficacy, high cost and continuous use of a single drug have complicated treatment against Helminthiasis (Shalaby, 2013); (Nixon *et al.,* 2020). In this study, the multimammate mouse (*Mastomys natalensis*) was used as an important bridge species (model) to investigate the effect of Ivermectin® as

anthelminthic in rodents. It is hypothesized that, this anthelminthic can significantly reduce the gastrointestinal helminth burden in *M. natalensis* due to its safety, availability, ease of administration and mechanism of action (Foletto *et al.,* 2015). It is also a commonly used anthelmintic in laboratory, domestic animals and humans, and it is widely used for the treatment of gastro-intestinal nematode infections as well as ectoparasite infestation (Wolstenholme & Rogers, 2006).

Ivermectin® targets a glutamate-gated chloride channel in some invertebrates as well as the chloride channel of gamma-aminobutyric acid type A (GABA-A) receptors, promoting cellular hyperpolarization and worm paralysis (Foletto *et al.,* 2015; Bosco *et al.*, 2020) and (Cullin *et al.,* 2017). Peridomestic small mammals, such as *M. natalensis* are important reservoirs and *cum vectors* of helminths, however, little data is available on the effective treatments against gastrointestinal helminths in small mammals. This study aims to achieve the following objectives; to determine the overall prevalence of gastrointestinal helminths (GIT) in *M. natalensis* and the burden of helminths eggs before and after treatment with Ivermectin® as well as to determine the distribution of helminths eggs per sex of *M. natalensis.* The findings of this study will help to reduce environmental contamination with zoonotic helminths transmitted by *M. natalensis*. Also, will be a useful reference in developing an appropriate research model to study coinfections involving rodent transmitted helminths and other pathogens.

## 2.0 Materials and Methods

### 2.1 Study Area

This study was conducted in an enclosed area, measuring 1ha field (also referred to as fence). Two plots measuring 0.25 ha were demarcated in the fence by sub partitioning with rodent proof iron sheets. The fence was previously used for rodent ecological

studies and is located near Sokoine University of Agriculture at Edward Moringe Sokoine campus in Morogoro, Tanzania (6°50′34.9794″S; 37°38′8.232″E) (Fig.1).

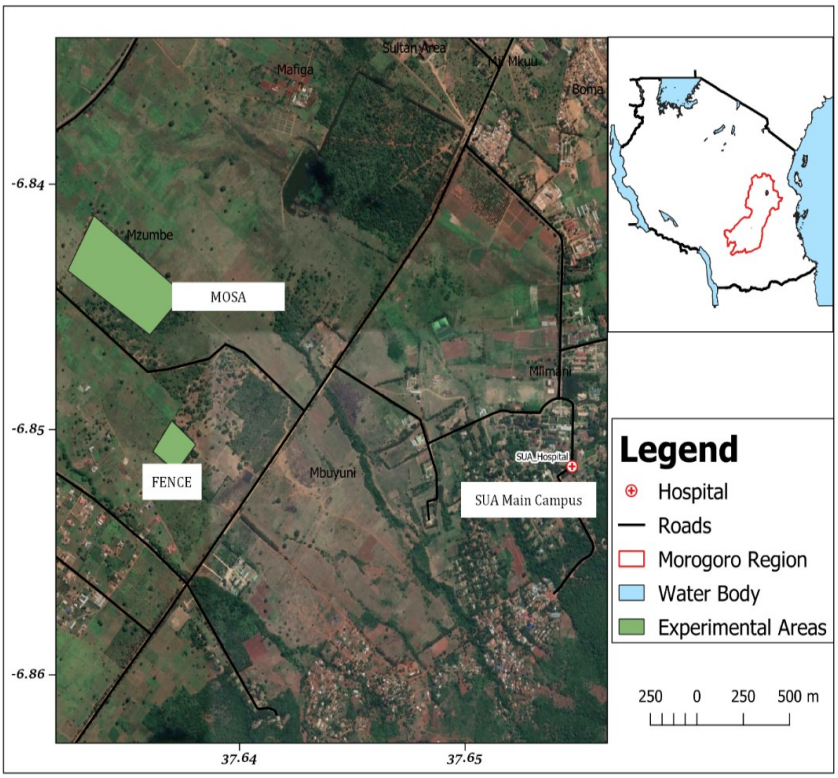


**Figure 1:** Map of FENCE - SUA main campus

## 2.2 Data collection

### 2.2.1 Removal trapping

Rodents were trapped from the fence using live Sherman® traps (Standard medium size LFA: 7.6 X 8.9 X 23 cm). The plots were considered depleted of rodents after seven consecutive days of trapping without capturing any animal as recommended by (H. Leirs, personal communication, January, 2021). A total of 300 traps were used during rodent trapping, in each trapping sites

(areas outside the fence and around the SUA campus), 10 transect lines were set, each laid with 30 Sherman® ® traps. A 10m space was set from line to line and from trap to trap. A mixture of peanut butter and maize bran was used as the bait. A total of 90 rodents were captured and considered to be adequate for the study (Seras, 2003; Foletto *et al.,* 2015b). Each trapped animal was weighed and checked for sex (Maric *et al.,* 2022).

