# ROLE OF CYTOKINES IN VARYING *Trypanosoma brucei rhodesiense* INFECTION OUTCOMES IN VERVET MONKEYS

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## DECLARATION AND RECOMMENDATION

### Declaration

This thesis is my original work and has not been submitted or presented for examination in any institution.

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### Recommendation

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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## DEDICATION

I dedicate this thesis to God, who has been my sole provider, source of my strength, and knowledge throughout this work. All glory to Him.

I dedicate this work to my beloved parents, the late Mr. William Busienei and Mrs. Susana Busienei, who have always supported my dreams and ambitions. Their words of encouragement and support have taught me to handle matters one step at a time.

I dedicate this work to my lovely husband, Mr. Micah Lagat and our dear children Irene Jepkoech, Ryan Kimutai and Liam Kipchumba, whose presence in my life gives me strength and encouragement to do better.

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## ABSTRACT

The clinical manifestations of Rhodesian human African trypanosomiasis (rHAT), which is caused by *T. b. rhodesiense*, range from acute illness to chronic illness. The basis of this is poorly understood but is suggested to involve host and pathogen factors, and their interaction. Among other factors, host immune factors, including cytokines, play a role which has begun to unravel, and has been shown to vary depending on the host, pathogen species and strain, and mode of infection. These factors have also been suggested to be potential diagnostic biomarkers. With clinical diagnosis important in HAT, understanding the underlying basis of varying infection outcomes is important. In this study, the vervet monkey (*Chlorocebus aethiops*), a non-human primate (NHP) model of HAT, was employed to investigate the involvement of cytokines in the manifestation of different clinical outcomes of rHAT. The work utilised pathological data along with cryopreserved, archived serum and cerebrospinal fluid (CSF) samples obtained from previously infected animals. Two groups of vervet monkeys were infected with strains KETRI 3801 and KETRI 3928 to represent acute and chronic disease forms, respectively, alongside an uninfected control group. Three animals per group (n=3) for plasma analysis and two animals per group(n=2) for CSF analysis were selected due to limited resources available. Quantities of immune modulators, namely INF-γ, TGF-β, TNF-α, IL-1β, IL-6, IL-10, IL-12, IL-13 and a brain damage biomarker protein, S100B were determined, and pathological data, including survival time, parasitaemia, packed cell volume (PCV), temperature, weight and food intake, were analysed. The levels of individual cytokines showed variations in the course of infection. Levels of IL-12, IL-6 and IL-1β cytokines were significantly increased ($p<0.05$) from the early stage through to the onset of late-stage disease. Additionally, cerebrospinal fluid (CSF) parasite counts and white blood cell (WBC) levels were higher in KETRI 3801 infections compared to KETRI 3928. IL-12, IL-6 and IL-1β cytokines were particularly higher in acute infection, possibly contributing to the severity of the disease in KETRI 3801 infections, while the interaction between IL-1β, IL-6 and IL-10 played a typical pro- and counter-inflammatory response during infection progression. Fluctuations in parasitaemia were observed in infected animals, with the KETRI 3801 cohort exhibiting a higher parasitaemia (peak antilog 8.7) than KETRI 3928 (peak antilog 7.8). In addition, infected animals had higher febrile temperature, lower body weight and PCV, which these much pronounced in acute as compared to chronic infections. Monkeys infected with KETRI 3801 and KETRI 3928 had a mean survival time of 28 and 95 days, respectively. The findings suggest strain-directed and host-dependent immunomodulation as the basis of the different infection outcomes. Also, cytokines are key regulators of disease progression and severity in the NHP model of HAT, and they are essential for understanding the differences in infection outcomes

