THE IMPACT OF *SCHISTOSOMA MANSONI* ON DISEASE SEVERITY OF SECONDARY INFECTION WITH *TRYPANOSOMA BRUCEI RHODESIENSE* IN A MOUSE MODEL

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DECLARATION

This thesis is my original work and has not been submitted to any institution for a degree award or other qualification.

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DEDICATION

I dedicate this work to the Almighty God for the strength and grace during the entire period. I Also dedicate this to my parents, Mr. and Mrs. Alphonse Mitalo for their endless prayers and encouragement. My daughter Rhema Ritah for being an understanding one while busy working on this. Finally, my siblings and friends who morally supported me.

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
BBB	Blood brain barrier
CSF	Cerebral spinal fluid
DNA	Deoxyribonucleic acid
DPI	Days post infection
GSH	Glutathione
HAT	Human African Trypanosomiasis
HCT	Hematocrit
HDL	High density Lipoprotein
ICAM-1	Intercellular adhesion molecule-1
IFN-γ	Interferon gamma
IL4–/– mice	IL-4 Knockout Mice
IL-1	Interleukin-1
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-13	Interleukin-13
MCH	Mean concentration hemoglobin
MCHC	Mean Corpuscular hemoglobin concentration
MCV	Mean corpuscular volume

MDA	Malondialdehyde
MPV	Mean Platelet Volume
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
PBMCs	Peripheral-blood mononuclear cells
PCT	Plateletcrit
PCV	Packed cell volume
PDW	Platelet Distribution Width
P-LCR	Platelet Large Cell-Ratio
RBC	Red blood cell
RDW-SD	Red cell distribution Width-Standard deviation
RMCBS	Rapid Murine Coma and Behavior Scale
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
S. mansoni	*Schistosoma mansoni*
SDG" s	Strategic development goals
SEA	Soluble egg antigen
SEM	Standard Error of Mean
SRA	Serum resistance-associated gene
T.b. rhodesiense	*Trypanosoma brucei rhodesiense*
TH1	T helper 1 cells
TH2	T helper 2 cells
TLTF	Trypanosome-lymphocyte triggering factor
TNF	Tumor-necrosis factor

TNF- α	Tumor necrotic factor-alpha
VCAM-1	Vascular cell adhesion molecule-1
VSG	Variant surface glycoprotein

ABSTRACT

Human African Trypanosomiasis (HAT) and Schistosomiasis are neglected parasitic diseases found in the African continent. There's paucity of data on the outcome during co-infection of *S. mansoni* and *Trypanosoma brucei rhodesiense*. This study was conducted to determine how primary infection with *Schistosoma mansoni* affects HAT diseases progression with a secondary infection with *Trypanosoma brucei rhodesiense* (*T.b.r)* in a mouse model. Female BALB-c mice (6-8 weeks old) were randomly divided into four groups of 12 mice each. The different groups were infected with *Schistosoma mansoni* (100 cercariae) and *Trypanosoma brucei rhodesiense* ($5.0x10^4$) separately or together. Twenty-one days' post infection with *T.b.r*, mice were sacrificed and samples collected for analysis. The primary infection with *S. mansoni* significantly enhanced successive infection by *T.b.r*; consequently, promoting HAT disease severity and curtailing host survival time. *T.b.r*-induced neurological integrity impairment and breach of the blood brain barrier were markedly pronounced on co-infection with *S. mansoni*. Co-infection with *S. mansoni* and *T.b.r* resulted in microcytic hypochromic anemia characterized by suppression of RBCs, hematocrit, hemoglobin and red cell indices. Moreover, co-infection of the mice with the two parasites resulted in leukocytosis which was accompanied with elevation of basophils, neutrophils, lymphocytes, monocytes, and eosinophils. More importantly, co-infection resulted in significant elevation of alanine amino transferase (ALT), alkaline phosphatase (ALP), aspartate amino transferase (AST), total bilirubin, creatinine, urea and uric acid, markers of liver and kidney damage. Meanwhile, *S. mansoni*-driven dyslipidemia was significantly enhanced by co-infection of mice with *T.b.r*. Moreover, co-infection with *S. mansoni* and *T.b.r* led to a strong immune response characterized by significant increase in serum IFN-γ and TNF-α. *T.b.r* infection enhanced *S. mansoni*-induced depletion of cellular reduced glutathione (GSH) in the brain and liver tissues, indicative of lethal oxidative damage. Similarly, co-infection resulted in significant rise in nitric oxide (NO) and malondialdehyde (MDA) levels. In conclusion, primary infection with *S. mansoni* exacerbates disease severity of secondary infection with *T.b.r* in a mouse model that is linked with harmful inflammatory response, oxidative stress and organ injury.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Schistosomiasis also known as bilharzia, snail fever, kayayama fever, is an acute and chronic infection caused by parasitic flat worms known as schistosomes. It is a neglected tropical disease affecting hundreds of people posing a major public health problem globally (WHO, 2022). The species of schistosomes that are known to cause schistosomiasis include *Schistosoma mansoni, Schistosoma japonicum, Schistosoma haematobium, Schistosoma intercalatum* and *Schistosoma mekongi* (U Olveda, 2013). At least 700 million people live in endemic areas, and it has been estimated that more than 240 million individuals are infected globally each year (WHO, 2022). About 279,000 succumb to the disease annually and the burden of disability rated is at 3.2 million worldwide (Ogongo *et al.,* 2022). It's mainly spread through immigration and water-based development projects (Nelwan, 2019).

Schistosomiasis presents itself in two clinical stages: chronic and acute (Katayama syndrome). The incubation time for Katayama syndrome is thought to be between 14 and 85 days and its symptoms include dry cough, fever, myalgia, headache, respiratory symptoms and rash (muculopapular skin lesions) (Jauréguiberry *et al.,* 2010). On the other hand, the chronic disease, in *S. haematobium*, can result in hematuria and dysuria, and injury to the genital tract leading to other susceptible infections. Chronic disease can also trigger cancer of the bladder and its symptoms include bloody stool, diarrhea, and constipation (Thijs *et al.,* 2018). Specifically, individuals suffering from schistosomiasis due to *S. mansoni and S. japonicum* manifest clinical symptoms linked to chronic inflammation, splenomegally, hyperplasia, fibrosis, polyposis, bowel wall ulceration, and portal hypertension (Nelwan, 2019).

The most prominent pathological event that is connected with severe organ injury among Schistosoma infected individuals is granulomatous inflammation (Figliuolo *et al.,* 2019; *Zuim et al.,* 2012). Notably, the antigens of parasite eggs which primarily are retained in the host's tissues, particularly the liver, spleen, and lungs, trigger granulomatous inflammation as a defense mechanism (Alam, 2007); Lambertucci, 2010). This is a vigorous process coordinated by the immunological response of the host (Alam, 2007). Neutrophils and monocytes are principally involved in the development of Schistosoma granulomas, accompanied by the production of IFN-γ , IL-12, and TNF-α (Costain *et al.,* 2018). Nevertheless, a typical T_H2 response is developed by the host as the infection progresses to being chronic, as a result, more activated macrophages and eosinophils are recruited (McGovern & Wilson, 2014; De Oliveira *et al.,* 2010).

Sleeping sickness, well known as Human African trypanosomiasis (HAT), is a neglected tropical disease that mostly affect people from sub-Saharan Africa. It is spread through the biting of infected tsetse flies of the Glossina species (Kennedy, 2004). HAT caused by *Trypanosoma brucei gambiense* results to a chronic form of infection in the West and Central Africa while *Trypanosoma brucei rhodesiense* is associated with the acute form in Eastern and Southern Africa (Kato *et al.,* 2015). HAT due to *T. brucei rhodesiense* is known to cause acute infection and is widespread in tropical parts of Africa, with estimated 64 million persons being at risk of infection (WHO, 2022). Additionally, HAT manifests in two disease stages: the first stage, also known as the haemo-lymphatic stage, in which the parasite grows and multiplies along with an invasion of the lymph nodes (Kioy *et al.,* 2004). The clinical signs and symptoms associated with the haemo-lymphatic stage aren't specific but they include malaise, headache, anorexia, and enlarged lymphadenopathy. Noteworthy is the presence of a chancre at the location of a tsetse fly bite, which represents the sign of the first stage that shows a *T. brucei rhodesiense* infection (Brun *et al.,* 2010). The second

stage also known as meningoencephalitis is associated with symptoms demonstrated by leukocytosis (>5mm WBC/mm3) in the cerebral spinal fluid (CSF) and motor-neuropsychiatric symptoms (Kagira *et al.,* 2011). Research has demonstrated that during infection with *T. brucei rhodesiense*, the host immune system comes into contact with a variety of antigens, including trypanosome-lymphocyte triggering factor (TLTF) and variant surface glycoprotein (VSG) among others (Onyilagha & Uzonna, 2019) . Worthy to mention is that VSG is frequently linked with T and B lymphocytes and macrophages priming resulting in the induction of pro-inflammatory cytokines IFN-γ, TNF-α, IL-6 and also nitric oxide (NO) production (Ponte-Sucre, 2016). The secretion of pro-inflammatory cytokine IL-6 during HAT has been observed to induce the production of Interferon-γ (IFN-γ) through early activation of natural killer cells (Sanches-Vaz *et al.,* 2019). Anemia, immunosuppression, and tissue lesions are some of the features that have been observed to aggravate the pathogenesis of HAT (Eze & Okonkwo, 2013). In trypanosomiasis infection, anemia has been reported as a major laboratory and clinical finding, characterized by a marked reduction in red blood cells, packed cell volume (PCV) and haemoglobin (Hb). This anemia could be attributed to erythrophagocytosis, hemolytic factor, hemodilution, hematopoietic response and bone marrow dyserythropoiesis (Noyes *et al.,* 2009). Anemia as a result of oxidative stress has been equally linked to driving deleterious effects associated with HAT (Eze *et al.,* 2016). Oxidative hemolysis has been associated with the high production of free radicals in infected animal models (Slater, 1984) and reduced body's endogenous antioxidant reserves. In addition, reports link trypanosome infection to elevated levels of lipid peroxidation (Niranjan *et al.,* 2013) and depleted antioxidants (Omobowale *et al.,* 2015). Considerable evidence has demonstrated that during HAT, elevated levels of pro-inflammatory cytokines are also major players that exacerbate the progression of anemia (Stijlemans *et al.,* 2018). Therefore, it is evident that the rapid increase

in inflammatory mediators, harmful free radicals, nitric oxide, and reactive oxygen species) is characteristic in the pathophysiology of HAT.

Treatment of HAT due to *T.b. rhodesiense* currently depends on a limited number of potentially toxic drugs, but if the disease progresses without being treatment can prove fatal. The kind and stage of HAT determine the kind of medication are used to be administered. For the early stage of HAT against *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* infection, respectively, pentamidine and suramin are very effective (Monica *et al.,* 2020). However, these drugs are not able to cross the blood-brain barrier hence not recommended for treatment of the second stage of HAT. Melasoprol is regarded as the most effective drug for treating stage two infections due to *T.b.r*, though it poses dreadful side effects with 5-10% of the patients succumbing as a result of post-treatment reactive encephalopathy (Babokhov *et al.,* 2013). Eflornithine and nirfutimox drugs combined are considered cheaper but effective only against *Trypanosoma brucei gambiense* disease. Resistance to both eflornithine and melarsoprol has been documented hence the need for novel drugs (Bouteille & Buguet, 2012).

Several studies have given contradicting information on co-infections with either HAT or schistosomiasis. For instance, results from a study by Waknine *et al. (*2010) clearly illustrated that simultaneous *P. berghei* ANKA and *S. mansoni* infection resulted in the amelioration of the severity of cerebral malaria in a mouse model. In another study, co-infection of *Schistosoma mansoni* with *Plasmodium coatney* impaired antimalarial treatment and immune responses in Rhesus Macaques (Semenya *et al.*, 2012). Additionally, a study by Sanches *et al.* (2019) observed that a persistent *T. brucei* infection results in elevated lymphocyte-derived IFN-γ inside the liver, limiting the establishment of a successive hepatic disease by sporozoites of *P. berghei*.

Despite the natural existence between the parasites that are the major causative agents of HAT and Schistosomiasis, the mutual effect of a co-infection by *S. mansoni* and *T. b. rhodesiense* had hitherto not been investigated. The goal of the current study was to clarify how *S. mansoni* affected the severity of *T. b. rhodesiense* as a secondary infection in a mouse model.

1.2 Statement of the problem

Human African Trypanosomiasis in the developing countries still persists therefore efforts to ease the economic burden and reduced productivity are being sought. Previous studies have demonstrated that *S. mansoni* significantly inhibit malaria parasite in the liver (Moriyasu *et al.,* 2018). Other studies have shown that *S. mansoni* attenuates *T. gondii* induced ileitis by preserving mucosal integrity and downregulating local inflammatory response (Pêgo *et al.*, 2019). Additionally, *Trypanosoma brucei* infection protects mice against malaria, by *T. brucei* eliciting strong immune response with a potential to limit plasmodium parasites subsequently infecting a similar host (Sanches *et al.,* 2019). Experimentally, a number of investigations have presented varying information on the outcome of co-infection in both schistosomiasis and HAT. Given the importance of Schistosomiasis and HAT; there is paucity of data on the outcome during co-infection of *S. mansoni* and *Trypanosoma brucei rhodesiense* despite them being tropical diseases. Moreover, despite the natural existence between the two parasites that are the major causative agents of HAT and Schistosomiasis, the resultant effect of a co-infection by *S. mansoni* and *T. b. rhodesiense* hitherto is poorly understood.

1.3 Justification

Trypanosoma brucei rhodesiense and *S. mansoni* are diseases associated with poverty which are highly prevalent in most parts of Kenya (Mott *et al.,* 1990). The outcome of co-infections could

either result in the improvement or the reduction of the development of either parasite(s), leading to the exacerbation or enhancement of the pathology of both diseases. Currently, little information is available regarding the immune responses and oxidative stress that is integral in determining the outcome of co-infection by *S. mansoni* and *T. b. rhodesiense.* On the other hand, organ pathology and profiles of cytokines associated with the ensuing disease progression in view of the significance of these diseases in human is less known. This study therefore sought to determine whether the immune response elicited by *S. mansoni* can confer immunity due to secondary infection by a *T.b. rhodesiense.* Information generated from this study will lend critical, novel and comprehensive insight on the immunological, biochemical and oxidative processes involved in the pathogenesis of schistosomiasis-HAT infection.

Additionally, the findings from this study will be significant in triggering more studies on higher animal models or human subjects, geared towards safeguarding healthy lives and endorse well-being for the people, which is one of the sustainable development goals (SDG" s) of the Vision 2030, considering that these are neglected diseases.

1.4 Objectives

1.4.1 General Objective

To determine the impact of *S. mansoni* on disease severity of secondary infection with *T. b. rhodesiense* in a mouse model

1.4.2 Specific objectives

1. To determine the effects of *S. mansoni* on the host physiological and hematological events following *T. b. r* induced infection in a mouse model

2. To investigate the role of *S. mansoni* on HAT driven biochemical and immune regulation in a mouse model
3. To determine the impact of *S. mansoni* and *T. b. r* on inflammation and oxidative stress in a mouse model

1.5 Research questions

1. What are the effects of *S. mansoni* on the host physiological and hematological events following *T. b. r* induced infection in a mouse model?
2. What is the role of *S. mansoni* on HAT driven biochemical and immune regulation in mouse model?
3. What is the impact of *S. mansoni* and *Trypanosoma brucei rhodesiense* on inflammation and oxidative stress in a mouse model?

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of Schistosomiasis

Blood flukes of the genus *Schistosoma* are known to cause schistosomiasis, *with S. mansoni, S. haematobium, S. japonicum, S.mekongi and S. intercalatum* being the species that are well-known to cause infection in humans (U Olveda, 2013). It is estimated that more than 250 million people are affected annually, and at least 770 million people are at risk of getting infected (WHO, 2022). About 279,000 succumb to the disease annually and a burden of disability rated at 3.2 million worldwide (Mouahid *et al.,* 2018). Human schistosomiasis is a widespread human parasitic infection, ranked second after malaria and it occurs in about 76 countries mainly from developing countries in Africa, South America, several Caribbean islands and the disease is generally spread through immigration and water-based development projects (Nelwan, 2019).

2.2 Life cycle of Schistosomiasis

Schistosoma eggs are released with feces or urine, depending on species of schistosomiasis (figure 2.1). Usually, the eggs hatch in water thereby releasing miracidia under suitable conditions, which swim and enter definite snails which are intermediate hosts. In the snail, it undergoes two generations of sporocysts hence developing into cercariae. Upon release from the snail, infective cercariae swim, penetrates the skin of a mammalian host. The cercariae become schistosomulae after shedding of their forked tails. The schistosomulae move through blood circulation, then to lungs, to the heart, maturing in the liver. Upon maturity they leave the liver through the portal vein system. Female and male adult worms' mate and live in the mesenteric venules, depending on different species. They deposit eggs either in the small venules of the entry and also perivesical systems. The deposited eggs are transported gradually towards the intestine's lumen (*S. japonicum,*

S. intercalatum/guineensis, *S. mansoni*, *S. mekongi*) and that of the ureters and bladder (*S. haematobium*). These eggs are in turn removed in feces and urine. Global Health Division of Parasitic Diseases and Malaria

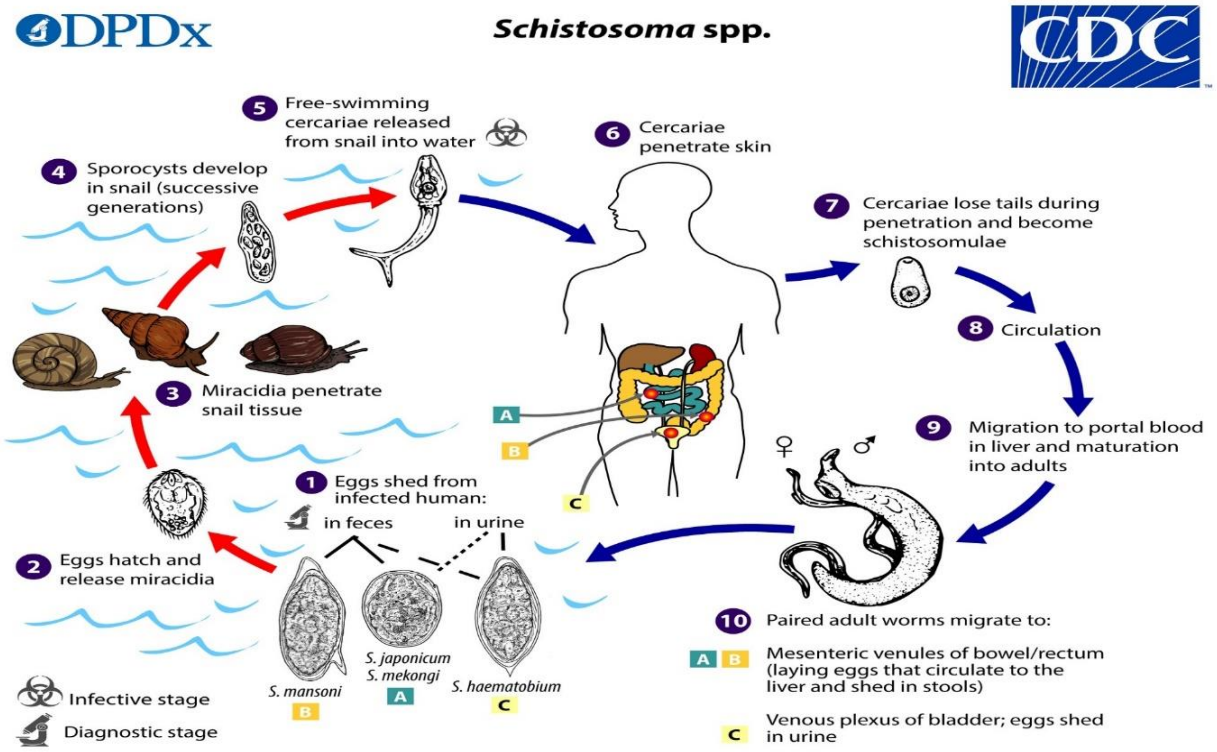

Figure 2.1: Life cycle of Schistosomiasis (image and information courtesy of CDC, 2019)

2.3 Immune response development in *S. mansoni* infection.

Normally, immune response is known to develop through three phases as the disease progresses (the early, intermediate and chronic schistosomiasis) (Alves *et al.*, 2016). It has been well documented that T helper 1 (T_H1) is the main response produced during schistosomiasis. Nevertheless, the T_H1 response diminishes when a strong T_H2 response is inducted, this takes place at weeks 5-6 when mature parasites pair and start producing eggs (Pearce & MacDonald, 2002).

Moreover, the presence of Schistosoma egg antigens is the main cause of T_H2 response. T helper 2 immune response is regulated during the disease's prolonged phase by granulomas that form around newly released eggs and are smaller than they were during the disease's early stages. (Castro *et al.,* 2018). T_H2- mediated granulomas guard hepatocytes but permit the fibrosis development (Colley & Secor, 2014).

2.4 Cytokine profile in Schistosomiasis

Acute schistosomiasis (also known as T_H1 disease) is an overwhelming burning disease referred to as Katayama fever arising before the eggs are observed in the stool. The presence of peripheral-blood mononuclear cells PBMCs, tumor necrosis factor (TNF) in plasma, IL-6 and interleukin-1 (IL-1) is evident during this stage (De Oliveira *et al.,* 2010). Remarkably, rather than T_H2, the (T_H1) response is dominant during acute illness with *S. mansoni*. However, the T_H2 response suppresses the effector activities, such as the synthesis of inflammatory mediators. IL-10 production plays a crucial role during this period. Febrile infection during the early phases of schistosome disease appears not to be common among persons who reside in areas where schistosomiasis is prevalent (Pearce & MacDonald, 2002). It only occurs to persons who had not previously been exposed but become infected after visiting the endemic area. This is a result of one being sensitized to schistosomes *in utero* due to maternal infection, and this permits them to respond otherwise from ‘naive’ persons when they become infected (Gryseels *et al.,* 2006).

They are less likely to experience a pro-inflammatory response during an initial schistosome infection because of their earlier T_H2 response. A study using a mouse model has demonstrated a relationship between acute schistosomiasis, which is regarded as deadly, and a failure to produce a T_H2 response for control of the first pro-inflammatory response (De Oliveira *et al.,* 2013).

Chronic schistosomiasis is classified according to severity, with the hepatosplenic disease being the most severe accompanied by severe portal hypertension and portosystemic hepatic and periportal fibrosis pushing of venous blood. Fibrosis is a quantitative tool used for assessing the disease severity (Tamarozzi *et al.,* 2021; Cheever *et al.,* 2002). Chronic morbidity and hepatic fibrosis are a result of prolonged T_H2 responses. Additionally, IL-13 which is a T_H2 cytokine has been shown to play a role in fibrosis (Vieira-de-Abreu *et al.,* 2012). Moreover, mediators of TH1 responses, including IL-12, TNF- α, interferon-γ (IFN-γ) and NO can prevent IL-13-mediated fibrosis (Costain *et al.,* 2018).

2.4.1 Role of Interferon gamma in Schistosomiasis

Interferon-gamma (IFN-γ) is a Th1 cytokine that has the ability to activate macrophages which in turn trigger the production of nitric oxide (NO) and several inflammatory mediators (Anne *et al.,* 2001). It's been documented that during acute schistosomiasis infection, elevated levels of IFN-γ are produced by IL-4/- mice especially in the liver, mesenteric lymph nodes (MLN) and spleen (Pearce *et al.,* 1996). Remarkably, this elevated IFN-γ production may justify the morbidity of mice during schistosomiasis infection. Additionally, interferon-gamma is a well-known anti-fibrogenic cytokine (Horras *et al.,* 2011; Fallon *et al.,* 2000) that has demonstrated the ability to protect against fibrosis among individuals with *S. mansoni* infection (Jobgen *et al.,* 2007). IFN- γ also inhibits the production of extracellular substances via the hepatic stellate cells (Hesse *et al.,* 2000) and it improves matrix metalloprotease (MMP) gene expression (Hoffmann *et al.,* 2000). Previously, reports have elucidated that reduced levels of this cytokine correlate with severe periportal fibrosis among individuals infected with *S. mansoni* (Hams *et al.,* 2013).

2.4.2 Role of Tumor Necrotic Factor alpha (TNF- α) in Schistosomiasis

The role played by tumor necrosis factor-alpha (TNF-α) in the regulation of schistosomiasis infection still remains unclear. While it has been documented in some studies that TNF- α plays a protective role against severe schistosomiasis disease (Hooper *et al.,* 1998), elevated levels have been shown to aggravate disease severity (Jankovic *et al.,* 2000).

2.4.3 Role of interleukin-10 (IL-10) in Schistosomiasis

The establishment and growth of liver granulomas are crucially regulated by CD41 cells, and a variety of cytokines control the inflammatory response occurring where schistosome eggs are located. After egg laying commences, Th2 cytokine manifestation becomes pronounced, with IL-5, IL-10, IL-4, and IL-13 being key cytokines that lymphoid cells produce (Wynn *et al.,* 1998). Therefore, the production of Th1 cytokines IL-2 and IFN- γ, is simultaneously down-regulated the moment Th2 responses reach their highest levels (Vecchio *et al.,* 2012).

2.5 Overview of oxidative Stress and inflammation during infection

Reactive oxygen species (ROS) are produced excessively in tissues under oxidative stress and cells making it impossible for the antioxidant system to neutralize them (Pizzino *et al.,* 2017). This imbalance between the antioxidant and ROS can destroy molecules of cells including proteins, lipids and DNA. ROS are usually produced inside the body in small quantities, they have demonstrated take part in regulating processes involved in cell homeostasis and functions including activation of receptors, signal transduction, and gene expression. However, in conditions where there's no oxygen, nitric oxide may also be generated during the respiratory chain reaction. Production of ROS in excess, in cells and tissues, may be harmful if not eliminated fast. When

ROS/RNS is produced in excess, may cause permanent damage to cells, causing cell death by the necrotic and apoptotic processes (Hussain *et al.,* 2016).

The term inflammation is an inborn protection means by the body immune system that counters pathogens, it is usually linked with microbial and viral disease, radiation, exposure to lethal chemicals, allergens, chronic and autoimmune diseases, obesity, tobacco use, alcohol consumption, and a high-calorie diet. A lot of prolonged diseases associated with elevated production of ROS can lead to oxidative stress and diversity of protein oxidations (Pizzino *et al.,* 2017).