### 2.3 Faecal Collection

Animals captured were sent to the laboratory at the Institute of Pest Management (IPM) animal house and caged individually to facilitate faecal sample collection. Each animal was marked by applying a permanent ink on their tails. Water and feed were provided *ad libitum*. Faecal samples were collected from every rodent directly for primary screening of helminths eggs. Collection was induced by pinching the skin/fur on the back where necessary. The faecal samples were weighed and preserved in eppendorf tubes containing ethanol 70% at room temperature (Pedersen & Antonovics, 2013).

### 2.4 Study Design

#### 2.4.1 Effect of ivermectin in M. natalensis

Rodents were separated into two groups, each containing 45 animals (i.e. treatment and control group).

a) Animals of the first group were inoculated subcutaneously using 1µL syringe as described by (Pedersen & Antonovics, 2013 and Foletto *et al.*, 2015a). Each animal received a single dose of aqueous suspension of Ivermectin® (Hebei Veyong Animal Pharmaceutical Co Ltd, Shijiazhuang, China) (1ml for 50kg animal body weight). This is equivalent to 0.0007ml/l Ivermectin®, assuming the average weight of each rodent was 35g.

b) For the second group of animals (control) were left untreated.

Each group was released into a separate fenced block; then faecal sample collection continued for secondary examination of helminths eggs, after every second week (14 days), between mid-May to mid-July, 2022 making a total of four sampling session for the entire study period according to Geurden *et al.* (2022). After the screening, animals were euthanatized in a jar using an overdose of halothane (Ribas *et al.,* 2020 and Vanden Broecke *et al.,* 2021).

T- test was used to compare the mean variation of gastrointestinal helminths burden in *M. natalensis* before and after treatment with Ivermectin® at a p-value of 0.05.

### 2.5 Screening for Helminths Eggs in Rodents

Helminths eggs screening was done using the faecal floatation technique (Foletto *et al.,* 2015), while faecal egg counts were conducted using a modified McMaster at SUA, Department of Veterinary Microbiology (Glover *et al.,* 2017). Briefly, faecal pellets were placed in 3-mL test tubes containing of floatation solution (analytical sodium chloride) (Fecasol, Vetoquinol USA, Fort Worth, TX) and once, softened, they were triturated to break up the pellets so as to facilitate the release of eggs. The test tubes were filled with floatation solution and covered with a coverslip for at least 15 min and then examined at a magnification of at least ×100 for the presence of eggs then followed by McMaster for quantification (Dole *et al.*, 2011; Foletto *et al.*, 2015).

## 3.0 Results

### 3.1 Overall prevalence of gastrointestinal helminths (GIT) in M. natalensis

A total of 90 *M. natalensis* were captured, 43 individuals were males and 47 were females. All the captured animals were adults. The prevalence of infection with GIT helminths of treated and

untreated groups was as presented in (Table 1). *Hymenolopsis diminuta* presented the highest prevalence of 39% followed by *Physaloptera* spp. (29%), *Hymenelopsis nana* (12%), *Strongiloides* spp. (11%) and *Trichuris* spp. (9%).

**Table 1:** Overall prevalence of GIT helminths in *M. natalensis* before treatment with Ivermectin®

| Helminth | Number of worm eggs per gram (EPG) | Prevalence (%) |
|---|---|---|
| *Strongiloides* spp. | 15100 | 11% |
| *Trichuris* spp. | 12500 | 9% |
| *H. diminuta* | 52600 | 39% |
| *H. nana* | 15900 | 12% |
| *Physaloptera* spp. | 38400 | 29% |

### 3.2 Helminths status among individuals treated with Ivermectin® (Group I)

Before treatment, eggs of different species of helminths detected from *M. natalensis* faecal samples were, *H. diminuta* 41800(43%), followed by *Physaloptera* spp. 27600(28%), *Strongiloides* spp. 12100(12%), *H. nana* 10000(10%) and *Trichuris* spp. 6600(7%) (Table 1). Following treatment, there was an overall reduction of worm eggs which was significant for *Trichuris* spp. t= 2.74, p= 0.0391, [SE]). (0.75+-20.24) and *H. diminuta* (P<0.05), t= 2.93, p= 0.0087, [SE]) (0.65+-19.20) suggesting that Ivermictin reduced *Trichuris* spp. and *H. diminuta* burden in *M. natalensis* (Table 2).

**Table 2:** Mean number of EPG for group I in four sampling sessions before and after treatment of *M. natalensis* by Ivermectin®

| SESSION 1 | Before treatment | After treatment Session I | After treatment Session II | After treatment Session III | After treatment session IV | p-value |
|---|---|---|---|---|---|---|
| *Strongiloides Spp.* | 12100 | 3900 | 2900 | 1300 | 1100 | 0.11 |
| *Trichuris Spp.* | 6600 | 300 | 250 | 200 | 250 | 0.03 |
| *H. diminuta* | 41800 | 4700 | 3400 | 2100 | 1500 | 0.008 |
| *H. nana* | 10000 | 6400 | 4200 | 3000 | 2000 | 0.64 |
| *Physalopteria Spp.* | 27600 | 13900 | 14800 | 12000 | 4000 | 0.29 |

### 3.3 Helminths status of individuals not treated with Ivermectin® (Group II)

The second untreated group (control), revealed no significant decrease in the number of eggs per gram during the study period when analyzed ($p>0.05$) (Table 3).