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| | |
|---|---|
| **AAT** | Animal African Trypanosomiasis |
| **ApoL1** | Apolipoprotein 1 |
| **AT** | African Trypanosomiasis |
| **BBB** | Blood Brain Barrier |
| **BCF** | Blood Cerebrospinal Fluid Barrier |
| **CATT** | Card Agglutination Test for Trypanosomiasis |
| **CNS** | Central Nervous System |
| **CSF** | Cerebrospinal Fluid |
| **CTL** | Cytotoxic Lymphocytes |
| **DALYs** | Disability Adjusted Life Years |
| **ELISA** | Enzyme-Linked Immunosorbent Assay |
| **FAO** | Food and Agriculture Organization |
| **GDB** | Global burden of disease |
| **GPI** | Glycosylphosphatidylinositol |
| **HAT** | Human African Trypanosomiasis |
| **HPR** | Haptoglobin Related Protein |
| **ICAM** | Intercellular Adhesion Molecule |
| **IL** | Interleukin |
| **MHCs** | Major Histocompatibility Complexes |
| **NTDs** | Neglected Tropical Diseases |
| **ODs** | Optical Densities |
| **PAMPS** | Pathogen Associated Molecular Patterns |
| **PATTEC** | Pan Africa Tsetse and Trypanosomiasis Eradication Campaign |
| **PCV** | Packed Cell Volume |

| | |
|---|---|
| **PRR** | Pathogen Recognition Receptors |
| **RNI** | Reactive Nitrogen Intermediates |
| **ROC** | Receiver Operating Characteristic |
| **ROI** | Reactive Oxygen Intermediates |
| **RT** | Room Temperature |
| **SAT** | Sequential Aerosol Technique |
| **SEM** | Standard Error of Mean |
| **SIT** | Sterile Insect Technology |
| **SPP** | Streptavidin-HRP Polymer |
| **SPSS** | Statistical Package for Social Sciences |
| **SRA** | Serum Associated Antigen |
| ***Tbg*** | *Trypanosoma brucei gambiense* |
| ***Tbr*** | *Tripanosoma brucei rhodesiense* |
| **Th** | T helper Cells |
| **TLF** | Trypanolytic Factor |
| **TLR** | Toll-Like Receptor |
| **TMB** | 3,3′,5,5′-Tetramethylbenzidine |
| **USD** | United State Dollar |
| **VCAM** | Vascular Cell Adhesion Molecule |
| **VSG** | Variable Surface Glycoprotein |
| **WBC** | White Blood Cells |
| **WHO** | World Health Organization |

# CHAPTER ONE
# INTRODUCTION

## 1.1 Background information

African trypanosomes (genus *Trypanosoma*) are flagellated parasitic protozoa responsible for a set of diseases collectively known as African trypanosomiasis (AT). In humans, the disease is commonly referred to as sleeping sickness (human African trypanosomiasis, HAT), while in cattle it is commonly called nagana (African animal Trypanosomiasis, AAT). Transmission occurs during a blood meal through a bite by an infected tsetse fly (*Glossina* spp.), a vector that is confined to 37 sub-Saharan African countries where the disease remains endemic. Within this region, over 70 million people and about 45 to 50 million cattle have been reported to be at risk, while approximately 21 million people are reported to reside in high-risk zones (Simarro *et al*., 2012; Salah *et al*., 2015). The cattle industry alone suffers annual economic losses estimated at USD 1.0–1.3 billion (Salah *et al*., 2015), while the Food and Agriculture Organization (FAO) estimates overall agricultural losses at about USD 4.75 billion annually in terms of GDP (FAO, 2012; Vreysen *et al*., 2013). Because the disease affects both humans and livestock, its socioeconomic burden is immense. The two main control strategies applied are chemotherapy and transmission disruption through vector control, both of which suffer several limitations. In addition, diagnostic methods remain inadequate. This underscores the pressing need for more effective and innovative approaches to disease control and diagnosis.