Avalanche body of evidence has documented the association between oxidative stress and inflammation. Previous and current literature has shown that oxidative stress plays a detrimental role in chronic inflammatory diseases (Biswas, 2016; Bogdan *et al.,* 2000). Oxidative stress damage including glycated products oxidized proteins, and lipid peroxidation leads to neuron deterioration in brain conditions. ROS produced in brain tissues can modify non-synaptic and synaptic messages between neurons leading to inflammation of neurons and death of cells, then memory loss and neurodegeneration. Tripeptide glutathione (GSH) is an intracellular thiol antioxidant; reduced GSH levels lead to elevated creation of ROS, hence inflammation, immune response that is not balanced, and predisposition to infection (Romão *et al.,* 2006). In diabetes studies, it was acknowledged that elevated nitrite levels, lipid peroxidation, total oxidants, and malondialdehyde status were decreased in total antioxidant marker enzymes in the diabetic rat's brain. Furthermore, studies have demonstrated that oxidative stress induced by diabetes elevates cytokines that are pro-inflammatory in nature such as interleukin-6 (IL-6) and TNF-α. Additionally, it causes inflammatory molecules such as intercellular adhesion molecule-1 (ICAM-

1), nuclear factor-kappa B (NF-κB), and vascular cell adhesion molecule-1 (VCAM-1) (Hussain *et al.,* 2016). Lipid peroxidation is a lethal process that is generated due to high levels of free radicals in an organism. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells (Khoubnasabjafari *et al.,* 2015). Elevated free radicals lead to excess production of MDA. MDA is a marker of antioxidant status and oxidative stress in cancer patients (van Crommenacker *et al.,* 2012).

2.6 Role of oxidative stress in Schistosomiasis

Studies have shown that oxidative stress plays a crucial part in the development of schistosomiasis, mostly via activating leukocytes in response to the parasite's presence (Elsammak *et al.,* 2008). When the balance between eosinophil peroxidase activity and antioxidant capacity is out of control, the involved organs including the liver, spleen, and kidneys move into a pro-oxidant condition. During schistosomiasis infection, the eggs of the parasite stay clogged in the sinusoids of the liver making an individual not able to clear the infection, a continuous series of healing and inflammation occurs and this leads to blockage of the fibrosis and vascular system (Andrade, 2004). This cycle was documented to rely on helper T (Th) type-2 cells in response to Schistosoma egg antigens (SEA), although some innate response is observed in the above process. Additionally, the T helper type-2 response is described by elevated levels of IL-5, IL-10, IL-4, and IL-13 (Pearce, 2005). In a study reported by La Flamme *et al.* (2001) excessive nitric oxide, peroxynitrite, and superoxide levels were identified in the livers of mice treated using IL-4 thus depleted antioxidant capability leading to the enlargement of the liver. Therefore this data indicate a clear connection between oxidative stress, tissue damage and pro-inflammatory stimulation during schistosomiasis infection (De Oliveira *et al.,* 2013).

2.7 Hematological changes in Schistosomiasis

Direct ingestion through the stomach or aggravating blood loss in the stool when blood vessels are broken due to the spine on the surface of the egg are two ways that schistosomiasis affects the host's hematological profiles. Indeed, it is noteworthy to mention that *S. mansoni*-infected patients present themselves with anemia (Dessie *et al.,* 2020). The type of anemia in *S. mansoni* is iron deficiency and its causes include amplified erythrocyte sequestration and extravascular hemolysis(Leir *et al.,* 2019). Findings from previous study had established that an adult worm pair (male and female) takes in about 100 and 900 µl of blood per day into their gut, respectively (Silva *et al.,* 2020). Another study had shown that hematological parameters including red blood cell (RBC) count, hemoglobin (Hgb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total white blood cell (WBC) count and differential leukocyte count, are significantly reduced in *S. mansoni*-infected patients (Wozniak *et al.,* 2021). Therefore, anemia experienced during *S. mansoni* infection may be due to either direct ingestion of red blood cells via the gut or exacerbation of blood loss through feces, with the aid of the *S. mansoni*'s egg spine that ruptures blood vessels (Lawrence, 1973).

2.8 Treatment of Schistosomiasis

Currently, the known treatment of choice for schistosomiasis infection is Praziquantel which is a derivative of pyrazine-isoquinoline (Coulibaly *et al.,* 2012). This drug acts against schistosoma adult worms but has reduced action against undeveloped schistosome larvae and the stipulated dosage is 40 mg/kg for both *S. mansoni* and *S. haematobium* but 60 mg/kg for *S. japonicum* for one day. The exact mechanism of action of this drug remains unclear (Cupit & Cunningham, 2015).

However, its documented that when schistosomes interact with Praziquantel in vitro, instantly they undergo a fast entry of Ca2+ accompanied by a strong muscular paralysis (Xiao *et al.,* 2009). Few alternatives to Praziquantel including metrifonate and Antimonial compounds were used before but due to their extreme toxicity, they are now regarded as obsolete (Nyakundi *et al.,* 2016). Outstandingly, Oxamniquine remains to be the other only drug available for schistosomiasis treatment (Foster, 1987). This drug is effective against *S. mansoni*, but has no known effect on *S. haematobium*. Well-known resistance to Praziquantel has been reported and currently, it is only used in Brazil on a large scale. Additionally, recent research has established the possible use of artemisinin derivatives, which can act only on the juvenile larval forms of schistosomes that are still developing(Pérez *et al.,* 2012). Interestingly, Praziquantel efficaciously treats other trematodes including, *Clonorchis sinensis, Fasciola hepatica,* and *Paragonimus westermani* as well as cestodes, comprising of *Taenia* species *Diphyllobothrium latum, Hymenolepis nana* and *Echinococcus granulosus* infections (Kyung *et al.,* 2011). Praziquantel is a drug mostly well accepted and common short-lived side effects include, among others, abdominal pain, headache, sleepiness dizziness, and diarrhea, and more so, it is safe and highly effective(Patel *et al.,* 2016).

2.9 Co-infection of Schistosomiasis with other parasitwes

A study by Pêgo *et al.* (2019) reported that *S. mansoni* Co-infection reduces murine *Toxoplasma gondii*-induced Crohn's-like ileitis by means of downregulating the inflammatory response and preserving the epithelial barrier. This is achieved by silencing of Th1 and Th17 pro-inflammatory T-cells subsets (Mbow *et al.*, 2013), and elevation of regulatory T cells (Tregs) (Wammes *et al.,* 2012), as well as interleukin (IL)-10 which is a regulatory cytokine (Sanin *et al.,* 2015). In another study, *Schistosoma mansoni* co-infection reactivates viruses in the blood of Rhesus Macaques

having Chronic Simian-Human Immunodeficiency Virus Clade C disease. This came about as a result of significantly elevated viral replication that induced variations in the subsets of T-cell in monkeys having chronic, clinically unwavering SHIV clade C disease (Ayash *et al.,* 2007). The hosts and organisms used in the studies, the timing and order of the first and second infections, the parasite strains and bloodlines, the dosage of the infectious agents are all factors that determines how co-infection affects a host (Abruzzi & Fried, 2011). Schistosoma infection, particularly a patent infection, frequently influences subsequent bacterial, protozoal, or helminth infections. In a small number of cases, a previous Schistosoma infection lessened the severity of the ensuing infection for example with *Helicobacter pylori*, *Echinostoma* and *Fasciola hepatica.* However, *Plasmodium* exhibits this behavior only when co-infected with *S. haematobium* (Abruzzi & Fried, 2011). In contrast, a co-infection with Schistosoma made the later illness worse as with, *Toxoplasma gondii, Leishmania, Staphylococcus aureus, Entamoeba histolytica,* or *Salmonella*. In the aforementioned co-infection trials, higher severity following a second infection was linked to a distinct, chronic type of the disease in people, with detrimental effects for patient management, treatment and recovery (Griffiths *et al.,* 2011).

2.10 Overview of Human African Trypanosomiasis

Human African Trypanosomiasis (HAT) is widely spread in sub-Saharan Africa with at least 60 million individuals being at risk, particularly in Eastern Africa, HAT due to *Trypanosoma brucei rhodesiense* is regarded to be acute (Franco *et al.,* 2014). It's known to occur in zones widespread of other tropical diseases like malaria since these infections are arthropods spread by mosquitoes and tsetse flies and these vectors inhabit the same location. HAT presents itself in two disease stages that are described as the first and second stages and characterized by extensive diverse clinical signs and symptoms (Kato *et al.,* 2015). The first stage symptoms and signs are non-

specific but may comprise body malaise, anorexia, headache and enlarged lymphadenopathy. A chancre at the spot of the tsetse fly bite is a first-stage sign that shows a *T. brucei rhodesiense* infection (Brun *et al.,* 2010). The second stage of the disease known as the meningo-encephalitis stage is associated with symptoms such as leukocytosis ($\geq$5mm WBC/mm^3) in the cerebral spinal fluid (CSF) and motor-neuropsychiatric symptoms (Kennedy, 2004).

Studies on mouse models have shown that African trypanosomes induce effective inflammatory responses (Maclean *et al.*, 2004). However, inadequate reports about cytokine reactions in HAT patients has made it hard to combine results, and high levels of both interleukin10 and TNF-alpha have been linked with HAT infection disease(Yoshihara *et al.*, 2007). Two subspecies of *Trypanosoma brucei* are known to cause HAT and these include *T. b. gambiense* mainly found in Central and Western parts of Africa and *T. b. rhodesiense* originates in East and Central Africa. The consequences of HAT infection by *T. b. rhodesiense* are more severe and lead to the development of faster late stage compared to that of T. *b. gambiense*. The two subspecies, *T. b. gambiense* and *T. b. rhodesiense* can be distinguished using genes for example the human serum resistance-associated (*SRA*) gene that describes the latter and takes part in permitting human infectivity. Despite *T. b. rhodesiense* causing acute infections in humans, the disease can progress to the central nervous system (Sternberg, 2004).

2.11 Life cycle of Human African Trypanosomiasis

The life cycle begins when a Glossina genus infected tsetse fly injects metacyclic trypomastigotes into the skin of a human host during a blood meal. After entering the lymphatic system, parasites eventually enter the bloodstream. In the bloodstream, they change into trypomastigotes and then progressively invade other parts of the body, reaching other fluids of the body including spinal

fluid lymph), where they multiply by binary division. African trypanosomes' entire lifecycle is characterized by extracellular stages. A tsetse fly picks up trypomastigotes as it consumes a blood meal from an infected human host. In the gut of the fly, parasites change into procyclic trypomastigotes, they then replicate by binary fission, move from the midgut, finally transform into epimastigotes. On reaching the salivary glands of the fly, epimastigotes continue replicating by binary fission (figure 2.2). In the tsetse fly, the cycle takes about 3 weeks. There's a possibility of *T. b. gambiense* being acquired congenitally especially during pregnancy if the mother is infected (CDC, 2020).

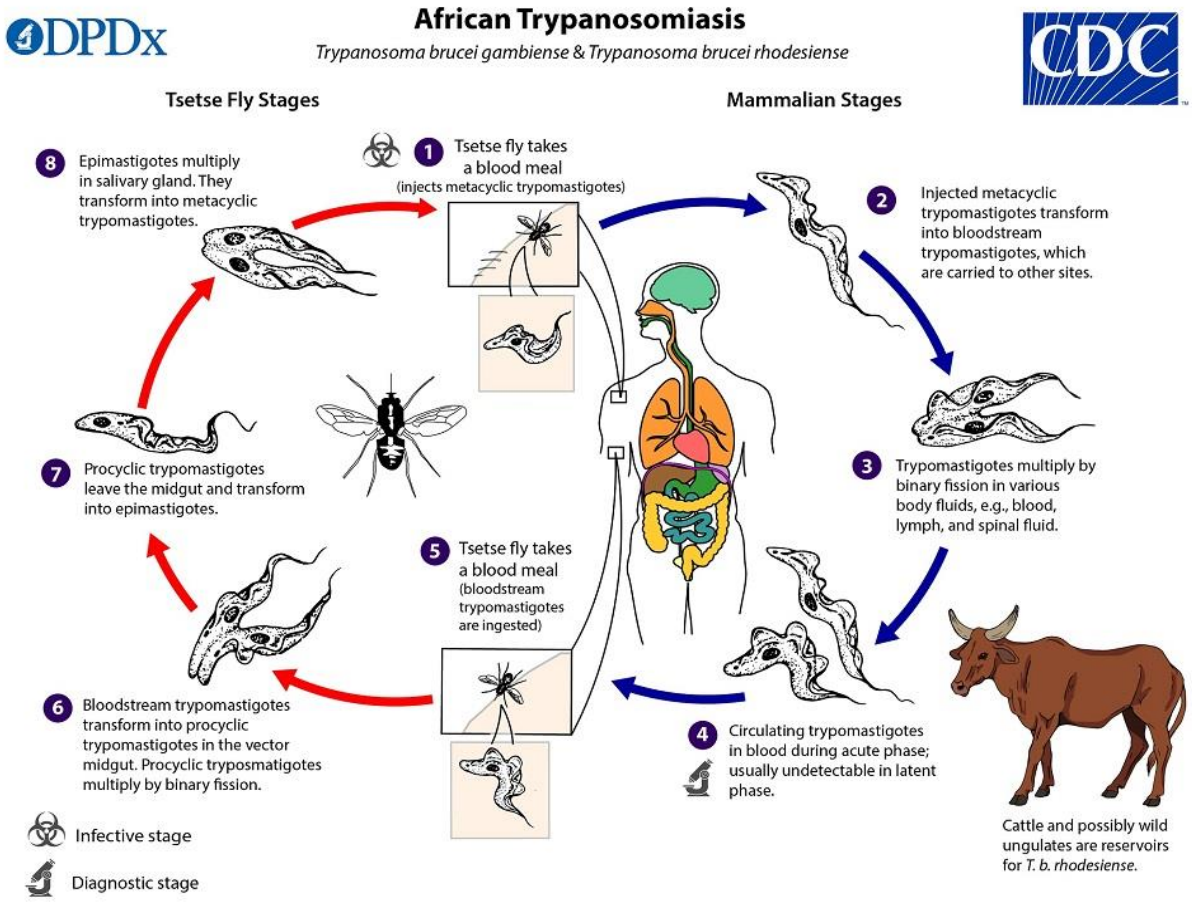

Figure 1.2: African Trypanosomiasis life cycle (image and information courtesy of CDC, 2019)

2.12 Epidemiology of Human African trypanosomes

Two subspecies of *Trypanosoma brucei* are known to cause HAT and these include *T. b. gambiense* mainly found in Central and Western parts of Africa and *T. b. rhodesiense* that originates in East Africa. According to WHO, 2021 report, the number of cases dropped below 10, 000 in the year 2009 for the first time in a span of 50 years. Approximately 992 and 663 cases were reported in 2019 and 2020 respectively. Additionally, higher cases of *T.b.r* have been reported in Uganda in the last ended 3 decades in comparison to Kenya. It has been reported that the duration and nature of interaction which could be either intimate or casual, determine the power of transmission to individuals (Rutto *et al.,* 2013). The consequences of HAT infection by *T. b. rhodesiense* is more severe and leads to development of faster late stage compared to that of T. *b. gambiense*. The two subspecies, *T. b. gambiense* and *T. b. rhodesiense* can be distinguished using genes for example the human serum resistance-associated (*SRA*) gene describes the latter and takes part in permitting human infectivity (Omobowale *et al.,* 2015). Despite *T. b. rhodesiense* causing acute infections in humans, the disease can progress to the central nervous system, especially during the late stage (Bolton *et al.,* 1998).

2.13 Hematological changes in Human African Trypanosomiasis

Human African trypanosomiasis is associated with plethora of factors that ultimately result in the alteration of the hematological profile with concomitant aggravation of the molecular pathogenesis of the disease. This disease is linked with anemia characterized by a decrease in red blood cell (RBC) counts, packed cell volume (PCV) and hemoglobin concentration. Anemia in animals has been classified as either macrocytic normochromic, normocytic normochromic (Lima *et al.*, 2012).

Variation in the type of anemia could be ascribed to the following factors, including pathogenicity of trypanosomes, the stage of disease, and host species. Inconsistent information on the pattern of white blood cell changes in African trypanosomiasis has been recorded. Distinct leukocytopenia which relates with lymphocytopenia and neutrocytopenia has been reported in livestock (Kamau *et al.,* 2021). In a number of studies, thrombocytopenia has been significantly reported in Trypanosomiasis, though, changes in the shape of the platelets during this infection have not yet been defined (Kagira *et al*., 2008).

2.14 Role of cytokines as inflammatory mediators in HAT

Experimental laboratory animals have been employed in most studies in order to understand the dysregulation of cytokines in HAT. This makes it possible to follow up on immune responses associated with disease development (Sternberg *et al.,* 2005; Namangala *et al.*, 2009). There's captivating proof documented those points to dysregulation in cytokine profiles as a key indicator in human African trypanosomiasis pathogenesis. During the first stage of human African trypanosomiasis infection, there's an elevation of pro-inflammatory cytokines including IFN-γ and TNF-α (Hertz *et al.,* 2022). Previous studies have confirmed that sustained survival of murine human African trypanosomiasis is thought to be dependent on the stage of infection, in that pro-inflammatory cytokine plays a great part in the initial stage of the infection, meanwhile, during the late stage of infection, counter-inflammatory cytokines govern survival (Namangala *et al.*, 2001). In addition, cytokines in the central Nervous system can be used as biomarkers for invasion of the CNS, because they have been demonstrated to go back to normal amounts after treatment (Kato *et al.*, 2016). African trypanosome resistance has been associated with type I cytokines, which inhibit parasite growth in the initial stages of infection (Fernandez, 2017; Hertz *et al.*, 2022). However, a

persistent type I cytokine response may be detrimental and accelerate the development of the HAT disease (Kuriakose *et al.,* 2016).

2.14.1 Role of IFN-γ in Human African Trypanosomiasis

Interferon-gamma (IFN-γ) plays an important role in combating African trypanosomes. Nevertheless, when extremely synthesized it can be detrimental to the human host. Overwhelming evidence points out that IFN-γ can induce immunopathology hence increasing susceptibility to trypanosomiasis (Fernandez, 2017). Therefore, IFN-γ production must be tightly controlled during trypanosomiasis infection to ensure that a strong immune response is stimulated without the destruction of tissue. Previous studies have demonstrated that IFN-γ production is downregulated by interleukin 10 (IL-10) (Bourke *et al.*, 2013). In this study, mice infected and treated with anti–Interleukin 10 receptor (anti-IL-10R) showed elevated levels of plasma pro-inflammatory cytokines and widespread crucial liver necrosis, suggesting that IFN-γ facilitates liver pathology (Shi *et al.,* 2003). Furthermore, liver damage due to IFN-γ was further established using interleukin (IL)-12p70−/− C57BL/6 mice, because interleukin (IL)-12p70−/− mice demonstrated outstandingly depleted IFN-γ production, corresponding with a fall of plasma aspartate transaminase and a substantially improved survival during *T. congolense* infection (Barkhuizen *et al.,* 2008). Besides liver pathology, more recent studies have confirmed that IFN-γ also causes acute anemia, this being one of the main features of African trypanosomiasis (Cnops *et al.*, 2015). This acute anemia in trypanosomiasis occurs as a result of enhanced erythrophagocytosis which is arbitrated by activated myeloid cells of the phagocytic system (Stijlemans *et al.*, 2015). Therefore, IFN-γ has been demonstrated to be disparagingly involved in the activation and recruitment of myeloid cells in erythrophagocytic system; hence interferon-gamma receptor null-mutant (IFN-γR−/−) mice were to some extent protected against acute anemia and inflammation associated

with trypanosomiasis indicating a harmful role of IFN-γ in enhancing erythrophagocytosis (Stijlemans *et al.,* 2015). IFN-γ is protective in trypanosomiasis infection, however, extreme synthesis of this cytokine may be detrimental.

2.14.2 Role of IL-10 in Human African Trypanosomiasis

Interleukin 10 (IL-10) being an immunoregulatory cytokine, plays a great role in limiting inflammation. IL-10 is crucial for preserving the immunological balance between protective and pathogenic immune responses throughout African trypanosomiasis, according to several investigations using mouse models (Bosschaerts *et al.,* 2011; Guilliams *et al.,* 2009). In addition, the anti-inflammatory role of IL-10 has also been confirmed in primate, cattle, and human infections by means of African trypanosomes (Ngotho *et al.,* 2009;Yoshihara *et al.,* 2007). Namangala *et al.* (2001) reported suppression of IFN-γ by IL-10 by demonstrating that interleukin (IL)-10−/− mice that were infected with *T. brucei brucei* had meaningfully elevated levels of plasma IFN-γ and succumbed earlier in comparison to wild-type infected mice. Jointly, IL-10, mostly secreted by myeloid cells and CD4+, deleteriously regulates the production of IFN-γ. It is possible that IL-10 impedes IFN-γ production via downregulation of the synthesis of TNF-α and IL-12 through uninterrupted variation of M1-type myeloid cells (Nyakundi *et al.,* 2016).

2.15 Organ pathology in Human African Trypanosomiasis

Man has been seen with enlarged liver and spleen with HAT infection (Cohen, 1973). For instance, experimentally, hepatocytes of *T. b. gambiense*-infected microtus contained trypanosomes integral though the inflammatory part of the cell response wasn't noticeably conflicting with inflammation reports in dogs infected with trypanosomes (Morrison *et al.,* 1981).

2.16 Treatment and management of Human African trypanosomiasis

Potentially hazardous medications are currently used to treat HAT caused by *T. b. rhodesiense*; however, the disease is almost always deadly if left untreated. Human HAT treatment drug selection is mostly influenced by the kind and stage of the disease. Specifically, pentamidine and suramin, are effective for the first stage of HAT against *Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense* infection respectively (Monica *et al.,* 2020). However, these drugs are not able to cross the blood-brain barrier hence not recommended for treatment of the second stage of HAT. Melasoprol is regarded as the most effective drug for treating stage two infections due to *T.b.r,* though it poses very dreadful side effects with 5-10% of the patients dying as a result of post-treatment reactive encephalopathy (Babokhov *et al.,* 2013). Eflornithine and nirfutimox drugs combined are considered cheaper but effective only against *gambiense* disease. Resistance to both melarsoprol and eflornithine is on record hence the need for novel drugs (Bouteille & Buguet, 2012).

2.17 HAT co-infection with other diseases

As a result of protecting mice with experimental cerebral malaria, a first *T. brucei brucei* infection has been shown to dramatically minimize an ensuing infection by the malaria parasite and increase host survival. Iinfection has been shown to dramatically minimize an ensuing infection by the malaria parasite and increase host survival. It was additionally observed that constant *T. brucei* infection brings about a buildup of lymphocyte-derived IFN-γ inside the liver hence restricting the establishment of an ensuing liver infection due to sporozoites of *P. berghei* (Sanches *et al.,* 2019).

In another study, *Trypanosoma brucei brucei* co-infection protected against Cutaneous Leishmaniasis (Pereira *et al.,* 2018). This resulted from trypanosomes' non-specific stimulation of

T lymphocytes. IFN-γ -production in the blood as well as at the bite sites indicated a robust immune response. This response led to an aggressive inflammatory situation for *L. major* parasites hence protecting the host from Cutaneous Leishmaniasis (Ikeogu *et al.,* 2020).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

School of Biological and Life Sciences laboratories at The Technical University of Kenya's (TU-K), served as the site of this study.

3.2 Ethical approval

Ethical clearance was sought from the Institutional Review Committee of the Institute of Primate Research (IPR) Karen, Kenya (ISERC/08/2017).

3.3 Experimental animal

A total of 70 female BALB/c mice, aged 6–8 weeks, were obtained from the Institute of Primate Research. These animals were habituated at a controlled room temperature (21-25^{0}C) with a 12-hour dark/light cycle for a period of one week for acclimatization before the start of the investigation. Pellets from (Unga Group Plc., Nairobi, Kenya) were used to feed the mice and access clean water *ad libitum* by the mice was put in place. Deworming was done using ivermectin, before the start of the experiment to get rid of endo-parasites.

3.4 Experimental design and animal infection

Four groups of 12 mice in each cage, were randomly assigned to receive three months of intraperitoneal treatment with the confirmed parasites (Table 3.1). The first was the control group that received vehicle only (water). The second group of mice was infected with *Trypanosoma brucei rhodesiense* at a dose of 50,000 parasites. The third group was innoculated with *S. mansoni* and *T.b. rhodesiense*. The fourth group of mice was treated with *S. mansoni cercariae* (100 cercariae per mouse). There were two survival analysis groups: *T.b.r* and *T.b. r+S. mansoni* (Table 3.2). Mice were followed up to 40 days' post-infection and then sacrificed.

Table 3.1: Experimental design

Group	Treatment	No. of mice per cage
1	Control (water)	12
2	*T.brucei rhodesiense* (Infected with 50,000 parasites)	12
3	*S.mansoni &T.brucei rhodesiense* (Exposed to 100 cercariae for 9 weeks, then inoculated with *T.b.r*)	12
4	*S. mansoni* (100 cercariae)	12

Table 3.2: Survival analysis

Group	Treatment	No. of mice
1	*T.b.r* (Infected with 50,000 parasites)	10
2	*S.mansoni* &*T.b.r* (Exposed to 100 cercariae for 9 weeks, then inoculated with *T.b.r*)	10
3	*S. mansoni* (100 cercariae)	10

3.5 Harvesting of *S. mansoni* cercariae from snails

Snails of *Biomphalaria pferiferi* shedding cercariae were put in a glass beaker under a strong light, and into the surrounding water, cercariae were shed. Snail records and the number of cercariae shed per snail were kept. Snails shedding cercariae were placed into 100-ml beakers with conditioned water. The beaker was then placed under an isolated, strong light source for one hour, overheating the snails was avoided. With featherweight forceps, the snails were returned to their aquarium from the beakers. The contents of the beaker were filtered into a clean beaker. The

cercarial suspension was gently swirled with a tip fastened to a pipette, and 20-µl aliquots were withdrawn. Each aliquot was placed in a separate counting dish and two mls of water was added together with a few drops of iodine solution to kill and stain the cercariae. All of the intact cercariae in the dish were counted using a dissecting microscope. The cercariae were used to infect mice within 5 hours of harvesting (Society & Journal, 2010).