**Table 3:** Mean number of EPG for group II before and after being released at FENCE

| SESSION 1 | Before released at FENCE | After release Session I | After release Session II | After release Session III | After release session IV | p-value |
|---|---|---|---|---|---|---|
| *Strongiloides Spp.* | 3000 | 3100 | 3050 | 3500 | 3600 | 0.92 |
| *Trichuris* spp. | 5900 | 5800 | 5900 | 5200 | 5400 | 0.53 |
| *H. diminuta* | 10800 | 10900 | 10900 | 10950 | 10700 | 0.32 |
| *H. nana* | 5900 | 6400 | 7200 | 7500 | 7200 | 0.41 |
| *Physaloptera* spp. | 10800 | 10800 | 10200 | 12000 | 10500 | 0.08 |

### 3.4 Distribution of helminths eggs per sex of *M. natalensis*

The overall prevalence of helminths eggs in male *M. natalensis* before and after treatment was significantly higher than in female rats (p=0.01) (Table 4).

**Table 4: Prevalence of helminths infection by host sex before and after treatment**

| Helminths eggs detected | Male rats before treatment | Male rats after treatment | Female rats before treatment | Female rats after treatment | p-value |
|---|---|---|---|---|---|
| *Strongiloides* spp. | 8000 | 2000 | 4100 | 1900 | 0.11 |
| *Trichuris* spp. | 4000 | 200 | 6600 | 100 | 0.03 |
| *H. diminuta* | 29800 | 2300 | 12000 | 2400 | 0.008 |
| *H. nana* | 7300 | 3200 | 2700 | 3400 | 0.646 |
| *Physaloptera* spp. | 15600 | 500 | 12000 | 400 | 0.29 |

## 4.0 Discussion

In this study, Ivermectin® was significantly effective as an anthelminthic against *H. diminuta* and *Trichuris* spp. but not against *Strongiloides* spp.*, H. nana* and *Physaloptera* spp. in *M. natalensis.* This finding is consistent with studies carried out elsewhere (Stojcevic *et al.,* 2012; Noradilah *et al.,* 2013; Mohtasebi, 2020). Ivermectin® has shown not to have effect over *H. nana*, this may be due to the fact that, Ivermectin® is effective against nematodes and arthropods, but not against cestodes and trematodes, because Ivermectin® acts as a GABA receptor agonist and cestodes and trematodes lack a GABA system (Martin *et al.,* 2021).This findings is contradicting with studies done by (Foletto *et al.,* 2015b) indicating that Ivermectin® is highly effective treatment for Giardia spp. and *Hymenolepis* spp. in laboratory rat colonies. However, further investigations are necessary on this disparity. For *Strongiloides* spp. and *Physaloptera* spp. Ivermectin® ineffectiveness can be due to the anthelminthic resistance (Shalaby, 2013, Bosco *et al.,* 2020).

The study has shown, *H. diminuta* and *Trichuris* spp. to be the predominant helminths in the studied species of rodents. These findings also agrees with studies done by (Noradilah *et al.,* 2013; Yang *et al.,* 2017 and Mohtasebi, 2020). In the untreated group, individuals showed no changes in the mean number of worm eggs per gram, showing a clear difference in the number of worms eggs in the Ivermectin® treated and control groups which is also in agreement with the studies by Davis *et al.* (1999); Malsawmtluangi & Tandon, (2009); Foletto *et al.* (2015); Bosco *et al.* (2020). Also, Richardson & Brink (2011) demonstrated a reduction of *H. diminuta* and *Trichuris* worms eggs of between 95.7% and 100% using a combination of Albendazole, Ivermectin®, pyrantel pamoate and Mebendazole (Richardson & Brink, 2011).

This study also showed that, the overall prevalence of helminths infection in *M. natalensis* was significantly higher in male than female rats. This could be explained by the fact that, males have larger home range compared to females, predisposing them to more frequent contact with helminths eggs found in the environment. Studies by Ferrari *et al.* (2015) indicated the infection rates of 62.06% and 37.94% for male and females animals respectively, suggesting that males have a dominant role to play in driving the dynamics of parasite transmission in this system. Experiments by (Pakdel *et al.*, 2013, Ferrari *et al.,* 2015 and Vanden Broecke *et al.,* 2021) demonstrated that host sex does not only affect the ability to modulate parasite but also their contribution to parasite transmission dynamics with individual males playing a dominant role in successful parasite infection.

Higher transmission in males can also be due to the habit of males grooming females more than the other way round. This observation is in agreement with the findings of Moore (1986), Pakdel *et al.* (2013); Ferrari *et al.* (2015); Kalueff *et al.* (2016); *Maric et al.* (2022). However, this disagrees with the report by

Milazzo *et al.* (2010) and Stojcevic *et al.* (2012) who found no significant differences between male and female worms infection in rodents.

In conclusion, this study has revealed a greater number of helminths infection in *M. natalensis* especially in male than female rodents. Also Ivermectin® has shown to be comparatively more effective against *H. diminuta* and *Trichuris* worms as compared to *Strongiloides* spp*., H. nana* and *Physaloptera* spp. Since rodents are usually infected with a number of detected zoonotic helminths, control of rodents is therefore important to safeguarding public health. *Mastomys natalensis* can also serve as a good model in the study of infection and coinfection by helminth and other pathogens. It is recommended to use Ivermectin® as anthelminthic against zoonotic infection transmitted by rodents especially soil borne helminths.

