Chemokines and haematological profile in Sprague-Dawley rats infected with *Trichinella zimbabwensis* and *Plasmodium berghei* ANKA

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A dissertation submitted in fulfillment of the requirements for the

Degree of Master of Science

School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Durban

South Africa

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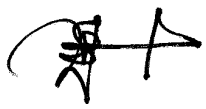
(2017)

PREFACE

The research contained in this dissertation/thesis was completed by the candidate while based in the College of Agriculture, Engineering and Science, School of Life Sciences, University of KwaZulu-Natal, Westville campus, South Africa.

The contents of this work have not been submitted in any form to another university.

The results reported are due to investigations by candidate, except where the work of others is acknowledged in the text.



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Signed: Supervisor

Prof. S. Mukaratirwa

Date: 01 March 2018

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Signed: Co-supervisor

DR. P. Murambiwa

Date: 01 March 2018

DECLARATION: PLAGIARISM

I **Yanga Mdleleni** declare that:

(i)The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

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(vii) Haematological data from this study have been shared among other two MSc students as part of a larger project on *Plasmodium berghei* ANKA/ *Trichinella zimbabwensis* co-infection in Sprague-Dawley.

Yanga Mdleleni

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Date: 01 March 2018

Abstract

Trichinellosis is an important re-emerging parasitic zoonosis caused by nematode species of the genus *Trichinella* and *Plasmodium falciparum* malaria is among the leading causes of mortality and morbidity in sub-Saharan Africa (SSA). Co-infection of the two diseases in rural communities is likely to be a common phenomenon although no reports have been made up to date. There is paucity of information on the consequence of co-infection regarding the clinical outcome of these diseases, especially malaria. Helminths, such as tissue-dwelling *Trichinella* spp larvae induce Th2 immune responses, while malaria induce Th1 immune responses as a survival strategy. On the other hand, chemokines are chemotactic cytokines produced by the host macrophages in order to elicit a protective immune response. Immuno-pathogenesis during co-infection remain obscure. It is against this background that this study aimed to determine host chemokine and haematological responses in male Sprague-Dawley rats during co-infection with *Trichinella zimbabwensis* and *Plasmodium berghei.* A 42 day follow up study was carried out, where 168 male Sprague-Dawley rats (90-150g) were randomly divided into four separate groups, control (n=42); malaria infected (n=42); *Trichinella* infected (n=42) and co-infected group (n=42). On day 0, male Sprague-Dawley rats were orally infected with 3 *T. zimbabwensis* muscle larvae per gram body weight. Followed at day 28 post-*Trichinella* infection with malaria induction using 10^5 *P. berghei* parasitized RBCs for the mono-infected group. While 42 male Sprague-Dawley rats were co-infected with *T. zimbabwensis* and *P. berghei* on day 0 and *P. berghei* infection occurred on day 28pi. Experimental animals were sacrificed on day 0,7,14,21,28,35 and 42 pi. Where whole blood and sera were collected. *Plasmodium berghei* percentage parastaemia, *T. zimbabwensis* adult worm count and muscle larvae load, haematological parameters and serum levels of IP-10, RANTES and EOTAXIN were determined. Co-infection with *P. berghei* and *T. zimbabwensis* showed elevated *P. berghei* percentage parastaemia, as well as upregulation of RANTES and EOTAXIN as a Th2 immune response, while IP-10 was downregulated as an effective immune response to parasitic infections. Mono-infection with *P. berghei* caused an upregulation of IP-10 as a Th1 immune response.

Acknowledgments:

Glory be to my Lord and savior Jesus Christ, for His love and grace.
My heartfelt gratitude goes to the following people, without whom this project would not have been a success:

Professor S. Mukaratirwa, thank you for imparting your great knowledge and guidance, most of all for allowing me to learn so much from you. Dr. P. Murambiwa, thank you for your patience and understanding and for being such an inspiration to me.
The staff at the biomedical resource unit with their assistance. To my colleagues Umo and Quinta, the department of human physiology UKZN, Westville campus and Medical School. Thank you, brother Dennis, Dr. Rene and everyone who lent a helping hand, may God bless you all.

To my amazing mother and best friend Bulelwa Mdleleni. Thank you for your constant love and support, for always providing selflessly for me, wiping every tear and cheering me on every step of the way. You have inspired me to be the best version of myself. Most of all thank you for teaching me the importance of education and independence. I love you with all my heart mom. To my siblings Sisispho and Nwabisa Mdleleni, my niece Zoey, thank you for your encouragement and support. To my great gran Lulama Rose Mdleleni, grandmother Nozipho Malinzi, Aunt Nelly, Thandi and entire family and friends, I love and appreciate all of you.

TABLE OF CONTENTS

List of Abbreviations:

BRU- Biomedical resource unit

CNS- central nervous system

ELISA - enzyme-linked immunosorbent assay

HRP- histidine-rich protein

iRBC- infected red blood cell

IFA - indirect fluorescent antibody

LPS- Activating lipopolysaccharide

MHC- major histocompatibility complex

NK- natural killer cells

NS- Not significant

PCR - Polymerase Chain Reaction

pLDH - lactate dehydrogenase

RBC- Red blood cells

RDTs- Rapid Diagnostic Tests

SSA- sub-Saharan Africa

TCR- T-cell receptor

TLRs- Toll-like receptors

Th- T helper

UKZN- University of KwaZulu-Natal

WHO- World Health Organization

Table of contents:

List of figures:

Chapter 1
Introduction

1.1. Background

Malaria is one of the major causes of mortality and morbidity in people residing in sub-Saharan Africa, with over 200-300 million infected people, however, the severity of each case differs from individual to individual (Yewhalaw *et al*., 2014). The disease is caused by a protozoan parasite from the genus *Plasmodium*, transmitted through a bite of the female *Anopheles* mosquito (De Souza and Riley, 2004). Drug resistant strains of *Plasmodium* as well as the state of the host immune system hinder the effectiveness of antimalarial drugs, indicating that impaired immune responses result in rapid evolution of drug-resistant parasite strains (Targett, 1992). The most effective strategy for the control of malaria is early diagnosis and treatment, and among the anti-malaria drugs is the use of artemisinin-combination therapy which is expensive and makes it difficult for the under-privileged in developing countries to have access (Yeung *et al*., 2004; WHO, 2006).

Another food-borne zoonotic parasitic disease often co-endemic with malaria in sub-Saharan Africa, of which there is paucity of information is trichinellosis. An estimated 11 million cases of trichinellosis infections have been recorded in over 66 countries (Yang *et al*., 2010). Trichinellosis is an important zoonotic parasitic disease caused by nematode species of the genus *Trichinella*, which have a worldwide distribution, infecting both domestic and wild animals (Pozio, 2007). The parasite circulates in either a sylvatic or domestic transmission cycle, with a flow existing between the two cycles (Campbell, 1983). The main source of human infection is through ingestion of raw or undercooked infected meat, contaminated with muscle larvae from pigs, horses and wild animals (Gottstein *et al*., 2009). *Trichinella spp* infections are of public health risk, even though there are currently no records of human infections by *T. zimbabwensis*, which is the most prevalent species in wildlife in sub-Saharan Africa (Mukaratirwa et al., 2013). Poverty, climate change, food insecurity as well as ineffective veterinary surveillance and control measures have increased the risk for future human *T. zimbabwensis* infections (Mukaratirwa et al., 2013).

Sub-Saharan Africa is predisposed to co-infection with both malaria and trichinellosis or with other tissue-dwelling nematodes, such as visceral larval migrans due to toxocariasis (Onkoba et al., 2015). There is scarcity of knowledge and understanding regarding immune response and protective immunity

due to malaria co-infections with tissue-dwelling nematodes in humans and this may therefore be deterring production of effective vaccines against malaria (Che *et al.*, 2015; Onkoba et al.,2015).

Progress in vaccine development and drug discovery is hindered by lack of full knowledge of the mechanism of the host-parasite interactions as well as immune response during polyparasitism and co-infection of malaria with tissue-dwelling nematodes such as *T. zimbabwensis* (Onkoba et al., 2015).

There has been a conspicuous interest in chemokines: a novel group of inflammatory mediators, which play a crucial role in arbitrating and recruiting definite leukocyte subsets during acute and chronic inflammatory immune-modulation during parasitic infections (Alberti et al., 1999). Therefore, the aim of this study was to experimentally determine the host immune response during co-infection of *P. berghei* and *T. zimbabwensis* in Sprague-Dawley rats.

1.2. Specific objectives

1.2.1 To determine the chemokines profile induced in Sprague-Dawley rats during mono-infection with *Trichinella zimbabwensis* and *Plasmodium berghei.*

1.2.2. To determine haematological profile during mono-infection with *Trichinella zimbabwensis* and *Plasmodium berghei.*

1.2.3. To determine the chemokines profile induced in Sprague-Dawley rats during co-infection with *Trichinella zimbabwensis* and *Plasmodium berghei.*

1.2.4. To determine haematological profile during co-infection with *Trichinella zimbabwensis* and *Plasmodium berghei.*

Chapter 2
Literature review

2.1 Introduction:

An estimated two billion people infected with malaria reside in poor backgrounds, limiting their ability to receive appropriate medical attention. Within malaria endemic regions, it can be noted that complicated and uncomplicated malaria infections exist (Che et al., 2015). Malaria is a parasitic infection with adverse complications in the functioning of the host immune system (Autino et al., 2012). This then makes it easier for patients with weak or negatively altered immune systems to be susceptible to co-infections or multi-parasitism (Onkoba et al., 2015) as seen in patients co-infected with multiple parasites simultaneously. It is therefore of great significance to study the nature of interactions of these parasites, the mechanisms in which the host immune system combat infections, as well as immunological mechanisms involved. During co-infections, one parasite does not have direct influence on disease outcome and establishment of another parasite, however, the concept of parasite-host-parasite interactions plays a significant role (Pozio et al., 2002; Che et al., 2015).

2.2. Malaria

2.2.1. *Plasmodium* species

The genus *Plasmodium* constitute of protozoan parasite species that cause malaria. Over 100 different *Plasmodium* species exist and are categorized by their specific host (human or non-human). *P. falciparum, P. malariae, P. vivax* and *P. ovale* are four *Plasmodium* species that result in human infections, under natural conditions (White et al., 1999; Singh et al., 2004; WHO, 2005 ;). In humans, malaria is responsible for mortality of two million people annually, as well as causing severe sickness in half a billion people (Teklehaimanot and Singer, 2005; Martinsen et al., 2007). *P. berghei* and *P. knowlesi* infect animals and *P. knowlesi* has been reported to occasionally infect humans (Tuteja, 2007).

P. berghei causes rodent malaria and is used in experimental laboratory animal models to study human malaria as it is regarded as safe since it does not infect humans (Janse and Waters, 1995).

According to WHO (2005), *P. knowlesi,* which causes 'monkey malaria' has been reported to occasionally cause human infections (Ozwara et al., 2003; Singh et al., 2004). The age of red blood cells has a restricting effect on malaria parasite, as stated by McQueen and McKenzie (2004). *P. vivax* and *P. ovale* are restricted to reticulocytes, while *P. malariae* is restricted to more mature red blood cells and *P. falciparum* infects red blood cells of all ages (McQueen and McKenzie, 2003; WHO, 2006). It is therefore for this reason that among the four species that infect humans, *P. falciparum* results in fatal infections (Barsoum, 2000; Singh et al., 2004).

2.2.2. Life cycle of *Plasmodium spp.*

The female *Anopheles* mosquito is the vector, which bites the human host. This results in the injection of sporozoites through the dermis. During the extra-erythrocytic cycle, these sporozoites travel through the blood stream and invade hepatocytes in the liver. Sporozoites mature to form schizonts, and when mature, release merozoites into the sinusoids of the liver. As seen in *P. vivax* and *P. ovale,* clinical relapse occurs when some sporozoites go into hibernation for years or months and this can lead to numerous complications when reactivation occurs (Mackintosh et al., 2004; Tuteja, 2007). Formation of trophozoites occurs in the erythrocytic cycle, where mature tissue schizonts enter erythrocytes (Clark and Schofield, 2000; Holt et al.,2002). The newly formed trophozoites mature and produce more merozoites.

Cryptobiotic phase occurs during hibernation of sporozoites; a phenomenon common in *P. ovale* and *P. vivax* infections, resulting in the formation of hypnozoites (Mackintosh et al.,2004). Hypnozoites can remain dormant for elongated periods of time (months); clinical relapse may occur when these are reactivated (Mackintosh et al., 2004, Tuteja, 2007). Resultant trophozoites further develop into schizonts, which subsequently develop into a new generation of merozoites. This cycle is repeated when another generation of merozoites is released into the blood stream through the rapture of parasitized red

blood cells (Mackintosh et al., 2004). The merozoites are differentiated into male and female gametocytes in the mammalian host which are subsequently transported by the vector during feeding, therefore completing the sexual cycle (Clark and Schofield, 2000; Mackintosh et al.,2004). The general life cycle of *Plasmodium* species is shown in the figure below;

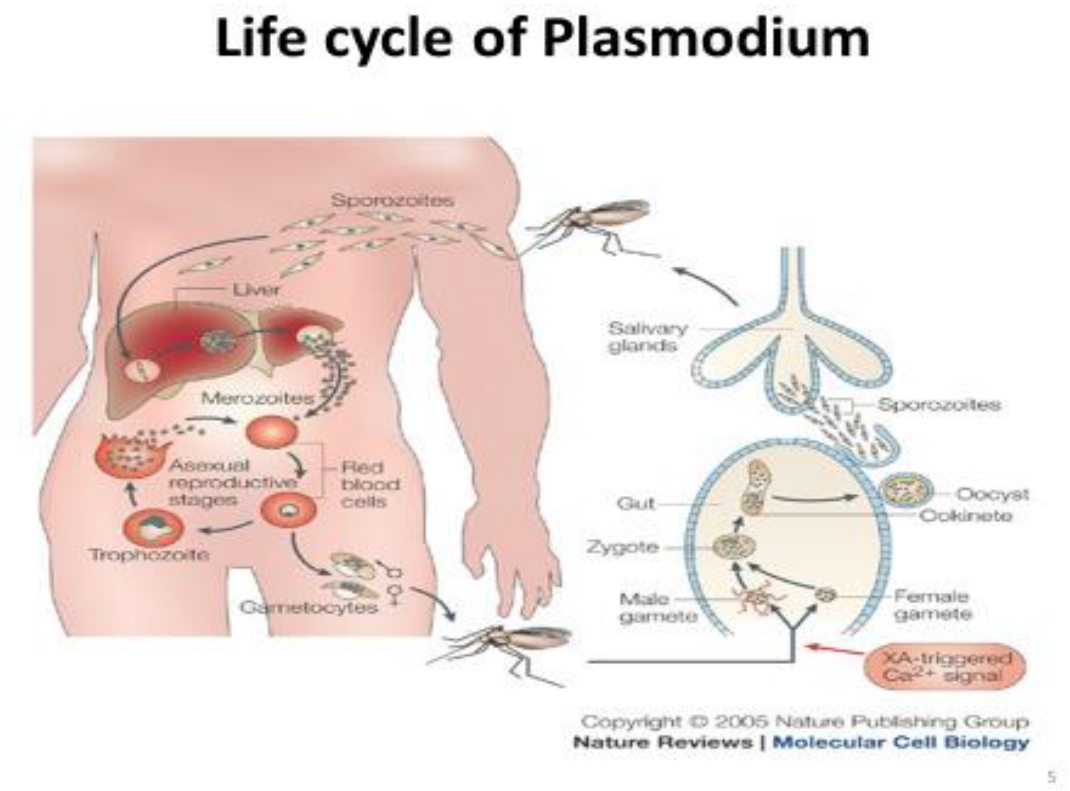

Figure 1: General life cycle of *Plasmodium* spp (WHO, 2008).

2.2.3. Diagnosis

Malaria infections result in millions of deaths and atrocious health complications. Therefore, to avoid such, accurate and prompt diagnosis and treatment must be carried out. Early signs and symptoms of malaria are not disease specific, as this can lead to misdiagnosis. It is for reason that more specific methods are used. These involve examining peripheral blood through microscopy, polymerase chain

reaction, as well as antigen detection (Bronzan et al.,2008). Light microscopy is the most commonly used quantitative method, as it can detect extremely low values of parasites such as 5 parasites/μL. This assists in the quantification of the parasite and identification of the specific *Plasmodium* species (Warrel and Giles,2002; Bronzan et al., 2008). This diagnostic procedure requires specific expertise, as low parasite counts can be misinterpreted as false negative. Immunochromatographic Rapid Diagnostic Tests (RDTs) are non-quantitative tests used to identify the presence of a *Plasmodium* species in peripheral blood using a monoclonal antibody to the parasite antigen, on a immunochromatographic strip (Bronzan et al., 2008). Presence of the parasite is denoted by a colour change of the dipstick or cassette on the strip. Some RDTs are species specific, such as one used to identify *P. falciparum.* This

focuses on histidine-rich protein 2 (HRP2), aldolase or lactate dehydrogenase (pLDH) and other RDTs recognize antigens present in all four *Plasmodium* species that cause human malaria (Bartoloni et al., 1998; Bronzan et al.,2008).

According to Han et al. (2007) there are complications and inaccuracies pertaining to the use of these tests in diagnosing non-*falciparum* malaria, including mixed infections, with a sensitivity of 44% for *P. vivax*. Polymerase Chain Reaction (PCR) is a quantitative test used to identify parasites below the threshold of microscopic identification, as it can detect as little as single parasites/mm^3 (therefore extremely useful in chemoprophylaxis patients with extremely low parastaemia) (Singh et al., 2004; Shillcutt et al., 2008).

Like microscopy identification, PCR can also be used in the detection specific *Plasmodium* species; where morphological characteristics may be distorted. PCR methods, targeting the small-subunit 18S rRNA gene, all four-human malaria inducing species could be identified and this method has high sensitivity (WHO 2005; Han et al.,2007). Serological diagnosis using enzyme-linked immunosorbent assay (ELISA) or indirect fluorescent antibody (IFA) test, is used to detect prior infections by malaria parasites, with the aim of identifying the specific species which caused the infection. This method cannot be used for acute cases due to the time required for the host immune system to form antibodies against infection (Bronzan et al.,2008). These techniques are prone to contamination during post-amplification product manipulation, they are time consuming and are technically demanding, therefore they require precision and highly skilled labor. Some patients may present with clinical signs that may result in easier diagnosis.

2.2.4. Clinical signs and pathological findings

Cerebral malaria can result from obstruction of blood vessels by erythrocytes infected with *P. falciparum*, and is a fatal condition, common in infants in Africa (Tuteja, 2007). *Plasmodium vivax* also causes anemia, even though this is not as fatal and common as *P. falciparum*. Relapses in malaria infection may be caused by *P. vivax* or *P. ovale*, due to hypnozoites (dormant liver stage) that may remain unnoticed for elongated phases, prior to the onset of pre- erythrocytic schizogony (Tuteja, 2007). Undetected blood infections that persist for prolonged periods may occur due to *P. malariae.* Improved diagnosis using the above-mentioned techniques will minimize infections combined with assessing possible risk factors that promote malaria infections and educating communities.

2.2.5. Epidemiology

2.2.5.1 Geographical distribution of malaria

The distribution of malaria transmission is based on biological constraints of climate on parasite population, morbidity and mortality data, climate and vector development (Craig et al.,1999) (Figure 2). Some variables such as mosquito density, human activities, human and vector genetics are not constant, therefore making it difficult to give a global distribution model (Craig et al., 1999; WHO,2006).

The fuzzy logic model of the distribution of stable malaria transmission in sub-Saharan Africa is based on the effect of mean rainfall and temperature on the biology of malaria transmission (Craig et al.,1999). Regions were malarial infections can be detected are conceptually classified into four categories. These are mainly (1) malaria free- these are areas where there are no records of infections. (2) epidemic – where long-term variation in climate results in conditions that are suitable for transmission on an irregular basis (could potentially lead to epidemic malaria), (3) seasonal – where conditions may be suitable for a short season annually (4) perennial – where conditions are always suitable for transmission (Ermert et al., 2012)

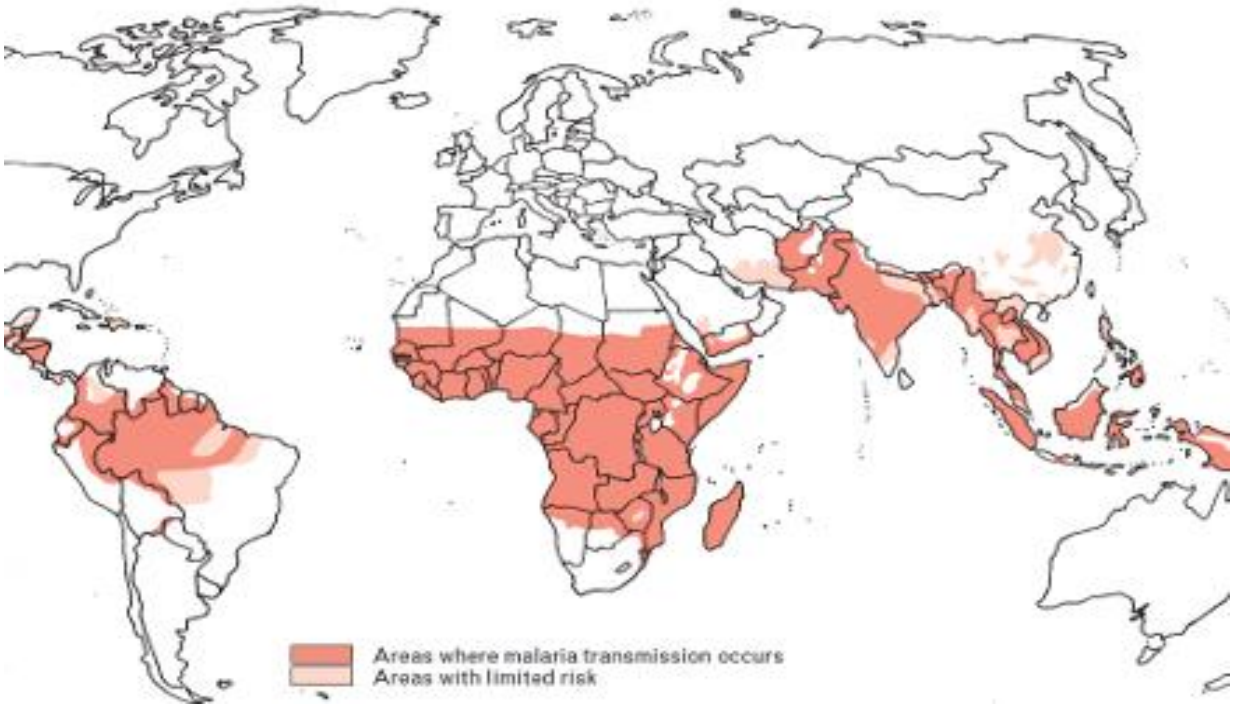

Figure 2: Map showing the geographical distribution of malaria (WHO, 2016). Light pink colour represents arears where risk of malaria infection is limited, while dark peach colour denotes areas where

the transmission of malaria is predominant. Unclassified areas (white) represent regions where malaria infections are extremely sporadic, as these are countries outside of the transmission limits and areas of population density are less than 1-person km^{-2} (WHO, 2016).