3.6 Exposure of mice to *S. mansoni* cercariae

Animals were anesthetized for about 1 hr. Dosage depended with the weight and the age of the mouse. Ketamine was used at a dosage of 0.05ml/Kg of a mouse. Mouse was weighed, anesthesia dosage calculated and the appropriate volume was injected intraperitoneally. The abdomen was shaved with a scalpel/scissors once sufficiently anesthetized and wiped with a moistened gauze sponge. The mouse was placed on its back in a restraining device that was slotted, so that unintentional movements won't interfere with the suspension containing cercariae. A ring of stainless-steel type was placed on the abdomen, and a suspension containing cercariae pipetted into the ring. The mouse was exposed to cercariae for one hr, followed by the removal of the cercarial suspension from the ring. The mouse was kept warm throughout the procedure, using a heated pad, then taken back to the cage without wiping the exposed site (Society & Journal, 2010).

3.7 Trypanosome expansion and immune suppression of donor mice

Two wild-type female mice were used to increase the trypanosome isolate stock of *Trypanosoma brucei rhodesiense* labelled KETRI 2537, recovered from a patient in Uganda, which is a derivative of EATRO 1989. Cyclophosphamide was used to immune suppress the donor mice at a dosage of 8.3mg/kg of body weight and inoculated using intraperitoneal method three days prior to infection. Cryopreserved *T. b. rhodesiense* isolate KETRI 2537 in 20µl capillary tubes were

collected from liquid nitrogen, left to defrost for the parasites to acclimatize to the room temperatures prior to inoculation. Cryopreserved Parasites in the capillary tube, were diluted using thawed phosphate buffered saline supplemented with glucose (PSG) and possibility of the parasites being alive was determined by placing a drop of the contents on a slide, cover slipped and viewed under a microscope to check whether trypanosomes were motile, this was followed by inoculating 0.2ml of the dilution to every donor mouse (Kipkorir *et al.,* 2021).

3.8 Tail Snipping procedure for parasitemia determination

Scissors were sterilized with 70% alcohol. A mouse was then retrieved from the cage and placed on top of the cage and the tip of the tail was snipped off. The tail was squeezed moderately, on a clean grease free slide, a drop of blood was collected and cover slipped as a wet preparation. The scissors were decontaminated using 70% alcohol for the next bleeding and gloves decontaminated with a disinfectant. For subsequent daily bleedings, one was needed to only disturb the previous wound.

3.9 Parasitemia Scoring (wet blood smear or buffy coat)

A microscope stage was set up for the slide and the microscope switched on, focused with x10 and examined with x40. The Scoring was done using Herbert's and Lumsden technique and parasitemia recorded (Table 3.3).

Table 3.3:Herbert and Lumsden parasitemia scoring chart

Trypanosomes/field	Antilog	Log
1/20	5.4	$2.51x10^5$
2-3/20	5.7	$5.01x10^5$
2-3/10	6.0	$1.0x10^6$
2-3//5	6.3	$2.0x10^6$
4-5/5	6.6	$3.98x10^6$
2/field	6.9	$7.94x10^6$
4/field	7.2	$1.56x10^7$
8/field	7.5	$3.16x10^7$
16/field	7.8	$6.3x10^7$
32/field (+)	8.1	$1.26x10^8$
64/field (++)	8.4	$2.51x10^8$
128/field (+++)	8.7	$5.0x10^8$
256/field (Massive)	9.0	$1.0x10^9$

3.10 Infection of mice with trypanosomes

Parasitaemia from the donor mice was completed by obtaining blood drop from the tail snip and microscopically examined by a wet preparation. The drops of blood obtained at the peak of the parasitemia, were then mixed with about 2 ml of EDTA saline-glucose (ESG) buffer (Kagira *et al.,* 2011). Improved Neubauer chamber was used to estimate the number of trypanosomes and viewed at x40 magnification under the microscope. ESG buffer at pH 8.0, using a leukopipette was used to dilute 10 times the blood solution to a last concentration of about $5.0x10^4$ trypanosomes per ml which was then injected intraperitoneally to each 12 experimental mouse of groups 2 and 3.

3.11 Behavioural assessment

In this study Rapid murine coma and behaviour scale was employed to determine the neuronal integrity and general health of mice following infection with the two parasites. Each of the 10 parameters that make up RMCBS is given a score between 0 and 2, with 0 being the lowest function and 2 representing the greatest. An animal can receive a total score ranging from 0 to 2. The mouse was positioned in the upper-left corner of an observation box with a grid floor during the first minute and thirty seconds of the assessment, and its behaviour in relation to hygiene, body position, gait, balance, and exploratory behaviour were assessed. The mouse's limb strength, reflexes, and self-preservation abilities were evaluated over the course of the following 90 seconds (Carroll *et al.,* 2010).

3.12 Evans blue assay

Evans blue assay was performed to assess the stability of the blood brain barrier. Mice were injected with 200µl of 2% Evans Blue in 0.95% NaCl (w/v) intravenously and left to sit for 1 hour

and then sacrificed. Brains were removed, photographs taken. The weight of these brains were taken and placed into 2 ml Formamide, incubated for 48 hours at 37°C on shaker. Evans Blue extravasation was quantified after 2 days as follows: 100µl of the incubated formamide/brain solution was placed in each well (triplicate). For the standard curve, it was diluted -Evans Blue to 200µg/ml (1:100) with formamide (starting concentration). Standard diluted 1:2 in each well (triplicates) with formamide. Concentration of Evans blue was measured using an ELISA reader at 620nm.

3.13 Determination relative organ weight

At the conclusion of the experiment, the mice and samples of their brains, livers, kidneys, and spleens were weighed. The ratio of the animal's weight of the organ, to its body, then multiplied by 100%, was then used to determine the relative organ weights. An analytical electronic balance was used for all weight measurements (Mettler PM34, DoltaRange®).

3.14 Hematological assay

Blood was drawn through a heart puncture at the conclusion of the experiment and placed in EDTA tubes for hematological parameter analysis using a hematology autoanalyser (Sysmex XS 1000i Hematology Analyzer, WA, USA).

3.15 Liver and kidney function assay and lipid profile elucidation

The serum from each mouse was collected in Eppendorf tubes then separated and used for estimation of liver and kidney function. Aspartate aminotransferase, Alanine aminotransferase bilirubin and Alkaline phosphatase transferase were used as markers for liver function, while creatinine, urea levels were estimated for kidney function. Additionally, lipid profile levels including triglycerides, cholesterol and high-density lipoprotein were estimated from the serum.

These estimations were done by use of an autoanalyser (Integra-400 plus analyzer, Roche, Basel, Switzerland).

3.16 Cytokines analysis by sandwich ELISA method

ELISA 96 well plates were bound with the capture antibody that is specifically for cytokines of concern. Plates were incubated overnight at 4°C then blocked with a blocking buffer of 150 µL/well for about 1 hour. Samples, Standards and the detection Ab cocktail were incubated same as the standard ELISA method. The plate was washed and streptavidin dye (interferon-gamma and tumor necrotic factor-alpha) and Avidin (interleukin-10) conjugate diluted using dilution buffer in a 50 µL/well and incubated for 30 min in the dark. The plate was washed and dried thoroughly. The bottom of the plate was cleaned and then placed into the plate reader at 590 and 465 nm and OD read.

3.17 Griess reaction (for Nitric oxide) Assay

Griess reagent (10 µl per microplate well) was made by mixing together same volumes of components A and B in an Eppi. 10 µl of Griess reagent + 75 µl of samples (duplicate or triplicate) + 65 µl of A. dest was mixed well together in the wells, then incubated for 30 min. Blank was prepared (row H1-3): by mixing together 10 µl of Griess reagent + 140 µl of A. dest. The standard was diluted: the sdt at 1/10 (e.g.: 50 µl sdt in 450 µl A. dest) in order to get a concentration of 100 µM in row A1-3 and a serial dilution made from row B-G1-3 (50-1 µM). Get in the sdt wells a total volume of 75. B-G1-3 75 µl of A1-3. dest and 150 µl of 100 µM sdt was put in row A and then the serial dilution performed. To the sdt wells 10 µl of Griess reagent +65 µl of A dest was added, and measured at 548 nm.

3.18 Sample preparation for glutathione estimation

Reduced glutathione (GSH) assay was used to detect the levels of oxidative stress in the brain, liver, kidney, and spleen organs. After being instantaneously frozen in dry ice, the organs were kept in liquid nitrogen until they could be used for biochemical studies in accordance with the goals of the study. Whole liver, spleen, brain, heart, kidney and lungs that was snap-frozen was made uniform on water with ice at (4^{o}C) in 0.5 mls of 0.25 M sucrose, Hepes-Tris 5mM, of a pH 7.4, with protease inhibitor mixture to a final concentration of 10% (w/v). Homogenates were then aliquoted into microfuge tubes of about 0.5 mls and stored under liquid nitrogen awaiting analysis. GSH concentration was calculated by adding 20µl each of the standards and the brain supernatant into a 96-well plate in triplicates, followed by 100µl of DNTB. The plate was then incubated at 37°C for 10 minutes, and the absorbance was read at 405 nm using a microplate reader at an interval of 30 seconds, i.e., 30sec, 60sec, 90sec, and 120sec. For the purpose of determining the GSH in the brain samples, the time window that produced the best GSH standard graph was chosen.

3.19 Malondialdehyde estimation

Thiobarbituric acid reactive species assays were used to evaluate malondialdehyde levels in mouse brains to determine the degree of lipid peroxidation (Draper and Hadley, 1990). Brains from mice were homogenized at day 6 in cold phosphate buffer, pH 7.4 with butylated hydroxytoulene (BHT) (final concentration 0.2%). Brain homogenate samples (0.5 ml) were mixed with equal volume of thiobarbituric acid 0.67% and heated at 92-96^{o}C for 30 min. Thiobarbituric acid reactive species production was quantified at 535nm using a spectrometer. A panel of chloroform: methanol (2:1, v: v) was used to extract lipids, and the resultant organic phase was measured at 234 nm. Results were presented in milligrammes of protein and malondialdehyde.

3.20 Histopathological examination

According to the experimental plan, mice from each group were sacrificed 60 days after treatment. With 10% ketamine, the mice were euthanized. For histopathology, the liver, spleen, and brain were removed, initially washed in PBS, kept in a 10% formalin fixative, and then frozen at -70° C until distribution. They were treated in arising grades of ethanol and fixed in paraffin wax in the automatic tissue processor. Tissue sections of 5µm width were sectioned in a microtome and placed on Mayer's egg albumin-coated glass slides. Every tissue section was dewaxed in twofold variations of xylene for two minutes, equally rehydrated over descending grades of ethanol for 30 minutes, and additionally rinsed in clean water to avoid abrupt contraction and busting of the cells. The tissue sections were treated with H and E stains (1%) for two minutes. The sections were subjected to ascending grades of alcohol for 30mins, washed in three changes of xylene and then fixed in DPX, and microscopically examined using oil immersion (x400)(Feldman & Wolfe, 2014).

3.21 Statistical analysis

Utilizing the software tool graph pad prism, statistical analysis was conducted (Version 5.0). To compare the mono-infected and co-infected groups with controls, a one-way ANOVA was conducted, trailed by Tukey's post hoc test designed for internal comparison. Survival analysis was performed using the Log-rank (Mantel-Cox) Test, while the examination of parasitemia used an unpaired, two-tailed student t-test. The results were given as a mean ± with significance set at $p<0.05$.

CHAPTER FOUR: RESULTS

4.1 Effects of *S. Mansoni* On the host physiological and Hematological events following *T. b. r* induced infection

4.1.1 Effects of Co-infection on Survival rate and parasitemia levels

This study sought to elucidate the impact of co-infection of Balb C mice with *S. mansoni* and *T.b.r* on the outcome of HAT through analysis of the survival time. It was observed that the group of mice co-infected with the two parasites succumbed to the infection within 16 to 31 days' post-infection, while *T.b.r* alone infected mice succumbed to the infection between 23 to 39 days' post infection. Accordingly, the *T.b.r* infected alone group of mice had the median survival time of 35 days' post-infection, in comparison to only 26 days' post-infection for the co-infected mice. However, the group of mice infected with *S. mansoni* alone registered no mortality, thus the mice in this group were sacrifice after 45 days' post infection due to the development of symptoms of anemia (Fig. 4.1A). It was surprising to observe that the survival was independent of peripheral parasitemia, as an analysis of parasitemia using Giemsa stain showed the percentage of iRBCs was comparable across all the groups (Fig. 4.1B).

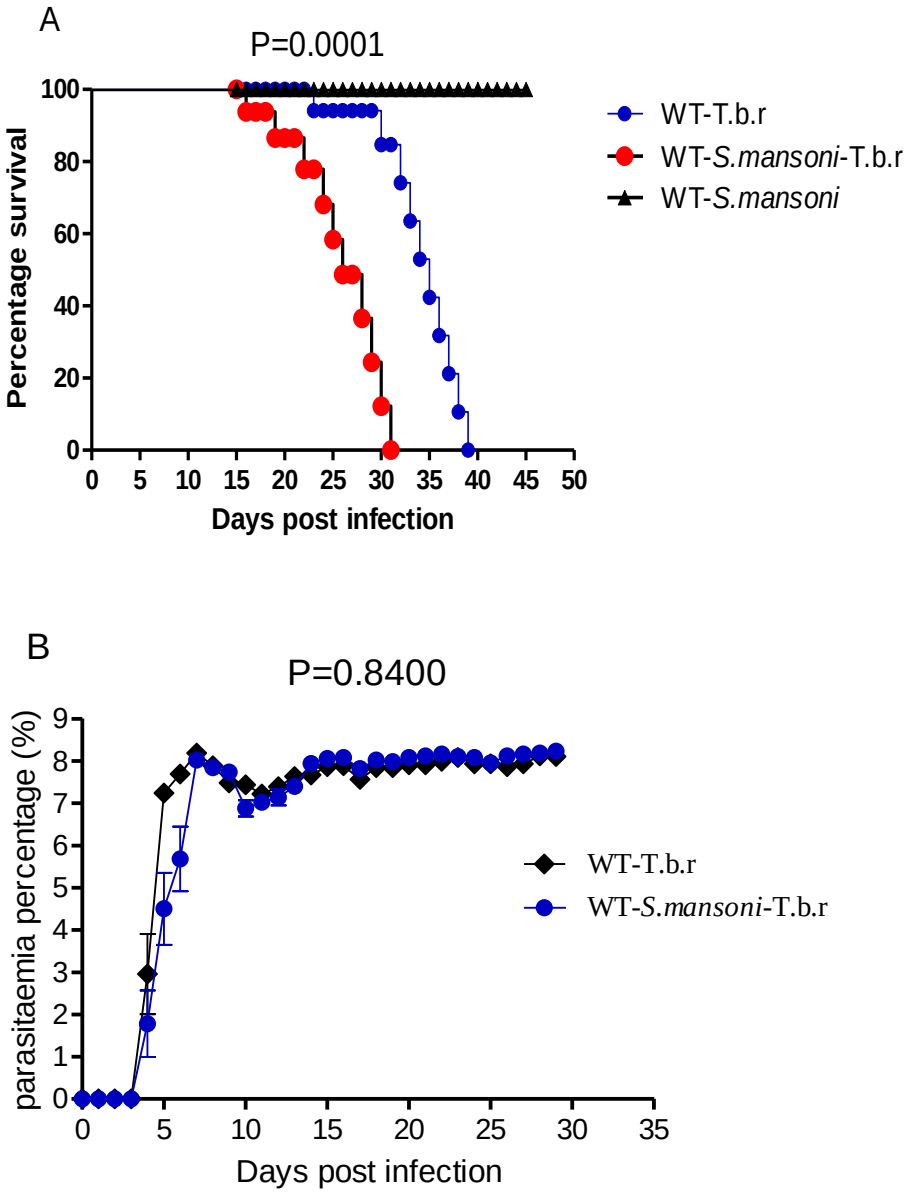

Figure 4.1: Effects of *S. mansoni* and *T.b.r* co-infection on Survival time and Parasitemia levels.

Survival time analyzed by Log- rank (Mantel- Cox) Test while parasitemia was analysed by unpaired, two tailed student t- test. Indicated significance level of *P< 0.05

4.1.2 Effects of *S. mansoni* and *T.b.r* co-infection on Rapid Murine Coma and Behavioral Scale

To demonstrate Neurological injury and health status of the mice, RMCBS analysis was employed in this study. It was observed that at day one, mice from all groups were healthy as depicted by high RMCBS scores. Notably, infection of mice with *T.b.r* alone resulted in a general RMCBS decline from 15 days' post infection; indicating compromised neurological integrity (Fig. 4.2). In addition, there was a sharp decline in the total RMCBS scores especially in the mice co-infected with *T.b.r* and *S. mansoni*. However, mice infected with *S. mansoni* registered minimal neurological injury relative to naïve group of mice.

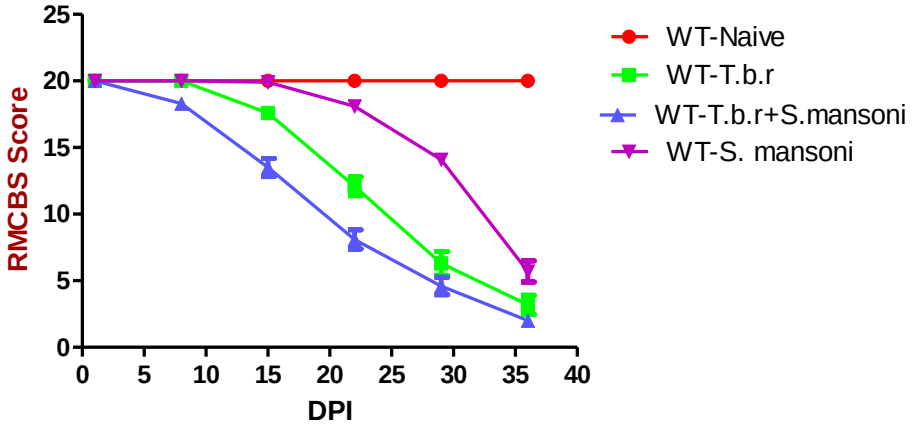

Figure 2.2A and B :Effects of *S. mansoni* and *T.b.r* co-infection on neurological parameters.

Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test. Bars represent mean ± SEM.

4.1.3: Effects of *S. mansoni* and *T.b.r* co-infection on individual RMCBS parameters

This study also evaluated deterioration of health among mice upon infection with parasites, by performing detailed analysis of individual parameters of the Rapid Murine Coma and Behavioral Scale. Hereby, mice infected with *T.b.r* or *S. mansoni* alone showed various clinical signs which included their inability to coordinate the body, motor performance related behaviors, changes in aggression and gait compared to the naïve group. However, mice co-infected with S. *mansoni* and *T.b.r* manifested marginal decline in clinical signs associated with body balance, motor performance, aggression and gait though not statistically significant (Fig. 4.3 A-D respectively).

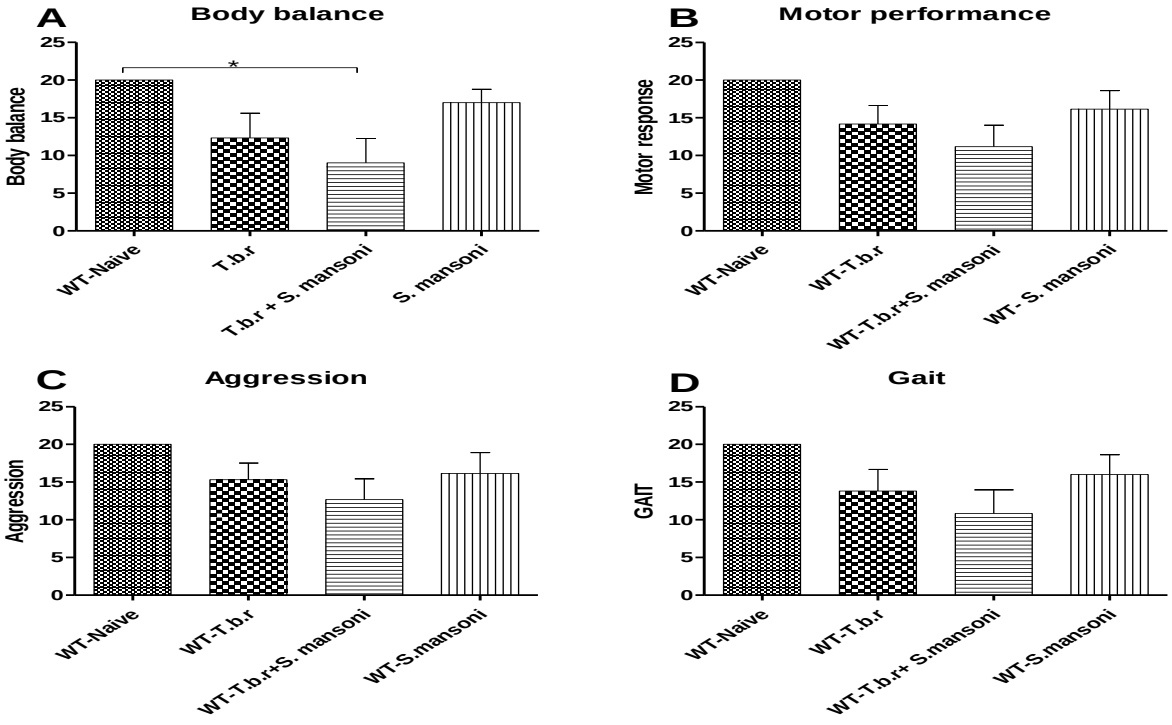

Figure 4.3: Effects of *S. mansoni* and *T.b.r* co-infection on individual RMCBS parameters.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05.

4.1.4 Effects of *S. mansoni* and *T.b.r* co-infection on Blood brain barrier (BBB)

Evans blue assay was performed to assess the stability of the blood brain barrier. Evans blue binds to serum albumin. Serum albumin cannot permeate the BBB if it is stable, however when there is permeability of the BBB, then there is infiltration of the albumin-dye from the blood into the brain resulting in staining the brains blue. Mice infected with *T.b.r* (Fig 4.4B) alone exhibited a compromised BBB as depicted by the staining of the brain blue. A similar outcome was observed in the group of mice co-infected with *S. mansoni* and *T.b.r* (Fig 4.4C), suggestive of a compromised BBB. In stark contrast, brains of mice infected with *S. mansoni* alone (Fig. 4.4D) and naïve group of mice (Fig. 4.4A) remained completely bright. Evidently, there was extravasation of the Evans blue dye in brains of *T.b.r* infected mice in comparison to the naïve group demonstrated by high colorimetric values (Fig. 4.5A). Of noteworthy, the amount of Evans blue dye that penetrated to the brains of the mice co-infected with the two parasites was significantly increased ($p<0.05$) relative to the *T.b.r* infected mice or *S. mansoni* group of mice, demonstrating a compromised BBB. There was no significance difference in the amount of Evans blue that was retained in the blood ($P< 0.0713$; Fig. 4.5B).

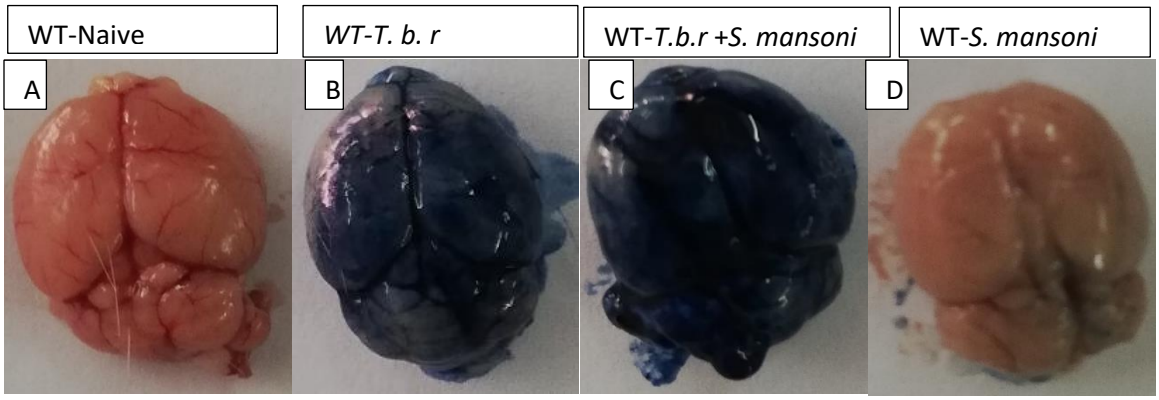

Figure 4.4: Effects of *S. mansoni* and *T.b.r* co-infection on Blood brain barrier.

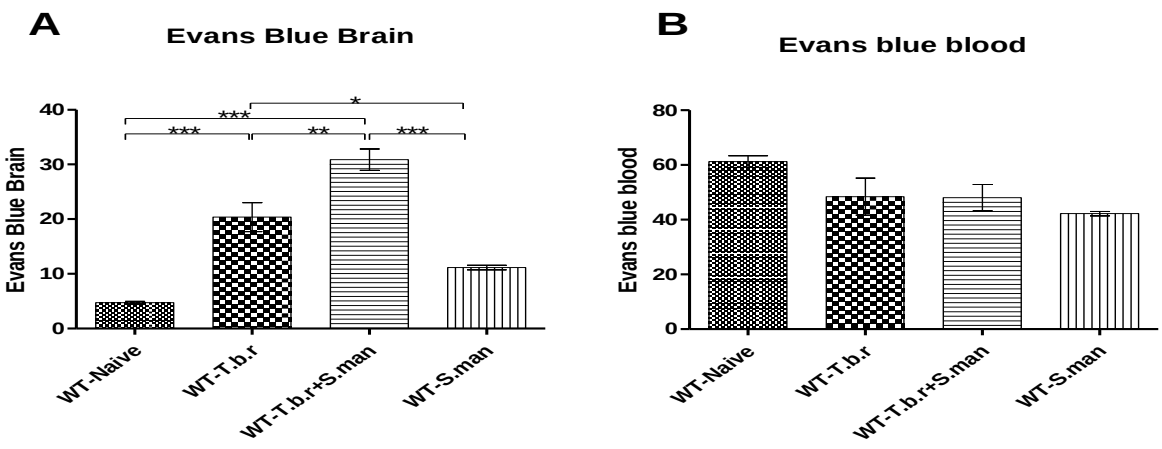

Figure 4.5: Effects *S. mansoni* and *T.b.r* on BBB by extravasation of Evans blue dye in the brain (A) and blood Evans blue dye levels (B).