**Ethical approval**

Research ethical approval was sought from ethical committee and decision board of Sokoine University of Agriculture [SUA], Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH), with permit number 2022-401-NA-2021-084.

**Competing interests**

Authors declare that they don't have competing interests.

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**REFERENCES**

Bosco, A., Kiebler, J., Amadesi, A., Varady, M., Hinney, B., Ianniello, D., Maurelli, M. P., Cringoli, G., & Rinaldi, L. (2020). The threat of reduced efficacy of anthelmintics against gastrointestinal nematodes in sheep from an area considered anthelmintic resistance-free. *Parasites & Vectors*, 13(1), 457. https://doi.org/10.1186/s13071-020-04329-2.

Broecke, B. V. (2021). Linking Behavior, Co-infection Patterns, and Viral Infection Risk With the Whole Gastrointestinal Helminth Community Structure in *Mastomys natalensis*. *Frontiers in Veterinary Science*, 8, 15.

Chaisiri, K., Siribat, P., Ribas, A., & Morand, S. (2015). Potentially Zoonotic Helminthiasis of Murid Rodents from the Indo-Chinese Peninsula: Impact of Habitat and the Risk of Human Infection. *Vector-Borne and Zoonotic Diseases*, 15(1), 73–85. https://doi.org/10.1089/vbz.2014.1619.

Cullin, C. O., Sellers, M. S., Rogers, E. R., Scott, K. E., Lee, D. N., Ophir, A. G., & Jackson, T. A. (2017). Intestinal Parasites and Anthelmintic Treatments in a Laboratory Colony of Wild-caught African Pouched Rats (*Cricetomys ansorgei)*. *Comparative Medicine*, *67*(5), Article 5.

Davis, J. A., Paylor, R., Mcdonald, M. P., Libbey, M., Ligler, A., Bryant, K., & Crawley, J. N. (1999). Behavioral Effects of Ivermectin® in Mice. *Laboratory Animal Science* 49(3), 9.

Dole, V. S., Zaias, J., Kyricopoulos-Cleasby, D. M., Banu, L. A., Waterman, L. L., Sanders, K., & Henderson, K. S. (2011). Comparison of Traditional and PCR Methods during Screening for and Confirmation of *Aspiculuris tetraptera* in

a Mouse Facility. *Journal of the American Association for Laboratory Animal Science* 50(6), 6.

Ferrari, N., Nespereira, J., Rizzoli, A., & Hudson, J. (n.d.). The role of host sex in parasite dynamics*: Field experiments on the yellownecked mouse Apodemus flavicollis*. 7.

Foletto, V., Vanz, F., Gazarini, L., Stern, C., & Tonussi, C. (2015). Efficacy and security of Ivermectin® given orally to rats naturally infected with *Syphacia* Spp.., *Giardia* Spp.. And *Hymenolepis nana*. *Laboratory Animals*, *49*(3), 196–200. https://³qdoi.org/10.1177/0023677214562850.

Geurden, T., Smith, E. R., Vercruysse, J., Yazwinski, T., Settje, T., & Nielsen, M. K. (2022). World association for the advancement of veterinary parasitology (WAAVP) guideline for the evaluation of the efficacy of anthelmintics in food-producing and companion animals: General guidelines. *Veterinary Parasitology*, 304, 109698. https://doi.org/10.1016/j.vetpar.2022.109698.

Glover, M., Clarke, C., Nabb, L., & Schmidt, J. (2017). Anthelmintic efficacy on sheep farms in south-west England. *Veterinary Record*, *180*(15), 378–378. https://doi.org/10.1136/vr.104151.

Herbreteau, V., Bordes, F., Jittapalapong, S., Supputamongkol, Y., & Morand, S. (2012). Rodent-borne diseases in Thailand: Targeting rodent carriers and risky habitats. *Infection Ecology & Epidemiology*, 2(1), 18637. https://doi.org/10.3402/iee.v2i0.18637.

Kalueff, A. V., Stewart, A. M., Song, C., Berridge, K. C., Graybiel, A. M., & Fentress, J. C. (2016). Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nature Reviews Neuroscience*, *17*(1), 45–59. https://doi.org/10.1038/nrn.2015.8.

Malsawmtluangi, C., & Tandon, V. (2009). Helminth parasite spectrum in rodent hosts from bamboo growing areas of Mizoram, North-east India. *Journal of Parasitic Diseases*, *33*(1–2), 28–35. https://doi.org/10.1007/s12639-009-0004-5.

Maric, I., Krieger, J.P., van der Velden, P., Börchers, S., Asker, M., Vujicic, M., Wernstedt Asterholm, I., & Skibicka, K. P. (2022). Sex and Species Differences in the Development of Diet-Induced Obesity and Metabolic Disturbances in Rodents. *Frontiers in Nutrition*, 9, 828522. https://doi.org/10.3389/fnut.2022.828522.