Nagana is caused by different African trypanosome species, namely *T. brucei*, *T. congolense*, and *T. vivax*. Other forms in domestic animals also exist and include surra disease in camels caused by *T. evansi*, and dourine, a venereal disease of equines caused by *T. equiperdum.* AAT has a huge economic impact, including reduced milk and meat production in cattle, as well as reduced draught power, which is important in mixed farming (Oluwafemi *et al*., 2007; Thumbi *et al*., 2010). In addition, there are deaths, reduced calving, abortions and poor quality of hide of infected animals. This hugely impacts the livelihood of people living in tsetse-infested areas, the majority of whom are among the world's poorest (Mpanya *et al*., 2012; Shaw *et al*., 2010; WHO, 2013). Notably, both livestock and wild animals serve as reservoirs for African trypanosomes that can infect humans (Ng'ayo *et al.*, 2005; von Wissmann, 2011).

Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is caused by two subspecies of *Trypanosoma brucei*: *T. b. rhodesiense* (*Tbr*) and *T. b. gambiense* (*Tbg*). The Rhodesiense form (rHAT), associated with *Tbr*, generally causes an acute and rapidly progressing illness in eastern and southern Africa, whereas the Gambiense form (gHAT), linked to *Tbg*, leads to a more chronic disease in western and central Africa. Following the bite during a blood meal by an infected tsetse fly, the infection develops in two distinct clinical stages. The first, referred to as the

early or haemolymphatic stage, is characterised by parasite proliferation in blood, lymphatic fluid and other tissues. Subsequently, the parasites traverse the blood-brain barrier (BBB) and/or the blood cerebrospinal fluid (BCF) barrier, infiltrating the central nervous system (CNS) and cerebrospinal fluid (CSF). This progression marks the onset of the second, late stage of the disease, also known as the meningoencephalitic or CNS stage. The disease symptoms are similar to other tropical diseases. Therefore, timely diagnosis is essential to prevent neurological complications. Currently, the World Health Organization (WHO) identifies microscopic detection of parasites in blood or lymph node aspirates as the preferred diagnostic standard (Brun *et al*., 2010; WHO, 2022). If the test is positive, disease staging involves performing a lumbar puncture to examine cerebrospinal fluid for trypanosomes and/or a white blood cell (WBC) count above 5 cells/µL. These procedures suffer several limitations, including low sensitivity, inapplicability in remote settings where the disease is endemic and the need for skilled personnel and health infrastructure that are absent or limited in endemic foci (Mwanakasale *et al*., 2013). These limitations hinder the elimination of the disease. Therefore, there is a pressing need for diagnostic methods that are both more reliable and widely accessible. Here, diagnostic and staging biomarkers are needed.

The disease progression and pathogenesis have been associated with the host and pathogen factors, environment and a combination of these (MacLean *et al*., 2010). The host's immune response is a key determinant in shaping disease progression, influencing pathogenicity and ultimately affecting the overall outcome of infections. Notably, African trypanosomes deploy an elaborated mechanism of immune evasion termed antigenic variation. The parasites undergo sequential and periodic changes in their surface coat protein, known as the variable surface glycoprotein (VSG), which enables them to evade the host's immune system. This mechanism allows the parasites to persist for extended periods, sometimes even decades, increasing the window for cyclic transmission. VSG also affects the immune elements through the induction of cytokines and antibodies (Magez *et al*., 2002). Other parasite factors, including trypanosome-derived lymphocyte triggering factor (TLTF), trigger T cells to produce interferon (IFN)-γ and modulate the immune system (Abdulla *et al*., 2013). Due to the parasite's highly sophisticated immune evasion strategies, timely diagnosis and rapid initiation of treatment are critical for effective disease control and management. This is especially vital for achieving global goals of elimination and eventual eradication. In addition, early detection before progression into stage 2, which is more challenging to manage and involves the use of less effective and toxic drugs, is more desirable. Consequently, the identification of novel detection and staging biomarkers is urgently needed. The cytokines produced during immune system activation show a significant variation in levels throughout the early and late phases of the disease (Kato *et al*., 2015; Maranga *et al*., 2013). This knowledge on variations is important in understanding pathogen-host interactions and host immune response in

infection outcome in *Tbr* infection, which poses a significant challenge in disease management and control.