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *$p \leq 0.05$; **$p<0.01$; *** $p<0.001$.

4.1.5: Effects of *S. mansoni* and *T.b.r* co-infection on organ weight

A significant increase in the weight of the spleen was noted following infection of mice with *T.b.r* (Fig. 4.6A). Similarly, there was a significant increase in the weight of the spleen following co-infection of mice with *S. mansoni* and *T.b.r.* A comparative analysis of mice infected with *T.b.r* alone revealed that there was a significant increase in the relative weight of the liver, however there was no statistical difference in the relative weight of the liver among the *S. mansoni* and co-infected group of mice (Fig 4.6B). However, there was no significance difference in the weights of the lungs, kidney and heart (Fig. 4.6C- E respectively).

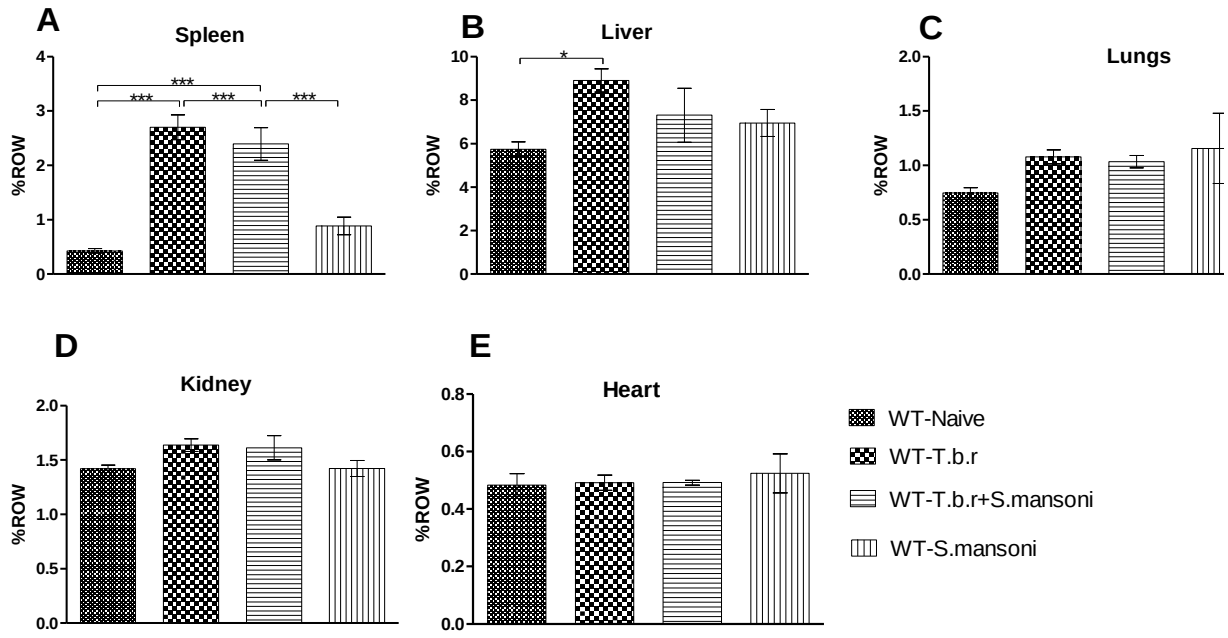

Figure 4.6: Effects of *S. mansoni* and *T.b.r* co-infection on organ weight.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; *** p<0.001.

4.1.6 Effects of *S. mansoni* and *T.b.r* co-infection on hematocrit levels

Mice infected with *T.b.r* or *S. mansoni* alone significantly (P < 0.0001) suppressed hematocrit levels relative to the naïve group (Fig. 4.7). However, co-infection of mice with the two parasites resulted in a further significant (P < 0.0001) depletion of the hematocrit levels.

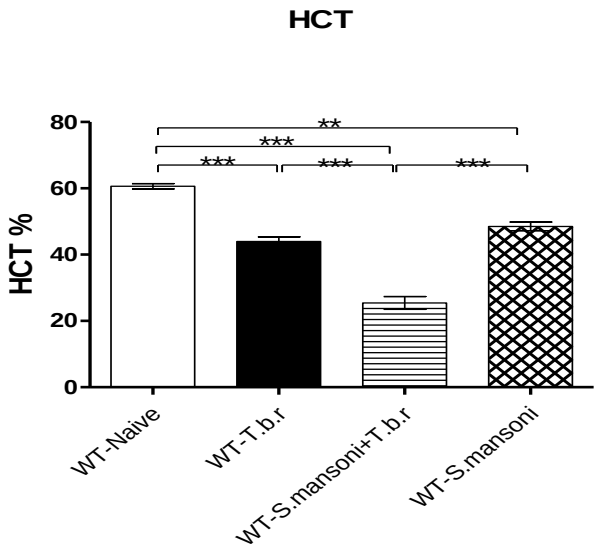

Figure 4.7: Effects of *S. mansoni* and *T.b.r* co-infection on Hematocrit levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of **p<0.01; *** p<0.001.

4.1.7 Effects of *S. mansoni* and *T.b.r* co-infection on Red blood cells and Hemoglobin levels

Infection of mice with *T.b.r* or *S. mansoni* alone resulted in a significant (P<0.0001) decrease in the levels of RBC's and hemoglobin (Fig. 4.8A, B respectively). Similarly, the co-infected mice with both *S. mansoni* and *T.b.r* registered a further significantly decreased levels of RBC's (P<0.0001; Fig. 4.8A) and Hemoglobin (P<0.0001; Fig. 4.8B) respectively.

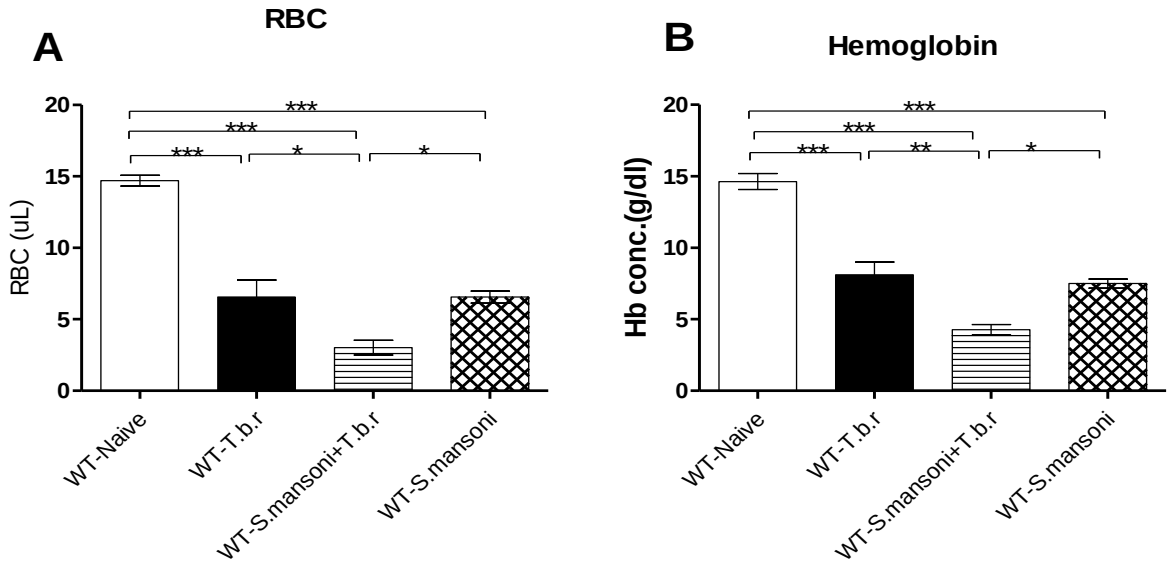

Figure 4.8: Effects of *S. mansoni* and *T.b.r* co-infection on Red blood cells and Hemoglobin levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.1.8: Effects of *S. mansoni* and *T.b.r* co-infection on MCV, MCH, MCHC, RDW-SD and RDW-CV levels

There was a significant reduction in the levels of MCV, MCH, MCHC in mice infected with *T.b.r* or *S. mansoni* alone (Fig.4.9A-C). Consequently, mice co-infected with *S. mansoni* and *T.b.r* resulted in a markedly significant low levels of these RBC indices (P<0.0001), indicative of microcytic hypochromic anemia. Exposure of mice to *T.b.r* or *S. mansoni* alone lead to a significantly lower RDW-SD in comparison to the levels of naive mice (Fig.4.9D). However, mice co-infected with *S. mansoni* and *T.b.r* caused marked elevation in the levels of RDW-SD (P<0.0365). No significance difference in the levels of RDW-CV (Fig.4.9E) among all groups of mice was noted.

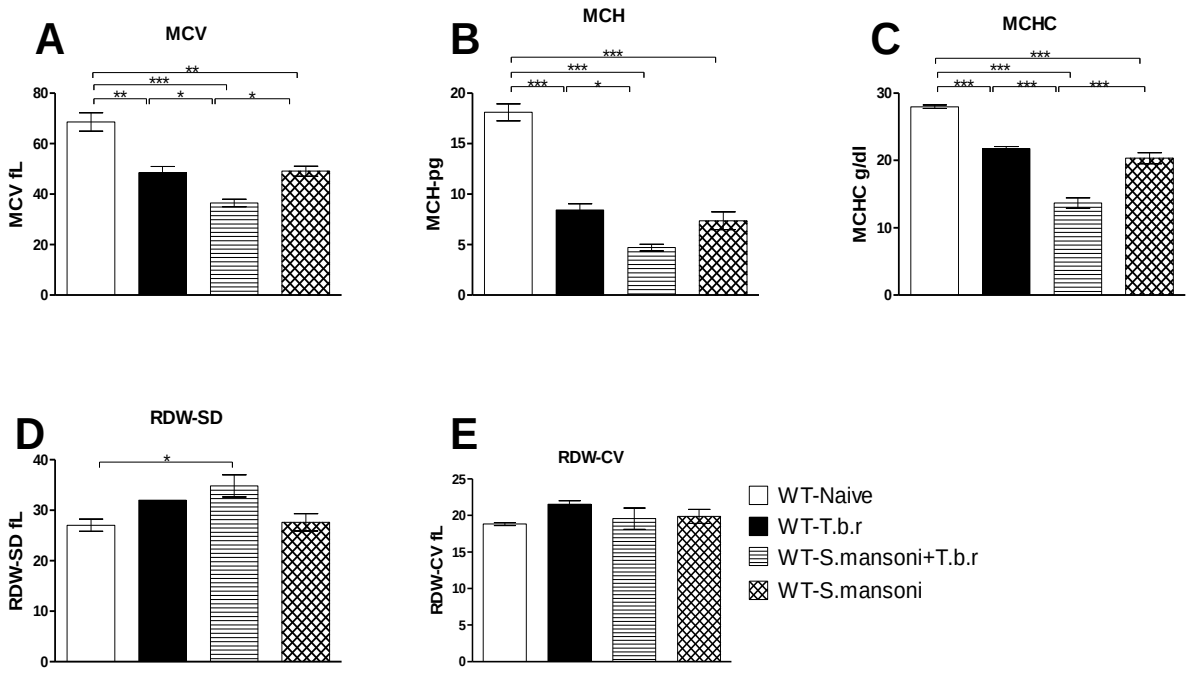

Figure 4.9: Effects of *S. mansoni* and *T.b.r* co-infection on MCV, MCH, MCHC, RDW-SD and RDW-CV levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.1.9: Effects of *T.b.r* and *S. mansoni* co-infection on white blood cells levels

WBC levels were significantly elevated (P<0.0047) in a group of mice infected with *T.b.r* or *S. mansoni* alone compared to the naïve group of mice. On the other hand, co-infected group of mice had a significant elevated levels of WBC compared to the naïve group of mice (P<0.0047, Fig. 4.10).

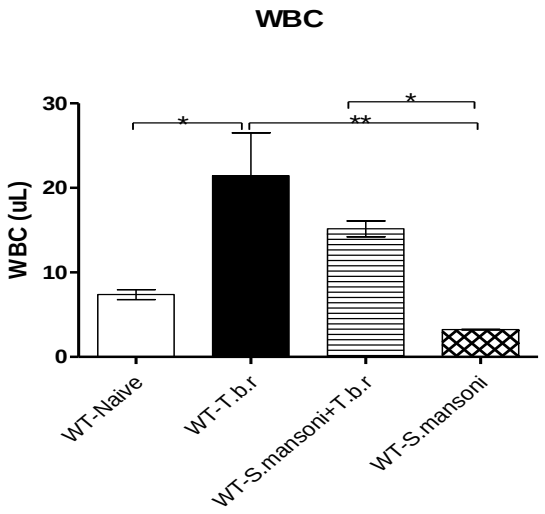

Figure 4.10: Effects of *S. mansoni* and *T.b.r* co-infection on white blood cells levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01.

4.1.10: Effects of *S. mansoni* and *T.b.r* co-infection on WBC differential count levels

Mice infected with *T.b.r* or *S. mansoni* alone resulted in a significantly elevated levels of WBC differential count of lymphocytes (P ≤0.0002); neutrophils (P< 0.0001); monocytes (P< 0.0001) and eosinophils (P ≤0.0004) (Fig. 4.11A- D) respectively compared to the naïve group. These WBC sub-types were also significantly elevated in mice co-infected with *S. mansoni* and *T.b.r*. Intriguingly, *T.b.r* or *S. mansoni* alone infected group of mice, resulted in elevated levels of basophils (P ≤0.0177) relative to the naïve group of mice. However, mice co-infected with *S. mansoni* and *T.b.r* showed a significant (P ≤0.0177) depletion in the levels of basophils count (Fig. 4.11E).

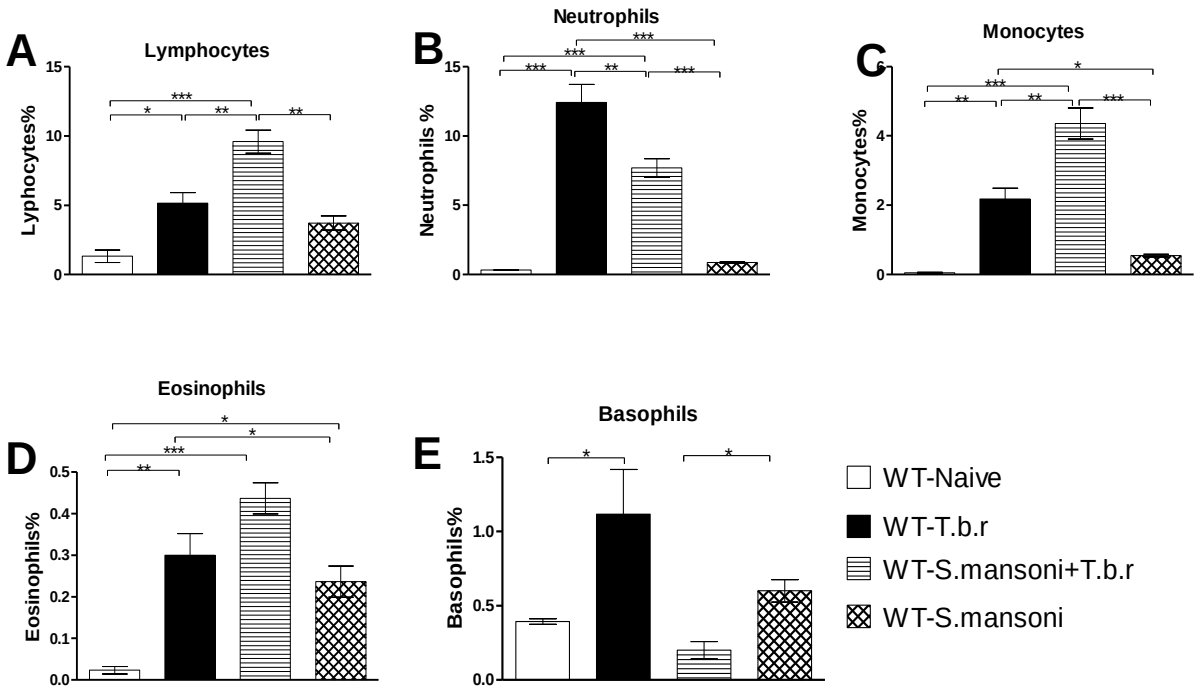

Figure 4.11: Effects of *S. mansoni* and *T.b.r* co-infection on WBC differential count levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.1.11 Effects of *S. mansoni* and *T.b.r* co-infection on platelets and its subtypes levels

The effect of infection of mice with the two parasites on the levels of platelets and its subtypes was analyzed in this study. It was noted that *T.b.r* or *S. mansoni* alone infected mice exhibited significantly elevated (P≤ 0.0112) levels of platelet count relative to the naive group mice (Fig. 4.12A). However, co-infection of mice with *S. mansoni* and *T.b.r* significantly (P≤ 0.0112) suppressed the total platelet count indicative of thrombocytopenia. The other subtypes/indices including Platelet Distribution Width (PDW), plateletcrit (PCT), Platelet Large Cell-Ratio (P-LCR) and Mean Platelet Volume (MPV) values were significantly (P≤ 0.0001; P≤ 0.0081; P≤ 0.0229; P< 0.0001) elevated in the *T.b.r* or *S. mansoni* alone infected mice in comparison to the

naïve group of mice. However, the same values were significantly depleted in the co-infection group of mice (Fig. 4.12 B-E respectively).

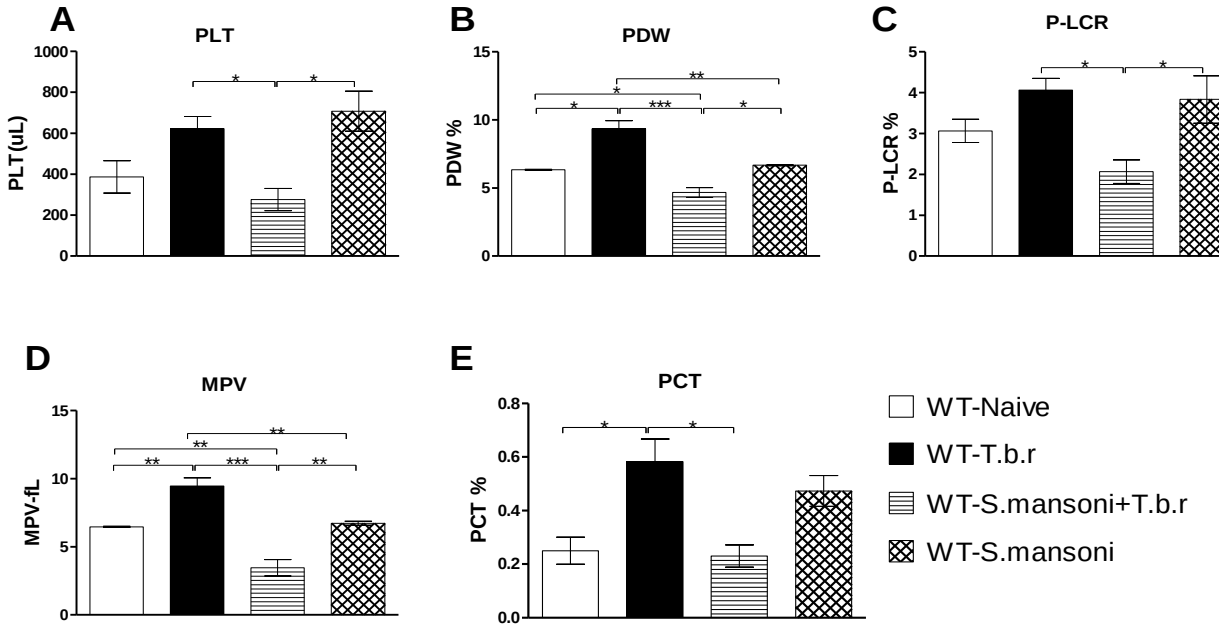

Figure 4.12: Effects of *S. mansoni* and *T.b.r* co-infection on platelets and its subtypes levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.2 The role of *S. Mansoni* on HAT driven biochemical and immune regulation in a mouse model

4.2.1 Effects of *S. mansoni* and *T.b.r* co-infection on creatinine and urea levels

In this study, creatinine and urea levels were significantly elevated in *T.b.r* or *S. mansoni* alone infected group of mice in comparison to naive group. Consequently, mice co-infected with *S. mansoni* and *T.b.r* resulted in a more significantly higher levels of creatinine and urea indicating severe kidney damage (P< 0.0001; P ≤0.0004) (Fig. 4.13A and B) respectively.

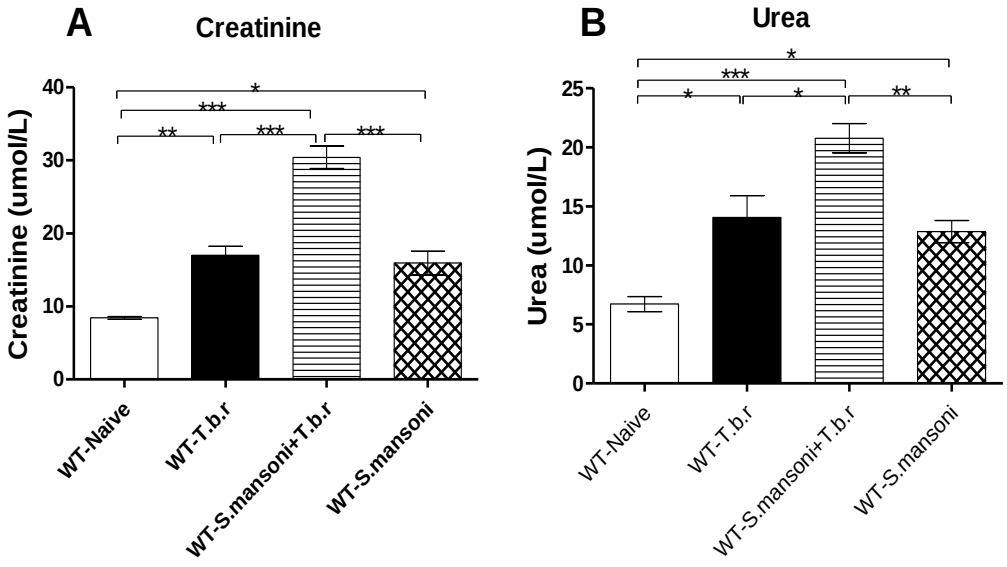

Figure 4.13: Effects of *S. mansoni* and *T.b.r* co-infection on creatinine (A) and urea(B) levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.2.2 Effects of *S. mansoni* and *T.b.r* co-infection on uric acid levels

Significant (P< 0.0001) elevation of serum uric acid levels was observed in *T.b.r* or *S. mansoni* alone infected mice compared to the naïve group. However, upon co-infection of mice with *S. mansoni,* and *T.b.r* uric acid levels were double fold elevated, suggestive of severe kidney and liver damage (P < 0.0001; Fig. 4.14).

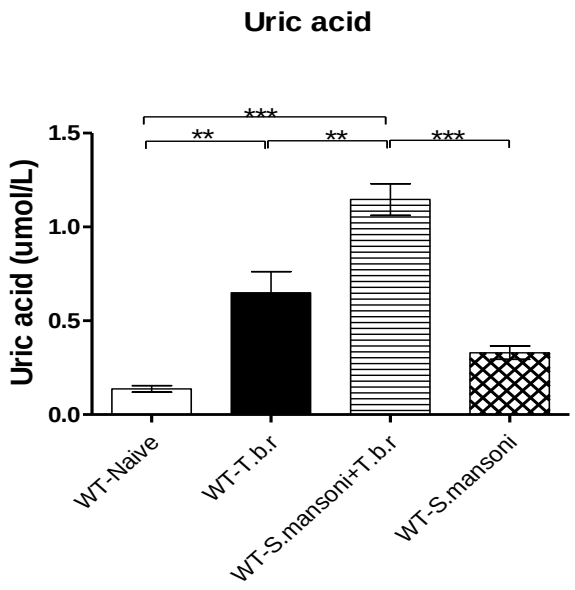

Figure 4.14: Effects of *S. mansoni* and *T.b.r* co-infection on uric acid levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of **p<0.01; *** p<0.001.

4.2.3 Effects of *S. mansoni* and *T.b.r* co-infection on AST and ALT levels and ratio

Infection of *T.b.r* or *S. mansoni* significantly elevated levels of serum ALT and AST in comparison to naive group mice (P≤ 0.0181 and P< 0.0001; [Fig. 4.15A and B] respectively). In a similar manner, significantly higher levels of ALT and AST were noted among the group of mice co-infected with *T.b.r* and *S. mansoni* (P≤0.0181 and P<0.0001); [Fig. 4.15A and B] respectively), denoting liver pathology Additional findings showed that the ratio of AST to ALT was comparable across all the groups (P= 0.2703; Fig.4.15C).