Martin, R. J., Robertson, A. P., & Choudhary, S. (2021). Ivermectin®: An Anthelmintic, an Insecticide, and Much More. *Trends in Parasitology*, *37*(1), 48–64. https://doi.org/10.1016/j.pt.2020.10.005.

Meerburg, B. G., Singleton, G. R., & Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*, 35(3), 221–270. https://doi.org/10.1080/10408410902989837

Milazzo, C., Ribas, A., Casanova, J., Cagnin, M., Geraci, F., & Bella, C. (2010). Helminths of the brown rat (Rattus norvegicus) (Berkenhout, 1769) in the city of Palermo, Italy. *Helminthologia*, *47*(4), 238–240. https:/ /doi. org/10. 2478/s11687-010-0037-4.

Mohtasebi, S. (2020). Intestinal helminthic parasites of rodents in the central region of Iran: *First report of a capillariid nematode from Dryomys nitedula*. 7.

Moore, C. L. (1986). Sex differences in self-grooming of rats: Effects of gonadal hormones and context. *Physiology & Behavior*, 36(3), 451–455. https://doi.org/10.1016/0031-9384(86)90314-8.

Mulungu, L. S., Mahlaba, T. A., Massawe, A. W., Kennis, J., Crauwels, D., Eiseb, S., Monadjem, A., Makundi, R. H., Katakweba, A. A. S., Leirs, H., & Belmain, S. R. (2011). Dietary differences of the multimammate mouse, *Mastomys natalensis* (Smith, 1834), across different habitats and seasons in Tanzania and Swaziland. *Wildlife Research*, 38(7), 640. https://doi.org/10.1071/WR11028.

Nixon, S. A., Welz, C., Woods, D. J., Costa-Junior, L., Zamanian, M., & Martin, R. J. (2020). Where are all the anthelmintics? Challenges and opportunities on the path to new anthelmintics. *International Journal for Parasitology: Drugs and Drug Resistance*, 14, 8–16. https://doi.org/10.1016/j.ijpddr.2020.07.001.

Pakdel, N., Naem, S., Rezaei, F., & Chalehchaleh, A.-A. (2013). A survey on helminthic infection in mice *(Mus musculus)* and rats *(Rattus norvegicus and Rattus rattus) in Kermanshah, Iran*. 5.

Paul, B., Kyari, F., Gadzama, M., Zango, M., Ejeh, E., Ndahi, J., & Mana, H. (2016). Gastrointestinal helminths and external parasites of domestic rats trapped from residential areas within Maiduguri Municipality, Nigeria. *Sokoto Journal of Veterinary Sciences*, 14(1), 10. https://doi.org/10.4314/sokjvs.v14i1.2.

Pedersen, A. B., & Antonovics, J. (2013). Anthelmintic treatment alters the parasite community in a wild mouse host. *Biology Letters*, *9*(4), 20130205. https://doi.org/10.1098/rsbl.2013.0205.

Peña-Espinoza, M. (2018). Drug resistance in parasitic helminths of veterinary importance in Chile: Status review and research needs. *Austral Journal of Veterinary Sciences*, 50(2), 65–76. https://doi.org/10.4067/S0719-81322018000200065.

Ranjbar, M. J., Sarkari, B., Mowlavi, G. R., Seifollahi, Z., Moshfe, A., & Khabisi, S. A. (2017). Helminth Infections of Rodents and Their Zoonotic Importance in Boyer-Ahmad District, Southwestern Iran. *Iran J Parasitol*, 12(4), 8.

Ribas, A., Wells, K., Morand, S., Chaisiri, K., Agatsuma, T., Lakim, M. B., Yuh Tuh, F. Y., & Saijuntha, W. (2020). Whipworms of south-east Asian rodents are distinct from *Trichuris muris. Parasitology International*, 77, 102128. https://doi.org/10.1016/j.parint.2020.102128.

Shalaby, H. A. (2013). *Anthelmintics Resistance; How to Overcome it? 8*.

Stojcevic, D., Mihaljevic, Z., & Marinculic, A. (2012). Parasitological survey of rats in rural regions

of Croatia. *Veterinární Medicína*, 49(No. 3), 70–74. https://doi.org/10.17221/5679-VETMED.

Vanden Broecke, B., Bernaerts, L., Ribas, A., Sluydts, V., Mnyone, L., Matthysen, E., & Leirs, H. (2021). Linking Behavior, Co-infection Patterns, and Viral Infection Risk With the Whole Gastrointestinal Helminth Community Structure in *Mastomys natalensis*. *Frontiers in Veterinary Science*,

Wolstenholme, A. J., & Rogers, A. T. (2006). *8*, 669058. https://doi.org/10.3389/fvets. 2021. 669058.Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology*, 131(S1), S85. https://doi.org/10.1017/S0031182005008218.

Yang, D., Zhao, W., Zhang, Y., & Liu, A. (2017). Prevalence of *Hymenolepis nana* and H. diminuta from Brown Rats *(Rattus norvegicus)* in Heilongjiang Province, China. *The Korean Journal of Parasitology*, 55(3), 351–355. https://doi.org/10.3347/kjp.2017.55.3.351.