2.2.5.2 Risk of infection

During transmission season in at risk countries, all non-immune travelers that may be exposed to mosquito bites, between dusk and dawn, are at risk of malaria infection. Some previously immune individuals who have vacated at risk countries for over six months, may lose their immunity or become partially immune. Children who have migrated to countries and areas of no risk, become partially at risk and partially susceptible to malaria infection (WHO, 2006). Failure of treatment or the use of inappropriate malaria drugs and failure of travellers to take precautionary measures prior to visiting at risk countries increases cases of *P. falciparum* infections (; WHO, 2006; Pozio et al., 2007).

There is uneven distribution of malaria in regions where it is prevalent, and the degree and severity

differ and therefore travellers should seek advice regarding the most at-risk zones. In the case of lack of current information regarding these risk zones, travellers must take appropriate precautions until such information can be adjusted when more information becomes available on arrival. This applies particularly to individuals backpacking to remote places and visiting areas where diagnostic facilities and medical care are not readily available. Travellers staying overnight in rural areas may be at highest risk, this implies that geographic locations play a pivotal role in the spread of malaria (WHO,2006).

2.2.6. Control of malaria transmission

According to WHO (2006) there are four key interventions. These are (i) introduction of insecticide-treated nets for people at risk, (ii) the use of indoor residual spraying of insecticide in at risk households, (iii) appropriate antimalarial drugs for patients with probable or confirmed malaria as well, (iv) sporadic preventive treatment in pregnancy (DeBach and Rosen,1999). All these are personal protective measures to limit or eradicate infection through the inhibition of vector proliferation and increased parasitic adaptability within the host. Other personal control measures include using mosquito repellent skin products (ointments or creams), closing windows in endemic regions (DeBach and Rosen,1999; WHO,2008). Individuals travelling to malaria endemic regions need to take chemoprophylaxis of anti-

malarial drugs, even though this may be difficult in sub-Saharan Africa due to poor medical treatment facilities (WHO, 2006; WHO, 2008). It is also the responsibility of those who are infected or who suspect possible exposure to this parasite to go to clinics and hospitals for early treatment. As much as these control strategies are applicable, parasitic infections such malaria compromise patient's immune system, making it easier for co-infections or poly-infections to occur. Some of these co- infecting parasites are *Plasmodium* and tissue-dwelling nematodes such as *Trichinella* species, which may enhance or diminish each other's co-existence, depending on the parasite-host interaction. In sub-Saharan Africa, co-infections with tropical parasitic infections has been neglected, even though it is very common with malaria (Onkoba et al., 2005).

2.3. Trichinellosis

2.3.1. Classification of *Trichinella*

There are two categories distinguishing between *Trichinella* species and these are encapsulated or non-encapsulated groups (La Grange et al., 2009). Encapsulated species include *T. nativa,* which infects

sylvatic carnivores, *T. spiralis* which infects domestic and sylvatic mammals, *Trichinella* genotype T6 which infects sylvatic carnivores, *T. britovi* which infects sylvatic mammals and seldom domestic pigs, *T. murrelli* which infects sylvatic carnivores, *T. nelsoni* which infects sylvatic mammals, *Trichinella* T8 is found in South Africa and Namibia, infecting sylvatic carnivores (Mukaratirwa et al.,2013),*Trichinella* genotype T9 infects sylvatic carnivores as well as *Trichinella* genotype T12, which infects cougars (Gottstein et al.,2009). The non-encapsulated taxa on the other hand includes *T. papuae,* and *T. zimbabwensis,* which infects mammals and reptiles and *T. psuedospiralis* which mammals and infects birds and mammals (Gottstein et al., 2009; Pozio et al., 2013). According to La Grange et al. (2009) *T. britovi*, *Trichinella* T8 and *T. nelsoni* are encapsulated taxa that have been reported to only infect mammals in sub-Saharan Africa.

2.3.2. *Trichinella* life cycle

Human infections occur during ingestion of muscle larvae in undercooked or raw infected meat. This is followed by the release of hydrochloric acid and pepsin from the stomach, causing the release of larvae from the cyst into the stomach. In a period of 5 or 6 days after a host has ingested meat infected with *Trichinella* larvae, the larva develops into an adult form in the gut (Pozio *et al.*,2001). The adult form attaches to the intestinal mucosa and releases larvae in one week (Pozio et al., 2001; Yang et al., 2010). These adults survive in the small intestine and may release more than 1000 larvae (Pozio *et al*.,2001).

After 2 to 4 weeks post-fertilization, larvae is discharged and disseminated through the blood stream or lymphatic system into organs but only persists in skeletal muscle fibers, where they encyst in individual cells also known as nurse cells where the larvae becomes encapsulated through the development of capillary network around these cells (Pozio *et al.*, 2001; Mitreva *et al*., 2005). In some cases, alteration of the host immune system may allow infective larvae to survive for years within the host (Pozio et al., 2001). Host immune response limits the infection at numerous stages in the *Trichinella* life cycle (Reale *et al.*,1998).

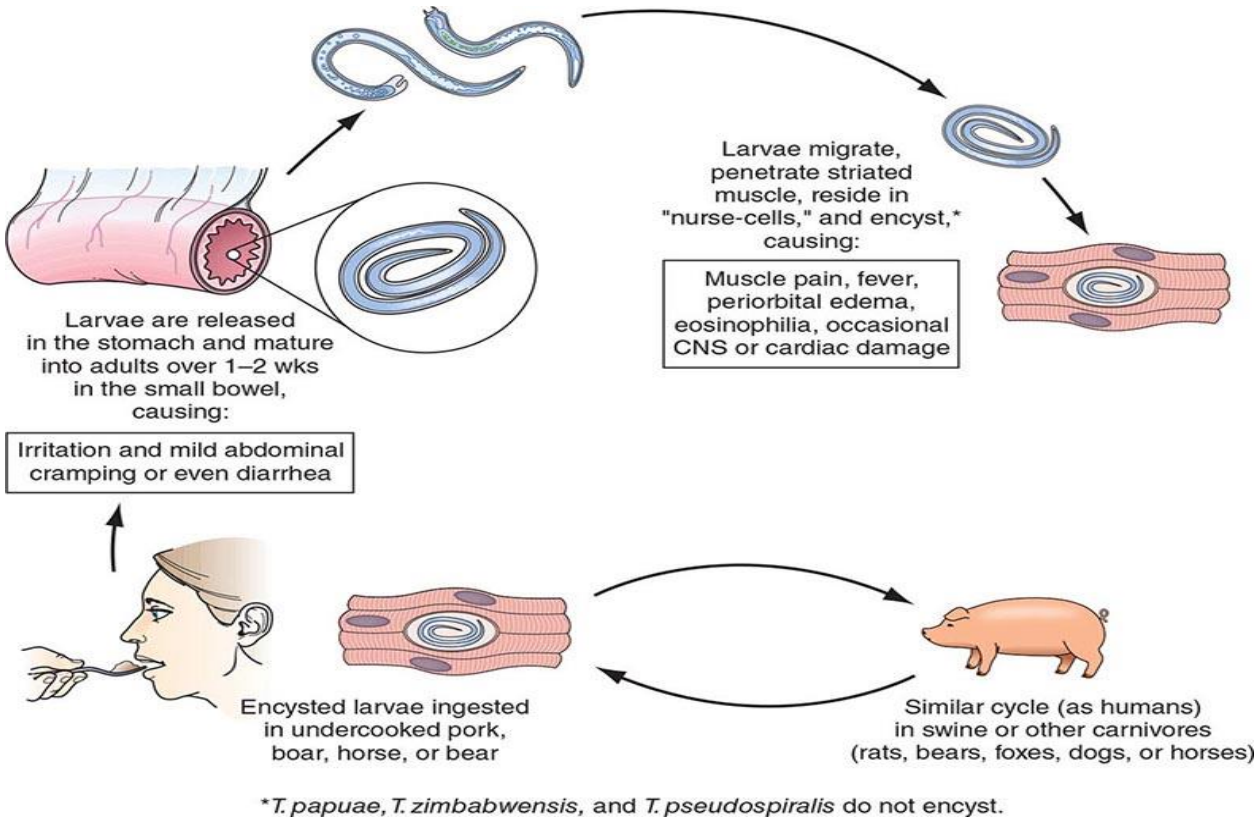

Figure 3. *Trichinella* spp life cycle (Wilson et al., 2015)

2.3.3. Diagnosis of trichinellosis

Early diagnosis is complicated, due to lack of pathognomonic signs (Dupouy-Camet and Murrel. 2007; Gottstein et al.,2009). Medical practitioners from non-endemic regions have difficulty diagnosing trichinellosis due to unfamiliarity. Diagnosis is based on three criteria; clinical manifestations (signs and symptoms), laboratory results (muscle enzyme and eosinophilia), detection of antibodies or muscle biopsy, as well as epidemiological investigations (this focuses on tracing back the source of infection, the type of species and its geographical distribution) (Kociecka, 2000).

2.3.4. Pathogenicity

Trichinellosis symptoms may manifest as either mild or complicated, depending on the species, as seen in the enteral and parenteral phase.

(i) Enteral

This phase affects the intestines and symptoms depends on species, gender, age, host immunity and intensity of infection (Pozio et al., 2013). Clinical symptoms present two to seven days post-infection and diarrhea, nausea, dyspepsia and heartburn are some of the symptoms including eosinophilia which presents early and increases rapidly (WHO, 2006).

(ii) Parenteral phase:

The quantity of larvae produced and migration of the larvae to vessels and tissues determines the severity of symptoms, and inflammatory response in humans and non-human primates include, arthralgia, facial oedema, fever, diarrhea, myalgia and prostration fever, weakness and muscle pain (Mitreva and Jasmer,2006; Mukaratirwa *et al.*, 2008)). A classic sign of trichinosis is periorbital edema, due to vasculitis and splinter hemorrhage. The most severe case of *Trichinella* infection may be due to worms entering the central nervous system (CNS) (Duprey and Schantz,2003). These are unable to survive the environment in the CNS, but they may promote sufficient damage to produce serious neurological deficits (ataxia, respiratory paralysis or stroke) or even death (Mitreva and Jasmer, 2006; Pozoi ,2013). Mortality of patients is within 4-6 weeks post-infection due to pneumonia, encephalitis or myocarditis. Intercontinental travelling of infected patients and animals may cause an increase in the spread of trichinellosis, as geographic locations of *Trichinella* species influences the severity of the disease and response to treatment (Pozio et al.,2013).

2.3.5. Geographical distribution of *Trichinella*

T. spiralis life cycle is well adapted to wild and domestic swine, as well as synanthropic rats. It is well distributed globally, resulting in higher records of human infections (Gottstein et al.,2009). Unlike the domestic cycle, the sylvatic cycle of *T. spiralis* includes a wide range of wild carnivores which play a crucial role in the introduction of this species to the domestic host during the life cycle (Pozio et al.,2006). *T. nativa,* is well adapted to terrestrial (black bears, raccoons, wolverine, fox) and marine animals (seals, polar bears) as hosts. While affecting sylvatic carnivores residing in Northern America, Asia, Northeastern Europe as well as North America (Gottstein et al.,2009). This species is well adapted to survive in extreme environmental conditions such as frozen muscles of carnivores, for a maximum of five years (Pozio et al.,2004; Pozio et al., 2013). *T. britovi* is the second-most common species of *Trichinella* that may affect human health, infecting domestic pigs. This is the most widely distributed parasite species in Asia Northern, Europe, and Western Africa (Pozio et al., 2004; Pozio et al., 2006; Gottstein et al., 2009). Like *T. nativa*, this species can endure low temperature for extensive periods of time. *T. zimbabwensis* has been reported in Zimbabwe, Mozambique, Ethiopia and South Africa in Nile crocodiles (Pozio et al., 2006).

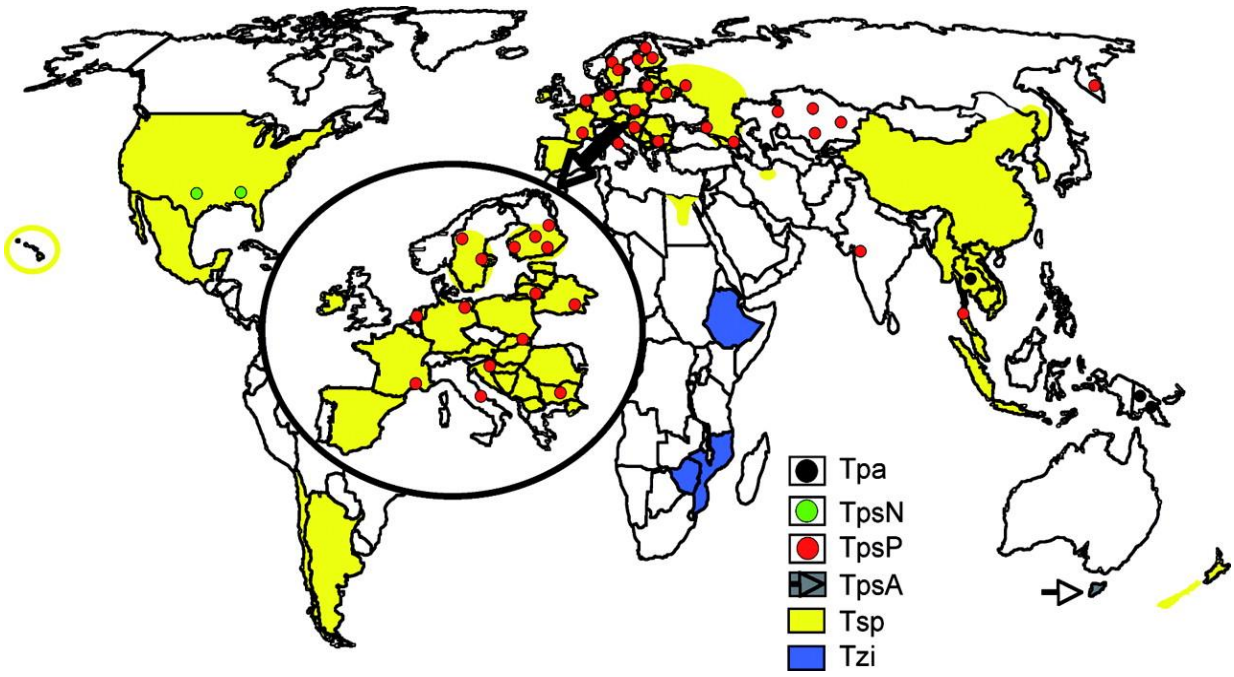

Figure 4**:** Map showing the global distribution of *Trichinella* species; *Trichinella zimbabwensis* (Tzi), *Trichinella spiralis* (Tsp), *Trichinella papuae* (Tpa), *Trichinella pseudospiralis* from north America (TpsN), *T. pseudospiralis* from Europe and Asia (TpsP), *T. pseudospiralis* from Tasmania (TpsA),) and *T. pseudospiralis* from Europe(TpsA). Image sourced from (www.iss.it/site/Trichinella/index.asp)

2.3.6. Pathological findings

In countries where human trichinellosis is endemic, physicians are more likely to recognize the infection early than in countries where infections are only sporadic, where treatment is often delayed (Pozio et al., 2001). Economic and political changes, revolutions as well as wars contribute to an increase in prevalence of trichinellosis among the human population (Bolpe and Boffi, 2001; Marinculic *et al.*, 2001; Djordjevic *et al*., 2003). Reliable epidemiological information on animal and human infections is not uniformly collected around the world (Onkoba et al., 2015). Only a limited number of countries have implemented an official recording system for human and/or animal infections in the last 50 years (Marinculic *et al*., 2001). In most countries, reporting of infection has been and continues to be on a voluntary basis and relies on physicians, veterinarians, biologists, zoologists or epidemiologists who are working on these parasites (Naotunne *et al*., 1991; Djordjevic *et al*., 2003). This often results in fragmented information (Naotunne et al., 1991).

The occurrence of trichinellosis in humans is strictly related to cultural food practices, including the consumption of raw or undercooked meat of animal origin (Yang et al., 2010). Therefore, most of the epidemiological data and ad hoc surveys on *Trichinella* species such as *T. zimbabwensis* and *T. spiralis* in domestic and/or wild animals are related to outbreaks in humans (Gottstein *et al.*, 2009).

2.3.7. Prevention of *Trichinella* Infections in humans

Three strategies are used to prevent trichinellosis infections in humans and these include community education on the danger of consuming uncooked or semi-raw meat products. These can either be sylvatic (e.g., walruses, cougars, bears badgers, wild boars, crocodiles, foxes, jackals, armadillos, and monitor lizards) or domestic (e.g., horses, pigs and dogs) as these may be hosts to *Trichinella spp* (Gottstein et al., 2009). Pigs are the major source of human *Trichinella* infections, therefore there must be strict veterinary inspections of pork.

Three methods have been shown to reliably inactivate *Trichinella* meat larvae; (i). Cooking to reach a core temperature of not less than 71 ^{0} C (159. 8 ^{0} F) for at least 1-minute (i.e. the meat must change from a pink to grey colour, muscle fibers must be easily separated from each other), (ii) freezing, (iii) irradiation, curing, drying, using microwave ovens or smoking suspected meat products, assists in the preparation of meat products that are safe to consume (Dupouy-Camet and Murrel, 2007; WHO, 2008). The way a host's immune system responds to infections, may limit or enhance the disease outcome. Understanding the functioning of the immune systems may bring light to the comprehension, control and treatment of parasitic diseases.

2.4.1. Host immune response to infections

There are two separate but integrated arms of the host immune system and these are mainly the innate (non-specific but can recognize foreign invading bodies) as well as the adaptive immune response (mediated by the T and B cells) (Luster, 2002). The innate response provides the first form of defense, as it is antigen-independent, responding within hours or immediately to invading antigen (Leckie et al., 2000; Warrington et al., 2011). It lacks immunologic memory, therefore making it unable to recognize the same antigen in future exposure. Both innate and adaptive responses are integrated, as the innate response instructs the adaptive response to elicit an appropriate response (Gallucci et al., 2001; Luster, 2002).

(i) Innate immunity

This type of immune response primarily functions in recruiting immune cells such as various cytokines (small proteins involved in inter-cellular communication) to sites of inflammation and infection, where these cytokines further release proteins, glycoproteins and antibodies to activate a biochemical cascade, complement systems and opsonize foreign antigens, for antigen phagocytosis to occur (Warrington et

al.,2011). Expulsion and clearance of antibody complexes, foreign bodies or dead cells in the lymphatic system, organs and tissues, is a result of innate immunity (Turvey and Broide, 2010). There are numerous cells involved in innate immune response and these include basophils, mast cells, dendritic cells, eosinophils, natural killer cells (NK), lymphocytes, mast cells and phagocytes (neutrophils and macrophages).

Phagocytes are further differentiated into distinct groups i.e. (i) neutrophils; short-lived cells, containing pathogen eliminating granules, (ii) macrophages; are long-lived cells, named according to the type of cells in which they reside, e.g. histiocytes are macrophages found in the connective tissue, (iii) Dendritic cells; these cells function as important messengers and antigen-presenting cells between adaptive and innate immunity (Warrington et al., 2011). Basophils and mast cells are involved in initiating acute inflammatory responses, these cells both share crucial features, even though they differ in their site of residence, as mast cells are found within the connective tissue, while basophils are found within the circulation (Bonilla et al.,2010; Turvey and Broide, 2010). Eosinophils are polymorphic granulocytes, functioning in the elimination of parasites, with phagocytic properties, expelling parasites from the host (Maizels et al., 2004). NK cells are involved in distracting virally infected cells, through the release of granzymes and perforins from NK-cell granules to induce apoptosis (Warrington et al., 2011).