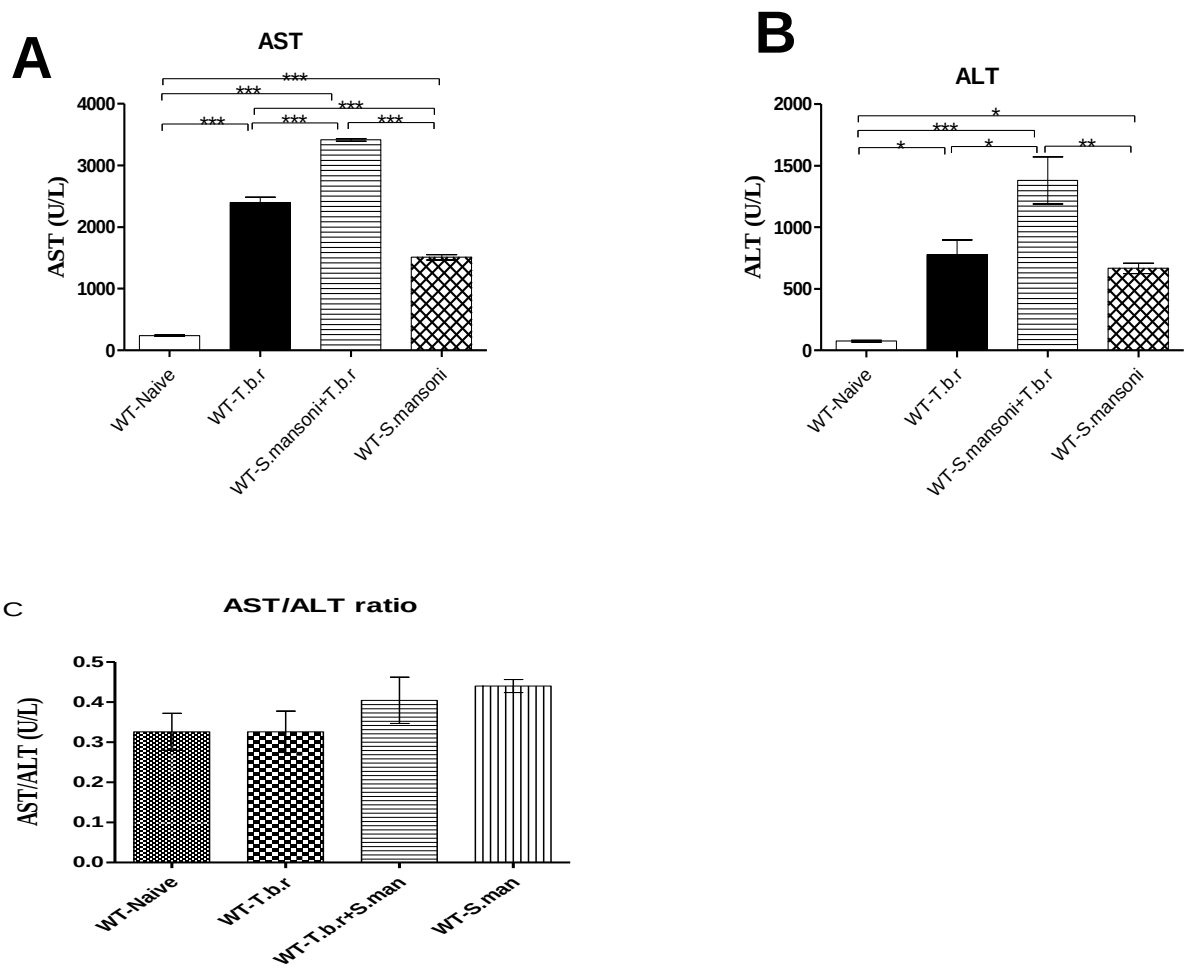

Figure 4.15: Effects of *S. mansoni* and *T.b.r* co-infection on AST and ALT levels and ratio.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.2.4: Effects of *S. mansoni* and *T.b.r* co-infection on direct, total bilirubin, ALP and total protein levels

Significantly heightened levels of serum direct bilirubin, total bilirubin and alkaline phosphatase levels were observed in *T.b.r* or *S. mansoni* infected group of mice compared to naïve group mice

(P≤ 0.0008, P≤ 0.0002, and P< 0.0001 respectively; Fig. 4.16A- C). Of north worthy, the mice co-infected with *S. mansoni* and *T.b.r* had significantly elevated levels of direct bilirubin, total bilirubin and ALP (P≤0.0008, P≤ 0.0002 and P< 0.0001 respectively. However, there was no statistical difference in total protein across all the groups (Fig. 4.16D).

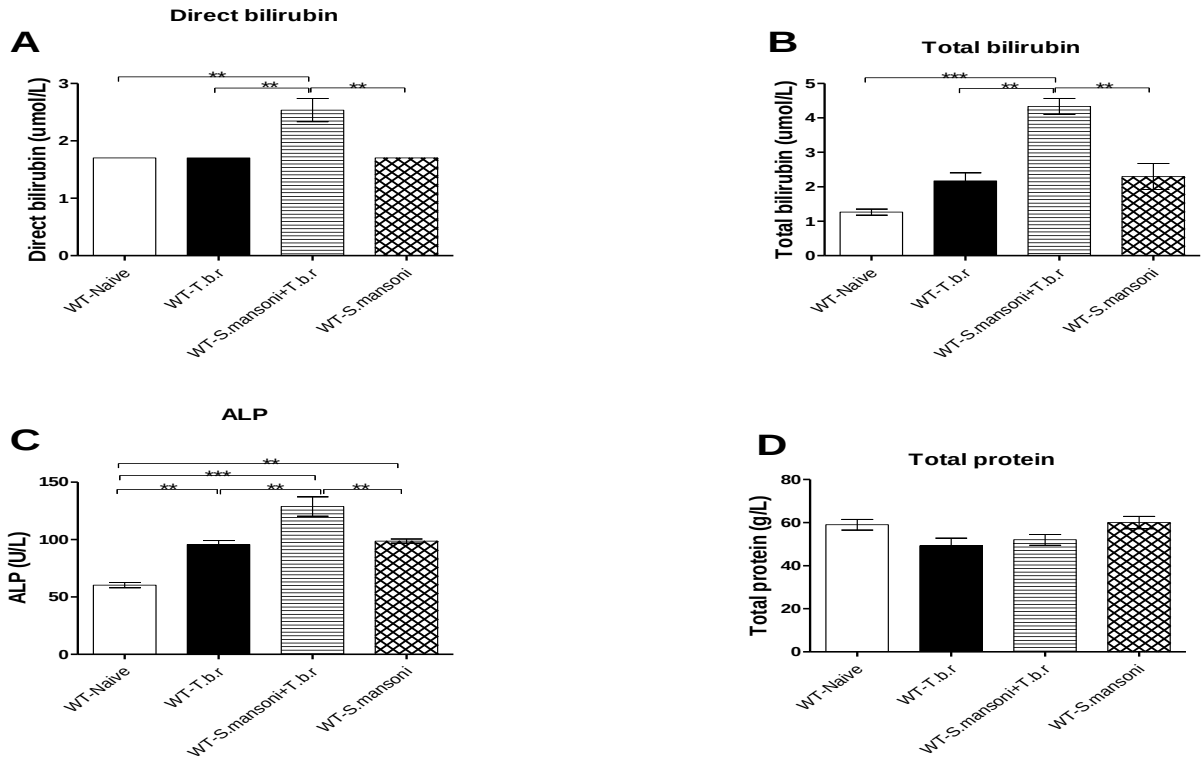

Figure 4.16: Effects of *S. mansoni* and *T.b.r* co-infection on Direct, total bilirubin, ALP and total protein levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.2.5 Effects of *S. mansoni* and *T.b.r* co-infection on albumin levels

Albumin levels decreased significantly in *T.b.r* or *S. mansoni* alone infected mice in comparison to naive group (P≤ 0.0001; Fig. 4.17). Similarly, co-infection of mice with *S. mansoni* and *T.b.r*,

resulted to markedly depleted albumin levels indicative of both liver and kidney damage (Fig. 4.17).

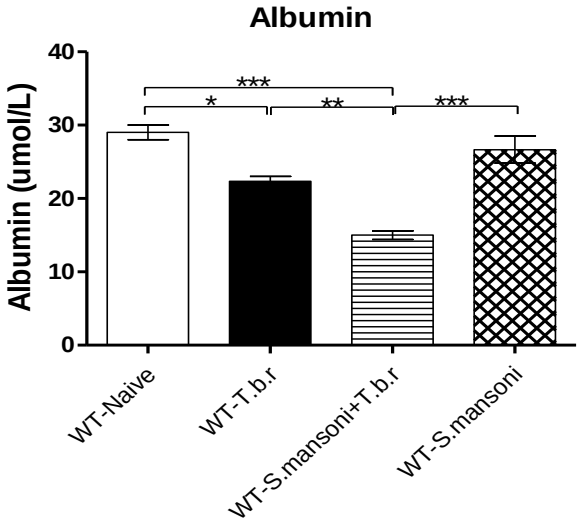

Figure 4.17: Effects of *S. mansoni* and *T.b.r* co-infection on albumin levels

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.2.6 Effects of *S. mansoni* and *T.b.r* co-infection on Lipid profile levels

Findings from this study, depicted that *T.b.r* or *S. mansoni* alone infected mice registered a significant elevation in cholesterol and triglycerides levels in comparison to naive group of mice (P≤0.0002 and P≤ 0.0015 respectively; Fig. 4.18A and B). Noticeably, mice co-infected with the two parasites had significantly elevated levels of cholesterol and triglycerides (P≤0.0002; P≤ 0.0015) respectively. Meanwhile, *T.b.r* or *S. mansoni* alone infected mice showed significantly (P<0.0001) diminished high density lipoproteins in comparison to the naive group of mice. Mice co-infected with *S. mansoni* and *T.b.r,* however resulted in a more significant (P<0.0001; Fig.4.18C) decrease in HDL levels.

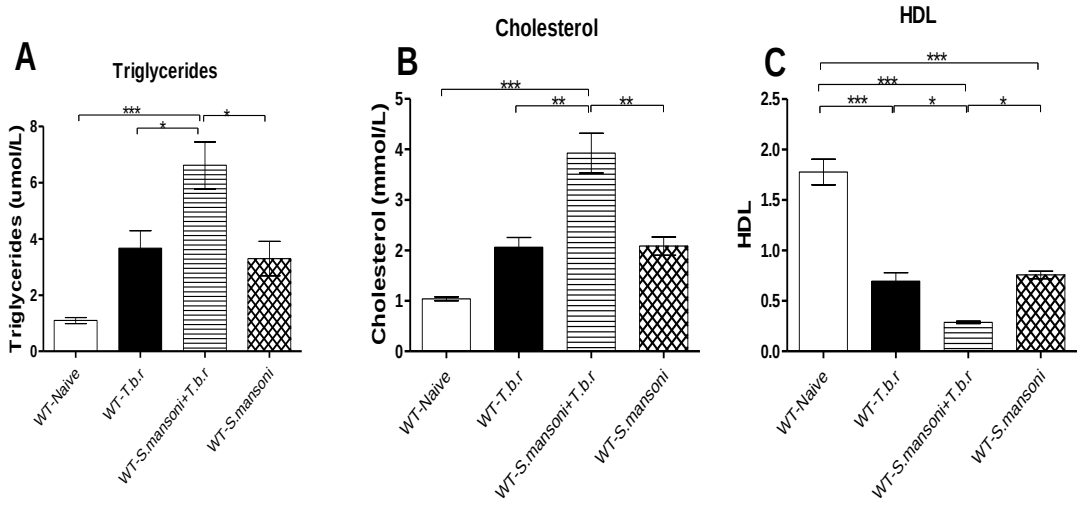

Figure 4.18: Effects of *S. mansoni* and *T.b.r* co-infection on Lipid profile levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.2.7: Effects of *S. mansoni* and *T.b.r* co-infection on Pro and anti-inflammatory cytokines levels and ratio

Serum levels of IFN-γ and TNF-α was measured from the serum samples to assess the extent of inflammation during the infection process. Results from this study clearly indicates a significant elevation (P< 0.0001) of serum IFN-γ and TNF-α in mice infected with either *T.b.r* or *S. mansoni* alone relative to naive group of mice (Fig. 4.19A and B). Interestingly, mice co-infected with *S. mansoni* and *T.b.r* showed a markedly pronounced rise of these pro-inflammatory cytokines (P< 0.0001). Additionally, *T.b.r* or *S. mansoni* alone infected mice exhibited significant reduction of serum IL-10 relative to the naïve group of mice (P≤0.0008; Fig. 4.19C). Remarkably co-infection of mice with *S. mansoni* and *T.b.r*, resulted in significantly (P≤ 0.0008) downregulation of serum IL-10 (P≤0.0008; Fig. 4.19C). A further analysis of the ratio of the pro-inflammatory to anti-inflammatory cytokines TNF-α:IL-10 (Fig.4.19D) and INF-γ-:IL-10 ratio (Fig. 4.19E) in *T.b.r* or

S. mansoni alone infected mice was significantly elevated (P<0.0001) in comparison to naive group of mice. Moreover, mice co-infected with *S. mansoni* and *T.b.r* registered a pronounced imbalance between the pro and anti-inflammatory cytokines (P<0.0001; Fig. 4.19D and E).

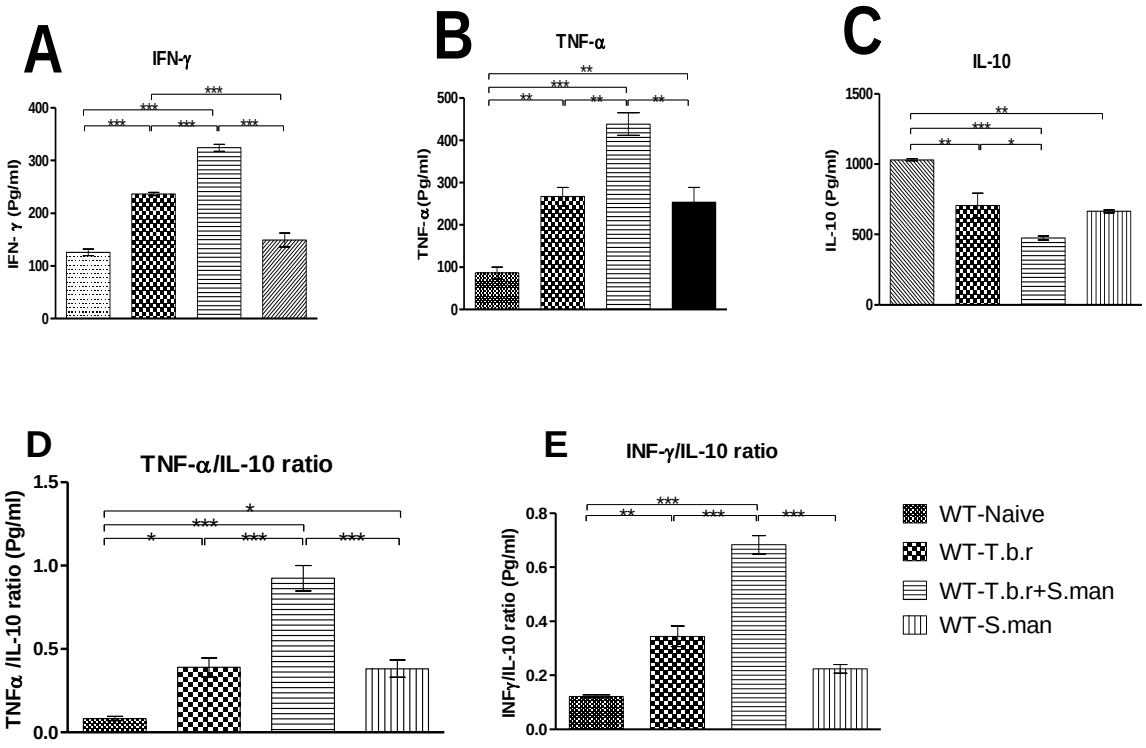

Figure 4.19: Effects of *S. mansoni* and *T.b.r* co-infection on Pro and anti-inflammatory cytokines levels and ratio.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.2.8 Effects of *S. mansoni* and *T.b.r* co-infection on Immunoglobulin levels

Immunoglobulin levels were significantly elevated in *T.b.r* infected mice in comparison to the naïve group of mice (P<0.0037). Notably, the group of mice infected with either *S. mansoni* alone

or co-infected with the two parasites registered a statistically significant decrease in the levels of immunoglobulin relative to *T.b.r* infected group of mice (P<0.0037; Fig. 4.20).

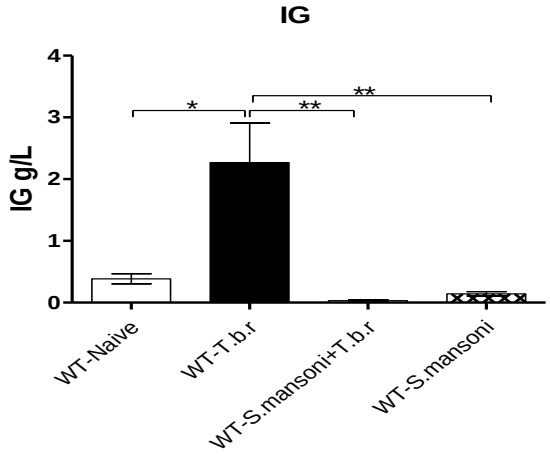

Figure 4.20: Effects of *S. mansoni* and *T.b.r* co-infection on Immunoglobulin levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01.

4.3 The impact of *S. mansoni* and *T.b.r* on inflammation and oxidative stress in a mouse model

4.3.1 Effects of *S. mansoni* and *T.b.r* co-infection on Nitric oxide levels

In this study, *T.b.r* or *S. mansoni* alone infected group of mice exhibited a significant elevation of nitric oxide (NO) levels when compared to naïve group mice (P< 0.0001). On the other hand, *S. mansoni* and *T.b.r* infected group of mice portrayed more significantly elevated levels of nitric oxide (NO) (P< 0.0001); (Fig. 4.21).

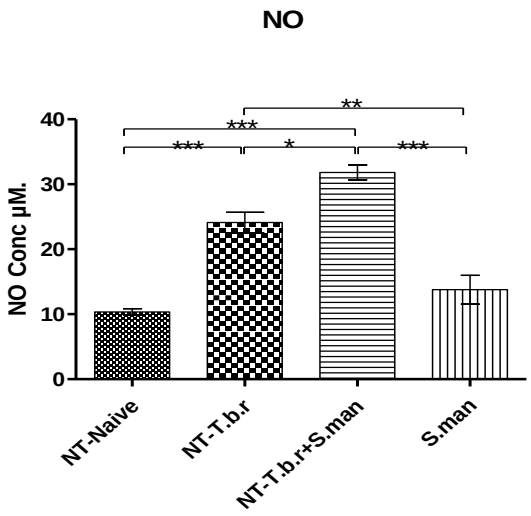

Figure 4.21: Effects of *S. mansoni* and *T.b.r* co-infection on Nitric oxide levels.

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.3.2 Effects of *S. mansoni* and *T.b.r* co-infection on glutathione levels in the brain, liver, lungs, kidney and spleen

Infection of mice with *T.b.r* or *S. mansoni* alone resulted in significant (P < 0.0001) reduction of glutathione (GSH) levels in the brain and liver (P < 0.0001; P < 0.0001) compared to the naïve group of mice. Herein, co-infection of mice with *S. mansoni* and *T.b.r* resulted in a more marked decline in liver and brain cellular levels of GSH (P < 0.0001; P < 0.0001); Fig. 4.22A and B), denoting active oxidative stress. Interestingly, lungs, kidney and spleen cellular levels of GSH were significantly elevated in *T.b.r* or *S. mansoni* alone infected mice compared to the levels in the naive group of mice (P≤ 0.0003; P< 0.0001). An elevated level of cellular GSH was equally observed in the lungs, kidney and spleen from mice infected with both *S. mansoni* and *T.b.r* (P≤ 0.0003; P< 0.0001; Fig. 4.22 C- E).

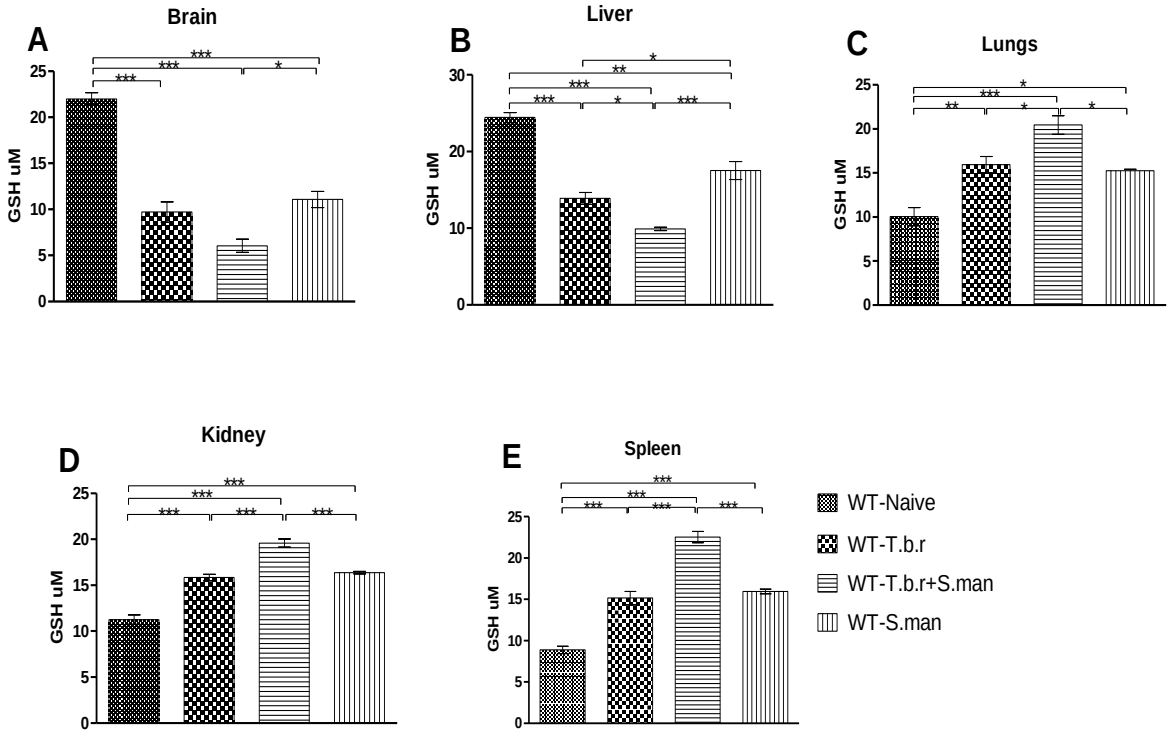

Figure 4.22: Effects of *S. mansoni* and *T.b.r* co-infection on the levels of GSH in the brain (A), liver (B), lungs (C), kidney (D) and spleen (E).

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.3.3: Effects of *S. mansoni* and *T.b.r* co-infection on malondialdehyde levels

T.b.r or *S. mansoni* infected mice showed significantly elevated levels of MDA when compared to naïve group of mice (P≤0.0001). Notably, markedly elevated levels of serum MDA was observed in the group of mice co-infected with *S. mansoni* and *T.b.r* compared to the naïve group (P≤0.0001; fig. 4.23), signaling active lipid peroxidation event.

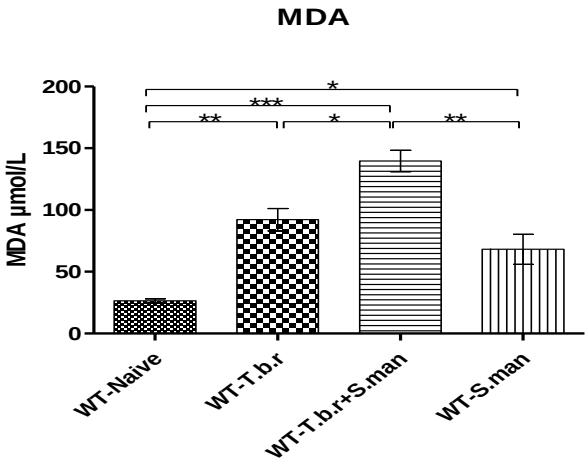

Figure 4.23: Effects of *S. mansoni* and *T.b.r* co-infection on malondialdehyde levels

One-way ANOVA used to compare between various groups followed by Tukey multiple comparisons post hoc test. Bars represent mean ± SEM. Indicated significance level of *p≤ 0.05; **p<0.01; *** p<0.001.

4.3.4 Effects of *S. mansoni* and *T.b.r* on pathology of mice liver tissues

The liver sections from naïve group of mice demonstrated no form of inflammatory lesions or injury (Fig. 4.24A). Nevertheless, *T.b.r* or *S. mansoni* alone infected mice showed hepatic injury characterized with multifocal granulomas surrounding parasite eggs (arrow) (Fig. 4.24B) and multifocal granulomas, marked by connective tissue proliferation (star), infiltration by lymphocytes, kupffer cells, and hepatocytes necrosis (arrow head) (Fig. 4.24 B-D respectively). Livers sections from mice co-infected with *S. mansoni* and *T.b.r*, revealed chronic liver injury characterized by granulomas surrounding parasite eggs(arrow), connective tissue proliferation, infiltration by lymphocytes(star), and kupffer cells, and hepatocytes necrosis (arrow head) (Fig. 4.24C).

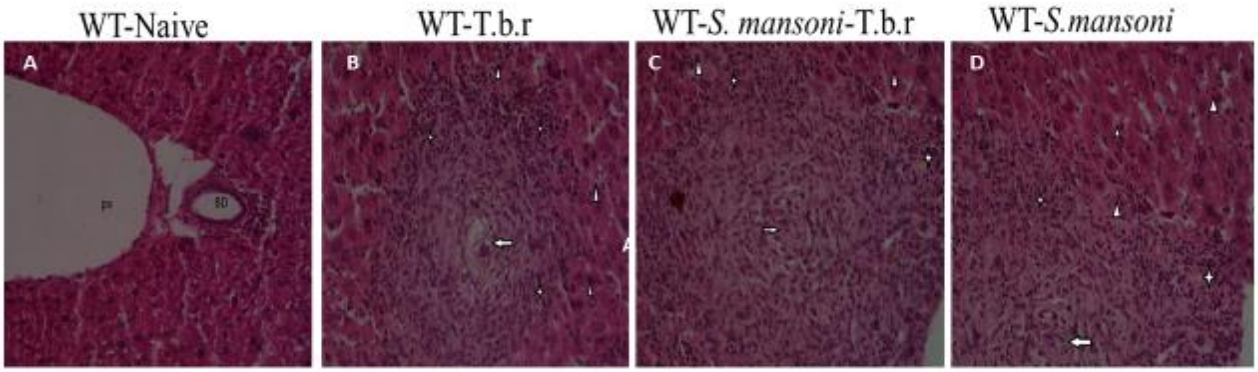

Figure 4.24: Effects of *S. mansoni* and *T.b.r* on inflammation of mice liver tissues

4.3.5 Effects of *S. mansoni* and *T.b.r* on in brain tissues pathology

Histopathological examination of the brain was further performed in this study to determine if there was any inflammation or pathology. Brains from naive group of mice did not show any sign of injury (Fig. 4.25A). Whereas, brains from mice infected with *T.b.r* or *S. mansoni* alone showed features of brain injury that were characterized with congestion of meningeal blood vessels (Fig. 4.25B) and infiltration of meninges with mononuclear cells (Fig. 4.25D) respectively. Additionally, mice co-infected with *S. mansoni* and *T.b.r* showed chronic brain injury characterized by focal areas of brain hemorrhages (arrow) and infiltration of meninges with mononuclear cells (star) (Fig. 4.25C).