# CHAPTER FIVE

## 5.0 GENERAL DISCUSSION

### 5.1 General Discussion

The current study aimed to investigate rodent borne pathogens infecting *M. natalensis* in selected areas of Morogoro and Iringa. The following were determined: Prevalence and seasonal variation of *Trichuris* worms infections in *M. natalensis* in two climatically different regions of Tanzania (Morogoro and Iringa), molecular detection of *Leptospira* spp. and *Bartonella* spp. in *M. natalensis* and their ectoparasites (*Laelaptine* spp. and *Xenopsylla cheopis*) in Morogoro and the effectiveness of Ivermectin® against gastrointestinal helminths in *M. natalensis.* The following were the findings: The difference in rainfall pattern, temperature and humidity in both regions caused variation in infection in *M. natalensis* and ultimately these climatic factors were noted to be important determinants of helminths and bacterial infections. It was further noted that rodents from areas with higher altitude and more rainfall were at a higher risk of being infected with *Trichuris* worms than areas with lower altitude and little rains, suggesting that zoonotic infection can be affected by changes in humidity and rainfall patterns. It was shown that, Iringa region had a higher prevalence of helminths infection than in Morogoro region. This finding is similar with studies done elsewhere by Mekonnen *et al.* (2019) and Nandi & Allen (2021) on a crucial role played by seasonality in the transmission of zoonotic diseases. Our study, further revealed that the prevalence of *Trichuris* worms and other helminths in both regions was higher during the rainy than the dry seasons, this can be due to the fact that, rainfall may facilitate a wider spreading of contaminated faecal matters deposited on the ground and hence increases rate of helminths infection. In addition to that, a large number of bacteria and helminths ova deposited in the ground may probably not withstand the higher temperature of the environment during the dry season.

These observations agrees with the number of studies on seasonal variations of *Trichuris* spp. and other intestinal helminth

infections by Stojcevic *et al.* (2012) and Yevstafieva *et al.* (2019). However, studies by Kanojiya *et al.* (2016) and Mekonnen *et al.* (2019) conducted in India and Ethiopia respectively, conflicted with this study by showing that, *Trichuris* worm infections and other helminths were higher during the dry season than the rainy season. This could be due to the fact that, *Trichuris* worm ova can adapt easily under different environmental conditions, thus enabling them to survive well even during the dry seasons, however, more studies that will involve a larger sample size and advanced molecular techniques are required to explain this disparity.

Overall, more males *M. natalensis* were infected by helminths than females. A higher prevalence of helminths and bacterial infections in male than females could be explained by the fact that males have larger home range compared to females, hence predisposing them to frequent contact with helminths eggs found in the environment. These findings are consistent with studies done by Ferrari *et al.* (2015) showing a relatively higher infection rates in males than females, this suggests that males have a dominant role in driving the dynamics of parasite transmission. Our findings contradicted with the study by Dahmana *et al.* (2020) who found that females were overall more infected than males, which is against the common trend of higher parasitism rates in males (Ferrari *et al.*, 2015). Furthermore, findings of Pakdel *et al.* (2013); Kalueff *et al.* (2016); Vanden Broecke *et al.*(2021) and Maric *et al.* (2022) demonstrated that, host sex does not only affect the ability to modulate parasite but also their contribution to parasite transmission dynamics, with individual males playing a dominant role in successful parasite infection. Higher infection rates in males can also be due to the habit of males grooming females more than the other way round.

However, the above findings disagrees with the report by Milazzo *et al*. (2010) and Stojcevic *et al.* (2012) who found no significant difference in worms infection between males and females. Generally our findings agrees with the fact that host sexual

differences can influence both the susceptibility and the exposition to different rodent pathogens, although, further investigation on the influence of rodent sex in helminths and bacterial infection is highly recommended. Further to this, the number of zoonotic bacterial infection especially *Bartonella* spp. and *Leptospira* spp. is generally increasing. More than 40 species are currently described, and interestingly, more than half are harbored by rodents (Dahmana *et al.,* 2020). In this study, a relatively low prevalence of *Leptospira* spp. and higher prevalence of *Bartonella* spp. in *M. natalensis* and in mites respectively was recorded. The current findings are consistent with studies done in Tanzania by Machang'u *et al*. (2004) and Mgode *et al.* (2021) who found a lower prevalence of *Leptospira* spp. in *M. natalensis* but higher prevalence in *Cricetomys* spp. and *R. rattus*. This difference in prevalence among rodent species could be due to the host ecology and habitats differences (Massawe *et al.,* 2005; Mgode *et al.,* 2021). Studies by Mulungu *et al.* (2011) and samiji *et al.* (2021) have shown that, *M. natalensis* differ with *R. rattus* and *Cricetomys* spp. interms of food categories, nesting behavior, habitat, distribution and susceptibility to various infection. Regardless of the fact that, *M. natalensis* is the most adaptable and widespread rodent in East Africa and with a larger sample size in these studies, still it presents a minimum rate of infection compared to *Rattus rattus and Cricetomys* spp. Moreover, future studies to investigate this disparity and variation of infection in relation to rodent or host species have to be conducted.

For *Bartonella* spp. infection, a higher prevalence was recorded in mites from adults than juvenile rodents, this can be due to a higher rate of exposure to the environment of adult than the juveniles. *Bartonella* infection specifically *Uncultured Bartonella*

specie was detected in mites but not in fleas, this could be due to the limited number of fleas infesting rodents in the study areas and study period. These findings are consistent with studies done by Alsarraf *et al.* (2017) showing that mites can carry *Bartonella* infection. However, further studies on pathogens and their ectoparasites are also encouraged.