(i) Adaptive immunity

Innate immunity may be ineffective in eliminating some pathogens, this leads to the development of adaptive immunity. Adaptive immunity has the primary role of generating pathogen-specific immunological pathways and recognizing specific foreign antigens, to develop an immunological memory that easily recognizes and eliminates subsequent infections (Turvey and Broide, 2010; Warrington et al., 2011). Adaptive immunity comprises of antigen-presenting (APCs) T cells as well B cells. These cells are derived from hematopoietic stem cells, found in the bone marrow, which later develop and mature within the thymus (Warrington et al., 2011). To recognize specific antigens, the outer membrane of these cells consists of an antigen-binding receptor known as T-cell receptor (TCR), which requires the presence of APCs to express major histocompatibility complex (MHC). There are two distinct classes of MHCs; these are class I, human leukocyte antigen (A, B and C) and class II, found on immune cells such as dendritic cells, macrophages and B cells (Turvey and Broide, 2010). Peptides on antigens become expressed by MHCs when a pathogen infects a cell or when foreign bodies are phagocytosed (Turvey and Broide, 2010).

When an APC digests an antigen, displaying antigen properties bound to its specific MHC molecule, T

cells become activated. According to Warrington et al. (2011) activation of T cells and TCR occurs because of the formation of the MHC-antigen complex. These cells further induce secretion of cytokines, which control host immunity. The presence of an antigen stimulates differentiation of T cells into T helper (Th or CD4+) or cytotoxic T cells (CD8+ cells), which are responsible for expulsion of foreign bodies. Interaction between peptide-bound MHC class I molecules and TCR of these cells activates these cells (Turvey and Broide, 2010).

Lysis and apoptosis of target cells occurs through the release of granzyme, granulysin and perforin from clonally expanded cytotoxic T cells (Warrington et al., 2011). Once infection has been resolved, various effector cells die, and some are removed through phagocytosis, while others are retained in the immune system as memory cells, to rapidly differentiate into effector cells, upon future infection by the same antigen (Bonilla et al., 2010, Turvey and Broide, 2010). T helper (Th) cells are involved in maximizing and establishing immunity, as they lack phagocytic and cytotoxic activity, therefore are unable to cause pathogen clearance or lysis of infected cells, as cytotoxic T cells can. These cells mediate immune responses. When TCRs recognize an antigen bound to class II MCH molecules, Th cells become activated (Warrington et al., 2011). Activated Th cells release cytokines which influence various cells types, including APCs that initially activated them. APCs can induce two Th cell responses, these are Th1 and Th2 responses, characterized by the ability to produce interforn-gamma (IFN-γ) this is responsible for activating bactericidal activities of macrophages, B cell inducing cytokines, as well as neutralizing antibodies (Bonilla et al.,2010; Warrington et al., 2011).

Th2 immune response can be denoted by the release of specific cytokines (interleukin-4, 5 and 13), which play a role in the recruitment and activation of immunoglobulin E (IgE) antibody-producing B cells, eosinophils and mast cells. As stated above, IgE, eosinophils and mast cells initiate acute inflammatory response, this is apparent in allergic reactions, which result in the imbalance of Th2 (Warrington et al., 2011). Regulatory T cells (T reg) are the third type of T cells, which suppress and limit immune response by controlling aberrant responses to self-antigens and autoimmune diseases. B cells originate from hematopoietic stem cells found within the bone marrow, subsequent to maturation, migrate from the marrow, expressing specific antigen-binding receptor, found on their membrane. These cells unlike T cells, can recognize free antigen directly, in the absence of APCs (Warrington et al.,2011). B cells produce antibodies against foreign antibodies, undergoing differentiation and proliferation, forming memory B cells or antibody-secreting plasma cells or memory B cells (Bonilla et al., 2010, Warrington et al., 2011). Memory cells can endure past infections, as they are long-lived cells, which can express antigen-binding receptors, to elicit a prompt immune response to invading foreign antigens, which differs from short-lived plasma cells (Bonilla et al., 2010).

B cells are involved in antibody-mediated or humoral response, unlike cell-mediated responses which are primarily controlled by T cell) (Warrington et al., 2010**).** Signaling of the adaptive response results in the release of phagocytes, such as neutrophils and macrophages by the innate response, to eliminate the parasite within the tissues. Dendritic cells respond within the lymph nodes and activation of T and B cells occurs and these are then targeted to the site of inflammation. During pathogenic infections, intermediate responses are initiated in epithelial tissues, such as intestinal/urogenital epithelium and skin; through insect vectors, where first entry of pathogens occurs (John and Abraham, 2013). Pattern recognition receptors (PRRs), expressed by mast cells (MCs) are responsible for host immune surveillance to intra extracellular pathogens. Local immune cells such as macrophages(MFs), monocytes and dendritic cells (DCs) are induced by PRR-initiated signaling to release appropriate antimicrobial agents, chemokines and cytokines (Meylan et al., 2006; Kawai and Akira, 2010; John and Abraham, 2013).

2.4.1 Chemokines

Chemokines are chemotactic cytokines that play important roles in bridging the innate and the adaptive immune systems (Boström *et al*., 2012). Functionally, chemokines can be divided into two broad categories: constitutively expressed chemokines that maintain homoeostatic functions, and inducible chemokines that are usually upregulated in response to infection and/or inflammation (Sallusto et al., 2000; Moser et al., 2004). They orchestrate the migration of leucocytes and other cells by activating corresponding receptors on responsive cells, thereby inducing chemotaxis of immune cells to sites of infection (Luster, 2002; Boström *et al*., 2012). There are currently 46 chemokine ligands and 18 receptors that have been described to date (Miu et al., 2006).

Chemokine superfamily has been divided into four distinct subgroups, based on structural differences, on whether the first two N-terminal cysteine residues are separated by a single amino acid or if these N-terminal cysteine groups are positioned adjacent to each other (Mahalingam and Karupiah, 1999). These subgroups are broadly defined as CC (β) chemokines; this group encompasses dead cells in the lymphatic system, organs and tissues, as a result of innate immunity (Turvey and Broide,2010). There are numerous cells involved in innate immune response as explained, these include basophils, mast cells, dendritic cells, eosinophils, natural killer cells (NK), lymphocytes and phagocytes (neutrophils and macrophages).

2.4.2. The role of chemokines in host immunity

Chemokines play a crucial role in leukocyte recruitment and trafficking; a highly controlled process that also involves molecules such as integrins and selectin adhesion (Von Andrian, 2000; Miu et al., 2006). Chemokine signaling, and their receptors are important in leukocyte migration which occurs during normal immune system surveillance in inflammatory reactions. Cytokines are examples of pro-inflammatory stimulus that upregulate chemokines such as CXCL10 (IP-10), CCL (RANTES) and ant-inflammatory chemokines e.g. CCL11 (Eotaxin), which are of importance in this study to direct the migration of leukocytes (Adams and Rlloyd, 1997).

B cells produce antibodies against foreign antigens, undergoing differentiation and proliferation, forming memory B cells or antibody-secreting plasma cells or memory B cells (Bonilla et al., 2010; Warrington et al., 2011). Memory cells can endure past infections, as they are long-lived cells, which can express antigen-binding receptors, to elicit a prompt immune response to invading macrophage inflammatory protein-1 (MIP-1) macrophages and CXC (α)chemokines, C and CX3C chemokines with differences between the overall dimers between the two subgroups (Baglionili, 2001; Ochiel et al., 2005). Leukocyte activation, hematopoiesis, angiogenesis, chemotactic recruitment of inflammatory cells and antimicrobial effects are biological events that are culminated by the binding of chemokines to their cognate receptors (Baglionili, 2001).

RANTES; a CC chemokine produced by lymphocytes, epithelial cells and platelets consisting of mainly CCR1, CCR3, CCR4 as well as CCR5 receptors. This chemokine functions as a chemo-attractant for NK cells, eosinophils, memory T cells and monocytes (Mackay, 2001). The mechanisms that RANTES uses to influence host mucosal immune responses remains elusive (Lillard et al., 2001). IP-10 is produced by NK cells, through induction of IFN-γ from resident tissue cells (Luster, 2002). It acts as a chemo-attractant of antigen-specific CD4+, CD8+ and lymphocytes to sites of infection, thereby inducing cellular responses. This chemokine encodes for receptors such as CXCL9, CXCL10 and CXCL11 respectively (Frigerio et al., 2002). Eotaxin (CCL11) is a CC anti-inflammatory chemokine, and is highly sensitive, binding G-protein linked receptors such as CCR2, CCR3 and CCR5. It is responsible for recruiting eosinophils, through chemotaxis induction to eliminate infections (Nagarkar, 2010). These chemokines are constitutively expressed by cells of secondary lymphoid organs and are primarily involved in the homing of dendritic cells (DCs) and lymphocytes, which therefore elicits a host immune response (Miu et al., 2006).

Leukocytes undergo differentiation and antigen- dependent proliferation in secondary lymphoid organs, enabling them to respond to inflammatory stimuli. This varies from homeostatic chemokines, as they possess the ability to bind numerous chemokine receptors of a specific class (Sallusto et al., 2000; Miu et al., 2006). This phenomenon was observed in experiments by Moser et al. (2004) involving mice with a genetic defect except for CXCL12/SDF-1-/- and CXCR4-/- mice, which lacked multitudes of phenotypic abnormalities, therefore suggesting that chemokine receptors can compensate for such defects. Th1 immune response controls the expression and polarization of specific chemokine receptors such as CCR, CXCR3 and CXCR6, while Th2 immune responses are responsible for the expression of CCR4 and CCR8 chemokine receptors on specific effector cells (Miu et al., 2006). The host response to parasitic infections requires the coordinated action of both the innate and acquired arms of the immune system (Luster, 2002).

2.4.3. The role of chemokines during malaria infection

Chemokines mediate the trafficking of dendritic cells, B cells and T cells required to generate an immune response (Boström et al., 2012; Aleksandra et al., 2013). Activating lipopolysaccharide (LPS), Toll-like receptors (TLRs) on the surface of bacterial pathogens causes stimulation of local release of chemokines such as MIP-1α, MIP-1β, MIP-3α and IP-10 (Luster, 2002; Hunter et al., 2006). Activation of chemokine receptors such as CCR1, CCR5 and CCR6 causes attraction of immature dendritic cells to a local milieu and are efficient at picking-up antigens (Salazar-Mather et al.,1998; Luster, 2002) but must mature and differentiate into cells that can activate naive T cells. In this local milieu, there are pathogen-associated molecular pathogens such as LPS which induce the differentiation and maturation of dendritic cells into potent antigen-presenting cells (Khan et al., 2002). This downregulates the expression of CCR1, CCR5 and CCR6 while upregulating the expression of CCR7 (Luster, 2002) causing its migration into the afferent lymphatic system.

Many cell types such as DCs, B and T lymphocytes express CCR7, CCL21 receptor. The CCR7 ligand SLC is expressed on the endothelium of the afferent lymphatic system (Forster et al., 1999; Griffith et al., 2014; Nchangnwi *et al.*, 2015). CR7 plays an important role in directing the migration of the antigen-loaded mature dendritic cells (Khan *et al*., 2000; Luster, 2002). Naïve T and B cells from blood are brought in contact with activated dendrite cells and into lymph nodes by chemokines (Banchereau and Steinman, 1998; Luster, 2002). It is likely that chemokines such as DC-CK1 and ELC (produced from stromal cells within T-cell zone) play a crucial role in the juxta-positioning of T and B cells within

lymph nodes (Banchereau and Steinman, 1998). Some activated T cells downregulate expression of CCR7 but upregulate CXCR5 and so become directed towards the follicle to deliver help to B cells, whereas other activated T cells upregulate CXCR3 and are attracted into inflamed tissue (Reale et al., 1996; Luster, 2002). T cells that become activated in regional lymph nodes, after encountering antigen-loaded dendritic cells, subsequently return to sites of inflammation by sensing chemokine gradients established at these local sites (Luster, 2002; Lazzeri and Romagnani, 2005). Chemokines such as IP-10, which is induced by LPS and IFN-γ, and a ligand for CXCR3, which is highly expressed on activated T cells, are believed to be important in this process in immune responses mediated by Th1 cells (Reale et al., 1996; Salazar-Mather et al., 1998; Luster, 2002).

Chemokines during malaria infection play a crucial role in the control of malaria in humans, as they contribute to immunotherapy and parasite clearance (Favre et al., 1997; Lazzeri et al., 2005; Boström et al., 2012). Resistance to malaria infection and protection from onset of clinical episodes is inferred by the early production of IFN-γ (Artavanis-Tsakonas and Riley, 2002). Some pro-inflammatory cytokines such as tumour necrosis factor (TNF) and interleukin (IL)-12 have also shown to be essential mediators in immune protection against malaria infection (Torre et al., 2002; Boström et al., 2012). Chemokines as well as cytokines inhibit parasite growth and stimulation of phagocytosis to enhance clearance of parasitized erythrocytes (Boström et al., 2012). According to Ateba-Ngoa et al. (2015), INFγ and TNF levels increase during the acute phase of *P. falciparum* infection, as these are pro-inflammatory response and Th1 markers. Parasite clearance and impediment of parasite multiplication and clearance is due to the activity of pro-inflammatory responses.

Chemokine levels in children with acute *P. falciparum* infection vary with disease severity and some, such as IP-10 and RANTES, have been associated with mortality during cerebral malaria (Boström et al., 2012). Even though the innate immune system is essential during malaria infections, the importance of antibody mediated response against *Plasmodium* parasites was shown 50 years ago in passive transfer studies, in which immunoglobulin (IgG antibodies from immune African adults) reduced the level of parasites in Gambian children (Cohen et al., 1961; Boström et al., 2012). Cytophilic antibodies IgG1 and IgG3 play an important role in protective immunity against *Plasmodium* infection, while the role of IgG2 and IgG4 is less understood and has been related to protective mechanisms seen by the cytophilic antibodies (Groux et al., 1990; Fava et al., 2009; Boström et al., 2012). Protection against malaria depends on early, and intense but carefully balanced pro-inflammatory response to control or reduce the parasite numbers in the human host (Fava et al., 2009; Boström et al., 2012).

2.4.4. Chemokines in response to trichinellosis

During trichinellosis, the host immune response combats infection by producing a variety of immune cells, such as Chemokines, macrophages and cytokines (Pozio et al., 2001). Chemokines are chemotactic cytokines that play important roles in bridging the innate and the adaptive immune system (Boström *et al*., 2012). They orchestrate the migration of leucocytes and other cells by activating corresponding receptors on responsive cells, thereby inducing chemotaxis of immune cells to sites of infection (Luster, 2002; Boström *et al*., 2012).

Macrophages play a pivotal role in the functioning of the immune system to eliminate parasitic infections (Reale et al., 1998). According to Reale et al. (1998), mice that were infected with *Trichinella* released macrophages which were then activated, which produced several monokines. Monokines are involved in the resolution and exacerbation of parasitic infections (Reale et al., 1998; Mitreva et al., 2005). As stated by Scuderi et al. (1986) TNFα mainly produced by activated macrophages is an inducible cytokine with multiple biological activities. Raised TNFα levels have been found in parasitic and bacterial infections (Scuderi et al., 1986; Reale et al., 1998).

According to Rallis et al. (2000) the most commonly studied β-chemokines are monocyte chemotactic protein-1(MCP-1), which is a mediator of chronic and acute inflammatory diseases. Studies conducted on rats in vivo demonstrated that proteins and m-RNA for MIP-2 were upregulated during lung inflammation (Rallis at el., 2000). However, there is scarcity of information regarding chemokines and *Trichinella* infections. There are numerous parasitic infections in Sub-Saharan Africa, these have become a major cause of mortalities throughout the years. There has been a rise in these infections and this can be attributed for by poverty, lack of vaccines, unavailability of reliable diagnostic tools for early diagnosis (Yépez-Mulia et al., 2007). In countries where human trichinellosis is endemic, physicians are more likely to recognize the infection early; in countries where infections are only sporadic, the diagnosis and, consequently, the treatment are often delayed (Pozio et al., 2001).

Blood is the most accessible diagnostic tissue, as it is used for diagnosis of numerous diseases. Malaria and Trichinellosis both affects host heamapoetic physiology and contribute to disease severity. Patients with P. falciparum malaria depicted high haematological abnormalities such as changes in leucocyte count, haemoglobin and platelet abnormalities resulting in defective thromboplastin (Maina et al., 2009).

Chapter 3

Materials and Methods

3.2. Ethical consideration

Ethical approval was obtained from at the University of KwaZulu-Natal Animal Research Ethics Committee, under ethics number AREC/018/016PD.

3.3. Experimental animals.

This study was carried out at the University of KwaZulu-Natal, Westville campus, using one hundred and eighty-six male Sprague-Dawley rats, initially weighing between 90-150g. Experimental animals were bred and maintained at the Biomedical Resource Unit (BRU) of the University if Kwazulu-Natal, Westville campus, under pathogen free conditions, with constant room temperature of 25-27^{0}C, under 12-hour light (0800-2000hrs) and dark cycles (2000-0800hrs). Clean water and food was supplied *ad libitum*. All procedures conducted on the animals were strictly according to the rules and regulations of the University of KwaZulu-Natal Animal Ethics committee, under ethics number AREC/018/016PD.

3.4. Experimental design

Figure 1 shows the experimental study design, where one hundred and sixty-eight rats were randomly separated into 4 experimental groups (n=42), mainly the *P. berghei* ANKA infected group (M); *T. zimbabwensis* infected group (T); co-infected group (TM) and control group (C) (non-infected). *T. zimbabwensis* was induced on day 0 for the T group, *P. berghei* was induced on day 28 post-*Trichinella* infection for the TM group and *P. berghei* was induced as a mono-infection for the M group. On day 0 baseline parameters were recorded and animals were sacrificed on day 0, 7, 14, 21, 28, 35 and 42 post-*Trichinella* infection for T and TM groups, where the brain and spleen were harvested and haematological parameters were measured. Organs, serum and plasma samples were stored in a Bio Ultra freezer (Snijers Scientific, Tilburg, Netherlands) at -70 °C until assayed.

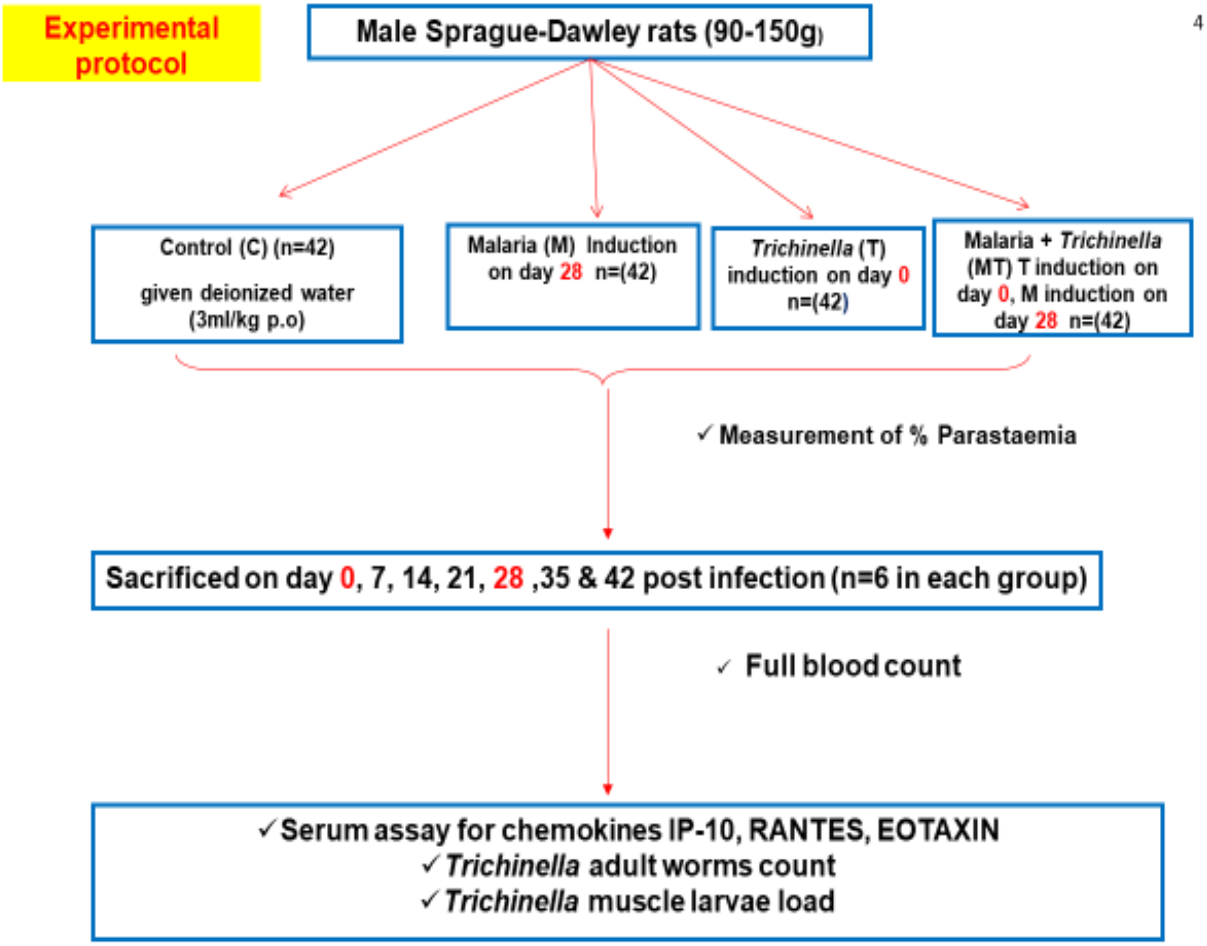

Figure 5: A schematic diagram illustrating a 42-day experimental protocol design. Where *Trichinella zimbabwensis* was induced on day 0, while malaria induction occurred on day 28 of the 42-day experimental study.