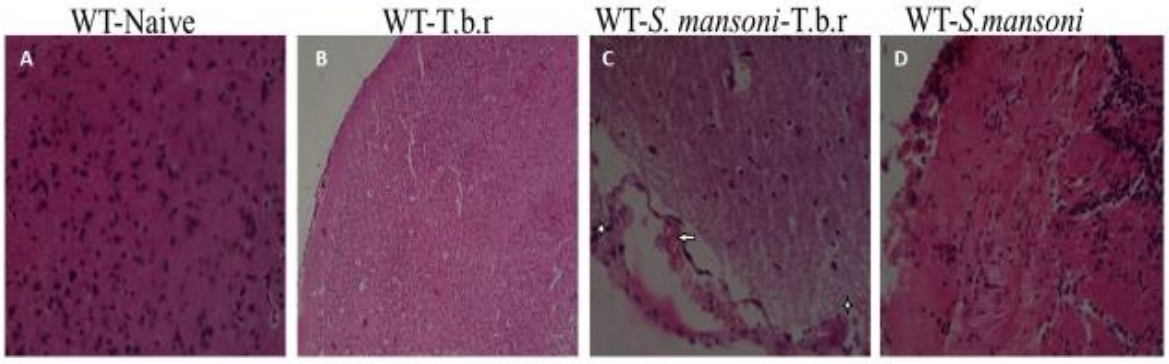

Figure 4.25: Effects of *S. mansoni* and *T.b.r* on inflammation of mice brain tissues.

4.3.6 Effects of *S. mansoni* and *T.b.r* on inflammation of spleen tissues

There was no injury noted in spleen samples across all groups of mice (Fig. 4.26A- D) as demonstrated by lymphocyte proliferation in all groups of mice.

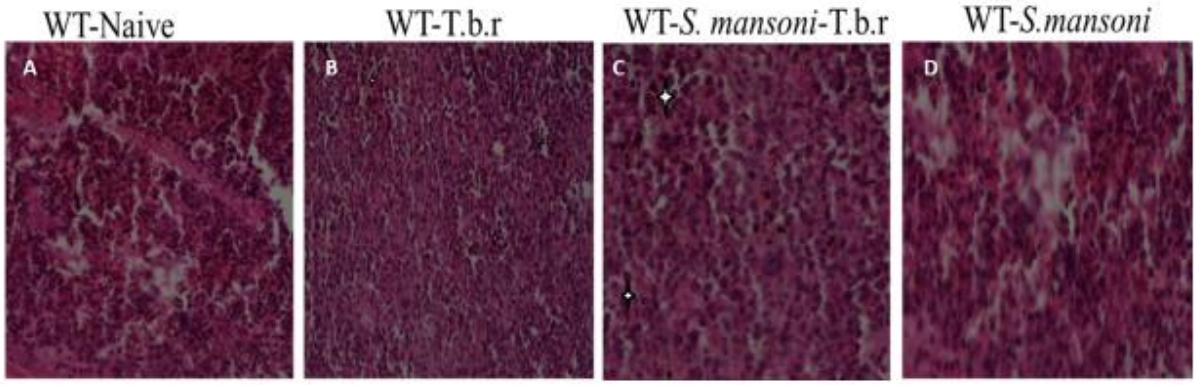

Figure 4.26: Effects of *S. mansoni* and *T.b.r* on inflammation of spleen tissues

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Research studies focusing on co-infections are on the rise and for several years it has proven to be a topic of interest. Indeed, much attention has been focused on the immunological impact of schistosomes in the induction and aggravation of Th2 response on the human host (Semenya *et al.,* 2012). For the first time, this study investigated whether immune response elicited by a primary infection i.e. *Schistosoma mansoni*, can potentially reduce or enhance disease severity with a secondary infection i.e. *Trypanosoma brucei rhodesiense.*

5.1: DISCUSSION

5.1.1. Effects of *S. mansoni* on the host physiological and hematological events following *T. b. r* induced infection

From this study, it was established that mice co-infected with the two parasites succumbed to the infection earlier before any of the other groups. However, mice infected with *S. mansoni* alone registered no mortality. Notably, it was observed that the mice survival was independent of peripheral parasitemia, as parasitemia levels across all the groups were comparable. This means that there could be other factors that led to lower survival rate especially in the co-infected group of mice.

This study used the Rapid Murine Coma and Behavior Scale (RMCBS) to assess how the two parasites affected the mice's overall health and brain integrity (Carroll *et al.,* 2010). The findings from this study demonstrated a drop in neuronal integrity of mice co-infected with the two parasites. Notably, the most significant drop in neuronal integrity was recorded in the co-infected group. The *T.b.r* infected group presented with low RMCBS scores from 15 days' post-infection (dpi) while the *S. mansoni*-infected group did not register a significant change in RMCBS scores.

However, a sharp decline in the combined RMCBS scores was recorded in the co-infected group indicating compromised neuronal integrity and poor health. On exploration of individual RMCBS parameters, there was no significant difference in body balance, changes in aggression, gait and motor response-related behaviors. These findings demonstrate the likelihood of a severe injury to the central nervous system especially in the *S. mansoni* and *T.b.r* infected group of mice.

Meningoencephalitic or late stage quite often is described by the passage of the blood-brain barrier (BBB) by parasites and also with the central nervous system (CNS) invasion by the same parasites (Rodgers, 2010; Frevert *et al.,* 2012). In this study, to evaluate the stability of the blood-brain barrier, Evans blue assay was performed. This dye binds to serum albumin, this serum albumin cannot permeate the BBB if it is stable, however when there is breakage of the BBB, then there is infiltration of the albumin dye into the brain resulting in staining the brains blue. From the results obtained, it was clear that the co-infected group exhibited a compromised blood-brain barrier as depicted by the deep blue staining of the brain. A similar outcome was observed in the group infected with *T.b. r*hodesiense suggestive of a compromised blood-brain barrier. The quantity of Evans blue dye that penetrated the mice' brains in the co-infected group was significantly high relative to the group of mice infected with *T.b. rhodesiense* or *S. mansoni*, demonstrating a severely compromised blood-brain barrier. However, there was no significant difference in the amount of Evans blue that was retained in the blood. Possibly, a rise in nitric oxide and pro-inflammatory cytokines that mediate inflammation could play a role in the breach of the blood-brain barrier in the current study. In a study by Amrouni *et al.,* (2011) activation of iNOS was described to elevate during the infection process in a central brain compartment particularly in the hypothalamus and thalamus, where regulation of the wake/ sleep is located (Amrouni *et al.,* 2011). Hence, preferred loss and interruption of trypanosomes in the above areas along with toxins release and mediators

of inflammation along with the cytotoxic consequence of nitric oxide including its byproducts may lead to neurological damage and transformed signaling, this would contribute to distinctive HAT pathogenesis (Tesoriero *et al.*, 2018). On the contrary, little iNOS expression and nitric oxide synthesis areas such as the cortex may sustain the survival of parasites.

Notably from this study, there was the development of splenomegaly, especially in the *T.b. rhodesiense* alone infected mice and *S. mansoni* and *T.b. rhodesiense* co-infected group of mice. The proliferation of lymphocytes and other blood cells, as well as the accumulation of activated macrophages in the splenic sinusoids that cause intense inflammation, may all contribute to the observed splenomegaly (Lewis, 2019). On the other hand, in schistosomiasis splenomegaly has been linked to two diverse pathological processes. For instance, in adolescents and young children, an inflammatory response is prominent as an early response to eggs that were trapped in the liver perisinusoidal periportal spaces, and nodular enlargement of the spleen (Paran, 2018). However, among adults, a long-lasting extreme infection results in massive deposition of diffuse collagen in the liver periportal space that leads to Symmer's pipestem or pathognomonic periportal fibrosis. The resulting fibrosis contributes to spleen enlargement and portal hypertension with surety venous circulation (Kioy *et al.*, 2004). Indeed, all these factors may be associated with massive splenomegaly among *S. mansoni* and *T.b.r.* co-infected mice.

(Bradley, 1972) reported the possibility that immunological response to the eggs of *Schistosoma mansoni* that have been cleared by the circulation system to the liver, where they get trapped, is the main fundamental component of immunological inflammation that occurs in hepatomegaly. This inflammation is categorized by lymphoid and reticuloendothelial hyperplasia. However, in this study, associating hepatosplenomegaly with elevated levels of pro-inflammatory reaction to schistosome antigens was not possible. Nevertheless, TNF-α as a pro-inflammatory cytokine was

one major response associated with *Schistosoma* egg antigen stimulation, and small IL-6 and TGF reaction levels in young ones with hepatomegaly point out that these children would be unable to satisfactorily regulate a pro-inflammatory reaction (Wilson *et al.,* 2008). In this study, a comparative analysis of infection of mice with *T.b.r* alone and co-infected group revealed that there was a significant increase in the relative weight of the liver indicative of hepatomegaly, likely to be linked to an increase in pro-inflammatory cytokines reported in this study. However, there was no significant difference in the weights of the lungs, kidneys and heart.

Derangement of blood parameters has been shown to have serious consequences in blood-related conditions (Eze & Okonkwo, 2013). In this study, it was evident that in *S. mansoni* or *T.b.r* alone infected group of mice, anemia was apparent as shown by the depletion of red blood cells, hemoglobin, and HCT. This was replicated in the co-infected group of mice, where anemia was more prominent. This outcome is in agreement with other previous studies in which anemia, in children co-infected with Schistosomiasis and malaria was observed (Leonard *et al.,* 2020; Waknine *et al.,* 2010). The anemia seen in this study may be caused by ROS produced during infection, which can cause oxidative stress and target the erythrocyte membrane, starting its oxidation and ultimately causing hemolysis (Greca & Magez, 2011). Additionally, the results of this study show that the mice's co-infection with the two parasites specifically caused microcytic hypochromic anemia, which is shown by the decreased levels of RBC indices (MCV, MCH, MCHC, and RDW-SD).

The white blood cell levels were elevated in all infected groups in this study. However, this increase was more in the *T.b.r* and *S. mansoni* co-infected group. Therefore, this observation may suggest that the co-infection of *T.b.r* and *S. mansoni* is immunostimulatory rather than

immunosuppressive. This stimulation of the components of the immune system would be of benefit in any kind of infection, especially in trypanosomiasis and Schistosomiasis co-infection (Islam *et al.*, 2012). Levels of monocytes, lymphocytes and eosinophils were more elevated in the co-infected group compared to the *T.b.r* or *S. mansoni* infected groups, with basophils levels depleted in the group. Similarly, just like WBCs, neutrophils level was highly elevated in the *T.b.r* or *S. mansoni* alone infected group. Notably, other studies have revealed that elevated levels of neutrophils and monocytes activate the production of pro-inflammatory cytokines including IFN-γ, TNF-α, IL-6 and IL-8 leading to inflammation-linked conditions (Wright *et al.*, 2010) and this may elucidate the function of a co-infection in exacerbating disease conditions and inflammation. Additionally, monocyte proliferation has been connected with the simultaneous synthesis of pro-inflammatory cytokines TNF-α, IL-6 and IFN-γ.

Platelets are useful effectors of inflammation, hemostasis, and also immune activity. They perform specific functions including injury response, defense of the host, and immune surveillance (Vieira *et al.*, 2012). Additionally, platelets are fundamental effector cells in the immune field and also in inflammation, which incorporate adaptive and innate immune responses (Iwasaki & Medzhitov, 2010; Weyrich & Zimmerman, 2004). In addition, platelets have signaling roles that prompt crucial responses of other myeloid cells including lymphocytes and leukocytes, of which are key effector immune cells in addition to endothelial cells, that contribute to major immune and inflammatory responses (Danese *et al.*, 2007). Therefore, any change in platelets disrupts these processes, creating a serious risk for anyone using blood thinners. From this study, *T.b.r* and *S. mansoni* co-infected group of mice exhibited extremely low platelet count. Thrombocytopenia could be associated with disease severity and hemodynamic instability levels. This thrombocytopenia may predispose individuals to blood coagulation abnormalities. This finding

corroborates with a co-infection study that reported thrombocytopenia to be prevalent among participants co-infected with malaria and schistosomiasis compared to Malaria or schistosomiasis-infected group of participants (Kamau *et al.,* 2021). Notably, elevation in P-LCR percentage, has been linked with myocardial infarction and ischemia (Gawlita *et al.,* 2015). Furthermore, in this study, the percentage of platelet indices were significantly depleted in *S. mansoni* and *T.b.r* alone infected mice. This depletion was more marked in mice that was co-infected with both *S. mansoni* and *T.b.r*. Decreased platelet and its indices are a clear demonstration of *S. mansoni* and *T.b.r* induced thrombocytopenia.

5.1.2. The role of *S. mansoni* on HAT driven biochemical and immune regulation in a mouse model.

Levels of urea and creatinine are usually measured in serum samples to determine the degree of the kidney functionality, with elevated levels denoting kidney disease or damage (Oladipo *et al.,* 2016). Creatinine is a byproduct of creatine phosphate in the muscle while urea is a product of nitrogenous protein catabolism and amino acid secreted by the liver. Estimation of serum urea is useful in the diagnosis of both acute renal failure and pre-renal condition (Gowda *et al.,* 2010). In this study, *T.b.r* or *S. mansoni* alone infected mice resulting in elevated levels of urea and creatinine signaling kidney damage. Remarkably, *S. mansoni* and *T.b.r* co-infection led to double-fold elevation of urea and creatinine. Increased levels of creatinine and urea are usually indicative of kidney damage.

Furthermore, to assess the extent of the kidney and liver injury, levels of uric acid were estimated. In this study, increased uric acid levels were evident in *T.b.r* or *S. mansoni* alone infected mice. These levels were markedly elevated in the co-infected mice group. Elevated levels of uric acid

could indicate kidney and liver damage hence the inability to competently eliminate it. Uric acid is an unwanted product released during purine catabolism (Johnson *et al.,* 2018;Bursill *et al.,* 2019). Therefore, uric acid buildup can be due to elevated creation of purines with unavailability of uricase, the degradation enzyme (Johnson *et al.,* 2011). At normal physiological levels, uric acid possesses antioxidant properties in body fluids. In contrast, at higher concentrations, it appears to have pro-inflammatory properties (Alcaíno *et al.,* 2011). This uric acid is mostly excreted via kidneys and also the intestines (Martinon *et al.,* 2006). Additionally, the phagocytes' uptake of uric acid causes a pro-inflammatory reaction and monosodium urate (MSU) accumulation in the joints, which results in chondrocyte death and is therefore associated with gouty arthritis (Martinon *et al.,* 2006). Therefore, uric acid is a weak acid generated in the liver, intestines and muscles (Sharaf *et al.,* 2017).

Estimation of liver enzymes is critical in the diagnosis of the extent of liver damage or injury due to toxic chemicals or diseases. Remarkably, increase in these markers are key indicators of liver damage (Wang, 2015). Herein, *T.b.r* or *S. mansoni* alone infected group of mice resulted in elevated levels of ALT, ALP, AST, total bilirubin and direct bilirubin. Intriguingly, co-infection with *T.b.r* and *S. mansoni*, resulted in prominent elevated of ALT, ALP, AST and bilirubin indicating hepatocellular injury. It is well documented that ALT is present in diverse organs including the in heart, kidney, muscle, with a higher concentration in liver in comparison to other body tissues. An increase in ALT signifies liver cell damage. On the other hand, AST is found in greatest concentration in heart in comparison to the liver, kidney and skeletal muscle. AST elevation in the mitochondria is linked to widespread tissue necrosis in chronic liver diseases and myocardial infarction (Gowda *et al.,* 2009). For the first time, this study has established that *T.b.r*

and *S. mansoni* co-infection exacerbates liver damage as indicated by marked elevated serum transaminases level.

Bilirubin is a byproduct of hemoglobin from damaged RBCs made by the reticuloendothelial system, released as indirect/unconjugated then moves to the liver where it's changed to conjugated form. In this study, levels of direct and total bilirubin in *S. mansoni* and *T.b.r* alone infected mice were elevated. On the other hand, the levels were double fold increased in the group of mice infected with *S. mansoni* and *T.b. r*hodesiense. The induction hemolysis of the RBCs was the reason for elevated bilirubin levels. Elevated serum bilirubin suggests hepatic diseases. Quick destruction of the red blood cells within the bone marrow yields bilirubin (Liu *et al.,* 2016) . Bilirubin elevation and buildup in the liver leads to inflammation, causing tissue damage (Gowda *et al.,* 2009).

Serum albumin is synthesized in the liver and has several physiological roles. In this study, the levels of albumin were depleted among mice infected with *S. mansoni* or *T.b. rhodesiense*. However, in the co-infected group, albumin levels extremely depleted. The depleted albumin levels are usually connected to end-stage liver disease, though low albumin levels occur at early stages of the disease due to reduced production (*Garcovich et al.,* 2009). Some of the roles of albumin include facilitation of coagulation, microvascular permeability and pH maintenance (Ait *et al.,* 2010). Additionally, it has antioxidant properties (Quinlan *et al.,* 2004). This could be an indicator of end stage liver and kidney disease.

In this study, *T.b.r* or *S. mansoni* alone infected mice resulted in alteration of lipid metabolism as shown by elevation of triglycerides, cholesterol and a decrease in high density lipoproteins. On the other hand, a co-infection of *T.b.r* and *S. mansoni* resulted into extremely elevated levels of the

above lipids. This could indicate a possibility of liver injury/damage. Earlier studies have demonstrated that individuals suffering from schistosomiasis depicted depleted serum cholesterol levels than healthy controls. (Rong *et al.,* 2016) observed a substantial depletion in levels of serum lipid profile among mice that were infected with *S. mansoni*. On the other hand, Grundy *et al* detected a reasonably elevated triglyceride levels though, LDL and HDL were depleted. Furthermore, the reduced mean levels of high-density lipoprotein cholesterol and low-density lipoprotein cholesterol in the same individuals may be partially explained by a considerable decrease in the mean total cholesterol among infected subjects as found in Grundy's study. This is because of a progressive relationship between HDL-C, total cholesterol and LDL-C that has been extensively described also among normal individuals. Low triglyceride levels among subjects that are infected with *S. mansoni* remains unclear but (Felici *et al.,* 2021) had proved the capability of *S. mansoni* in synthesizing of triacylglycerols and phospholipids from precursors acquired from the host.

For the immune system to function properly and effectively, pro-inflammatory and anti-inflammatory cytokines must be in homeostasis (Fry *et al.,* 2007). This study aimed to find out how *T.b.r* and *S. mansoni* co-infection of mice will influence the immuno-stimulatory or immune-suppressive responses on anti and pro-inflammatory cytokines. It was evident from this study that pro-inflammatory cytokine TNF-α in mice upon coinfection was significantly elevated in infected group of mice with *T.b.r* or *S. mansoni* alone relative to the control. Crucially, there was a double fold increase of this cytokine in the *T.b.r* and *S. mansoni* co-infected group of mice. This could be due to the impact of the two parasites in combination. This study also observed elevation in IFN-γ, associated with long-lasting inflammatory responses. These results corroborate with the findings reported by (Lima *et al.,* 2012) involving co-infection of *S. mansoni* and *Paracoccidioides*

brasiliensis that reflected the reorganization of cells of the schistosomiasis granulomatous as a spontaneous effect of the start of an involute phase of granulomatous. On the other hand, IL-10 was significantly depleted by *T.b.r* or *S. mansoni* infections and further depleted due to co-infection. This resulted in an imbalance in the anti- and pro-inflammatory cytokines hence necessitating the need to analyze the ratio between pro-inflammatory and anti-inflammatory cytokines to denote inflammation status (Hesse *et al.,* 2000). Excessive pro-inflammatory response due to *T.b.r* or S. *mansoni* alone infected mice was evident as demonstrated by this ratio. Furthermore, coinfection with both *T.b.r* and *S. mansoni* created greater imbalance between pro and anti-inflammatory cytokines.

Immunoglobulins also known as antibodies are glycoproteins produced by plasma cells. Specific immunogens including bacterial proteins instruct B cells to differentiate into plasma cells that are involved in humoral responses to various pathogens including viruses, bacteria, parasites etc. (Vasilev *et al.,* 2016). In this study, the mice infected with *T.b.r* resulted in elevation of immunoglobulins, while those infected with *S. mansoni* depicted depletion of the same. However, in the co-infected mice there was further depletion of immunoglobulins. This could be due to immune suppression because of the two parasites, especially in *S. mansoni* alone group and co-infected group.

5.1.3 The impact of *S. mansoni* and *T.b. r* on inflammation and oxidative stress in a mouse model

GSH has a crucial function in protecting cell macromolecules from both exogenous and endogenous reactive nitrogen and oxygen species. Co-infection of *T.b.r* and *S. mansoni* resulted

in elevated GSH levels in the kidney, lungs and the spleen, indicating oxidative stress. Estimation of cellular GSH determines levels of cells antioxidant capacity and also oxidative damage to cells. Though it directly reduces free radicals, it's greatest role is dealing directly with the causative agents of oxidative stress for example persistent organic pollutants (POPs) and mercury (Pizzino *et al.,* 2017). Additional findings in regards to oxidative stress indicated complete suppression of GSH in the brain and liver, indicating oxidative stress as a result of rigorously overwhelmed antioxidant capacity in brain and liver cells. Oxidative stress could cause brain and liver pathology. Prolonged oxidative stress has the ability to form numerous free radicals in the liver, leading to liver cirrhosis, fibrosis and carcinogenesis. Therefore, this study may clearly indicate that *T.b.r* and *S. mansoni* co-infection tend to induce severe oxidative stress in the liver, spleen, lungs, kidney and brain.

To further validate GSH results for oxidative stress, malondialdehyde (MDA) and nitric oxide (NO) estimation was conducted. In this study, elevation of MDA and nitric oxide was manifested in the *T.b.r* or *S. mansoni* infected group. Interestingly, on coinfection with *T.b.r* and *S. mansoni*, the levels were extremely increased indicating massive oxidative stress/injury to the organs. Elevated levels of NO are also an indicator of inflammation. The heightened destruction of erythrocytes and lymphocytes may lead to the release of MDA as well as reduced GSH (Ivanov *et al.,* 2016). The commonly used oxidative stress biomarker is MDA, mostly in diseases such as chronic obstructive pulmonary disease, psychiatry, cardiovascular diseases, asthma, and cancer (Khoubnasab *et al.,* 2015). Malondialdehyde production has been shown as a relevant indicator to prove the occurrence of lipid peroxidation in situ (Saad *et al.,* 2020). Nitric oxide plays a great role as an endothelium and endogenous relaxing factor, as a free radical. NOS enzyme produces NO

which is an excellent free radical in malignancies. NO can also lead to a response with superoxide hence creating nitrogen dioxide and nitrite causing cause cellular DNA damage (Papi *et al.*, 2019).

Additionally, by use of standard histopathological analysis, this study sought to determine inflammatory effects of *S. mansoni* or *T.b.r* infections and the impact of a co-infection on the liver, spleen and brain sections. The co-infected group demonstrated liver injury that exacerbated into chronic liver damage characterized by granulomas, connective tissue proliferation, infiltration by lymphocytes, Kupffer cells and hepatocytes necrosis (Arch *et al.,* 1997). This outcome is reasonable having seen that bilirubin and liver enzymes were exacerbated in the *S. mansoni* and *T.b.r* alone mice and also in the co-infected group of mice. Extraordinarily, the finding in this study is reasonable, given the fact that bilirubin and liver enzymes were exacerbated in the *S. mansoni* and *T.b.r* alone mice and also in the co-infected group of mice. On examination of histological brain sections, tissues from a group of mice infected with *T.b.r* or *S. mansoni* alone exhibited Congestion of meningeal blood vessels. Besides, *T.b.r* and *S. mansoni* co-infected group of mice exhibited meningitis characterized by focal areas of brain hemorrhages and infiltration of meninges with mononuclear cells. These findings contradict a study by Bucher *et al.* (2011) which reported *Schistosoma* co-infection being able to confer protection against brain pathology and the fatal outcome of brain pathology was associated with parasitemia. On the other hand, spleen section analysis revealed increased lymphocyte proliferation which is a clear indication of no effect on the spleen in all groups of infected mice.

5.2 CONCLUSIONS

Outcomes from the present study provide compelling evidence that *S. mansoni* and *T.b.r* co-infected mice result in adverse effects linked to

I. alteration of biochemical and physiological functions.

II. Moreover, the findings show the impact of co-infection to exacerbate neurobehavioral deficits, organ pathology and compromised immune response.

III. In addition, the outcomes provide sufficient evidence for the first time that co-infections were able to alter the clinical course of infections with the relatively chronic schistosomiasis, accelerating inflammation, oxidative stress and causing potentially fatal and severe human African trypanosomiasis disease.

Overall, primary infection with *Schistosoma mansoni* exacerbates the severity of the disease of a secondary infection with *Trypanosoma brucei rhodesience* in a mouse model that is associated with harmful inflammatory response, oxidative stress, and organ injury.

5.3 RECOMMENDATIONS

1. Bearing in mind the significance and the geographical distribution of schistosomiasis and Trypanosomiasis in tropical areas, additional studies on immunomodulatory mechanisms during co-infection are paramount to understanding how co-infection can impact the treatment and preclusion of both.
2. Co-infection with the two parasites resulted in the disruption of the BBB, hence further studies need to be conducted to elucidate whether the infiltration of effector immune cells contributes to the brain pathology also analysis for the differential vulnerability of neurons versus astrocytes merits investigation.
3. Give the importance of these two diseases, this study could be exteded to higher animal models.

REFERENCES

Abruzzi, A., & Fried, B. (2011). Coinfection of Schistosoma (Trematoda) with Bacteria, Protozoa and Helminths. In *Advances in Parasitology* (Vol. 77). https://doi.org/10.1016/B978-0-12-391429-3.00005-8

Ait-Oufella, H., Maury, E., Lehoux, S., Guidet, B., & Offenstadt, G. (2010). The endothelium: Physiological functions and role in microcirculatory failure during severe sepsis. *Intensive Care Medicine*, *36*(8), 1286–1298. https://doi.org/10.1007/s00134-010-1893-6

Alam, U. (2007). Immunity: The Immune Response to Infectious and Inflammatory Disease. *The Yale Journal of Biology and Medicine*, *80*(3), 137. http://www.worldcat.org/title/immunity-the-immune-response-in-infectious-inflammatory-disease/oclc/487281978

Alcaíno, H., Greig, D., Castro, P., Verdejo, H., Mellado, R., García, L., Díaz-Araya, G., Quiroga, C., Chiong, M., & Lavandero, S. (2011). Ácido úrico: Una molécula con acciones paradójicas en la insuficiencia cardiaca. *Revista Medica de Chile*, *139*(4), 505–515. https://doi.org/10.4067/S0034-98872011000400014

Alves, C. C., Araujo, N., Cassali, G. D., & Fonseca, C. T. (2016). Parasitological, Pathological, and Immunological Parameters Associated with Schistosoma mansoni Infection and Reinfection in BALB/c and C57BL/6 Mice. *Journal of Parasitology*, *102*(3), 336–341. https://doi.org/10.1645/14-664

Amrouni, D., Meiller, A., Gautier-Sauvigné, S., Piraud, M., Bouteille, B., Vincendeau, P., Buguet, A., & Cespuglio, R. (2011). Cerebral changes occurring in arginase and dimethylarginine dimethylaminohydrolase (DDAH) in a rat model of sleeping sickness.