This study also aims to determine the effect of Ivermectin® against gastrointestinal helminths of the multimammate mouse (*Mastomys natalensis*) under the field condition. Ivermectin® has shown to be comparatively more effective anthelminthic against *H. diminuta* and *Trichuris* worms as compared to *Strongiloides* spp., *H. nana* and *Physaloptera* spp. during the study. The findings were similar with studies carried out elsewhere by Stojcevic *et al. (*2012); Noradilah *et al.* (2013) and Mohtasebi, (2020). This anthelminthic has also shown not to have effect over *H. nana* and this may be due to the fact that, Ivermectin® is effective against nematodes and arthropods, but not against cestodes and trematodes, because of its ability to acts as a GABA receptor meanwhile, cestodes and trematodes lack a GABA system (Martin *et al.,* 2021). In addition to this, ineffectiveness of Ivermectin® against *Strongiloides* spp. and *Physaloptera* spp. can be due to the anthelminthic resistance as presented in the studies by Shalaby, (2013) and Bosco *et al.* (2020).These findings also contradicts with studies done by (Foletto *et al.,* 2015b), indicating that Ivermectin® is highly effective treatment for *Giardia* spp. and *Hymenolepis* spp. in laboratory rat colonies. However, further comparative studies with other group of anthelminthics against the broad spectrum of helminths should be done.

It is also been reported that these bacterial and helminthic infections can compromise the animal's health by impairing the digestive function and consequently its efficiency in absorbing nutrients from the gut. This general observation is in agreement with studies done elsewhere (Katakweba *et al.,* 2012; Stojcevic

e*t al.,* 2012; Ribas *et al.,* 2013; Noradilah *et al.*, 2013; Kaminskienė *et al.,* 2017; Mohtasebi, 2020 *;* Mariën *et al.,* 2022). Since these pathogens are zoonotic, they may inevitably cause adverse impact on human health as well (Jones, 2021). Further to this, molecular characterization of all studied pathogens and ectoparasites would have given a broader picture of infection in rodents and their ectoparasites.

This study have further revealed that *M. natalensis* can be coinfected with more than one pathogen and this reality is not adequately captured, especially in sub-Saharan Africa, considering the existing interaction between rodents and human dwellings which may pose a great risk of acquiring infectious diseases. This study calls upon further molecular studies to screen for other rodent borne pathogens which have not been covered in the present study, especially viruses and protozoa in areas of Tanzania where interactions between rodents and humans are highly reported.

Generally, this study with others (Pakdel *et al.,* 2013; Yevstafieva *et al.,* 2019; Jones, 2021) have shown that, *M. natalensis* is involved in transmitting a series of bacterial and helminthic infections which are potential pathogens also to humans. Therefore, medical, veterinary, wildlife, environmental health professionals and policy makers should encourage One Health approaches in strategic control of rodents and their endo/ectoparasites as well as effectively use of anthelminths in order to reduce their adverse impact on humans and other animals.

# CHAPTER SIX

## 6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

### 6.1 General Conclusion

This study has provided detailed information regarding the rodent borne pathogens, specifically, *Trichuris* worms, *Leptospir*a spp., *Bartonella* spp. It has also provided evidence on the effective anthelminthic against specific gastrointestinal parasites in *M. natalensis* which could be applicable in other small mammals as well. This information is useful for researchers in public health, environmentalists and policy makers. It will also serve as baseline for the future studies on coinfection with rodent borne helminths and other parasites of small mammals including guinea pigs, bats and rats. *M. natalensis* has shown to be an important vector of bacteria and intestinal parasites, known to be potential pathogens to humans and other animals.

### 6.2 Contribution of my PhD

This study serves as evidence-based information for public health purposes particularly in the management of rodent borne pathogens (*Leptospira* spp., *Bartonella* spp) and treatment of intestinal parasites, specifically *Trichuris* spp. and *H. diminuta.* This study has also shown that, the difference in altitude, rainfall, temperature and humidity could influence infection patterns by rodent borne pathogens. Furthermore, infection by the intestinal parasites in this study was higher in male than female *M. natalensis* and Ivermectin® has proven to be relatively more effective against *H. diminuta* and *Trichuris* worms. *Leptospira* spp. infection appears to be insignificant in *M. natalensis* in this study. However, *Bartonella* spp. prevalence has been shown to be relatively high in mites, suggesting mites to be considered as a potential reservoirs *cum* vectors of bartonellosis.

### 6.3 Recommendation

**Based on the findings of this study, the following recommendations are made;**

It is important to carry out periodic screening of pathogens in rodents in order to determine the carrier status for endo/ectoparasites of public health importance.

Further molecular characterization should be carried out on the *Trichuris* spp. isolates to show the link between the *M. natalensis* and human isolate of this helminths.

Comparative studies involving Ivermectin® versus other anthelminthics against gastrointestinal helminths should be done.

Further studies on the role of mites as reservoirs or vectors of bartonellosis are recommended.