3.5 Methods

3.5.1 Induction of *Plasmodium berghei*

Plasmodium berghei was maintained through passage in male Sprague-Dawley stock rats, and the rats were sacrificed once infection was successfully induced. Blood was drawn through cardiac puncture into serum and serum-gel separator blood collection tubes. These were then spun using a Sorvall Legend Micro 17 Microcentrifuge (ThermoFisher scientific.za) machine for 5 minutes. Blood was separated from serum and placed in -80^0 C bio freezer for future use. Induction of *P. berghei* on experimental rats was initiated using the previously stored blood. Forty-two male Sprague-Dawley rats (90-150g) were

infected with *P. berghei* through a single intraperitoneal injection of *P. berghei* (diluted to form 10^5 parasitized RBC) as described by (Beaudoin et al., 1977) Phosphate buffered saline vehicle was injected to the control animals. Infection was confirmed through thin-smears of tail blood stained with Giemsa of the infected animals.

3.5.2 Induction of *Trichinella zimbabwensis*

Crocodile-derived *T. zimbabwensis* (ISS1209) parasite strain was used to induce *Trichinella* infection. Muscle larvae were obtained from infected stock rats whole carcasses digested at 42 days post infection (dpi) were used to infect experimental animals. Stock rats were euthanized, skinned, and eviscerated. Whole carcasses were weighed and deboned individually and the HCL-pepsin method (Kapel and Gamble, 2000) was used to digest the muscle tissue to recuperate the muscle larvae. Each experimental animal in group T and TM was orally infected with 3 muscle larvae per gram of body weight as described by Mukaratirwa et al. (2001). Six animals from the T and TM groups were humanly sacrificed on a weekly basis and blood was drawn through cardiac puncture, organs such as the spleen, muscle and the brain were harvested and later used for haematological analysis.

3.5.3 Haematology measurements

A full blood count, giving the following parameters; Red blood cell count (RBC), White blood cell count (WBC), haemoglobin (HGB), haematocrit (HCT), Monocytes (MO), Lymphocytes (LY), and basophils (BA) was carried out using Beckman Coulter counter (USA-Miami Florida) according to the manufacturer's instructions.

3.5.4. Serum chemokines determination

IP-10, RANTES and EOTAXIN were measured using commercially available kits (Duo Set ELISA Development System; RsD Sys- tems). Protocol was carried out according to the manufacturer's instructions. Briefly, NUNC Maxisorp® 96-well ELISA flat bottom plates were marked and labelled. Magnetic beads were vortexed for 30s, 50µl bead solution was added using a multichannel pipette into wells of the plate. The plate was securely placed on the handheld magnetic washer, the beads were left to settle at the bottom of the plate. This plate was then carefully inverted over a sink and blotted over paper towel. 25µl of universal Assay buffer was added into each well, which was allowed to settle for 30s and 25µl of standard and samples were added into the well. The plate was incubated 60-120minutes

at room temperature. The beads were washed 3X. 25µl of detection antibody mix (X1) was added. The plates were cautiously sealed and placed on the shaker for 30min and washed 3X. 50µl of Streptavidin-PE. Plates were sealed and incubated on the shaker for 30min at room temperature and washed 3X. The beads were resuspended by addition of 25µl of reading buffer. Plates were properly sealed and shaken for 5min at room temperature. Data was acquired using Luminex® 100/200. Chemokine concentrations were extrapolated from standards using a 4parameter logistic equation in Graph Pad PRISM version 5.04 for windows (Graph Pad Software, San Diego, CA).

3.7. Data analyses

Malaria percentage parastaemia, *Trichinella* muscle larvae load, number of adult worms and haematological parameters measurements were expressed as means ± standard error (SE), were analyzed using repeated measure analysis of variance (ANOVA). Levels of significance were determined using Kruskal Wallis for comparison of more than two non-parametric means using Graph pad PRISM version 5.0 for windows (Graph pad software, San Diego, CA, USA), where a *p*-value of < 0.05 was considered as significant.

Chapter 4

Results

4.0 Results

4.1 General

This chapter describes the following:

4.2 Percentage parasitaemia in malaria induced experimental animals

4.3 Trichinella Muscle larvae load

4.4 Haematology parameters concentration of the experimental groups

4.5. Serum chemokines concentration

4.5.1 Serum CXCL10 (IP-10) concentration

4.5.2 Serum CCL (RANTES) concentration

4.5.3 Serum CCL11 (Eotaxin) concentration

4.2 Parasitaemia reduction after treatment

Levels of parastaemia were attained as percentage of parasitized red blood cells measured in male Sprague-Dawley rats (figure 6). An increase in percentage *P. berghei* parastaemia was observed in both *P. berghei* mono and co-infected groups, with a higher percentage parastaemia in the mono infected group on day four of the experimental protocol. Where there was a significant difference (*$p<0.05$) on day 4 post infection, when comparing *P. berghei* and co-infected group. Figure 1 shows that the peak percentage parasitaemia of the mono and co-infected groups on day 7, subsequently followed by a gradual decrease thereafter. There was an increase in percentage parastaemia on day 12 post infection of the mono-infected group, however this did not result in a significant difference between the *P. berghei* mono and co-infected groups. There were no rat mortalities throughout the duration of the experiment.

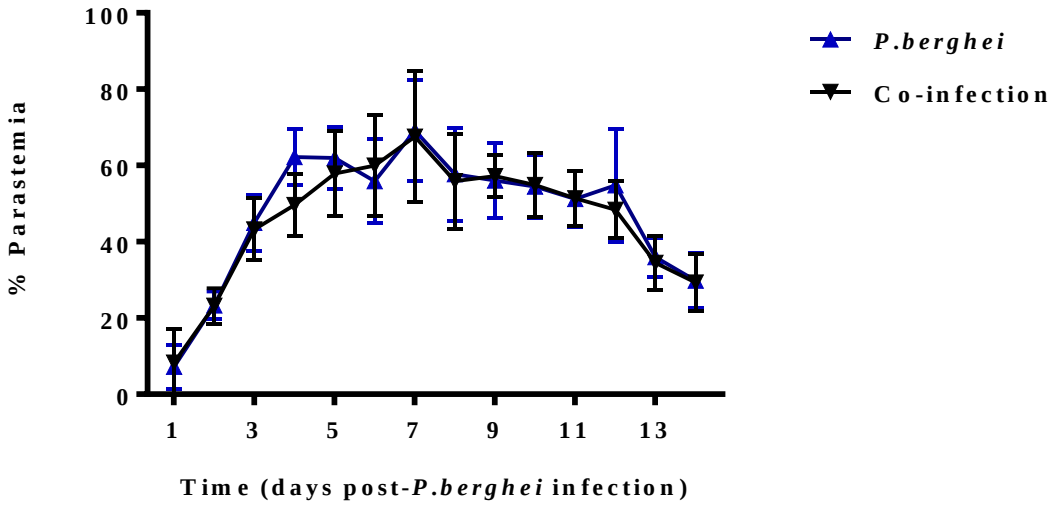

Figure 6. Mean percentage parasitaemia of male Sprague-Dawley rats, mono or co-infected with *Plasmodium berghei* and *Trichinella zimbabwensis.* Day 0 represents the day of *P. berghei* induction and day 28 post-*Trichinella* infection in the 42-day experimental protocol, when *Trichinella* larvae has migrated to muscles of the rats. Values are presented as means and vertical bars indicate ± SEM (n = 6 for each group).

4.3. Trichinella *zimbabwensis* muscle larvae load

Parasite establishment

The effects of *P. berghei* and *T. zimbabwensis* co-infection on *Trichinella* muscle larvae load were observed in figure 7. *T. zimbabwensis* adult worms (AW) were recovered within the intestines of the male Sprague-Dawley rats up to day 14 pi (figure 2). No AW were recovered on day 21 post infection. On day 7pi, there was a higher number of AW recovered, relative to day 14, although there was no significant statistical difference observed between the *P. berghei* mono and co-infected groups. Muscle larvae (ML) was obtained from day 28 to day 42 pi. The number of ML increased gradually from day 35 to day 42 pi, with slight variations between the *T. zimbabwensis* mono and co-infected groups, however this variation was not statistically significant, as depicted by Figure 7 below:

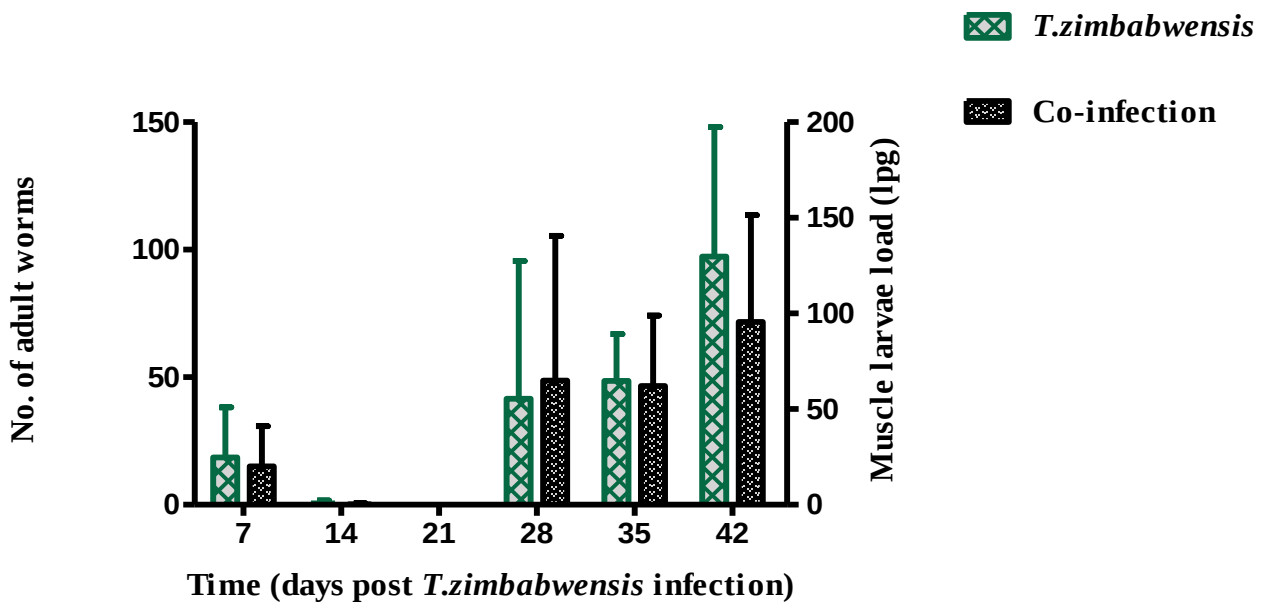

Figure 7. Mean number of intestinal adult worms (AW) and counts of muscle larvae (ML) per gram of muscle (lpg) recovered from Sprague-Dawley rats co-infected with *Plasmodium berghei* and *Trichinella zimbabwensis*. Values are presented as means and vertical bars indicate SEM (n = 6 of each group).

4.4.0 Concentration of haematological parameters in male Sprague-Dawley rats non-infected, mono, co-infected with *Plasmodium berghei* and *T. zimbabwensis*.

4.4.1. Red blood cell count

The effects of malaria and *Trichinella* co-infection on red blood cell count (cell/mL) were investigated in separate groups of non-infected, *P. berghei* mono-infected, *T. zimbabwensis* mono-infected and co-infected experimental animals as shown in Figure 8. There were significant differences in the red blood count for the *T. zimbabwensis* mono-infected group from day 0 to day 14. Whereas RBC count between control group and *P. berghei* mono-infected group was significantly higher on day 7pi (** $P<0.01$), RBC count was significantly lower (*** $P<0.001$) by comparison of control and co-infected group. On day 14 (* $P<0.05$) was observed when comparing *P. berghei* mono infected and control group (figure 8).

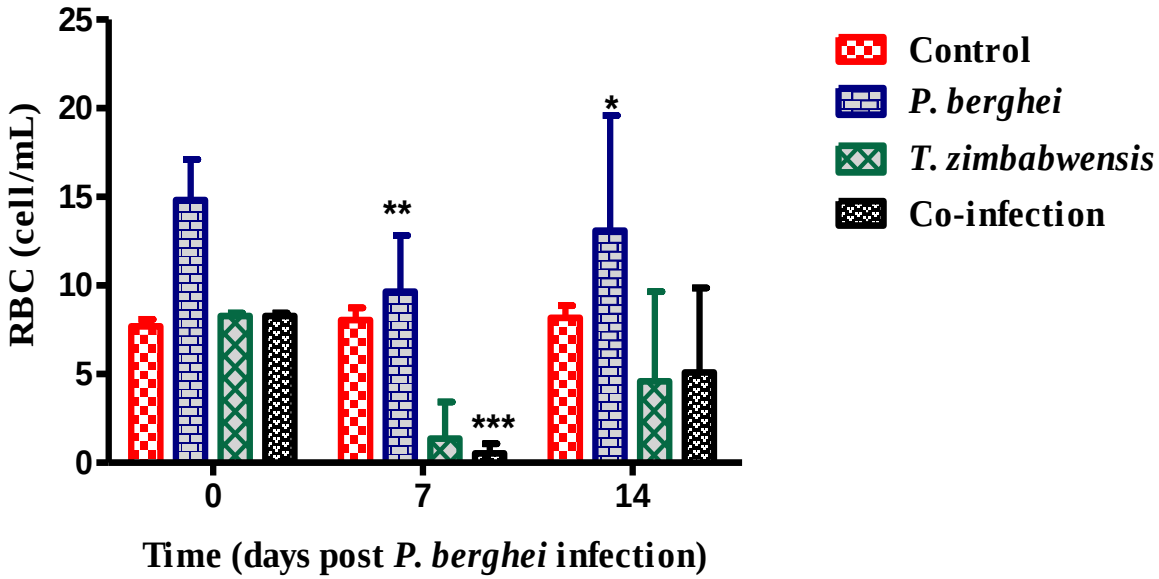

Figure 8. Mean red blood cell count of non-infected, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis*. Day 0 represents the day of malaria induction in the 42-day experimental protocol, when *Trichinella* muscle larvae has migrated to the rat muscle). Values are presented as means and vertical bars indicate SEM (n=6 in each group).

4.4.2 Percentage haemoglobin concentration

Percentage haemoglobin levels were observed in the control, *P. berghei* and *T. zimbabwensis* mono and co-infected groups (Figure 8). On day 0 of the experimental protocol, there was no significant difference in haemoglobin values throughout the four groups respectively. A steady decrease in percentage values was observed (figure 3), with *P. berghei* mono infected group having the lowest percentage on day 7pi, which was significantly lower (* $p<0.05$) than the control group. The highest concentrations were observed on day 14pi in both *P. berghei* and *T. zimbabwensis* mono infected groups, with a significant difference (** $p<0.01$) by comparison with the control group with both *Trichinella* and co-infected group (figure 9).

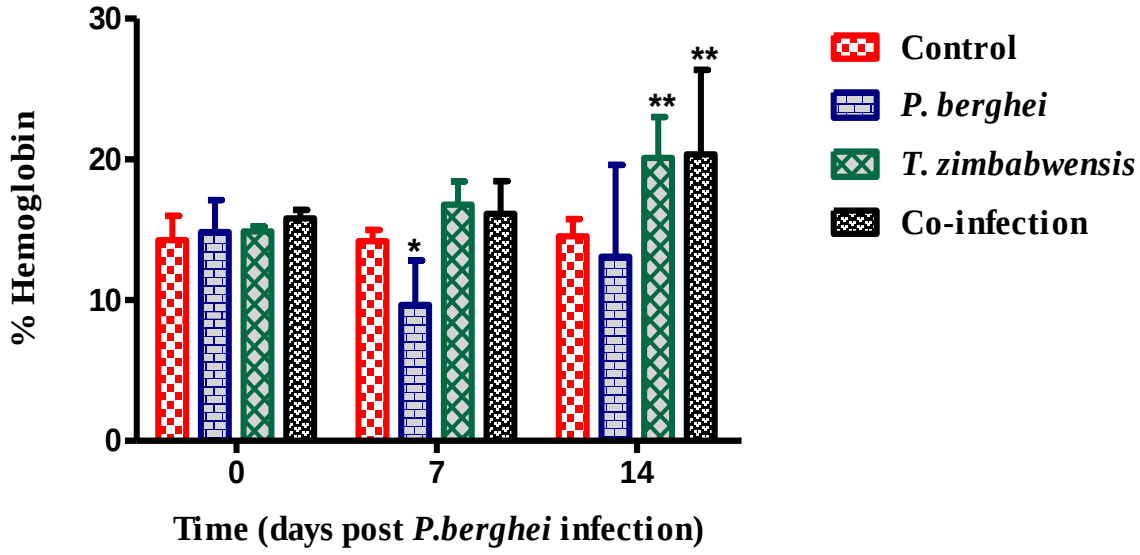

Figure 9. Mean haemoglobin (%) of control, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis.* Day 0 represents the day of *P. berghei* induction which was day 28 post-*Trichinella* infection in the 42-day experimental protocol, when Trichinella muscle larvae has migrated to the rat muscle. Values are presented as means and vertical bars indicate SEM (n = 6 of each group).

4.4.3. Percentage haematocrit

The effects of *P. berghei* and *T. zimbabwensis* co-infection on percentage haematocrit were investigated on male Sprague-Dawley rats (figure 10). On day 0 *P. berghei* mono infected group depicted a significantly high percentage haematocrit (***$p<0.001$) by comparison with the control group. Gradual decrease in percentage haematocrit was observed on day 14 pi. *P. berghei* and *T. zimbabwensis* mono infected groups depicted significantly low percentages (***$p<0.001$) by comparison with the control group in figure 10 below:

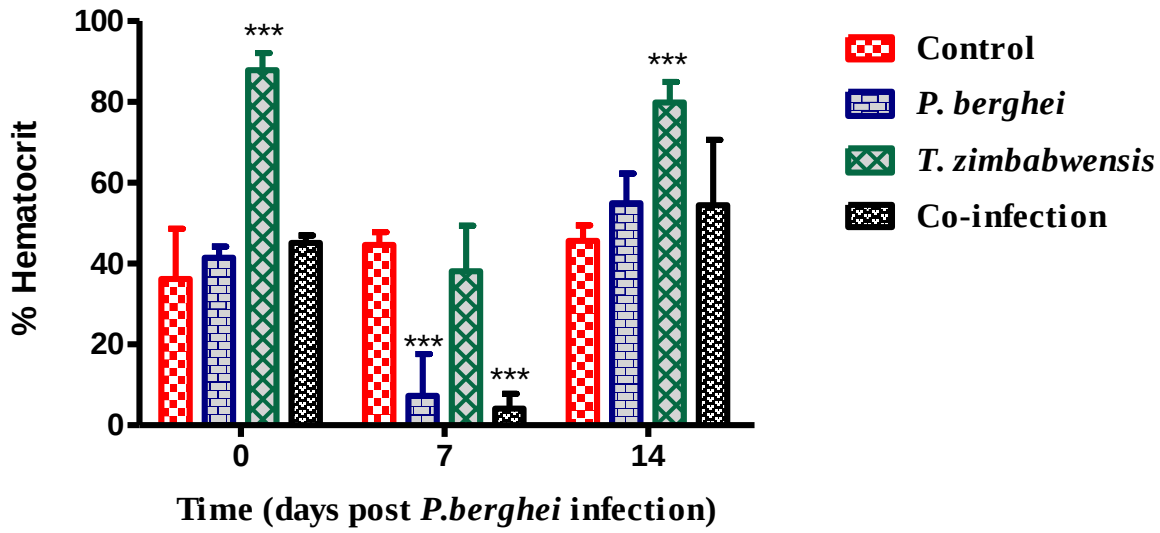

Figure 10. Mean haematocrit (%) of control, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis.* Day 0 represents the day of *P. berghei* induction which is day 28 post-*Trichinella* induction in the 42-day experimental protocol, when *Trichinella* muscle larvae had migrated to the rat muscle. Values are presented as means and vertical bars indicate ± SEM (n = 6 of each group.

4.4.4. White blood cell count

The effects of malaria and *Trichinella* co-infection on white blood cell count of male Sprague-Dawley rats was investigated in separate groups of non-infected, *P. berghei* mono-infected, *T. zimbabwensis* mono-infected and co-infected experimental animals as shown in Figure 11. The was a gradual increase in white blood cells during day 0 to 14pi in *T. zimbabwensis* mono, co-infected and a trivial decrease in *P. berghei* group on day 14pi during the experiment. However, this was not significantly different among all experimental groups.