PLoS ONE, *6*(3), 1–15. https://doi.org/10.1371/journal.pone.0016891

Andrade, Z. A. (2004). Schistosomal hepatopathy. *Memorias Do Instituto Oswaldo Cruz*, *99*(5), 51–57. https://doi.org/10.1590/s0074-02762004000900009

Arch, V., Hiv, A., Cdc, I. I. I., Cdc, I. I., Tel, G., Brackmann, G. H. H., & Haematology, E. (1997). *Liver histopathology in patients with concurrent chronic hepatitis C and HIV infection*. 271–277.

Ayash-Rashkovsky, M., Chenine, A. L., Steele, L. N., Lee, S. J., Song, R., Ong, H., Rasmussen, R. A., Hoffmann-Lehmann, R., Else, J. G., Augostini, P., McClure, H. M., Secor, W. E., & Ruprecht, R. M. (2007). Coinfection with Schistosoma mansoni reactivates viremia in rhesus macaques with chronic simian-human immunodeficiency virus clade C infection. *Infection and Immunity*, *75*(4), 1751–1756. https://doi.org/10.1128/IAI.01703-06

Babokhov, P., Sanyaolu, A. O., Oyibo, W. A., Fagbenro-Beyioku, A. F., & Iriemenam, N. C. (2013). A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis. *Pathogens and Global Health*, *107*(5), 242–252. https://doi.org/10.1179/2047773213Y.0000000105

Barkhuizen, M., Magez, S., Ryffel, B., & Brombacher, F. (2008). Interleukin-12p70 deficiency increases survival and diminishes pathology in Trypanosoma congolense infection. *Journal of Infectious Diseases*, *198*(9), 1284–1291. https://doi.org/10.1086/592048

Biswas, S. K. (2016). Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox? *Oxidative Medicine and Cellular Longevity*, *2016*, 17–19. https://doi.org/10.1155/2016/5698931

Bogdan, C., Röllinghoff, M., & Diefenbach, A. (2000). Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Current Opinion in Immunology*, *12*(1), 64–76. https://doi.org/10.1016/S0952-7915(99)00052-7

Bolton, S. J., Anthony, D. C., & Perry, V. H. (1998). Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood-brain barrier breakdown in vivo. *Neuroscience*, *86*(4), 1245–1257. https://doi.org/10.1016/S0306-4522(98)00058-X

Bosschaerts, T., Morias, Y., Stijlemans, B., Hérin, M., Porta, C., Sica, A., Mantovani, A., De Baetselier, P., & Beschin, A. (2011). IL-10 limits production of pathogenic TNF by M1 myeloid cells through induction of nuclear NF-κB p50 member in Trypanosoma congolense infection-resistant C57BL/6 mice. *European Journal of Immunology*, *41*(11), 3270–3280. https://doi.org/10.1002/eji.201041307

Bourke, C. D., Nausch, N., Rujeni, N., Appleby, L. J., Mitchell, K. M., Midzi, N., Mduluza, T., & Mutapi, F. (2013). Integrated analysis of innate, Th1, Th2, Th17, and regulatory cytokines identifies changes in immune polarisation following treatment of human schistosomiasis. *Journal of Infectious Diseases*, *208*(1), 159–169. https://doi.org/10.1093/infdis/jis524

Bouteille, B., & Buguet, A. (2012). The detection and treatment of human African trypanosomiasis. *Research and Reports in Tropical Medicine*, 35. https://doi.org/10.2147/rrtm.s24751

Bradley, D. J. (1972). *THE EPIDEMIOLOGY AND CONSEQUENCES OF SCHISTOSOMA I* .

FIELD STUDIES OF A COMMUNITY AT PANYAGORO. *66*(6).

Brun, R., Blum, J., Chappuis, F., & Burri, C. (2010). Human African trypanosomiasis. *The Lancet*, *375*(9709), 148–159. https://doi.org/10.1016/S0140-6736(09)60829-1

Bucher, K., Dietz, K., Lackner, P., Pasche, B., Fendel, R., Mordmüller, B., Ben-smith, A., & Hoffmann, W. H. (2011). Schistosoma co-infection protects against brain pathology but does not prevent severe disease and death in a murine model of cerebral malaria. *International Journal for Parasitology*, *41*(1), 21–31. https://doi.org/10.1016/j.ijpara.2010.06.008

Bursill, D., Taylor, W. J., Terkeltaub, R., Kuwabara, M., Merriman, T. R., Grainger, R., Pineda, C., Louthrenoo, W., Edwards, N. L., Andrés, M., Vargas-Santos, A. B., Roddy, E., Pascart, T., Lin, C. T., Perez-Ruiz, F., Tedeschi, S. K., Kim, S. C., Harrold, L. R., McCarthy, G., … Dalbeth, N. (2019). Gout, Hyperuricemia, and Crystal-Associated Disease Network Consensus Statement Regarding Labels and Definitions for Disease Elements in Gout. *Arthritis Care and Research*, *71*(3), 427–434. https://doi.org/10.1002/acr.23607

Carroll, R. W., Wainwright, M. S., Kim, K. Y., Kidambi, T., Gómez, N. D., Taylor, T., & Haldar, K. (2010). A rapid murine coma and behavior scale for quantitative assessment of murine cerebral malaria. *PLoS ONE*, *5*(10), 1–12. https://doi.org/10.1371/journal.pone.0013124

Castro, V. N., Rodrigues, J. L., Cardoso, D. T., Resende, S. D., Magalhães, F. C., Souza, D. C., Requeijo, M. H., Negrão-Corrêa, D., & Geiger, S. M. (2018). Systemic Cytokine and Chemokine Profiles in Individuals With Schistosoma mansoni Infection and Low Parasite

Burden. *Frontiers in Immunology*, *9*(December), 1–12. https://doi.org/10.3389/fimmu.2018.02975

CDC, 2019. Control and surveillance of Human African trypanosomiasis.

CDC, 2020. Control and surveillance of Human Schistosomiasis.

Cheever, A. W., Lenzi, J. A., Lenzi, H. L., & Andrade, Z. A. (2002). Experimental models of Schistosoma mansoni infection. *Memorias Do Instituto Oswaldo Cruz*, *97*(7), 917–940. https://doi.org/10.1590/S0074-02762002000700002

Cnops, J., De Trez, C., Stijlemans, B., Keirsse, J., Kauffmann, F., Barkhuizen, M., Keeton, R., Boon, L., Brombacher, F., & Magez, S. (2015). NK-, NKT- and CD8-Derived IFNγ Drives Myeloid Cell Activation and Erythrophagocytosis, Resulting in Trypanosomosis-Associated Acute Anemia. *PLoS Pathogens*, *11*(6), 1–19. https://doi.org/10.1371/journal.ppat.1004964

Cohen, C. (1973). Trypanosomiasis on the Witwatersrand. *South African Medical Journal*, *47*(11), 485–491.

Colley, D. G., & Secor, W. E. (2014). Immunology of human schistosomiasis. *Parasite Immunology*, *36*(8), 347–357. https://doi.org/10.1111/pim.12087

Costain, A. H., MacDonald, A. S., & Smits, H. H. (2018). Schistosome Egg Migration: Mechanisms, Pathogenesis and Host Immune Responses. *Frontiers in Immunology*, *9*(December), 1–16. https://doi.org/10.3389/fimmu.2018.03042

Coulibaly, J. T., N'Gbesso, Y. K., Knopp, S., Keiser, J., N'Goran, E. K., & Utzinger, J. (2012). Efficacy and Safety of Praziquantel in Preschool-Aged Children in an Area Co-Endemic for Schistosoma mansoni and S. haematobium. *PLoS Neglected Tropical Diseases*, *6*(12).

https://doi.org/10.1371/journal.pntd.0001917

Cupit, P. M., & Cunningham, C. (2015). What is the mechanism of action of praziquantel and how might resistance strike? *Future Medicinal Chemistry*, *7*(6), 701–705. https://doi.org/10.4155/fmc.15.11

Danese, S., Dejana, E., & Fiocchi, C. (2007). Immune Regulation by Microvascular Endothelial Cells: Directing Innate and Adaptive Immunity, Coagulation, and Inflammation. *The Journal of Immunology*, *178*(10), 6017–6022. https://doi.org/10.4049/jimmunol.178.10.6017

De Oliveira Fraga, L. A., Torrero, M. N., Tocheva, A. S., Mitre, E., & Davies, S. J. (2010). Induction of type 2 responses by schistosome worms during prepatent infection. *Journal of Infectious Diseases*, *201*(3), 464–472. https://doi.org/10.1086/649841

De Oliveira, R. B., Senger, M. R., Vasques, L. M., Gasparotto, J., Dos Santos, J. P. A., De Bittencourt Pasquali, M. A., Moreira, J. C. F., Silva, F. P., & Gelain, D. P. (2013). Schistosoma mansoni infection causes oxidative stress and alters receptor for advanced glycation endproduct (RAGE) and tau levels in multiple organs in mice. *International Journal for Parasitology*, *43*(5), 371–379. https://doi.org/10.1016/j.ijpara.2012.12.006

Del Vecchio, G. C., Giordano, P., Tesse, R., Piacente, L., Altomare, M., & De Mattia, D. (2012). Clinical significance of serum cytokine levels and thrombopoietic markers in childhood idiopathic thrombocytopenic purpura. *Blood Transfusion*, *10*(2), 194–199. https://doi.org/10.2450/2011.0055-11

Dessie, N., Lema, W., & Aemero, M. (2020). Hematological and Biochemical Profile of Patients

Infected with Schistosoma mansoni in Comparison with Apparently Healthy Individuals at Sanja Town, Northwest Ethiopia: A Cross-Sectional Study. *Journal of Tropical Medicine, 2020*, 1–7. https://doi.org/10.1155/2020/4083252

Elsammak, M. Y., Al-Sharkaweey, R. M., Ragab, M. S., Amin, G. A., & Kandil, M. H. (2008). IL-4 and reactive oxygen species are elevated in Egyptian patients affected with schistosomal liver disease. *Parasite Immunology*, *30*(11–12), 603–609. https://doi.org/10.1111/j.1365-3024.2008.01061.x

Eze, J. I., Ajanwachukwu, N., Animoke, P. C., Onoja, S. O., Anosa, G. N., & Eze, U. U. (2016). Immune response, anaemia and oxidative stress in Trypanosoma brucei brucei infected rats fed vitamin E supplemented diet. *Anti-Infective Agents*, *14*(1), 28–37.

Eze, J. I., & Okonkwo, C. A. (2013). Effect of dietary probiotic (Saccharomyses cerevisiae) supplementation on the severity of Trypanosoma brucei infection in rats. *Global Veterinaria*, *11*(4), 459–464. https://doi.org/10.5829/idosi.gv.2013.11.4.76176

Fallon, P. G., Richardson, E. J., McKenzie, G. J., & McKenzie, A. N. J. (2000). Schistosome Infection of Transgenic Mice Defines Distinct and Contrasting Pathogenic Roles for IL-4 and IL-13: IL-13 Is a Profibrotic Agent. *The Journal of Immunology*, *164*(5), 2585–2591. https://doi.org/10.4049/jimmunol.164.5.2585

Feldman, A. T., & Wolfe, D. (2014). Tissue processing and hematoxylin and eosin staining. *Methods in Molecular Biology*, *1180*, 31–43. https://doi.org/10.1007/978-1-4939-1050-2_3

Felici, N., Liu, D., Maret, J., Restrepo, M., Borovskiy, Y., Hajj, J., Chung, W., & Laudanski, K. (2021). Long-Term Abnormalities of Lipid Profile After a Single Episode of Sepsis.

Frontiers in Cardiovascular Medicine, *8*(November), 1–14. https://doi.org/10.3389/fcvm.2021.674248

Fernandez, M. M. (2017). *Interferon Gamma in African Trypanosome Infections : Friends or Foes ? 8*(September), 4–10. https://doi.org/10.3389/fimmu.2017.01105

Figliuolo da Paz, V. R., Figueiredo-Vanzan, D., & dos Santos Pyrrho, A. (2019). Interaction and involvement of cellular adhesion molecules in the pathogenesis of Schistosomiasis mansoni. *Immunology Letters*, *206*(August 2018), 11–18. https://doi.org/10.1016/j.imlet.2018.11.011

Foster, R. (1987). A review of clinical experience with oxamniquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *81*(1), 55–59. https://doi.org/10.1016/0035-9203(87)90282-3

Franco, J. R., Simarro, P. P., Diarra, A., & Jannin, J. G. (2014). Epidemiology of human African trypanosomiasis. *Clinical Epidemiology*, *6*(1), 257–275. https://doi.org/10.2147/CLEP.S39728

Frevert, U., Movila, A., Nikolskaia, O. V., Raper, J., Mackey, Z. B., Abdulla, M., McKerrow, J., & Grab, D. J. (2012). Early Invasion of Brain Parenchyma by African Trypanosomes. *PLoS ONE*, *7*(8). https://doi.org/10.1371/journal.pone.0043913

Fry, R. C., Navasumrit, P., Valiathan, C., Svensson, J. P., Hogan, B. J., Luo, M., Bhattacharya, S., Kandjanapa, K., Soontararuks, S., Nookabkaew, S., Mahidol, C., Ruchirawat, M., & Samson, L. D. (2007). Activation of inflammation/NF-κB signaling in infants born to arsenic-exposed mothers. *PLoS Genetics*, *3*(11), 2180–2189. https://doi.org/10.1371/journal.pgen.0030207

Garcovich, M., Zocco, M. A., & Gasbarrini, A. (2009). Clinical use of albumin in hepatology. *Blood Transfusion*, *7*(4), 268–277. https://doi.org/10.2450/2008.0080-08

Gawlita, M., Osadnik, T., & Bujak, K. (2015). *Mean platelet volume and platelet-large cell ratio as prognostic factors for coronary artery disease and myocardial infarction Średnia objętość płytek krwi i wskaźnik dużych komórek jako czynniki prognostyczne choroby wieńcowej i zawału serca*. *6*, 418–422. https://doi.org/10.5603/FC.2015.0079

Gowda, S., Desai, P. B., Hull, V. V, Math, A. A. K., Vernekar, S. N., & Kulkarni, S. S. (2009). A review on laboratory liver function tests. *The Pan African Medical Journal*, *3*(November), 17. http://www.ncbi.nlm.nih.gov/pubmed/21532726%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2984286

Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V, Math, A. A. K., & Vernekar, S. N. (2010). Markers of renal function tests. *North American Journal of Medical Sciences*, *2*(4), 170–173. http://www.ncbi.nlm.nih.gov/pubmed/22624135%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3354405

Greca, F. La, & Magez, S. (2011). Vaccination against trypanosomiasis: Can it be done or is the trypanosome truly the ultimate immune destroyer and escape artist? *Human Vaccines*, *7*(11), 1225–1233. https://doi.org/10.4161/hv.7.11.18203

Griffiths, E. C., Pedersen, A. B., Fenton, A., & Petchey, O. L. (2011). The nature and consequences of coinfection in humans. *Journal of Infection*, *63*(3), 200–206.

https://doi.org/10.1016/j.jinf.2011.06.005

Gryseels, B., Polman, K., Clerinx, J., & Kestens, L. (2006). Human schistosomiasis. *Lancet*, *368*(9541), 1106–1118. https://doi.org/10.1016/S0140-6736(06)69440-3

Guilliams, M., Movahedi, K., Bosschaerts, T., VandenDriessche, T., Chuah, M. K., Hérin, M., Acosta-Sanchez, A., Ma, L., Moser, M., Van Ginderachter, J. A., Brys, L., De Baetselier, P., & Beschin, A. (2009). IL-10 Dampens TNF/Inducible Nitric Oxide Synthase-Producing Dendritic Cell-Mediated Pathogenicity during Parasitic Infection. *The Journal of Immunology*, *182*(2), 1107–1118. https://doi.org/10.4049/jimmunol.182.2.1107

Hams, E., Aviello, G., & Fallon, P. G. (2013). The Schistosoma granuloma: Friend or foe? *Frontiers in Immunology*, *4*(APR), 1–8. https://doi.org/10.3389/fimmu.2013.00089

Hertz, C. J., Filutowicz, H., & Mansfield, J. M. (2022). *Resistance to the African Trypanosomes Is IFN- γ Dependent*.

Hesse, M., Cheever, A. W., Jankovic, D., & Wynn, T. A. (2000). NOS-2 mediates the protective anti-inflammatory and antifibrotic effects of the Th1-inducing adjuvant, IL-12, in a Th2 model of granulomatous disease. *American Journal of Pathology*, *157*(3), 945–955. https://doi.org/10.1016/S0002-9440(10)64607-X

Hoffmann, K. F., Cheever, A. W., & Wynn, T. A. (2000). IL-10 and the Dangers of Immune Polarization: Excessive Type 1 and Type 2 Cytokine Responses Induce Distinct Forms of Lethal Immunopathology in Murine Schistosomiasis. *The Journal of Immunology*, *164*(12), 6406–6416. https://doi.org/10.4049/jimmunol.164.12.6406

Hooper, D. C., Spitsin, S., Kean, R. B., Champion, J. M., Dickson, G. M., Chaudhry, I., &

Koprowski, H. (1998). Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(2), 675–680. https://doi.org/10.1073/pnas.95.2.675

Horras, C. J., Lamb, C. L., & Mitchell, K. A. (2011). Regulation of hepatocyte fate by interferon-γ. *Cytokine and Growth Factor Reviews*, *22*(1), 35–43. https://doi.org/10.1016/j.cytogfr.2011.01.001

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C. B., & Rahu, N. (2016). Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? *Oxidative Medicine and Cellular Longevity*, *2016*. https://doi.org/10.1155/2016/7432797

Ikeogu, N. M., Akaluka, G. N., Edechi, C. A., Salako, E. S., Onyilagha, C., Barazandeh, A. F., & Uzonna, J. E. (2020). Leishmania immunity: Advancing immunotherapy and vaccine development. *Microorganisms*, *8*(8), 1–21. https://doi.org/10.3390/microorganisms8081201

Islam, M. R., Roh, Y. S., Cho, A., Kim, J., Kim, J. H., Eo, S. kug, Lim, C. W., & Kim, B. (2012). Immune modulatory effects of the foodborne contaminant citrinin in mice. *Food and Chemical Toxicology*, *50*(10), 3537–3547. https://doi.org/10.1016/j.fct.2012.06.050

Ivanov, A. V., Valuev-Elliston, V. T., Ivanova, O. N., Kochetkov, S. N., Starodubova, E. S., Bartosch, B., & Isaguliants, M. G. (2016). Oxidative Stress during HIV Infection: Mechanisms and Consequences. *Oxidative Medicine and Cellular Longevity*, *2016*(2). https://doi.org/10.1155/2016/8910396

Iwasaki, A., & Medzhitov, R. (2010). Regulation of adaptive immunity by the innate immune

system. *Science*, *327*(5963), 291–295. https://doi.org/10.1126/science.1183021

Jankovic, D., Kullberg, M. C., Noben-Trauth, N., Caspar, P., Paul, W. E., & Sher, A. (2000). Single Cell Analysis Reveals That IL-4 Receptor/Stat6 Signaling Is Not Required for the In Vivo or In Vitro Development of CD4 + Lymphocytes with a Th2 Cytokine Profile . *The Journal of Immunology*, *164*(6), 3047–3055. https://doi.org/10.4049/jimmunol.164.6.3047

Jauréguiberry, S., Paris, L., & Caumes, E. (2010). Acute schistosomiasis, a diagnostic and therapeutic challenge. *Clinical Microbiology and Infection*, *16*(3), 225–231. https://doi.org/10.1111/j.1469-0691.2009.03131.x

Jobgen, W. S., Jobgen, S. C., Li, H., Meininger, C. J., & Wu, G. (2007). Analysis of nitrite and nitrate in biological samples using high-performance liquid chromatography. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *851*(1–2), 71–82. https://doi.org/10.1016/j.jchromb.2006.07.018

Johnson, R. J., Bakris, G. L., Borghi, C., Chonchol, M. B., Feldman, D., Lanaspa, M. A., Merriman, T. R., Moe, O. W., Mount, D. B., Sanchez Lozada, L. G., Stahl, E., Weiner, D. E., & Chertow, G. M. (2018). Hyperuricemia, Acute and Chronic Kidney Disease, Hypertension, and Cardiovascular Disease: Report of a Scientific Workshop Organized by the National Kidney Foundation. *American Journal of Kidney Diseases*, *71*(6), 851–865. https://doi.org/10.1053/j.ajkd.2017.12.009

Johnson, R. J., Lanaspa, M. A., & Gaucher, E. A. (2011). Uric Acid: A Danger Signal From the RNA World That May Have a Role in the Epidemic of Obesity, Metabolic Syndrome, and Cardiorenal Disease: Evolutionary Considerations. *Seminars in Nephrology*, *31*(5), 394–

399. https://doi.org/10.1016/j.semnephrol.2011.08.002

Kagira, J. M., Maina, N., Njenga, J., Karanja, S. M., Karori, S. M., & Ngotho, J. M. (n.d.). Prevalence and types of coinfections in sleeping sickness patients in Kenya (2000/2009). *Journal of Tropical Medicine*. https://doi.org/10.1155/2011/248914

Kagira, J., Thuita, J., Ngotho, M., Mdachi, R., Mwangangi, D., & Ndung'u, J. (2008). Haematology of experimental *Trypanosoma brucei rhodesiense* infection in vervet monkeys. *African Journal of Health Sciences*, *13*(3). https://doi.org/10.4314/ajhs.v13i3.30837

Kamau, E., Yates, A., Maisiba, R., Singoei, V., Opot, B., Adeny, R., Arima, C. O., Otieno, V., Sumbi, C. S., Okoth, R. O., Abdi, F., Mwalo, M., Ochola, J., Otieno, J., Ake, J., Imbach, M., Turley, H. A., Juma, D., Akala, H. M., … Tran, C. (2021). Epidemiological and clinical implications of asymptomatic malaria and schistosomiasis co-infections in a rural community in western Kenya. *BMC Infectious Diseases*, *21*(1), 1–13. https://doi.org/10.1186/s12879-021-06626-2

Kato, C. D., Matovu, E., Mugasa, C. M., Nanteza, A., & Alibu, V. P. (2016). The role of cytokines in the pathogenesis and staging of Trypanosoma brucei rhodesiense sleeping sickness. *Allergy, Asthma and Clinical Immunology*, *12*(1), 1–10. https://doi.org/10.1186/s13223-016-0113-5

Kato, C. D., Nanteza, A., Mugasa, C., Edyelu, A., Matovu, E., & Alibu, V. P. (2015). Clinical profiles, disease outcome and co- Morbidities among T.b. rhodesiense sleeping sickness patients in uganda. *PLoS ONE*, *10*(2), 1–13. https://doi.org/10.1371/journal.pone.0118370

Kennedy, P. G. E. (2004). Human African trypanosomiasis of the CNS: Current issues and challenges. *Journal of Clinical Investigation*, *113*(4), 496–504. https://doi.org/10.1172/JCI200421052

Khoubnasab Jafari, M., Ansarin, K., & Jouyban, A. (2015). Comments on "use of malondialdehyde as a biomarker for assesing oxidative stress in different disease pathologies: A review." *Iranian Journal of Public Health*, *44*(5), 714–715.