Given the fact that, *Leptospira* spp. and *Bartonella* spp. are zoonotic pathogens transmitted by rodents and their ectoparasites respectively, surveillance for these bacteria should also be considered alongside studies of other rodent borne pathogens such as, *Borrelia* spp., *Babesia* spp. and *Anaplasma* spp.

Management of rodents in communities should be appropriately regulated to avoid the danger of spreading diseases to humans and other animals through the pathogens they carry.

*Mastomys natalensis* should be considered as a suitable model in the study of disease transmission due to its abundance.

**REFERENCES**

Ferrari, N., Nespereira, J., Rizzoli, A., & Hudson, J. (2015). *The role of host sex in parasite dynamics: Field experiments on the yellownecked mouse Apodemus flavicollis*. 7.

Jones, K. R. (2021). *Trichuris* spp. In Animals, with Specific Reference to Neo-Tropical Rodents. *Veterinary Sciences*, *8*(2), Article 2.

Kalueff, A. V., Stewart, A. M., Song, C., Berridge, K. C., Graybiel, A. M., & Fentress, J. C. (2016). Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nature Reviews Neuroscience* 17(1), 45–59.

Kaminskienė, E., Radzijevskaja, J., Balčiauskas, L., Gedminas, V., & Paulauskas, A. (2017). *Laelapidae mites* (Acari: Mesostigmata) infesting small rodents in the Curonian Spit, Lithuania. *Biologija* 63(2).

Katakweba, A. A. S., Mulungu, L. S., Eiseb, S. J., Mahlaba, T. A., Makundi, R. H., Massawe, A. W., Borremans, B., & Belmain, S. R. (2012a). Prevalence of Haemoparasites, Leptospires and Coccobacilli with Potential for Human Infection in the Blood of Rodents and Shrews from Selected Localities in Tanzania, Namibia and Swaziland †. *African Zoology* 47(1), Article 1.

Katakweba, A. A. S., Mulungu, L. S., Eiseb, S. J., Mahlaba, T. A., Makundi, R. H., Massawe, A. W., Borremans, B., & Belmain, S. R. (2012b). Prevalence of Haemoparasites, Leptospires and Coccobacilli with Potential for Human Infection in the Blood of Rodents and Shrews from Selected Localities in Tanzania, Namibia and Swaziland †. *African Zoology* 47(1), 119–127.

Maric, I., Krieger, J.-P., van der Velden, P., Börchers, S., Asker, M., Vujicic, M., Wernstedt Asterholm, I., & Skibicka, K. P. (2022). Sex and Species Differences in the Development of Diet-Induced Obesity and Metabolic Disturbances in Rodents. *Frontiers in Nutrition* 9, 828522.

Mariën, J., Vanden Broecke, B., Tafompa, P. J. J., Bernaerts, L., Ribas, A., Mnyone, L. L., Mulugu, L. S., & Leirs, H. (2022). Host related factors determine co-occurrence patterns between pathogenic bacteria, protozoa, and helminths in populations of the multimammate mouse, *Mastomys natalensis* [Preprint]. Ecology.

Milazzo, C., Ribas, A., Casanova, J., Cagnin, M., Geraci, F., & Bella, C. (2010). Helminths of the brown rat (*Rattus norvegicus*) (Berkenhout, 1769) in the city of Palermo, Italy. *Helminthologia* 47(4), 238–240.

Mohtasebi, S. (2020). Intestinal helminthic parasites of rodents in the central region of Iran: *First report of a capillariid nematode from Dryomys nitedula*. *7.*

Mekonnen, Z., Getachew, M., Bogers, J., Vercruysse, J., & Levecke, B. (2019). Assessment of seasonality in soil-transmitted helminth infections across 14 schools in Jimma Town, Ethiopia. *Pan African Medical Journal*, *32*(1).

Mulungu, L. S., Massawe, A. W., Kennis, J., Crauwels, D., Eiseb, S., Mahlaba, T. A. A., & Belmain, S. R. (2011). Differences in diet between two rodent species, Mastomys natalensis and Gerbilliscus vicinus, in fallow land habitats in central Tanzania. *African Zoology*, *46*(2), 387-392.

Noradilah, S. A., Anuar, T. S., & Lee, I. L. (2013). Role of seasonal variation on the prevalence and risk factors of trichuriasis among the aboriginal community in Malaysia. 9.

Nandi, A., & Allen, L. J. (2021). Probability of a zoonotic spillover with seasonal variation. *Infectious Disease Modelling*, *6*, 514-531.

Pakdel, N., Naem, S., Rezaei, F., & Chalehchaleh, A.A. (2013). A survey on helminthic infection in mice *(Mus musculus)* and rats *(Rattus norvegicus and Rattus rattus) in Kermanshah, Iran*. 5.

Ribas, A., López, S., Makundi, R. H., Leirs, H., & de Bellocq, J. G. (2013). *Trichuris* spp. (Nematoda: Trichuridae) from Two Rodents, *Mastomys natalensis* and *Gerbilliscus vicinus* in Tanzania. *Journal of Parasitology* 99(5): Article 5.

Stojcevic, D., Mihaljevic, Z., & Marinculic, A. (2012). Parasitological survey of rats in rural regions of Croatia. *Veterinární Medicína*, 49(No. 3), 70–74. https://doi.org/10.17221/5679-VETMED.