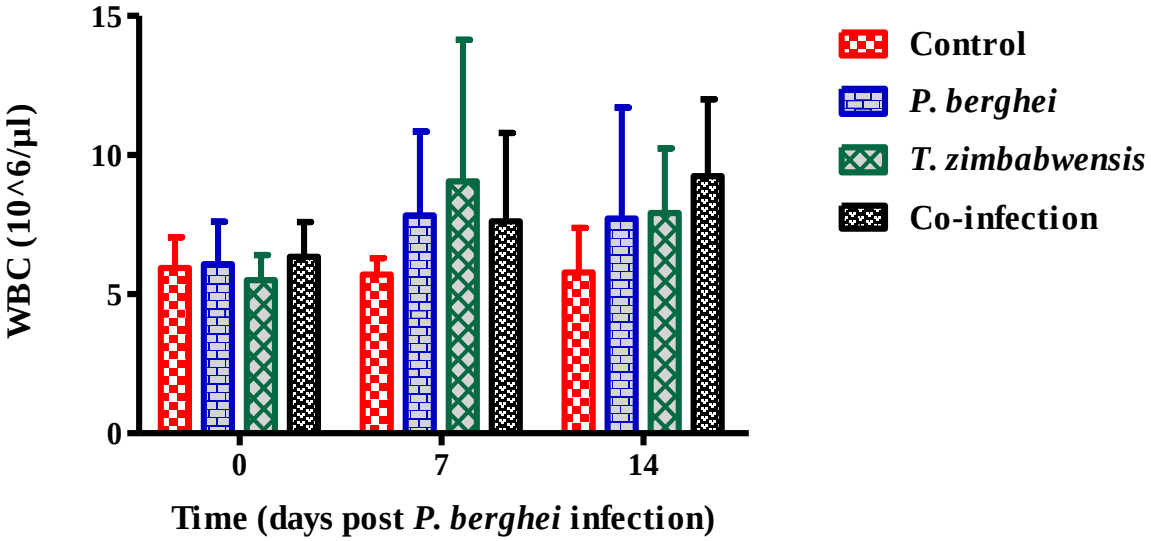

Figure 11. Mean white blood cell count of non-infected, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis.* Day 0 represents the day of malaria induction in the 42-day experimental protocol, when *Trichinella* muscle larvae has migrated to the rat muscle). Values are presented as means and vertical bars indicate SEM (n=6 in each group).

4.4.5. Percentage monocytes concentration

The effects of malaria and *Trichinella* co-infection on percentage monocytes was investigated in separate groups of non-infected, *P. berghei* mono-infected, *T. zimbabwensis* mono-infected and co-infected experimental animals as shown in Figure 11. There were no significant differences in percentage monocyte count on between day 0 and day 7pi in all experimental groups. However, day 14 showed a significant increase (***$p<0.001$) *P. berghei* and *T. zimbabwensis* mono infected groups, when compared with the control group. The co-infected group had a slight decrease in monocyte count, which was not statistically different from the control groups (figure 11).

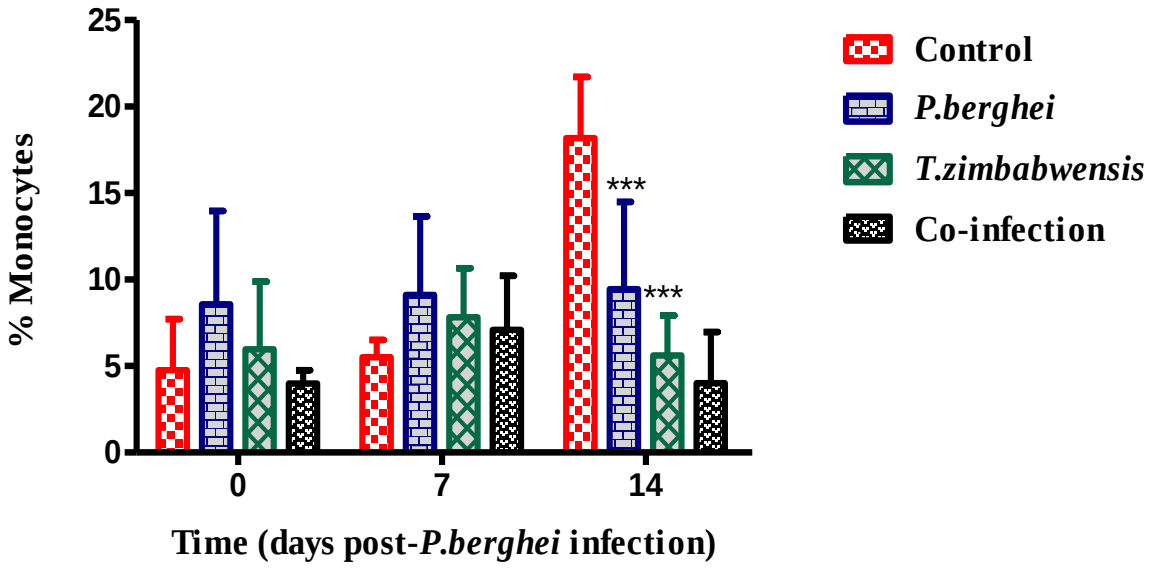

Figure 12. Mean monocytes of non-infected, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis*. Day 0 represents the day of malaria induction in the 42-day experimental protocol, when *Trichinella* muscle larvae has migrated to the rat muscle). Values are presented as means and vertical bars indicate SEM (n=6 in each group).

4.4.6. Percentage lymphocytes concentration

The effects of *P. berghei* and *Trichinella* co-infection on percentage lymphocytes are shown in Figure 12. Similar percentages of lymphocytes were observed with no significant difference across all experimental groups on day 0. However, a decrease on day 7pi for all experimental groups was shown in (figure 12). However, on day 7pi, *P. berghei* and *T, zimbabwensis* mono infected and co-infected groups significantly decreased (**$p<0.01$) by comparison with the control animals. A significant decrease (***$p<0.01$) in the *P. berghei* mono-infected group. On day 14pi, a significant increase (**$p<0.01$) by comparison of control with *T. zimbabwensis* and co-infected animals, while *P. berghei* mono infected group increased with (*$p<0.05$) when compared with the control.

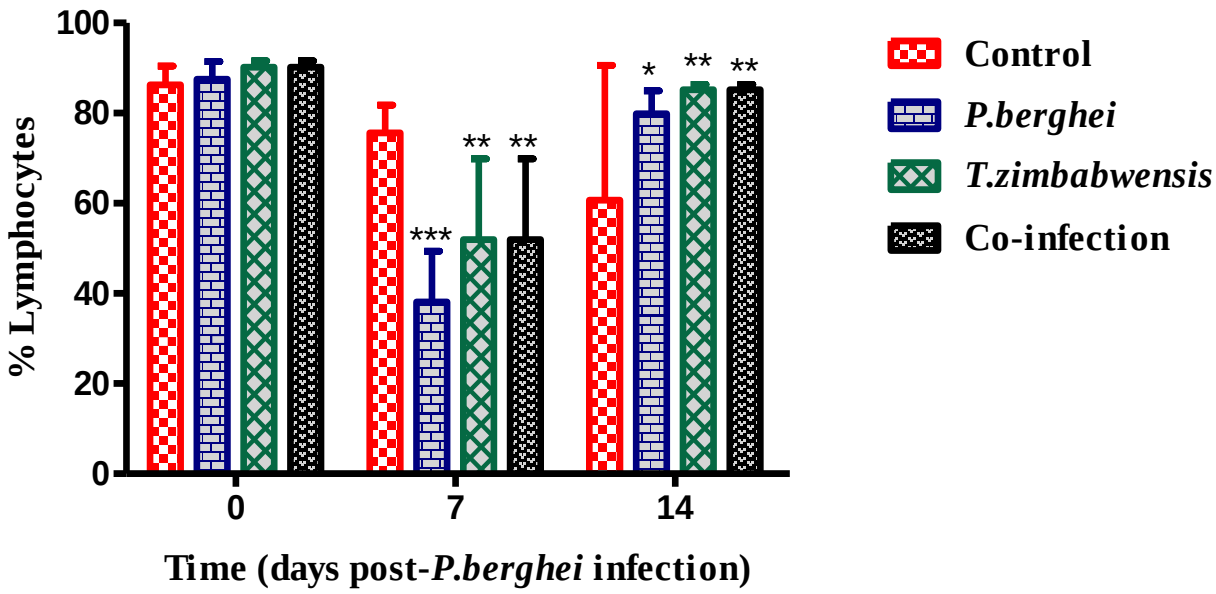

Figure 13. Mean Lymphocytes of non-infected, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis* Day 0 represents the day of malaria induction in the 42-day experimental protocol, when Trichinella muscle larvae has migrated to the rat muscle). Values are presented as means and vertical bars indicate SEM (n=6 in each group).

4.4.7. Percentage Basophils concentration

The effects of malaria and *Trichinella* co-infection on percentage basophil was investigated in separate groups of non-infected, *P. berghei* mono-infected, *T. zimbabwensis* mono-infected and co-infected experimental animals (figure 13). Day 7pi showed a significant increase in percentage basophils (***$p<0.001$) observed when comparing the control and *T. zimbabwensis* and *P. berghei* mono infected groups respectively.

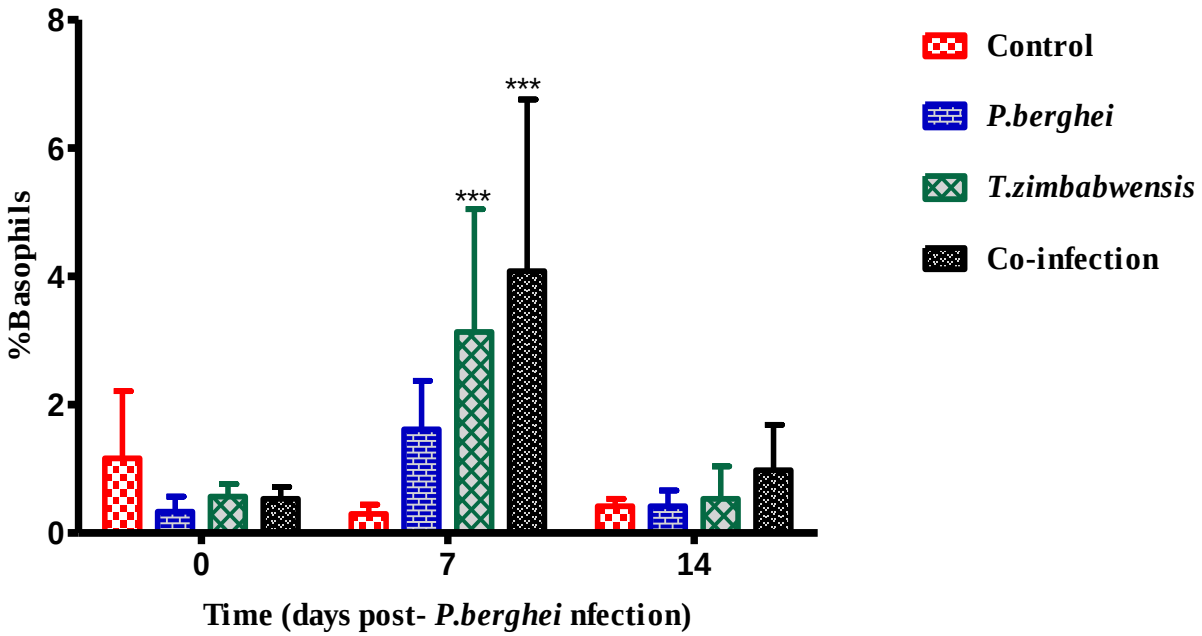

Figure 14. Mean basophil percentage of non-infected, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis* Day 0 represents the day of malaria induction in the 42-day experimental protocol, when *Trichinella* muscle larvae has migrated to the rat muscle). Values are presented as means and vertical bars indicate SEM (n=6 in each group).

4.4.8. Percentage neutrophil concentration

The effects of malaria and *Trichinella* co-infection on percentage neutrophil was investigated in separate groups of non-infected animals, malaria, *Trichinella* and co-infected male Sprague-Dawley rats (figure 14). On day 0 there was a significant decrease (*p<0.05) between the *P. berghei* mono infected, (**p<0.01) *T. zimbabwensis* mono infected and co-infected group in comparison with the control group.

This followed a significant increase in percentage neutrophils in the co-infected (**p<0.01) and (*p<0.05) *T. zimbabwensis* mono infected groups on day 12.

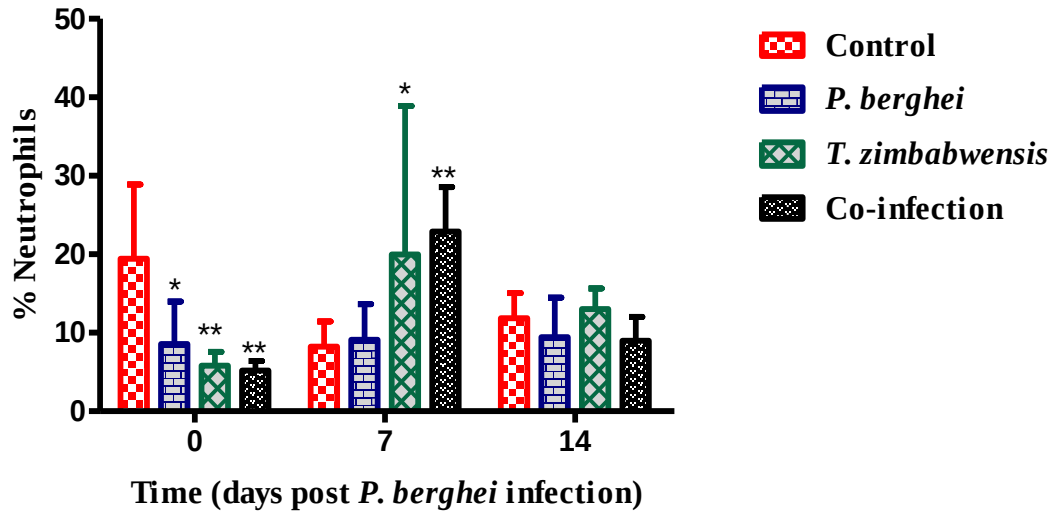

Figure 15. Mean neutrophil of non-infected, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis* (Day 0 represents the day of malaria induction in the 42-day experimental protocol, when *Trichinella* muscle larvae has migrated to the rat muscle). Values are presented as means and vertical bars indicate SEM (n=6 in each group).

4.5. Serum chemokines concentration

4.5.1. Serum CXCL10 (IP-10) concentration

The effects of malaria and *Trichinella* co-infection on serum CXCL10 (IP-10) concentration was investigated in separate groups of non -*P. berghei* mono-infected and co-infected experimental animals as shown in Figure 16. IP-10 concentrations were constant in the *T. zimbabwensis* mono and co-infected groups throughout the experiment. However, a significant increase (*** P<0.001) was noted when comparing *P. berghei* mono-infected group and the control group on day 7 followed by a significant decrease on day 14.

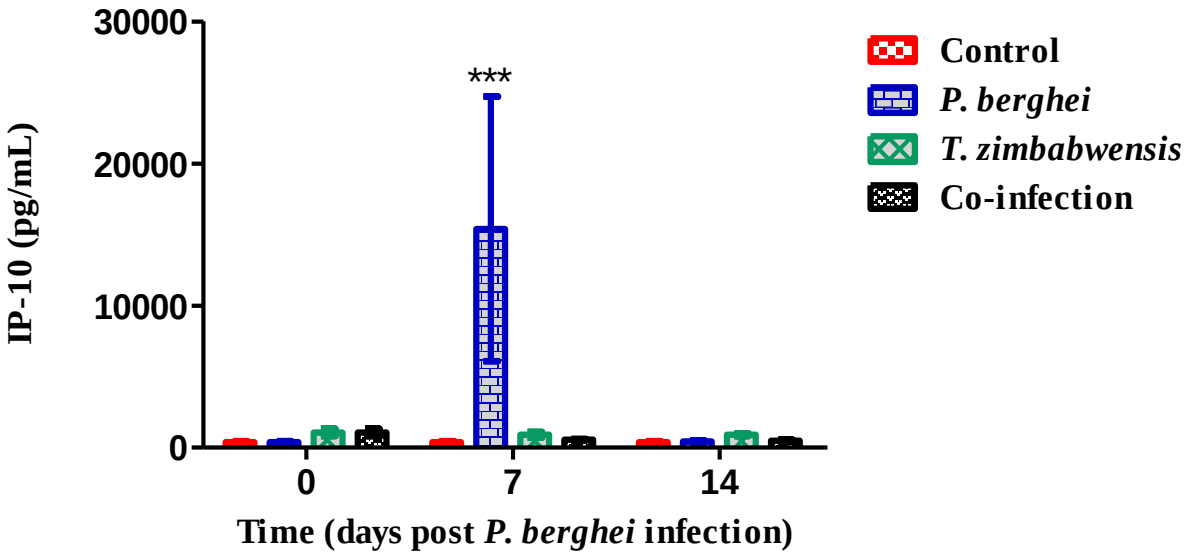

Figure 16. S**erum CXCL10 (IP-10) concentration** of control, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis*. Day 0 represents the day of malaria induction in the 42-day experimental protocol, when *Trichinella* muscle larvae is now in the rat muscle). Values are presented as means and vertical bars indicate SEM (n =6 in each group).

4.5.2. Serum CCL (RANTES) concentration

The effects of malaria and *Trichinella* co-infection on serum CCL (RANTES) concentration as investigated in separate groups of non- *P. berghei* mono-infected and co-infected experimental animals as shown in Figure 17. On day 0 there was a significant increase (*** P<0.001) in *T. zimbabwensis* mono and co-infected group, and a significant increase (*** P<0.001) of *P. berghei* mono infected group relative to the control group on day 7pi. RANTES counts decreased throughout the duration of the experiment in the co-infected group.

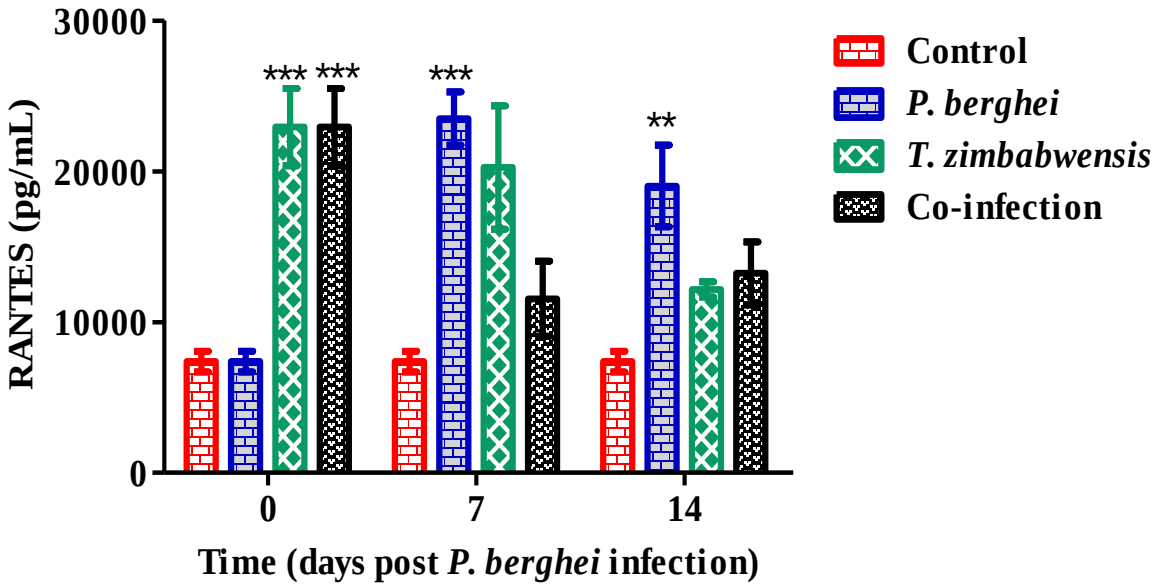

Figure 17. Serum CCL (RANTES) concentration of control, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis*. Day 0 represents the day of malaria induction in the 42-day experimental protocol, when *Trichinella* muscle larvae is now in the rat muscle). Values are presented as means and vertical bars indicate SEM (n=6 in each group).