In *Tbr* infection, the parasite can affect a broad spectrum of wild and domesticated animals, making interruption of transmission unrealistic. Recently, some regions have reported re-emergence of the disease despite not reporting any cases in the past 30 years (Abera *et al*., 2024). This can be attributed to the mutation of *Tbb* strain that infects animals and not humans (Gibson, 2005). Consequently, there is a need to understand host immunity and disease progression.

The host immune response significantly influences disease pathogenicity and progression of HAT. There are varying pathogenicity and infection outcomes in African trypanosome infections, with two forms of HAT being classic examples. Generally, rHAT, caused by *T. b. rhodesiense*, is considered acute, killing patients in weeks or months, while gHAT, caused by *T. b. gambiense*, can last up to a year and is chronic. However, rHAT with characteristic acute, chronic and asymptomatic (MacLean *et al*., 2004; Ormerod, 1967) outcomes have been recorded. This phenomenon has been associated with parasite and host genetics or a combination of both (MacLean *et al*., 2010), with intra-specific variation in the parasite and gene polymorphism of host immune factors, specifically cytokines (Courtin and Garcia, 2007), potentially involved. These immune factors, specifically IFN-γ and TNF-α, have also been implicated in parasite invasion of the CNS (Amin *et al*., 2012; Masocha *et al*., 2004; MacLean *et al*., 2004), indicating a role in progression and outcome. However, the comprehensive role of immune factors in varying disease outcomes in humans remains unclear. Improving our understanding in these areas is important, with insight gathered on potential applications in the identification of diagnostic and staging biomarkers, and also predicting the possible disease outcomes to allow better management. Notably, this can allow optimisation of clinical diagnosis, especially in areas with poor health infrastructure and other tropical diseases with similar clinical signs and their diagnosis is also poor.

Most of our current understanding of AT immunology is from mouse model studies. For HAT, a murine model as well as a non-human primate (NHP), particularly the vervet monkey (*Chlorocebus aethiops*, African Green Monkey) has been used (Brun *et al*., 2001). Other studies, which are quite informative, have involved sleeping sickness cases (MacLean *et al*., 2001; MacLean *et al*., 2004; MacLean *et al*., 2012) but suffer from serious limitations. For example, it is difficult to determine infection time and consequently disease onset in human cases. Additionally, monitoring host factors, including immune system factors, periodically with disease progression is not possible. Hence, the vervet monkey model of HAT (Schmidt & Sayer, 1982) is useful. Previously, studies described a vervet monkey HAT model in which infection occurred via the bite of a single infected tsetse fly, closely replicating the natural transmission of sleeping sickness (Thuita *et al*., 2008). Here, levels of cytokines in blood and CSF samples from vervet monkeys

previously infected with two strains of *Tbr* responsible for varying pathogenesis were evaluated. To understand variations of these factors under investigation, monkey clinical data were retrieved and analysed for comparison (Thuita & Masiga, 2016).

### 1.2 Statement of the problem

The rhodesiense infection kills in a matter of days or weeks if untreated. Current diagnostic and disease staging methods, more specifically, microscopic detection of the parasite in infected body fluids and/or elevated white blood cell counts in the CSF above 5 cells/ µL, are not sensitive enough. Controlling *Tbr* infection has posed a challenge due to re-emergence in regions where disease control had been achieved. This can be attributed to the fact that *Tbr* is a *Tbb* mutant that is animal-infective, making animal reservoirs important in disease transmission (Gibson, 2005). In addition, infections with *Tbr* can result in varying disease outcomes, with some strains and/or patients showing acute or chronic infections. The role of host immune factors in the varying infection outcomes and disease progression remains unclear and is important in revealing the dynamic host-pathogen interactions involved. Consequently, this study sought to reveal immune changes associated with *Tbr* infection and disease progression in the NHP HAT model that result in varying disease outcomes.