Khoubnasabjafari, M., Ansarin, K., & Jouyban, A. (2015). Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. *BioImpacts*, *5*(3), 123–127. https://doi.org/10.15171/bi.2015.20

Kioy, D., Jannin, J., & Mattock, N. (2004). Human African trypanosomiasis. *Nature Reviews Microbiology*, *2*(3), 186–187. https://doi.org/10.1038/nrmicro848

Kipkorir, L. W., John, T. K., Owino, O. B., John, O., Robert, S., Daniel, M., & Owino, A. V. (2021). Mouse experiments demonstrate differential pathogenicity and virulence of Trypanosoma brucei rhodesiense strains. *Experimental Parasitology*, *228*, 1–23. https://doi.org/10.1016/j.exppara.2021.108135

Kuriakose, S. M., Singh, R., & Uzonna, J. E. (2016). Host intracellular signaling events and pro-inflammatory cytokine production in African trypanosomiasis. *Frontiers in Immunology*, *7*(MAY), 1–10. https://doi.org/10.3389/fimmu.2016.00181

Kyung, S. Y., Cho, Y. K., Kim, Y. J., Park, J. W., Jeong, S. H., Lee, J. I., Sung, Y. M., & Lee, S. P. (2011). A Paragonimiasis Patient with Allergic Reaction to Praziquantel and Resistance to Triclabendazole: Successful Treatment after Desensitization to Praziquantel. *Korean*

Journal of Parasitology, *49*(1), 73–77. https://doi.org/10.3347/kjp.2011.49.1.73

La Flamme, A. C., Patton, E. A., & Pearce, E. J. (2001). Role of gamma interferon in the pathogenesis of severe Schistosomiasis in interleukin-4-deficient mice. *Infection and Immunity*, *69*(12), 7445–7452. https://doi.org/10.1128/IAI.69.12.7445-7452.2001

La Flamme, Anne Camille, Patton, E. A., Bauman, B., & Pearce, E. J. (2001). IL-4 Plays a Crucial Role in Regulating Oxidative Damage in the Liver During Schistosomiasis. *The Journal of Immunology*, *166*(3), 1903–1911. https://doi.org/10.4049/jimmunol.166.3.1903

Lambertucci, J. R. (2010). Acute schistosomiasis mansoni: Revisited and reconsidered. *Memorias Do Instituto Oswaldo Cruz*, *105*(4), 422–435. https://doi.org/10.1590/S0074-02762010000400012

Lawrence, J. D. (1973). The ingestion of red blood cells by Schistosoma mansoni. *Journal of Parasitology*, *59*(1), 60–63. https://doi.org/10.2307/3278572

Leir, S. A., Foot, O., Jeyaratnam, D., & Whyte, M. B. (2019). *Schistosomiasis and associated iron-deficiency anaemia presenting decades after immigration from sub-Saharan Africa*. https://doi.org/10.1136/bcr-2018-227564

Leonard, K. T. D., Khan, P. V, & Gustave, L. L. (2020). *Some Haematological Parameters among Urinary Schistosomiasis-Malaria Coinfected Children in Suburb of Malentouen Health District , West Region Cameroon*. *41*(7), 34–44. https://doi.org/10.9734/IJTDH/2020/v41i730295

Lewis, D. I. (2019). Animal experimentation: implementation and application of the 3Rs. *Emerging Topics in Life Sciences*, *3*(6), 675–679. https://doi.org/10.1042/etls20190061

Lima-Junior, J. da C., Rodrigues-da-Silva, R. N., Pereira, V. A., Storer, F. L., Perce-da-Silva, D. de S., Fabrino, D. L., Santos, F., Banic, D. M., & de Oliveira-Ferreira, J. (2012). Cells and mediators of inflammation (C-reactive protein, nitric oxide, platelets and neutrophils) in the acute and convalescent phases of uncomplicated Plasmodium vivax and Plasmodium falciparum infection. *Memorias Do Instituto Oswaldo Cruz*, *107*(8), 1035–1041. https://doi.org/10.1590/S0074-02762012000800012

Liu, H. T., Huang, Y. C., Cheng, S. Bin, Huang, Y. T., & Lin, P. T. (2016). Effects of coenzyme Q10 supplementation on antioxidant capacity and inflammation in hepatocellular carcinoma patients after surgery: a randomized, placebo-controlled trial. *Nutrition Journal*, *15*(1), 1–9. https://doi.org/10.1186/s12937-016-0205-6

Maclean, L., Chisi, J. E., Odiit, M., Gibson, W. C., Ferris, V., Picozzi, K., & Sternberg, J. M. (2004). *2004-Upgrading in Clusters and Value Chains in Latin America.pdf*. *72*(12), 7040–7044. https://doi.org/10.1128/IAI.72.12.7040

Martinon, F., Pétrilli, V., Mayor, A., Tardivel, A., & Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*, *440*(7081), 237–241. https://doi.org/10.1038/nature04516

Mbow, M., Larkin, B. M., Meurs, L., Wammes, L. J., De Jong, S. E., Labuda, L. A., Camara, M., Smits, H. H., Polman, K., Dieye, T. N., Mboup, S., Stadecker, M. J., & Yazdanbakhsh, M. (2013). T-helper 17 cells are associated with pathology in human schistosomiasis. *Journal of Infectious Diseases*, *207*(1), 186–195. https://doi.org/10.1093/infdis/jis654

McGovern, K., & Wilson, E. (2014). Role of Chemokines and Trafficking of Immune Cells in

Parasitic Infections. *Current Immunology Reviews*, *9*(3), 157–168. https://doi.org/10.2174/1573395509666131217000000

Monica, N., Gloria Grace, A., & George W, L. (2020). Early Blood Brain Barrier Traversal by Trypanosoma Brucei Brucei Strains: A Phenomenon of Laboratory Strains or Field Strains as Well? *International Journal of Tropical Diseases*, *3*(1), 1–7. https://doi.org/10.23937/2643-461x/1710027

Moriyasu, T., Nakamura, R., Deloer, S., Senba, M., Kubo, M., Inoue, M., Culleton, R., & Hamano, S. (2018). Schistosoma mansoni infection suppresses the growth of Plasmodium yoelii parasites in the liver and reduces gametocyte infectivity to mosquitoes. *PLoS Neglected Tropical Diseases*, *12*(1), 1–17. https://doi.org/10.1371/journal.pntd.0006197

Morrison, W. I., Murray, M., Sayer, P. D., & Preston, J. M. (1981). The pathogenesis of experimentally induced Trypanosoma brucei infection in the dog I. Tissue and organ damage. *American Journal of Pathology*, *102*(2), 168–181.

Mott, K. E., Desjeux, P., Moncayo, A., Ranque, P., & De Raadt, P. (1990). Parasitic diseases and urban development. *Bulletin of the World Health Organization*, *68*(6), 691–698.

Mouahid, G., Rognon, A., de Carvalho Augusto, R., Driguez, P., Geyer, K., Karinshak, S., Luviano, N., Mann, V., Quack, T., Rawlinson, K., Wendt, G., Grunau, C., & Moné, H. (2018). Transplantation of schistosome sporocysts between host snails: A video guide [version 1; referees: 2 approved]. *Wellcome Open Research*, *3*(0), 1–8. https://doi.org/10.12688/wellcomeopenres.13488.1

Namangala, B., de Baetselier, P., & Beschin, A. (2009). Both type-I and type-II responses

contribute to murine trypanotolerance. *Journal of Veterinary Medical Science*, *71*(3), 313–318. https://doi.org/10.1292/jvms.71.313

Namangala, B., Noël, W., De Baetselier, P., Brys, L., & Beschin, A. (2001). Relative contribution of interferon-γ and interleukin-10 to resistance to murine African trypanosomosis. *Journal of Infectious Diseases*, *183*(12), 1794–1800. https://doi.org/10.1086/320731

Nelwan, M. L. (2019). Schistosomiasis: Life Cycle, Diagnosis, and Control. *Current Therapeutic Research - Clinical and Experimental*, *91*(24), 5–9. https://doi.org/10.1016/j.curtheres.2019.06.001

Ngotho, M., Kagira, J. M., Jensen, H. E., Karanja, S. M., Farah, I. O., & Hau, J. (2009). Immunospecific immunoglobulins and IL-10 as markers for trypanosoma brucei rhodesiense late stage disease in experimentally infected vervet monkeys. *Tropical Medicine and International Health*, *14*(7), 736–747. https://doi.org/10.1111/j.1365-3156.2009.02285.x

Niranjan Banik, Adam Koesoemadinata, Charles Wagner, Charles Inyang, H. B. (2013). *No Title Стационарная медицинская помощь (основы организации)*. *June*, 834–837. https://doi.org/10.1190/segam2013-0137.1

Noyes, H. A., Alimohammadian, M. H., Agaba, M., Brass, A., Fuchs, H., Gailus-Durner, V., Hulme, H., Iraqi, F., Kemp, S., Rathkolb, B., Wolf, E., de Angelis, M. H., Roshandel, D., & Naessens, J. (2009). Mechanisms controlling anaemia in Trypanosoma congolense infected mice. *PLoS ONE*, *4*(4). https://doi.org/10.1371/journal.pone.0005170

Nyakundi, R. K., Nyamongo, O., Maamun, J., Akinyi, M., Mulei, I., Farah, I. O., Blankenship, D., Grimberg, B., Hau, J., Malhotra, I., Ozwara, H., King, C. L., & Kariuki, T. M. (2016). Protective effect of chronic schistosomiasis in baboons coinfected with Schistosoma mansoni and Plasmodium knowlesi. *Infection and Immunity*, *84*(5), 1320–1330. https://doi.org/10.1128/IAI.00490-15

Ogongo, P., Nyakundi, R. K., Chege, G. K., & Ochola, L. (2022). The Road to Elimination: Current State of Schistosomiasis Research and Progress Towards the End Game. *Frontiers in Immunology*, *13*(May), 1–23. https://doi.org/10.3389/fimmu.2022.846108

Oladipo, O. O., Ayo, J. O., Ambali, S. F., & Mohammed, B. (2016). Evaluation of hepatorenal impairments in Wistar rats coexposed to low-dose lead, cadmium and manganese: insights into oxidative stress mechanism. *Toxicology Mechanisms and Methods*, *26*(9), 674–684. https://doi.org/10.1080/15376516.2016.1223242

Omobowale, T. O., Oyagbemi, A. A., Oyewunmi, O. A., & Adejumobi, O. A. (2015). Chemopreventive effect of methanolic extract of Azadirachta indica on experimental Trypanosoma brucei induced oxidative stress in dogs. *Pharmacognosy Research*, *7*(3), 249–258. https://doi.org/10.4103/0974-8490.157972

Onyilagha, C., & Uzonna, J. E. (2019). *Host Immune Responses and Immune Evasion Strategies in African Trypanosomiasis*. *10*(November). https://doi.org/10.3389/fimmu.2019.02738

Papi, S., Ahmadizar, F., & Hasanvand, A. (2019). The role of nitric oxide in inflammation and oxidative stress. *Immunopathologia Persa*, *5*(1), e08–e08. https://doi.org/10.15171/ipp.2019.08

Paran, Y. (2018). Massive Splenomegaly: A Case Report of Visceral Leishmania and Schistosomiasis Co-infection and a Review of Infectious Causes of Massive Splenomegaly. *Clinical Case Reports and Reviews*, *4*(6), 1–3. https://doi.org/10.15761/ccrr.1000422

Patel, T. A., Lukawska, J., Rowe, J., & Bailey, R. L. (2016). Case report: Treatment of schistosomiasis in a patient allergic to praziquantel: A desensitization and treatment protocol. *American Journal of Tropical Medicine and Hygiene*, *95*(5), 1041–1043. https://doi.org/10.4269/ajtmh.16-0462

Pearce, E. J. (2005). Priming of the immune response by schistosome eggs. *Parasite Immunology*, *27*(7–8), 265–270. https://doi.org/10.1111/j.1365-3024.2005.00765.x

Pearce, E. J., Cheever, A., Leonard, S., Covalesky, M., Fernandez-Botran, R., Kohler, G., & Kopf, M. (1996). Schistosoma mansoni in IL-4-deficient mice. *International Immunology*, *8*(4), 435–444. https://doi.org/10.1093/intimm/8.4.435

Pearce, E. J., & MacDonald, A. S. (2002). The immunobiology of schistosomiasis. *Nature Reviews Immunology*, *2*(7), 499–511. https://doi.org/10.1038/nri843

Pêgo, B., Martinusso, C. A., Bernardazzi, C., Ribeiro, B. E., De Araujo Cunha, A. F., De Souza Mesquita, J., Nanini, H. F., Machado, M. P., Castelo-Branco, M. T. L., Cavalcanti, M. G., & De Souza, H. S. P. (2019). Schistosoma mansoni Coinfection Attenuates Murine Toxoplasma gondii-Induced Crohn's-Like Ileitis by Preserving the Epithelial Barrier and Downregulating the Inflammatory Response. *Frontiers in Immunology*, *10*(MAR), 1–16. https://doi.org/10.3389/fimmu.2019.00442

Pereira, L., Oliveira, F., Townsend, S., Metangmo, S., Meneses, C., Moore, I. N., Brodskyn, C.

I., Valenzuela, J. G., Magez, S., & Kamhawi, S. (2018). Coinfection With Trypanosoma brucei Confers Protection Against Cutaneous Leishmaniasis. *Frontiers in Immunology*, *9*, 2855. https://doi.org/10.3389/fimmu.2018.02855

Pérez del Villar, L., Burguillo, F. J., López-Abán, J., & Muro, A. (2012). Systematic Review and Meta-Analysis of Artemisinin Based Therapies for the Treatment and Prevention of Schistosomiasis. *PLoS ONE*, *7*(9). https://doi.org/10.1371/journal.pone.0045867

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, *2017*. https://doi.org/10.1155/2017/8416763

Ponte-Sucre, A. (2016). An overview of trypanosoma brucei infections: An intense host-parasite interaction. *Frontiers in Microbiology*, *7*(DEC), 1–12. https://doi.org/10.3389/fmicb.2016.02126

Quinlan, G. J., Mumby, S., Martin, G. S., Bernard, G. R., Gutteridge, J. M. C., & Evans, T. W. (2004). Albumin influences total plasma antioxidant capacity favorably in patients with acute lung injury. *Critical Care Medicine*, *32*(3), 755–759. https://doi.org/10.1097/01.CCM.0000114574.18641.5D

Rodgers, J. (2010). Trypanosomiasis and the brain. *Parasitology*, *137*(14), 1995–2006. https://doi.org/10.1017/S0031182009991806

Romão, P. R. T., Tovar, J., Fonseca, S. G., Moraes, R. H., Cruz, A. K., Hothersall, J. S., Noronha-Dutra, A. A., Ferreira, S. H., & Cunha, F. Q. (2006). Glutathione and the redox control system trypanothione/trypanothione reductase are involved in the protection of

Leishmania spp. against nitrosothiol-induced cytotoxicity. *Brazilian Journal of Medical and Biological Research*, *39*(3), 355–363. https://doi.org/10.1590/S0100-879X2006000300006

Rong, R., Cui, M. Y., Zhang, Q. L., Zhang, M. Y., Yu, Y. M., Zhou, X. Y., Yu, Z. G., & Zhao, Y. L. (2016). Anesthetic constituents of Zanthoxylum bungeanum Maxim.: A pharmacokinetic study. *Journal of Separation Science*, *39*(14), 2728–2735. https://doi.org/10.1002/jssc.201600295

Rutto, J. J., Osano, O., Thuranira, E. G., Kurgat, R. K., & Odenyo, V. A. O. (2013). Socio-Economic and Cultural Determinants of Human African Trypanosomiasis at the Kenya - Uganda Transboundary. *PLoS Neglected Tropical Diseases*, *7*(4). https://doi.org/10.1371/journal.pntd.0002186

Saad-Hussein, A., Ibrahim, K. S., Abdalla, M. S., El-Mezayen, H. A., & Osman, N. F. A. (2020). Effects of zinc supplementation on oxidant/antioxidant and lipids status of pesticides sprayers. *Journal of Complementary and Integrative Medicine*, *17*(1), 1–8. https://doi.org/10.1515/jcim-2019-0001

Sanches-Vaz, M., Temporão, A., Luis, R., Nunes-Cabaço, H., Mendes, A. M., Goellner, S., Carvalho, T., Figueiredo, L. M., & PrudêncioI, M. (2019). Trypanosoma brucei infection protects mice against malaria. *PLoS Pathogens*, *15*(11), 1–27. https://doi.org/10.1371/journal.ppat.1008145

Sanin, D. E., Prendergast, C. T., Bourke, C. D., & Mountford, A. P. (2015). Helminth Infection and Commensal Microbiota Drive Early IL-10 Production in the Skin by CD4+ T Cells That Are Functionally Suppressive. *PLoS Pathogens*, *11*(5), 1–23.

https://doi.org/10.1371/journal.ppat.1004841

Semenya, A. A., Sullivan, J. A. S., Barnwell, J. W., & Secor, W. E. (2012). Schistosoma mansoni infection impairs antimalaria treatment and immune responses of rhesus macaques infected with mosquito-borne Plasmodium coatneyi. *Infection and Immunity*, *80*(11), 3821–3827. https://doi.org/10.1128/IAI.00590-12

Sharaf El Din, U. A. A., Salem, M. M., & Abdulazim, D. O. (2017). Uric acid in the pathogenesis of metabolic, renal, and cardiovascular diseases: A review. *Journal of Advanced Research*, *8*(5), 537–548. https://doi.org/10.1016/j.jare.2016.11.004

Shi, M., Pan, W., & Tabel, H. (2003). Experimental African trypanosomiasis: IFN-γ mediates early mortality. *European Journal of Immunology*, *33*(1), 108–118. https://doi.org/10.1002/immu.200390013

Silva-Santana, G., Bax, J. C., Fernandes, D. C. S., Bacellar, D. T. L., Hooper, C., Dias, A. A. S. O., Silva, C. B., Souza, A. M., Ramos, S., Santos, R. A., Pinto, T. R., Ramão, M. A., & Mattos-Guaraldi, A. L. (2020). Clinical hematological and biochemical parameters in Swiss, BALB/c, C57BL/6 and B6D2F1 Mus musculus . *Animal Models and Experimental Medicine*, *3*(4), 304–315. https://doi.org/10.1002/ame2.12139

Slater, T. F. (1984). Free-radical mechanisms in tissue injury. *The Biochemical Journal*, *222*(1), 1–15. https://doi.org/10.1042/bj2220001

Society, T. A., & Journal, T. (2010). *An Efficient Method for Exposure of Mice to Cercariae of Schistosoma mansoni Author (s): Louis Olivier and M . A . Stirewalt Published by : The American Society of Parasitologists Stable URL : http://www.jstor.org/stable/3274166*.

38(1), 19–23.

Sternberg, J. M. (2004). Human African trypanosomiasis: Clinical presentation and immune response. *Parasite Immunology*, *26*(11–12), 469–476. https://doi.org/10.1111/j.0141-9838.2004.00731.x

Sternberg, J. M., Rodgers, J., Bradley, B., MacLean, L., Murray, M., & Kennedy, P. G. E. (2005). Meningoencephalitic African trypanosomiasis: Brain IL-10 and IL-6 are associated with protection from neuro-inflammatory pathology. *Journal of Neuroimmunology*, *167*(1–2), 81–89. https://doi.org/10.1016/j.jneuroim.2005.06.017

Stijlemans, B., Cnops, J., Naniima, P., Vaast, A., Bockstal, V., De Baetselier, P., & Magez, S. (2015). Development of a pHrodo-Based Assay for the Assessment of In Vitro and In Vivo Erythrophagocytosis during Experimental Trypanosomosis. *PLoS Neglected Tropical Diseases*, *9*(3), 1–21. https://doi.org/10.1371/journal.pntd.0003561

Stijlemans, B., De Baetselier, P., Magez, S., Van Ginderachter, J. A., & De Trez, C. (2018). African trypanosomiasis-associated anemia: The contribution of the interplay between parasites and the mononuclear phagocyte system. *Frontiers in Immunology*, *9*(FEB). https://doi.org/10.3389/fimmu.2018.00218

Tamarozzi, F., Fittipaldo, V. A., Orth, H. M., Richter, J., Buonfrate, D., Riccardi, N., & Gobbi, F. G. (2021). Diagnosis and clinical management of hepatosplenic schistosomiasis: A scoping review of the literature. *PLoS Neglected Tropical Diseases*, *15*(3), 1–25. https://doi.org/10.1371/journal.pntd.0009191

Tesoriero, C., Xu, Y. Z., Mumba Ngoyi, D., & Bentivoglio, M. (2018). Neural damage in

experimental trypanosoma brucei gambiense infection: The suprachiasmatic nucleus. *Frontiers in Neuroanatomy*, *12*(February), 1–10. https://doi.org/10.3389/fnana.2018.00006

Thijs, L., Messiaen, P., van der Hilst, J., Madoe, V., Melis, C., Van Eyken, P., Vanmoerkerke, I., & Janssens, F. (2018). Hepatic schistosomiasis with massive splenomegaly: A case report and literature review. *Acta Gastro-Enterologica Belgica*, *81*(1), 93–96.

U Olveda, D. (2013). Bilharzia: Pathology, Diagnosis, Management and Control. *Tropical Medicine & Surgery*, *01*(04), 1–19. https://doi.org/10.4172/2329-9088.1000135

van de Crommenacker, J., Richardson, D. S., Koltz, A. M., Hutchings, K., & Komdeur, J. (2012). Parasitic infection and oxidative status are associated and vary with breeding activity in the Seychelles warbler. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1733), 1466–1476. https://doi.org/10.1098/rspb.2011.1865

Vasilev, N., Smales, C. M., Schillberg, S., Fischer, R., & Schiermeyer, A. (2016). Developments in the production of mucosal antibodies in plants. *Biotechnology Advances*, *34*(2), 77–87. https://doi.org/10.1016/j.biotechadv.2015.11.002

Vieira-de-Abreu, A., Campbell, R. A., Weyrich, A. S., & Zimmerman, G. A. (2012). Platelets: Versatile effector cells in hemostasis, inflammation, and the immune continuum. *Seminars in Immunopathology*, *34*(1), 5–30. https://doi.org/10.1007/s00281-011-0286-4

Waknine-Grinberg, J. H., Gold, D., Ohayon, A., Flescher, E., Heyfets, A., Doenhoff, M. J., Schramm, G., Haas, H., & Golenser, J. (2010). Schistosoma mansoni infection reduces the incidence of murine cerebral malaria. *Malaria Journal*, *9*(1), 1–11. https://doi.org/10.1186/1475-2875-9-5

Wammes, L. J., Hamid, F., Wiria, A. E., Wibowo, H., Sartono, E., Maizels, R. M., Smits, H. H., Supali, T., & Yazdanbakhsh, M. (2012). Regulatory T cells in human lymphatic filariasis: Stronger functional activity in microfilaremics. *PLoS Neglected Tropical Diseases*, *6*(5). https://doi.org/10.1371/journal.pntd.0001655

Wang, J. (2015). *Inflammatory stress potentiates emodin-induced liver injury in rats*. *6*(October), 1–7. https://doi.org/10.3389/fphar.2015.00233

Weyrich, A. S., & Zimmerman, G. A. (2004). Platelets: Signaling cells in the immune continuum. *Trends in Immunology*, *25*(9), 489–495. https://doi.org/10.1016/j.it.2004.07.003

WHO, W. H. O. (2022). *WHO GUIDELINE on control and elimination of human schistosomiasis*.

Wilson, S., Jones, F. M., Mwatha, J. K., Kimani, G., Booth, M., Kariuki, H. C., Vennervald, B. J., Ouma, J. H., Muchiri, E., & Dunne, D. W. (2008). *Hepatosplenomegaly Is Associated with Low Regulatory and Th2 Responses to Schistosome Antigens in Childhood Schistosomiasis and Malaria Coinfection □*. *76*(5), 2212–2218. https://doi.org/10.1128/IAI.01433-07

Wozniak, D. M., Kirchoff, N., Hansen-Kant, K., Sogoba, N., Safronetz, D., & Prescott, J. (2021). Hematology and clinical chemistry reference ranges for laboratory-bred natal multimammate mice (Mastomys natalensis). *Viruses*, *13*(2). https://doi.org/10.3390/v13020187

Wright, H. L., Moots, R. J., Bucknall, R. C., & Edwards, S. W. (2010). Neutrophil function in inflammation and inflammatory diseases. *Rheumatology*, *49*(9), 1618–1631.

https://doi.org/10.1093/rheumatology/keq045

Wynn, T. A., Cheever, A. W., Williams, M. E., Hieny, S., Caspar, P., Kühn, R., Müller, W., & Sher, A. (1998). IL-10 regulates liver pathology in acute murine Schistosomiasis mansoni but is not required for immune down-modulation of chronic disease. *Journal of Immunology (Baltimore, Md. : 1950)*, *160*(9), 4473–4480. http://www.ncbi.nlm.nih.gov/pubmed/9574553

Xiao, S. H., Mei, J. Y., & Jiao, P. Y. (2009). The in vitro effect of mefloquine and praziquantel against juvenile and adult Schistosoma japonicum. *Parasitology Research*, *106*(1), 237–246. https://doi.org/10.1007/s00436-009-1656-x

Yoshihara, K., Morris, A., Iraqi, F., & Naessens, J. (2007). Cytokine mRNA profiles in bovine macrophages stimulated with Trypanosoma congolense. *Journal of Veterinary Medical Science*, *69*(4), 421–423. https://doi.org/10.1292/jvms.69.421

Zuim, N. R. B., Allegretti, S. M., Linhares, A. X., Magalhães, L. A., & Zanotti-Magalhães, E. M. (2012). A study of the granulomatous responses induced by different strains of Schistosoma mansoni. *Interdisciplinary Perspectives on Infectious Diseases*, *2012*, 21–27. https://doi.org/10.1155/2012/953524

APPENDICES

Appendix 1: Evans Blue Assay

Evans Blue Assay:

- 200µl 2% Evans Blue in 0,95% NaCl (w/v)
- inject intravenously
- let mice sit for 1 hour
- sacrifice mice
- remove brains
- photograph brains (good results on white background, e.g. paper)
- weigh brain and put into 2 ml Formamide
- incubate for 40-48 hours at 37°C on shaker

Quantification of Evans Blue extravasation after 2 days:

- 100µl of the incubated formamide/brain solution in each well (triplicates)
- For the standard curve dilute Evans Blue to 200µg/ml (1:100) with formamide (starting concentration).
- Dilute standard 1:2 in each well (triplicates) with formamide
- measure Concentration of Evans Blue in an ELISA reader at 620nm
- Calculate µg Evans Blue/ g brain tissue *2 (total EB in 2ml formamide)

Optional (Anmerkung: Hab ich noch nie gemacht ☺)
Detection of Evans Blue in brain sections: (not optimized)

- under the fluorescence microscope Evans Blue can be detected with the DAPI- or FITC-channel
- Evans Blue fluoresces red
- counterstain with other markers or HE can be done – not optimized

Appendix 2: Plagiarism report

THE IMPACT OF SCHISTOSOMA MANSONI ON DISEASE SEVERITY OF SECONDARY INFECTION WITH TRYPANOSOMA BRUCEI RHODESIENSE IN A MOUSE MODEL

ORIGINALITY REPORT

20%	15%	15%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to The Technical University of Kenya Student Paper	1%
2	www.frontiersin.org Internet Source	1%
3	Lynn Kitwan, Celestine Makobe, Raymond Mdachi, Dawn Nyawira Maranga, Alfred Orina Isaac, James Nyabuga Nyariki. "Coenzyme Q10 prevented Trypanosoma brucei rhodesiense-mediated breach of the blood brain barrier, inflammation and organ damage in late stage of Human African Trypanosomiasis", Journal of Parasitic Diseases, 2022 Publication	1%
4	www.hindawi.com Internet Source	1%
5	Michelle Carvalho de Rezende, João Marcelo Peixoto Moreira, Laura Liana Maggi	1%

Appendix 3: Ethical approval

Institute of Primate Research
Address: P. O. Box 24481-00502 Karen Nairobi Kenya I Tel: +254 02 2606235 | Fax: +254 02 260623 1
URL: www.primateresearch.OFF | Email : directoripr@ primateresearch.org

NATIONAL MUSEUMS OF KENYA
WILDLIFE HERITAGE DIVISION

INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: ISERC/08/2017

Dear Dr. Alfred Orina Isaac,

It is my pleasure to inform you that your proposal entitled "**The impact of *schistosoma mansoni* on disease severity of secondary infection with *trypanosoma brucei rhodesiense* in a mouse model**" has been reviewed by the Institutional Review Committee (IRC) at a meeting of 20" February 2018. The proposal was rex'iewed on the scientific merit and ethical considerations on the use of animals for research purposes.

This proposal was approved with the following recommendation;

The committee is guided by the Institutional guidelines as well as International regulations. Including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed... for Chairman IRC: Dr Lilly Olhola.

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED

Signed... Secretary IRC: Dr. MERCY AKINYI

Date 05/03/2018