4.5.3 Serum CCL11 (Eotaxin) concentration

The effects of malaria and *Trichinella* co-infection on serum CCL11 (Eotaxin) concentration as investigated in separate groups of non-infected, *P. berghei* mono-infected, *T. zimbabwensis* mono-infected and co-infected experimental animals as shown in Figure 18. There was a decrease in Eotaxin concentration in the control, *P. berghei* mono-infected and co-infected group from day 0 to day 14 pi. However, *T. zimbabwensis* mono-infected group showed a significant increase (** $P<0.01$) on day 7, and a significant decrease (*$P<0.05$) on day 14 relative to the control group (figure 18) below:

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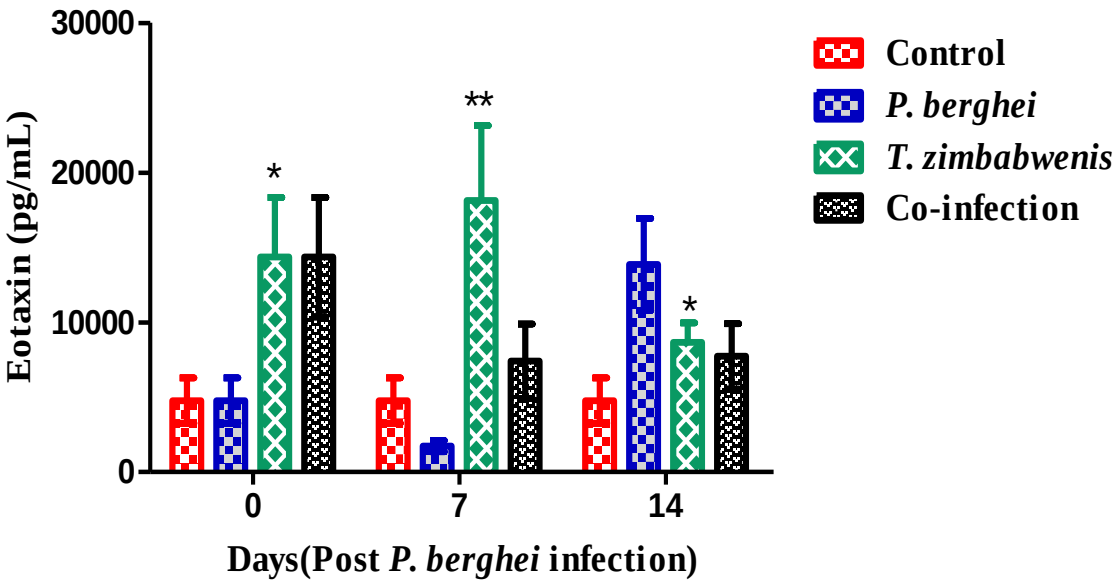


Figure 18. **Serum CCL11 (Eotaxin) concentration** of control, mono or co-infected male Sprague-Dawley rats infected with *Plasmodium berghei* and *Trichinella zimbabwensis* Day 0 represents the day of malaria induction in the 42-day experimental protocol, when *Trichinella* muscle larvae is now in the rat muscle). Values are presented as means and vertical bars indicate SEM (n=6 in each group)

Chapter 5

Discussion

From this study, it was observed that *P. berghei* and *T. zimbabwensis* co-infected and mono infected groups showed similar percentage parastaemia levels throughout the duration of the experiment. An increase in percentage parastaemia was observed in the early stages of infection in both mono and co-infected groups. This was supported by the upregulation of Th1 proinflammatory chemokine (IP-10) responses on day 7pi in the *P. berghei* mono-infected group. Th1 responses are associated with effective control of early phase parastaemia, followed by a significant decrease in 1P-10 from day 14pi, during chronic phases of the disease. However, a *P. chabaudi* and *Heligmosimoides polygrus (H. polygrus*) co-infection study, resulted in increased parastaemia and mortality in early phases of infection and this was associated with a reduction in Th1 cytokines Butcher et al., 2011, contrary to the current study. There was a correlation between the gradual decrease in parastaemia from mono and co-infected groups as well as Th1 chemokine (IP-10) concentration. This therefore confirmed that *T. zimbabwensis* predisposed male Sprague-Dawley rats to increased *P. berghei* percentage parastaemia. Which contrasted with other *Plasmodium* and helminth co-infection studies (Bucher et.al., 2011) where schistosomiasis repressed effective *Plasmodium* parasite control during early phases of infection in mice co-infected with *Schistosoma mansoni* and *P. yoelii*.

The present study also showed that *T. zimbabwensis* adult worms persisted for up to day 14 pi in the small intestines, with no adult worms recovered on day 21pi in both the *T. zimbabwensis* mono and co-infected groups. According to Onkoba et al. (2016), production of IgG and IgM antibodies occurs during intestinal phase of *T. zimbabwensis* infection, which implies that host intestinal epithelial mucosa elicits a protective innate immune response against new born larvae (NBL) and adult worms (AW). According to Fabre et al. (2009) Th2 intestinal immune response, leads to the expulsion of AW by eosinophil-dependent chemokines from the intestines, therefore coinciding with muscle invasion of *T. spiralis* larvae. Activated Th2 response results in the induction of T cell dependent inflammatory response during intestinal infection, and Th1 response is repressed (Wakelin & Goyal, 1996). However, a study by Onkoba et al. (2016) had trivial discrepancies from this study, as adult worms persisted up until day 21pi. From day 28, muscle larvae (ML) were recovered in high quantities in both groups, implying that larval migration occurs after 21 days pi.

Elevated RBC count (figure 8) on day 0, with a significant reduction on day 7pi, was observed. High *P. berghei* percentage parastaemia on day 7pi, resulted in a decrease in RBC count. This was due to the direct destruction of erythrocytes by *P. berghei* parasite and clearance of uninfected erythrocytes (Bennett, 2012). This may be due to reduction of erythropoietin activity, designated by reduced erythroblasts and augmented marrow cellularity (Sharma et al., 2008). Mice co-infected with *P. berghei and T. brucei* showed a reduction in RBC, which signified anemia and indicated disease severity (Ademola and Odeniran, 2016). During acute malaria with pre-eminent parastaemia, erythropoietin activity may be ineffective, while during the chronic phase; there is an upsurge in total erythropoietin activity as demonstrated by an amplified marrow cellularity collective with an augmented percentage of erythroblasts. This agreed with a study by Sharma et al. (2008) on patients with *P. vivax* and *P. falciparum*, malaria, where there was a suboptimal reticulocyte counter effect for the degree of anemia, and whereas chronic malaria was allied with erythroid hyperplasia. Subsequently, ferrokinetic studies in both these forms of malaria demonstrated that the plasma iron turnover, a measure of total erythropoiesis, was often amplified but that the red cell iron utilization, was reduced (Sharma et al., 2008). This may result either from peripheral hemolysis or ineffective erythropoiesis.

According to Löffler et al. (2000), *P. falciparum* and *P. vivax* impair erythropoiesis directly due to noxious effect on erythroid erythroblasts or progenitor cells, or both. This can be supported by a significant decrease in RBC count in both mono and co-infected groups during acute phase of the disease, on day 7pi due to a substantial demand of erythrocytes, despite a transient increase on day 14pi, probably due to a gradual increase in response to increasing levels of erythropoietin. *P. berghei* infected mice presented with an overall deterioration in erythroid progenitor cells in the bone marrow and spleen (Miller et al., 1988). These results suggest that despite the presence of significant levels of erythropoietin and an increased demand for erythrocytes, *P. berghei*-infected rats cannot sustain erythropoiesis. The decreased production of erythrocytes in malaria may be the result of events inhibiting the differentiation or proliferation or both of erythroid precursors (Miller et al., 1989).

An inverse correlation between Hb, haematocrit and parastaemia was observed in this study. As stated by Arese et al. (2006), Hb within RBC becomes digested during parasite development, therefore leading to the rapture of RBC, during the development of schizonts. There was a significant decrease in Hb and haematocrit concentration at day 7pi in both *P. berghei* mono and co-infected groups, subsequently followed by a significant increase on day 14pi as the hosts were restoring hematopoiesis. The marginal

parasitaemia undervalues the erythrocyte population at risk from haemolysis because of parasitization for two reasons. Firstly, as the parasites develop, many parasitized red cells are sequestered in the deep vasculature and secondly, red cells lacking intracellular parasites may have been deparasitized through the opposing function of the spleen and consequently damaged (Mitola et al., 2006).

High lymphocyte percentages were observed in figure 12 across all experimental groups on day 0 and day 14pi in this study. This percentage lymphocyte increase can be explained through study by Frydas et al. (1999) in mice infected with L1-*T. spirali*s. On day 20pi there was an upsurge in IgG and IgM levels, which demonstrated that *T. spiralis* induces Th2 immune responses as AWs are present on at least day 5 pi. The period from 5–15 days pi corresponded to the production and migration of the newborn L1 larvae to the muscle. B cells are stimulated to produce IgA after recognition of the cuticle's surface epitopes, between the 3rd and 13th day pi of T. spiralis (Frydas et al.,1999). IgG were also stimulated as a response to recognition of epitopes of the hypodermis and the intestinal gland cell granules of the L1 were distinguished 2–6 weeks subsequently to the infection (Frydas et al.,199). However, T cells were stimulated at day 20pi, similar to results obtained in this study.

Focal cellular immune response is prompted during striated muscle invasion of *T. spiralis*, where this parasite persists as nurse cells concurrently with CD8+, CD4+ T lymphocytes, B lymphocytes and macrophages. B lymphocytes secreting IgG and IgE antibodies result in antibody-dependent cell mediated cytotoxic reaction against *T. spiralis* larvae (Wang and Bell, 1998). As observed in the current study, immune complexes formed by specific antibodies bound to *Trichinella* antigens are noted in early stages of host infection. The protective isotypes IgG1 and IgG2 have an active role in the inflammatory response. In a *P. berghei* and *T. brucei* co-infection study, reduction in lymphocytes resulted in marked antigenic stimulation which led to accelerated transformation of lymphocytes to plasma cells and transferred lymphocytes resulting to lymphopenia (Ademola and Odeniran, 2016).

Results from the current study are further supported by a *P. falciparum* and *M. tuberculosis* co-infection study whereby *P. falciparum* was recognized by CD8 cytotoxic T-lymphocytes (CTLs) upon host invasion. Pathogen-derived proteins are synthesized within the host infected cells and processed through MHC class I antigen processing pathway, where short virus-derived peptides, amino acids in length, are generated and presented in association with MHC class I molecules at the cell surface. CD8 CTLs bearing T-cell receptors of appropriate specificity recognize these peptide–MHC class I molecule complexes through their T-cell receptors and are thus triggered to destroy infected host cells (Pathan, et al., 2001). A significant increase was further observed on day 14pi.

As observed in figure 11, there was no significant differences in monocyte concentrations across all experimental groups on day 7 and 14pi in all experimental groups. pRBCs adhere to numerous receptors such as E-selectin, CD36, ICAM-1 and vascular adhesion molecule-1 (VCAM-1), all of which are expressed by monocytes (Goldring and Ramoshebi,1999). These cells orchestrate cytokine secretions, cell-cell recognition, as well as produce inflammatory cytokines such as tumour necrosis factor (TNF), interleukin-6 and interleukin-1, which stimulate hepatic synthesis of acute phase inflammatory proteins, in response to parasitic infections (Strieter et al., 1993). This therefore triggers monocyte survival by inhibiting the apoptotic pathway, thus contributing to the maintenance of the inflammatory response. Secretory products of human monocytes can kill intracellular *P. falciparum* parasites in vitro and may provide some protection against malaria, (Ockenhouse et al., 1988).

On day 7pi we observed elevated levels of neutrophils and basophils. Higher levels of basophils and neutrophils denote the presence of high parasitaemia. Some neutrophils contained ingested merozoites and whole schizonts, to combat high parasite load. On day 14pi, we observed a reduction in the number of peripheral blood basophils and neutrophils in all experimental groups. This may be due to the phagocytosis of neutrophil precursors in the bone marrow and spleen (Ademola and Odeniran, 2016). The decrease of peripheral neutrophils and basophils may be due to the recruitment and accumulation of these cells in tissues. In host inflammatory reactions against malaria, Interleukin-3 (IL-3)- activated endothelial cells selectively enhance *in vitro* adhesion and interaction of basophils (Pelleau et al., 2012) and these subsequently move to the endothelium. This phenomenon is pertinent in malaria, where requisition of infected erythrocytes to microvascular sites is associated with local activation (Suzukawa et al., 2008) and basophils accrue to these sites of inflammation. Hence, the low basophils count observed in this study during disease progression. Another study in mouse model stated that a substantial number of basophils accumulated in the spleen (Suzukawa et al., 2008) and therefore accounting for the significant reduction in peripheral blood during malaria infection.

According to Giacomin et al. (2012) basophils contribute to optimal Th2 cytokine-mediated immune responses post parasitic helminth infection, which can be explained by the functional heterogeneity observed in basophil lineage. These CD4+ Th2 cytokine responses are generated as a crucial factor for immunity to parasitic helminths such as *Trichinella* (Giacomin et al., 2012). Data from the current study demonstrated elevated levels of basophils in both *T. zimbabwensis* mono and co-infected groups on day 7pi, which was during the intestinal invasion phase of the parasite as AW. A decrease in basophil concentration was observed on day 14pi. According to Noti et al. (2014) depletion of these cells results in impaired CD4+ Th2 cytokine responses post helminth infection. Which therefore implies that

basophils are critical for promoting optimal CD4+ Th2 cytokine responses after infection with a gastrointestinal helminth. This study confirms that parasitic infection results in the modification of the basophil activation status as well as altered response of hematological parameters.

According to Ochiel et al. (2005) there is paucity of information regarding molecular determinants of mild versus severe malaria. Current knowledge suggests a probable role of the virtual balance between pro- and anti-inflammatory cytokines (Liew et al., 2005). Disease progression is due to pre-eminent levels of pro-inflammatory cytokines observed during the acute phase of infection, although an anti-inflammatory response promotes pathogenesis (Fang et al., 2016). Chemokines are soluble inflammatory intermediaries which are important during parasitic infections, by regulating the innate and adaptive immune response (Maizels and Yazdanbakhsh, 2003).

IP-10 (CXCL10) is a proinflammatory chemokine, induced by IFN-γ, TNF-α, possessing chemotactic activity for activated Th1 lymphocytes in numerous inflammatory conditions (Jain et al., 2008). It also stimulates IFN-c production and T cell proliferation in response to alloantigen or to exogenous antigen implying a role in Th1 polarization (Nie et al., 2009). In this study, IP-10 levels were remarkably low in all experimental groups throughout the duration of the experiments. However, in the *P. berghei* mono-infected group, IP-10 levels were significantly elevated on day 7pi, which correlated with peak parastaemia. Liu et al. (2011) reported that the increased expression of IP-10 occurs prior to the development of clinical symptoms, as observed during HIV infection, in brain tissue of neonatal mice infected with virulent (Fr98) polytrophic murine retroviruses. This chemokine also functions as a biomarker that predicts severity of various diseases (Liu et al., 2011). In a cerebral malaria study, an increase in IP-10 was correlated with disease severity (Benesi, et al., 1998).

Production of IP-10 in brain capillaries and astrocytes may be activated by antigens after schizont rupture (Jain et al., 2008). IP-10 in concert with TNF-α can cause vascular injury resulting in breakdown in the blood brain barrier, which leads to accumulation of leukocytes that induce local hyper-inflammation (Sauti et al., 1998). Knockout mice which lacked IP-10 or its receptor CXCR3 demonstrated that these molecules played a critical role in the CM associated death caused by *P. berghei* ANKA infection, this not only alleviated intravascular inflammation but also reduced pRBCs sequestration in the brain and peripheral parasitaemia. It was further shown that IP-10 and monokines induced by gamma interferon (MIG) play a role in attracting CD8+T cells and NK cells to brains of infected mice and may be involved in causing death in the infected mice (Jain et al., 2008).

This was contrary to the current study, as extremely low IP-10 values were observed apart from day 7 pi, hence no rat mortalities occurred.

Th1 and Th2 cytokine responses depict contrasting effects during infections**.** Th1 cells produce IFN-γ, which induces the manufacture by dissimilar cell types of IP-10, permits IP-10 in sequence to attract and recruit Th1 cells, therefore signifying the presence of a positive feedback loop between IFN-γ producing Th1 cells and resident cells producing IP-10 (Liu et al., 2011). In mice infected with *T. gondii*, expression of IP-10 peaked by day 7pi in the gut, liver, spleen, and lung, in contrast with the current study, *T. zimbabwensis* mono and co-infected groups, constitutively showed extremely low levels of IP-10, throughout the duration of the experiments. *Trichinella zimbabwensis* is an extracellular pathogen, particularly helminthic parasites, drive polarized Th2 responses. This therefore depicts varied IP-10 responses in different helminth infections. Antigen-specific $CD4^+$ and $CD8^+$ T cells recruitment was controlled by IP-10, which evoked a robust cellular and humoral immune response during *Toxoplasma gondii* infection (Han et al., 200).

Immunity to *T. gondii* is strictly dependent on IFNγ production by CD81 T lymphocytes as well as NK and CD41 T cells (Cohen et al., 2013). Co-infections with HIV virus and other pathogens up regulated IP-10 beyond that observed in mono-infected patients. Which was contrary to the current study, as IP-10 levels remained extremely low in the *T. zimbabwensis* co-infected group. However, as described above, IP-10 levels were elevated on day 7pi in the *P. berghei* mono-infected group.

In this study, RANTES levels were elevated on day 7pi, in both *T. zimbabwensis* and *P. berghei* mono-infected groups and decreased significantly in the co-infected group, with peak *P. berghei* percentage parastaemia. This correlated with a human CM study by (Benesi, et al., 1998) where it was demonstrated that *P. falciparum* suppressed circulating RANTES levels due to naturally acquired monocytes, which was thought to be associated with increased mortalities in patients. As demonstrated in the present study, there was an inverse relationship between RANTES levels and monocyte concentration in all experimental groups. Normally, circulating monocytes are short-lived and undergo spontaneous apoptosis daily (Eubank et al., 2010), which explains the gradual decrease of monocytes in this study.

Parasites are cleared mainly by monocytes/macrophages either directly by phagocytosis and intracellular destruction or by the release of toxic mediators (Malaguarnera and Musumeci, 2002; Artavanis-Tsakonas et al., 2003). Circulating RANTES levels progressively decline in children with increasingly

severe malarial anemia, and this is significantly associated with suppression of erythropoiesis and thrombocytopenia (Were et al.,2006). The association between suppression of circulating RANTES and increasing severity of malarial anemia, along with the significantly positive correlation between RANTES and Hb, suggests that decreased production RANTES may play a role in the development of malarial anemia by promoting suppression of erythropoiesis (Perkins et al., 2011). Higher plasma concentrations of RANTES and IP-10 have been revealed to be related to elevated Hb concentrations during acute malarial infection (Jain et al., 2008). As stated by Casals-Pascual et al. (2006) malaria parasites digest Hb as they grow within erythrocytes, and insoluble heme residues are polymerized into malaria pigment.

Th1 cells are involved in the production IFN-γ and activation of mononuclear phagocytes, which subsequently expels microbes (Abbas et al., 2014). However, Th2 has a contrasting role and varying sites of invasion and variable migratory capacity to Th2 cells, as it produces IL-4 and IL-5, with responses dominated by eosinophils and basophils (Austrup et al., 1997). Eotaxin, produced by phagocytic and epithelial cells, is an efficient and selective chemo-attractant for eosinophils and basophils, consisting of a high affinity to CCR3 receptors present in basophils and eosinophils (Tsujimura et al., 2008). However, this was not the case in this study, as basophil levels inversely correlated with Eotaxin levels in *P. berghei* mono-infected group, except in the *T. zimbabwensis* mon-infected and co-infected groups, as levels were significantly elevated on day 7pi in both Eotaxin and basophil levels. This therefore suggests that there is a positive correlation between Eotaxin and basophil levels *Trichinella* infection. Eotaxin (CCL11) is involved vicissitudes occurring in T regulatory cells and B cell subsets (Vaqué et al., 2017). In his study, (CCL11) could be involved in the changes occurring in the B cell. Plasma Eotaxin concentration was lower in *P. berghei* mono-infected group compared with *T. zimbabwensis* mon-infected and co-infected groups on day 7pi, with a significant increase in the mono-infected group.

The low levels found in *P. berghei* mono-infected group on day 7pi, were unexpected considering the high parastaemia observed on this day, however according to Michizuri et al. (1998) Eotaxin and RANTES are produced by Th2 cells, not Th1 cells during *T. zimbabwensis* infection. This is not in agreement with findings by Onkoba et al. (2016), who stated that non-encapsulated *T. zimbabwensis* parasite plays a role in immunomodulating host Th1/Th17 type responses during chronic infection. In contrast, it suggests that the extent of Th1 responses induced by *Plasmodium* species is higher in mono-infected group, hence the significant negative correlation of Eotaxin with *P. berghei* parastaemia. There was no significant difference between Eotaxin levels in the co-infected and control groups on day 7 and

14pi. However, higher levels Eotaxin were observed on day 0 in the co-infected group, as a response to *T. zimbabwensis* parasite, as *Plasmodium* was only induced on this day. HIV and malaria co-infection studies reported that Eotaxin prevented hematopoietic cell differentiation by obstructing signaling, by suppressing cytokine expression (Ong'echa et al., 2011). Furthermore, in the same study, HIV-1-exposed and HIV-1-positive patients presented with elevated Eotaxin and anemia. Levels of Hb were inversely proportional to Eotaxin levels in all experimental groups, throughout the current study and this agreed with Ong'echa et al. (2011), whereby during malaria infection it was noted that IFN-y was a significant positive predictor of Hb, and Eotaxin had a significant inverse predictor of Hb (Ong'echa et al., 2011).

Th1 responses in mice infected with *Helicobacter. felis* showed elevated MIP-1β, RANTES, IP-10 levels in their stomachs, which were exponentially decreased by concurrent helminth infection (Fox et al., 2000). However, Th2 immunity resulted in preeminent expression of Eotaxin. There is paucity of information of concurrent *Trichinella* and *Plasmodium* co-infections, however co-infection studies have been previously reported between other helminths. As stated by Fox et al. (2000), *Helicobacter* infection in both humans and C57BL/6 mice is associated with a Th1-like immune responses, which correlates with *P. berghei* expression in the current study.

In contrast, BALB/c mice respond to the gastric *Helicobacter* infection with a Th2-like response and develop minimal gastritis despite the presence of dense colonization with *H. felis*. However, in *Schistosoma* and *Plasmodium* co-infection Th2 response to *Schistosome* eggs is likely extend to sporozoite- and liver stages of plasmodia. In analogy, it has been demonstrated that pre-treatment to tip the cytokine balance towards a Th1 response significantly reduces the malaria parasite load in the liver (Waknine-Grinberg et al., 2010). According to Ademola and Odeniran. (2016) mice co-infected with *P. berghei* and *Trypanosoma brucei* resulted in increased malaria and trypanosomiasis severity.

Conclusion:

From the current study, it was found that *T. zimbabwensis* predisposes male Sprague-Dawley rats to increased *P. berghei* percentage parastaemia, as observed in the co-infection, which may aggravate the course of disease in the host. However, mono-infected groups with *P. berghei* resulted in slower *P. berghei* development and decreased percentage parasitaemia. However, co-infection, resulted in *T. zimbabwensis* parasite load similar to that of the mono-infected group. *Plasmodium berghei* and *T. zimbabwensis* co-infection resulted in significantly elevated basophils and neutrophils levels during acute stages of infection, as a result of high phagocytosis activity to induce parasite clearance. Which coincided with upregulated Eotaxin in all groups as a Th2 immune response and a downregulation pro-inflammatory chemokine 1P-10. However, peak *P. berghei* percentage parastaemia resulted in an upregulation on IP-10 in the *P. berghei* mono-infected group as a Th1 immune response. An inverse correlation was observed between pro-inflammatory (IP-10) and anti-inflammatory (EOTAXIN) chemokines during co- and mono-infection with *T. zimbabwensis* and *P. berghei*.

References:

Ademola, I.O. and Odeniran, P.O., 2016. Co-infection with *Plasmodium berghei* and *Trypanosoma brucei* increases severity of malaria and trypanosomiasis in mice. *Acta tropica*, *159*, pp.29-35.

Artavanis-Tsakonas, K. and Riley, E.M., 2002. Innate immune response to malaria: rapid induction of IFN-γ from human NK cells by live Plasmodium falciparum-infected erythrocytes. *The Journal of Immunology*, *169*(6), pp.2956-2963.

Ateba-Ngoa, U., Adegnika, A.A., Zinsou, J.F., Kassa, R.F.K., Smits, H., Massinga-Loembe, M., Mordmüller, B., Kremsner, P.G. and Yazdanbakhsh, M., 2015. Cytokine and chemokine profile of the innate and adaptive immune response of schistosoma haematobium and plasmodium falciparum single and co-infected school-aged children from an endemic area of Lambaréné, Gabon. *Malaria journal*, *14*(1), p.94.

Austrup, F., Vestweber, D., Borges, E. and Lohning, M., 1997. P-and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. *Nature*, *385*(6611), p.81.

Autino, B., Noris, A., Russo, R. and Castelli, F., 2012. Epidemiology of malaria in endemic areas. *Mediterranean Journal of Hematology and Infectious Diseases*, *4*(1), p.2012060.

Autieri, M.V., 2012. Pro-and anti-inflammatory cytokine networks in atherosclerosis. *ISRN Vascular Medicine*, *2012*.Banchereau, J. and Steinman, R.M., 1998. Dendritic cells and the control of immunity. *Nature*, *392*(6673), pp.245-252.

Bartoloni, A., Strohmeyer, M., Sabatinelli, G., Benucci, M., Serni, U. and Paradisi, F., 1998. False positive ParaSight™-F test for malaria in patients with rheumatoid factor. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *92*(1), pp.33-34.

Barsoum, R.S., 2000. Malarial acute renal failure. *Journal of the American Society of Nephrology*, *11*(11), pp.2147-2154.

Beaudoin, R.L., Strome, C.P., Mitchell, F. and Tubergen, T.A., 1977. Plasmodium berghei: immunization of mice against the ANKA strain using the unaltered sporozoite as an antigen. *Experimental parasitology*, *42*(1), pp.1-5.

Bennett, B.L., 2012. *The Rodent Malaria Ortholog for Falcipain-1 is Required for Normocyte Invasion by Plasmodium berghei Merozoites* (Doctoral dissertation, New York University).

Benesi, I.R.M., Moyo, C.C., Mahungu, N.M., Mkumbira, J.M., Soko, M.M., Sauti, R.F.N. and Sandifolo, V.S., 1998, August. Progress in developing improved cassava varieties in Malawi. In *1999) Food Security and Crop Diversification in SADC Countries: The Role of Cassava and Sweet Potato. Proceedings of the scientific workshop of the Southern Africa Root Crops Research Network (SARRNET), Lusaka, Zambia* (pp. 17-19).

Bolpe, J. and Boffi, R., 2001. Human trichinellosis in Argentina. Review of the casuistry registered from 1990 to 1999. *Parasite*, *8*, pp. S78-S80.

Bonilla, F.A. and Oettgen, H.C., 2010. Adaptive immunity. *Journal of Allergy and Clinical Immunology*, *125*(2), pp. S33-S40.

Boström, P., Wu, J., Jedrychowski, M.P., Korde, A., Ye, L., Lo, J.C., Rasbach, K.A., Boström, E.A., Choi, J.H., Long, J.Z. and Kajimura, S., 2012. A PGC1-[agr]-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*, *481*(7382), pp.463-468.

Bronzan, R.N., McMorrow, M.L. and Kachur, S.P., 2008. Diagnosis of malaria: challenges for clinicians in endemic and non-endemic regions. *Molecular diagnosis & therapy*, *12*(5), p.299.

Brutus, L., Watier, L., Hanitrasoamampionona, V., Razanatsoarilala, H. and Cot, M., 2007. Confirmation of the protective effect of Ascaris lumbricoides on Plasmodium falciparum infection: results of a randomized trial in Madagascar. *The American journal of tropical medicine and hygiene*, *77*(6), pp.1091-1095.

Campbell, W.C., 1983. Epidemiology I Modes of Transmission. In *Trichinella and trichinosis* (pp. 425-444). Springer US.

Carroll, G., Bell, M., Wang, H., Chapman, H. and Mills, J., 1998. Antagonism of the IL-6 cytokine subfamily—a potential strategy for more effective therapy in rheumatoid arthritis. *Inflammation Research*, *47*(1), pp.1-7.

Che, J.N., Nmorsi, O.P.G., Nkot, B.P., Isaac, C. and Okonkwo, B.C., 2015. Chemokines responses to *Plasmodium falciparum* malaria and co-infections among rural Cameroonians. *Parasitology international*, *64*(2), pp.139-144.

Clark, I.A. and Schofield, L., 2000. Pathogenesis of malaria. *Parasitology Today*, *16*(10), pp.451-454.

Conti, P., Feliciani, C., Barbacane, R.C., Frydas, S., Placido, F.C., Cataldo, I. and Reale, M., 1999. Monocyte chemotactic protein-1 gene expression and translation in formed granulomatous calcified tissue in vivo. *Calcified tissue international*, *64*(1), pp.57-62.

Cohen, S.B., Maurer, K.J., Egan, C.E., Oghumu, S., Satoskar, A.R. and Denkers, E.Y., 2013. CXCR3-dependent CD4+ T cells are required to activate inflammatory monocytes for defense against intestinal infection. *PLoS pathogens*, *9*(10), p. e1003706.

Craig, M.H., Snow, R.W. and le Sueur, D., 1999. A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitology today*, *15*(3), pp.105-111.

DeBach, P. and Rosen, D., 1991. *Biological control by natural enemies*. CUP Archive.

Djordjevic, M., Bacic, M., Petricevic, M., Cuperlovic, K., Malakauskas, A., Kapel, C.M.O. and Murrell, K.D., 2003. Social, political, and economic factors responsible for the reemergence of trichinellosis in Serbia: a case study. *Journal of Parasitology*, *89*(2), pp.226-231.

Doligalska, M., 2000. Immune response to *Trichinella spiralis* larvae after treatment with the anti-allergic compound ketotifen. *Parasitology research*, *86*(3), pp.232-238.

Donnelly, S.C., Haslett, C.H.R.I.S.T.O.P.H.E.R., Strieter, R.M., Kunkel, S.L., Walz, A., Robertson, C.R., Carter, D.C., Pollok, A.J. and Grant, I.S., 1993. Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups. *The Lancet*, *341*(8846), pp.643-647.

Dupouy-Camet, J. and Murrell, K.D. eds., 2007. *FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis*. Food & Agriculture Org.

Dupouy-Camet, J., 2009. Presidential address of ICT12 Conference: "*Trichinella* and trichinellosis-A never ending story." Vet. Parasitol. 159, 194–196.

Duprey, Z.H. and Schantz, P.M., 2003. Toxocariasis and baylisascariasis. *North American Parasitic Zoonoses. Kluwer Academic Publishers, Boston, Massachusetts*, pp.23-40.

Ermert, V., Fink, A.H., Morse, A.P. and Paeth, H., 2012. The impact of regional climate change on malaria risk due to greenhouse forcing and land-use changes in tropical Africa. *Environmental Health Perspectives*, *120*(1), p.77.

Favre, B., Turowski, P. and Hemmings, B.A., 1997. Differential inhibition and posttranslational modification of protein phosphatase 1 and 2A in MCF7 cells treated with calyculin-A, okadaic acid, and tautomycin. *Journal of Biological Chemistry*, *272*(21), pp.13856-13863.

Fabre, M.V., Beiting, D.P., Bliss, S.K. and Appleton, J.A., 2009. Immunity to *Trichinella spiralis* muscle infection. *Veterinary parasitology*, *159*(3), pp.245-248.

Frigerio, S., Junt, T., Lu, B., Gerard, C., Zumsteg, U., Holländer, G.A. and Piali, L., 2002. β cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. *Nature medicine*, *8*(12), pp.1414-1420.

Frydas, S., Reale, M., Vacalis, D., Barbacane, R.C., Placido, F.C., Cataldo, I., Di Gioacchino, M., Karagouni, E., Dotsika, E., Anogiannakis, G. and Trakatellis, A., 1999. IgG, IgG1 and IgM response in Trichinella spiralis-infected mice treated with 4-deoxypirydoxine or fed a Vitamin B6-deficient diet. *Molecular and cellular biochemistry*, *194*(1-2), pp.47-52.

Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E. and Lipp, M., 1999. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell*, *99*(1), pp.23-33.

Fox, J.G., Beck, P., Dangler, C.A., Whary, M.T., Wang, T.C., Shi, H.N. and Nagler-Anderson, C., 2000. Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. *Nature medicine*, *6*(5), p.536.

Gallucci, S. and Matzinger, P., 2001. Danger signals: SOS to the immune system. *Current opinion in immunology*, *13*(1), pp.114-119.

Gamble, H.R., Pozio, E., Bruschi, F., Nöckler, K., Kapel, C.M. and Gajadhar, A.A., 2004. International Commission on Trichinellosis: recommendations on the use of serological tests for the detection of Trichinella infection in animals and man. *Parasite (Paris, France)*, *11*(1), pp.3-13.

Giacomin, P.R., Siracusa, M.C., Walsh, K.P., Grencis, R.K., Kubo, M., Comeau, M.R. and Artis, D., 2012. Thymic stromal lymphopoietin-dependent basophils promote Th2 cytokine responses following intestinal helminth infection. *The Journal of Immunology*, *189*(9), pp.4371-4378.

Gottstein, B., Pozio, E. and Nöckler, K., 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical microbiology reviews*, *22*(1), pp.127-145.

Goldberg, S.M., Johnson, J., Busam, D., Feldblyum, T., Ferriera, S., Friedman, R., Halpern, A., Khouri, H., Kravitz, S.A., Lauro, F.M. and Li, K., 2006. A Sanger/pyrosequencing hybrid approach for the generation of high-quality draft assemblies of marine microbial genomes. *Proceedings of the National Academy of Sciences*, *103*(30), pp.11240-11245.

Goldring, J.P.D. and Ramoshebi, L.N., 1999. Glucocorticoids, antioxidants and staurosporine modulate the adherence between monocytes and malaria infected erythrocytes. *Inflammation Research*, *48*(12), pp.657-661.

Griffith, J.W., Sokol, C.L. and Luster, A.D., 2014. Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annual review of immunology*, *32*, pp.659-702.

Groux, H. and Gysin, J., 1990. Opsonization as an effector mechanism in human protection against asexual blood stages of *Plasmodium falciparum*: functional role of IgG subclasses. *Research in immunology*, *141*(5), pp.529-542.

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., Cruchaga, C., Sassi, C., Kauwe, J.S., Younkin, S. and Hazrati, L., 2013. TREM2 variants in Alzheimer's disease. *New England Journal of Medicine*, *368*(2), pp.117-127.

Hahn, C.N., Chong, C.E., Carmichael, C.L., Wilkins, E.J., Brautigan, P.J., Li, X.C., Babic, M., Lin, M., Carmagnac, A., Lee, Y.K. and Kok, C.H., 2011. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nature genetics*, *43*(10), pp.1012-1017.

Han, E.T., Watanabe, R., Sattabongkot, J., Khuntirat, B., Sirichaisinthop, J., Iriko, H., Jin, L., Takeo, S. and Tsuboi, T., 2007. Detection of four *Plasmodium* species by genus-and species-specific loop-mediated isothermal amplification for clinical diagnosis. *Journal of clinical microbiology*, *45*(8), pp.2521-2528.

Hay, S.I., Omumbo, J.A., Craig, M.H. and Snow, R.W., 2000. Earth observation, geographic information systems and *Plasmodium falciparum* malaria in sub-Saharan Africa. *Advances in Parasitology*, *47*, pp.173-215.

Helleday, T., Petermann, E., Lundin, C., Hodgson, B. and Sharma, R.A., 2008. DNA repair pathways as targets for cancer therapy. *Nature reviews. Cancer*, *8*(3), p.193.

Holt, R.A., Subramanian, G.M., Halpern, A., Sutton, G.G., Charlab, R., Nusskern, D.R., Wincker, P., Clark, A.G., Ribeiro, J.C., Wides, R. and Salzberg, S.L., 2002. The genome sequence of the malaria mosquito Anopheles gambiae. *science*, *298*(5591), pp.129-149.

Hunt, N.H., Golenser, J., Chan-Ling, T., Parekh, S., Rae, C., Potter, S., Medana, I.M., Miu, J. and Ball, H.J., 2006. Immunopathogenesis of cerebral malaria. *International journal for parasitology*, *36*(5), pp.569-582.

Jain, R., Nandakumar, K., Srivastava, V., Kumar, V.S., Patet, S. and Kumar, P.M., 2008. Hepatoprotective activity of ethanolic and aqueous extract of Terminalia Bellerica in rats. *Pharmacologyonline*, *2*, pp.411-427.

Janse, C.J. and Waters, A.P., 1995. Plasmodium berghei: the application of cultivation and purification techniques to molecular studies of malaria parasites. *Parasitology today*, *11*(4), pp.138-143.

John, A.L.S. and Abraham, S.N., 2013. Innate immunity and its regulation by mast cells. *The Journal of Immunology*, *190*(9), pp.4458-4463.

Kapel, C.M.O. and Gamble, H.R., 2000. Infectivity, persistence, and antibody response to domestic and sylvatic Trichinella spp. in experimentally infected pigs. *International Journal for Parasitology*, *30*(2), pp.215-221.

Kawai, T. and Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nature immunology*, *11*(5), pp.373-384.

Khan, I.A., MacLean, J.A., Lee, F.S., Casciotti, L., DeHaan, E., Schwartzman, J.D. and Luster, A.D., 2000. IP-10 is critical for effector T cell trafficking and host survival in Toxoplasma gondii infection. *Immunity*, *12*(5), pp.483-494.

Khan, A., Khan, S. and Brown, W.A., 2002. Are placebo controls necessary to test new antidepressants and anxiolytics? *International Journal of Neuropsychopharmacology*, *5*(3), pp.193-197.

Kociecka, W., 2000. Trichinellosis: human disease, diagnosis and treatment. *Veterinary parasitology*, *93*(3), pp.365-383.

La Grange, L.J., Marucci, G. and Pozio, E., 2009. *Trichinella zimbabwensis* in wild Nile crocodiles (Crocodylus niloticus) of South Africa. *Veterinary parasitology*, *161*(1), pp.88-91.

Lazzeri, E., Rotondi, M., Mazzinghi, B., Lasagni, L., Buonamano, A., Rosati, A., Pradella, F., Fossombroni, V., La Villa, G., Gacci, M. and Bertoni, E., 2005. High CXCL10 expression in rejected kidneys and predictive role of pretransplant serum CXCL10 for acute rejection and chronic allograft nephropathy. *Transplantation*, *79*(9), pp.1215-1220.

Lazzeri, E. and Romagnani, P., 2005. CXCR3-binding chemokines: novel multifunctional therapeutic targets. *Current Drug Targets-Immune, Endocrine & Metabolic Disorders*, *5*(1), pp.109-118.

Leckie, M.J., ten Brinke, A., Khan, J., Diamant, Z., O'connor, B.J., Walls, C.M., Mathur, A.K., Cowley, H.C., Chung, K.F., Djukanovic, R. and Hansel, T.T., 2000. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *The Lancet*, *356*(9248), pp.2144-2148.

Liew, F.Y., Xu, D., Brint, E.K. and O'Neill, L.A., 2005. Negative regulation of toll-like receptor-mediated immune responses. *Nature reviews. Immunology*, *5*(6), p.446.

Liu, M., Guo, S., Hibbert, J.M., Jain, V., Singh, N., Wilson, N.O. and Stiles, J.K., 2011. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. *Cytokine & growth factor reviews*, *22*(3), pp.121-130.

Lillard, J.W., Boyaka, P.N., Taub, D.D. and McGhee, J.R., 2001. RANTES potentiates antigen-specific mucosal immune responses. *The Journal of Immunology*, *166*(1), pp.162-169.

Löffler, F.E., Sun, Q., Li, J. and Tiedje, J.M., 2000. 16S rRNA Gene-Based Detection of Tetrachloroethene-Dechlorinating Desulfuromonas and Dehalococcoides Species. *Applied and Environmental Microbiology*, *66*(4), pp.1369-1374.

Luster, A.D. and Ravetch, J.V., 1987. Biochemical characterization of a gamma interferon-inducible cytokine (IP-10). *Journal of Experimental Medicine*, *166*(4), pp.1084-1097.

Luster, A.D., 2002. The role of chemokines in linking innate and adaptive immunity. *Current opinion in immunology*, *14*(1), pp.129-135.

Mackintosh, C.L., Beeson, J.G. and Marsh, K., 2004. Clinical features and pathogenesis of severe malaria. *Trends in parasitology*, *20*(12), pp.597-603.

Mahalingam, S. and Karupiah, G., 1999. Chemokines and chemokine receptors in infectious diseases. *Immunology and cell biology*, *77*(6), pp.469-475.

Maizels, R.M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M.D. and Allen, J.E., 2004. Helminth parasites–masters of regulation. *Immunological reviews*, *201*(1), pp.89-116.

Maizels, R.M. and Yazdanbakhsh, M., 2003. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature reviews. Immunology*, *3*(9), p.733.

Malaguarnera, L. and Musumeci, S., 2002. The immune response to *Plasmodium falciparum* malaria. *The Lancet infectious diseases*, *2*(8), pp.472-478.

Marinculić, A., Gašpar, A., Duraković, E., Pozio, E. and La Rosa, G., 2001. Epidemiology of swine trichinellosis in the Republic of Croatia. *Parasite*, *8*, pp. S92-S94.

Martinsen, E.S., Perkins, S.L. and Schall, J.J., 2008. A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): evolution of life-history traits and host switches. *Molecular phylogenetics and evolution*, *47*(1), pp.261-273.

McQueen, P.G. and McKenzie, F.E., 2004. Age-structured red blood cell susceptibility and the dynamics of malaria infections. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(24), pp.9161-9166.

Medoff B.D., Sauty, A., Tager, A.M., Maclean, J.A., Smith, R.N., Mathew, A., Dufour, J.H. and Luster, A.D., 2002. IFN-γ-inducible protein 10 (CXCL10) contributes to airway hyperreactivity and airway inflammation in a mouse model of asthma. *The Journal of Immunology*, *168*(10), pp.5278-5286.

Meylan, E., Tschopp, J. and Karin, M., 2006. Intracellular pattern recognition receptors in the host response. *Nature*, *442*(7098), pp.39-44.

Mitola, S., Brenchio, B., Piccinini, M., Tertoolen, L., Zammataro, L., Breier, G., Rinaudo, M.T., den Hertog, J., Arese, M. and Bussolino, F., 2006. Type I Collagen Limits VEGFR-2 Signaling by a SHP2 Protein-Tyrosine Phosphatase–Dependent Mechanism 1. *Circulation research*, *98*(1), pp.45-54.

Mitreva, M., Blaxter, M.L., Bird, D.M. and McCarter, J.P., 2005. Comparative genomics of nematodes. *TRENDS in Genetics*, *21*(10), pp.573-581.

Mitreva, M. and Jasmer, D.P., 2006. Biology and genome of *Trichinella spiralis*. Comparative genomics. Nature genetics 43(12),pp 228-235.

Miller, S.A., Dykes, D.D. and Polesky, H.F.R.N., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic acids research*, *16*(3), p.1215.

Moser, B., Wolf, M., Walz, A. and Loetscher, P., 2004. Chemokines: multiple levels of leukocyte migration control☆. *Trends in immunology*, *25*(2), pp.75-84.

Mukaratirwa, S., Hove, T., Esmann, J.B. and Hoj, C.J., 2001. A survey of parasitic nematode infections of chickens in rural Zimbabwe. *The Onderstepoort journal of veterinary research*, *68*(3), p.183.

Mukaratirwa, S., Dzoma, B.M., Matenga, E., Ruziwa, S.D., Sacchi, L. and Pozio, E., 2008. Experimental infections of baboons (Papio spp.) and vervet monkeys (Cercopithecus aethiops) with *Trichinella zimbabwensis* and successful treatment with ivermectin. *Onderstepoort Journal of Veterinary Research*, *75*(2), pp.173-180.

Mukaratirwa, S., La Grange, L. and Pfukenyi, D.M., 2013. *Trichinella* infections in animals and humans in sub-Saharan Africa: a review. *Acta tropica*, *125*(1), pp.82-89.

Nagarkar, D.R., 2010. *Rhinovirus Induced Airway Inflammation in Naïve and Allergic Mouse Models* (Doctoral dissertation, University of Michigan). 185(4),pp.525-535.

Naotunne, T.D.S., Karunaweera, N.D., Del Giudice, G., Kularatne, M.U., Grau, G.E., Carter, R. and Mendis, K.N., 1991. Cytokines kill malaria parasites during infection crisis: extracellular complementary factors are essential. *J Exp Med*, *173*(3), pp.523-529.

Nie, J. and Sage, E.H., 2009. SPARC functions as an inhibitor of adipogenesis. *Journal of cell communication and signaling*, *3*(3-4), p.247.

Noti, M., Kim, B.S., Siracusa, M.C., Rak, G.D., Kubo, M., Moghaddam, A.E., Sattentau, Q.A., Comeau, M.R., Spergel, J.M. and Artis, D., 2014. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin–basophil axis. *Journal of allergy and clinical immunology*, *133*(5), pp.1390-1399.

Ochiel, D.O., Awandare, G.A., Keller, C.C., Hittner, J.B., Kremsner, P.G., Weinberg, J.B. and Perkins, D.J., 2005. Differential regulation of β-chemokines in children with Plasmodium falciparum malaria. *Infection and immunity*, *73*(7), pp.4190-4197.

Ockenhouse, C.F. and Chulay, J.D., 1988. Plasmodium falciparum sequestration: OKM5 antigen (CD36) mediates cytoadherence of parasitized erythrocytes to a myelomonocytic cell line. *The Journal of infectious diseases*, *157*(3), pp.584-588.

Onkoba, N.W., Chimbari, M.J. and Mukaratirwa, S., 2015. Malaria endemicity and co-infection with tissue-dwelling parasites in Sub-Saharan Africa: a review. *Infectious diseases of poverty*, *4*(1), p.35.

Ozwara, H., Langermans, J.A., Maamun, J., Farah, I.O., Yole, D.S., Mwenda, J.M., Weiler, H. and Thomas, A.W., 2003. Experimental infection of the olive baboon (Papio anubis) with Plasmodium knowlesi: severe disease accompanied by cerebral involvement. *The American journal of tropical medicine and hygiene*, *69*(2), pp.188-194.

Pang, T. and Blanden, R.V., 1977. Genetic factors in the stimulation of T cell responses against Ectromelia virus-infected cells; role of H-2K, H-2D and H-2I genes. *Australian Journal of Experimental Biology & Medical Science*, *55*(5).

Pathan, A.A., Wilkinson, K.A., Klenerman, P., McShane, H., Davidson, R.N., Pasvol, G., Hill, A.V. and Lalvani, A., 2001. Direct ex vivo analysis of antigen-specific IFN-γ-secreting CD4 T cells in

Mycobacterium tuberculosis-infected individuals: associations with clinical disease state and effect of treatment. *The Journal of Immunology*, *167*(9), pp.5217-5225.

Pelleau, S., Diop, S., Badiane, M.D., Vitte, J., Beguin, P., Nato, F., Diop, B.M., Bongrand, P., Parzy, D. and Jambou, R., 2012. Enhanced basophil reactivities during severe malaria and their relationship with the Plasmodium falciparum histamine-releasing factor translationally controlled tumor protein. *Infection and immunity*, *80*(8), pp.2963-2970.

Perkins, D.J., Were, T., Davenport, G.C., Kempaiah, P., Hittner, J.B. and Ong'echa, J.M., 2011. Severe malarial anemia: innate immunity and pathogenesis. *International journal of biological sciences*, *7*(9), p.1427.

Pozio, E., 2001. New patterns of Trichinella infection. *Veterinary parasitology*, *98*(1), pp.133-148.

Pozio, E., Foggin, C.M., Gelanew, T., Marucci, G., Hailu, A., Rossi, P. and Morales, M.A.G., 2007. Trichinella zimbabwensis in wild reptiles of Zimbabwe and Mozambique and farmed reptiles of Ethiopia. *Veterinary parasitology*, *143*(3), pp.305-310.

Pozio, E. and Murrell, K.D., 2006. Systematics and epidemiology of Trichinella. *Advances in parasitology*, *63*, pp.367-439.

Pozio, E., 2007. World distribution of Trichinella spp. infections in animals and humans. *Veterinary parasitology*, *149*(1), pp.3-21.

Reale, M., Frydas, S., Barbacane, R.C., Placido, F.C., Cataldo, I., Conti, P., Vacalis, D., Trakatellis, A., Anogianakis, G., Felaco, M. and Di Giocchino, M., 1998. Induction of monocyte chemotactic protein-1 (MCP-1) and TNF alpha by Trichinella spiralis in serum of mice in vivo. *Molecular and cellular biochemistry*, *179*(1), pp.1-5.

Roper, C., Pearce, R., Bredenkamp, B., Gumede, J., Drakeley, C., Mosha, F., Chandramohan, D. and Sharp, B., 2003. Antifolate antimalarial resistance in southeast Africa: a population-based analysis. *The Lancet*, *361*(9364), pp.1174-1181.

Salazar-Mather, T.P., Orange, J.S. and Biron, C.A., 1998. Early murine cytomegalovirus (MCMV) infection induces liver natural killer (NK) cell inflammation and protection through macrophage inflammatory protein 1α (MIP-1α)–dependent pathways. *Journal of Experimental Medicine*, *187*(1), pp.1-14.

Sallusto, F., Mackay, C.R. and Lanzavecchia, A., 2000. The role of chemokine receptors in primary, effector, and memory immune responses. *Annual review of immunology*, *18*(1), pp.593-620.

Scuderi, P., Lam, K., Ryan, K., Petersen, E., Sterling, K., Finley, P., Ray, C.G., Slymen, D. and Salmon, S., 1986. Raised serum levels of tumour necrosis factor in parasitic infections. *The Lancet*, *328*(8520), pp.1364-1365.

Shillcutt, S., Morel, C., Goodman, C., Coleman, P., Bell, D., Whitty, C.J. and Mills, A., 2008. Cost-effectiveness of malaria diagnostic methods in sub-Saharan Africa in an era of combination therapy. *Bulletin of the World Health Organization*, *86*(2), pp.101-110.

Singh, S.K., Hawkins, C., Clarke, I.D., Squire, J.A., Bayani, J., Hide, T., Henkelman, R.M., Cusimano, M.D. and Dirks, P.B., 2004. Identification of human brain tumour initiating cells. *nature*, *432*(7015), pp.396-401.

Suguitan Jr, A.L., Leke, R.G., Fouda, G., Zhou, A., Thuita, L., Metenou, S., Fogako, J., Megnekou, R. and Taylor, D.W., 2003. Changes in the levels of chemokines and cytokines in the placentas of women with Plasmodium falciparum malaria. *The Journal of infectious diseases*, *188*(7), pp.1074-1082.

Targett, G.A.T., 1992. Malaria: drug use and the immune response. *Parasitology*, *105*(S1), pp. S61-S70.

Teklehaimanot, A., Singer, B., Spielman, A., Tozan, Y. and Schapira, A., 2005. UN Millennium Project. *Coming to grips with malaria in the new millennium. Task Force on HIV/AIDS, malaria, TB, and access to essential medicines. Working Group on Malaria*, p.10.

Tsujimura, Y., Obata, K., Mukai, K., Shindou, H., Yoshida, M., Nishikado, H., Kawano, Y., Minegishi, Y., Shimizu, T. and Karasuyama, H., 2008. Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity*, *28*(4), pp.581-589.

Tuteja, R., 2007. Malaria− an overview. *FEBS Journal*, *274*(18), pp.4670-4679.

Turvey, S.E. and Broide, D.H., 2010. Innate immunity. *Journal of Allergy and Clinical Immunology*, *125*(2), pp. S24-S32.

Vaqué, J.P., Gómez-López, G., Monsálvez, V., Varela, I., Martínez, N., Pérez, C., Domínguez, O., Graña, O., Rodríguez-Peralto, J.L., Rodríguez-Pinilla, S.M. and González-Vela, C., 2014. PLCG1 mutations in cutaneous T-cell lymphomas. *Blood*, *123*(13), pp.2034-2043.

Von Andrian, U.H. and Mackay, C.R., 2000. T-cell function and migration—two sides of the same coin. *New England Journal of Medicine*, *343*(14), pp.1020-1034.

Wakelin, D. and Goyal, P.K., 1996. Trichinella isolates: parasite variability and host responses. *International Journal for Parasitology*, *26*(5), pp.471-481.

Walther, M., Tongren, J.E., Andrews, L., Korbel, D., King, E., Fletcher, H., Andersen, R.F., Bejon, P., Thompson, F., Dunachie, S.J. and Edele, F., 2005. Upregulation of TGF-β, FOXP3, and CD4+ CD25+ regulatory T cells correlates with more rapid parasite growth in human malaria infection. *Immunity*, *23*(3), pp.287-296.

Waknine-Grinberg, J.H., Gold, D., Ohayon, A., Flescher, E., Heyfets, A., Doenhoff, M.J., Schramm, G., Haas, H. and Golenser, J., 2010. Schistosoma mansoni infection reduces the incidence of murine cerebral malaria. *Malaria journal*, *9*(1), p.5.

Warrington, R., Watson, W., Kim, H.L. and Antonetti, F.R., 2011. An introduction to immunology and immunopathology. *Allergy, Asthma & Clinical Immunology*, *7*(1), p. S1.

Warrell, D.A. and Gilles, H.M., 2002. *Essential malariology* (No. Ed. 4). Arnold.

Were, T., Hittner, J.B., Ouma, C., Otieno, R.O., Orago, A.S., Ong'echa, J.M., Vulule, J.M., Keller, C.C. and Perkins, D.J., 2006. Suppression of RANTES in children with Plasmodium falciparum malaria. *Haematological*, *91*(10), pp.1396-1399.

White, N.J., Nosten, F., Looareesuwan, S., Watkins, W.M., Marsh, K., Snow, R.W., Kokwaro, G., Ouma, J., Hien, T.T., Molyneux, M.E. and Taylor, T.E., 1999. Averting a malaria disaster. *The Lancet*, *353*(9168), pp.1965-1967.

Wilson, N.O., Hall, R.L., Montgomery, S.P. and Jones, J.L., 2015. Trichinellosis surveillance—United States, 2008–2012. *MMWR Surveill Summ*, *64*(Suppl 1), pp.1-8.

World Health Organization, 2006. Malaria vector control and personal protection: report of a WHO study group.

World Health Organization, 2008. Global malaria control and elimination: report of a technical review. Geneva.

Xie, W., Fang, L., Gan, S. and Xuan, H., 2016. Interleukin-19 alleviates brain injury by anti-inflammatory effects in a mice model of focal cerebral ischemia. *Brain research*, *1650*, pp.172-177.

Yang, J., Gu, Y., Yang, Y., Wei, J., Wang, S., Cui, S., Pan, J., Li, Q. and Zhu, X., 2010. *Trichinella spiralis*: immune response and protective immunity elicited by recombinant paramyosin formulated with different adjuvants. *Experimental parasitology*, *124*(4), pp.403-408.

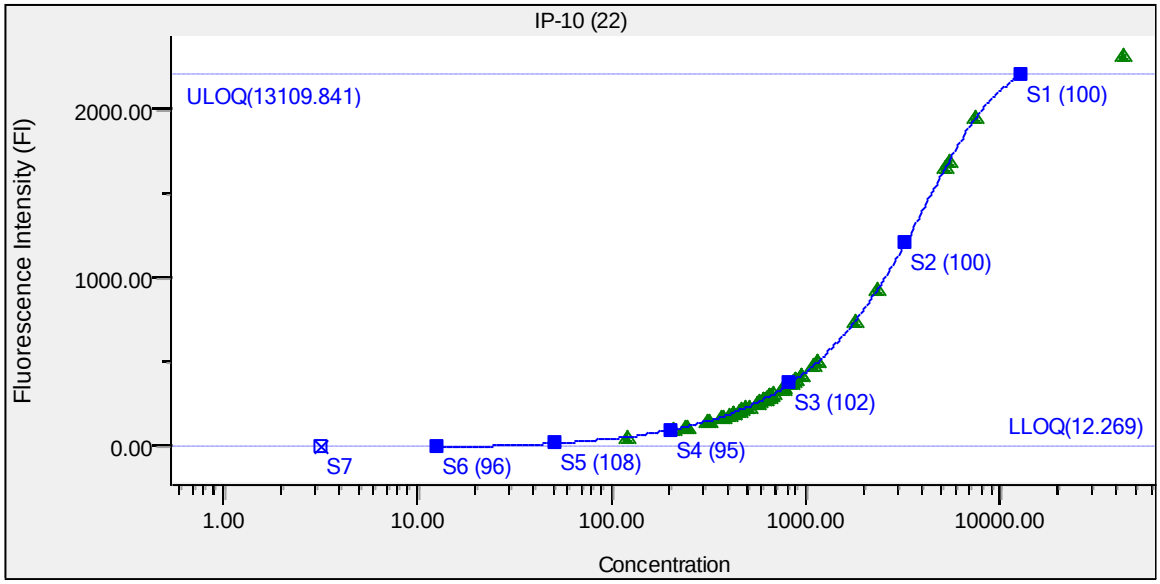
Yeung, S., Pongtavornpinyo, W., Hastings, I.M., Mills, A.J. and White, N.J., 2004. Antimalarial drug resistance, artemisinin-based combination therapy, and the contribution of modeling to elucidating policy choices. *The American journal of tropical medicine and hygiene*, *71*(2 suppl), pp.179-186.

Yépez-Mulia, L., Hernandez-Bello, R., Arizmendi-Puga, N, N, N., Fonseca-Linan, R. and Ortega-Pierres, G., 2007. Contributions to the study of Trichinella spiralis TSL-1 antigens in host immunity. *Parasite immunology*, *29*(12), pp.661-670.

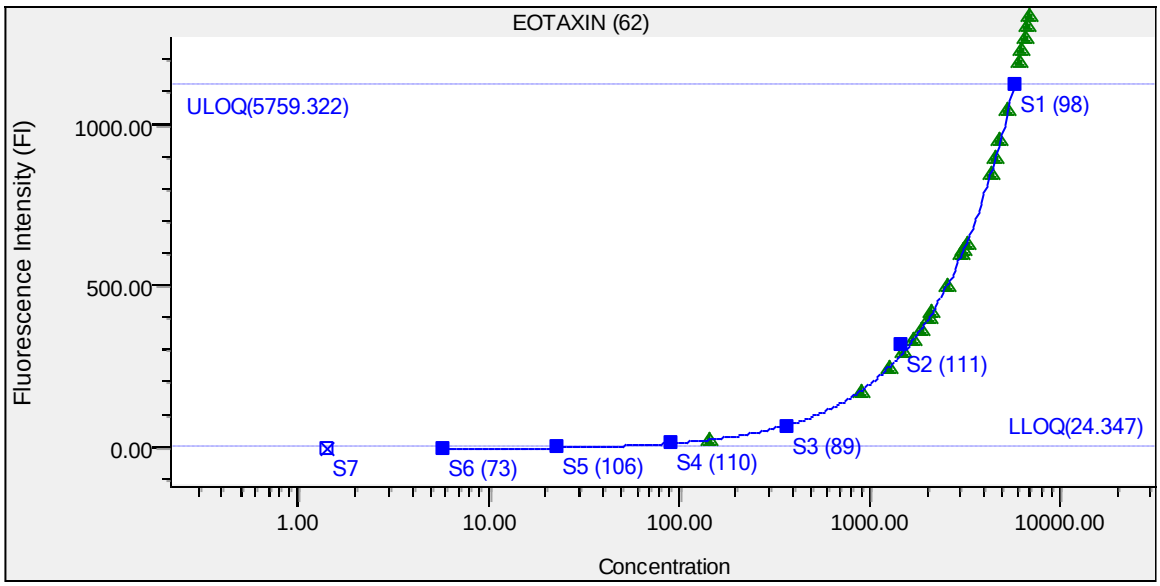
Yewhalaw, D., Hamels, S., Getachew, Y., Torgerson, P.R., Anagnostou, M., Legesse, W., Kloos, H., Duchateau, L. and Speybroeck, N., 2014. Water resource developments in Ethiopia: potential benefits and negative impacts on the environment, vector-borne diseases, and food security. *Environmental Reviews*, *22*(4), pp.364-371

Yang, J., Gu, Y., Yang, Y., Wei, J., Wang, S., Cui, S., Pan, J., Li, Q. and Zhu, X., 2010. *Trichinella spiralis:* immune response and protective immunity elicited by recombinant paramyosin formulated with different adjuvants. *Experimental parasitology*, *124*(4), pp.403-408.

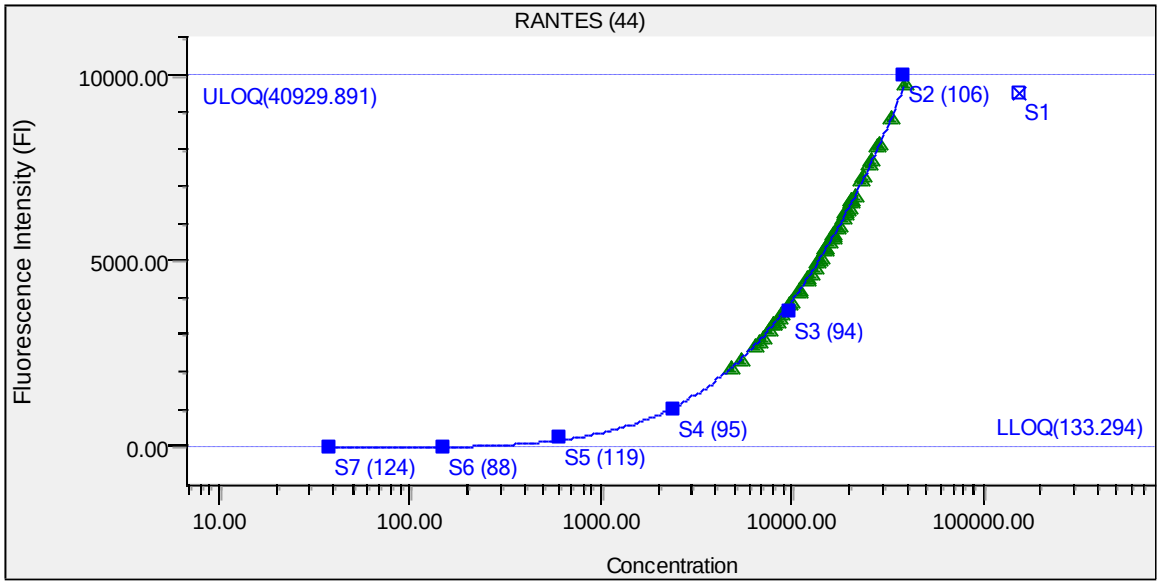
LIST OF APPENDICES



Appendix 1: IP-10 stadard curve



Appendix 2: Eoxtaxin standard curve



Appendix 3: RANTES standard curve

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
16 May 2016

Dr Pretty Murambiwa
School of Life Sciences Westville Campus

Dear Dr Murambiwa,

Protocol reference number: AREC/018/016PD
Project title: Immune responses in experimental animal paradigms co-infected with Trichinella. zimbabwensis and Plasmodium berghei

Full Approval — Research Application With regards to your revised application received on 05 May 2016. The documents submitted have been accepted by the Animal Research Ethics Committee and FULL APPROVAL for the protocol has been granted.
Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.
Please note: Research data should be securely stored in the discipline/department for a period of 5 years.
The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 16 May 2017.
I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....

Dr Sanil Singh
Deputy Chair: Animal Research Ethics Committee

/ms
Cc Supervisor: Professor S Mukaratirwa
Cc Academic Leader Teaching & Learning: Professor A Olaniran Cc Registrar: Mr Simon Mokoena cc NSPCA: Ms Jessica Light cc BRU - Dr Sanil Singh

Animal Research Ethics Committee (AREC)
Ms Marlette Snyman (Administrator)
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Website: http://research.ukzn.ac.za/Research-Ethics/AnimalEthics.aspx

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Appendix 4**:** UKZN ETHICS APPROVAL 2016

UNIVERSITY OF
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YAKWAZUW-NATALI

12 June 2017

Dr Pretty Murambiwa (16834)
School of Life Sciences Westville Campus
Dear Dr urambiwa,
Protocol reference number: AREC/018/016PD
Project title: Immune responses in experimental animal paradigms co-infected with Trichinella. zimbabwensis and Plasmodium berghei
Full Approval — Renewal Application With regards to your renewal application received on 31 May 2017. The documents submitted have been accepted by the Anima l Research Ethics Committee and APPROVAL for the renewal has been granted.
Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methcds must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.
Please note: Research data should be securely stored in the discipline/department for a period of 5 years.
The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 12 June 2018.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.
I take this opportunity of wishing you everything of the best with your study.
Yours faithfully

Animal Research Ethics Committee (AREC)
Ms Mariette Snyman (Administrator)
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Dr S Islam, PhD
Chair: Animal Research Ethics Committee
/ms
Cc Supervisor: Professor S Mukaratirwa
Cc Dean & HOS: Professor A Olaniran
Cc Registrar: Mr Simon Mokoena Cc NSPCA: Ms Stephanie Keulder cc BRU - Dr Sanil Singh

Appendix 5: UKZN ETHICS APPROVAL 2017