### 1.3 Objective

#### 1.3.1 General objective

To investigate the role of cytokines in varying rHAT disease outcomes in a vervet monkey model of HAT.

#### 1.3.2 Specific objectives

i. To identify cytokines in vervet monkeys infected with two strains of *T. b. rhodesiense* responsible for acute and chronic infections.
ii. To investigate the variations in cytokine levels with disease progression.
iii. To investigate the role of cytokines in *T. b. rhodesiense* disease pathogenicity.

### 1.4. Null hypotheses

i. There is no cytokine difference between acute and chronic infections.
ii. The variations in cytokine levels are not associated with disease progression.
iii. There is no relationship between cytokine levels and disease pathogenicity in *T. b. rhodesiense* infection.

## 1.5 Justification

Insights on the role of host immune factors in modulating disease outcomes reveal interactions between host and pathogen factors during infection. In rHAT, which is an acute infection that kills within a short period, this holds importance because of several reasons: First, it provides an understanding of the contribution of specific host immune factors in varied disease outcomes. Secondly, the knowledge of varying immune factor levels can permit the identification and subsequent validation of potential detection and/or stage biomarkers. These are particularly needed to improve diagnosis and eliminate the painful and invasive lumbar puncture process of disease staging. Thirdly, it contributes to the knowledge at the molecular level of host-pathogen interactions associated with varied disease outcomes and progression. This will further enable understanding of the basis of observed clinical signs, which can help resolve clinical diagnosis and hence improve disease management. Lastly, understanding the contribution of the immune system to the modulation of disease outcomes is important in the development of better and optimal treatment therapy, including anti-disease therapies as a part of a toolkit for effective disease management. In sum, insights on alterations associated with varying *Tbr* infection outcomes will reveal dynamic trypanosome-primate interaction that can be exploited in the discovery of candidate diagnostic and staging biomarkers and a novel disease management approach.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 African trypanosomiasis

African trypanosomiasis (AT) is a tropical disease caused by flagellated protozoan parasites known as African trypanosomes (genus *Trypanosoma*). Transmission occurs through the bite of the tsetse fly (*Glossina* spp.), which serves as the insect vector. In humans, the disease is referred to as sleeping sickness (human African trypanosomiasis, HAT), while in livestock it is known as nagana (African animal trypanosomiasis, AAT). AT is confined to 37 African countries where tsetse flies are present, though other forms of the disease that do not involve tsetse transmission are found beyond the continent. The endemic zone is a fertile region where agriculture is practiced by dwellers. Consequently, the disease exerts a substantial health and economic burden, with nearly 70 million people residing in endemic areas at risk of infection, an estimated 300,000 active cases and between 10,000 and 40,000 deaths reported annually (Franco *et al*., 2020; Simarro *et al*., 2012; WHO, 2013). The estimated losses in cattle production annually are approximately USD 1.3 billion (Salah *et al*., 2015). These losses are high, especially in a region inhabited by the world's poorest, who suffer from a myriad of other tropical diseases. Therefore, the control of the disease is essential for enhancing the quality of life of the population and aligns with the objectives of major global health initiatives, including the former Millennium Development Goals (MDGs) and the ongoing Sustainable Development Goals (SDGs).

The major control strategies for AT are vector control and chemotherapy. Vector control involves the prevention of a bite by tsetse, consequently disrupting the transmission of the parasite. In case of a bite by an infected tsetse and consequently an infection, chemotherapy remains the mainstay of the control approach applicable. If the disease is not treated, death is highly likely. Here, various drugs are used for treatment. However, most suffer from chemo-resistance and chemo-toxicity. Notably, diagnosis is also poor, significantly limiting the control and management strategies deployed. This necessitates improvement and/or development of novel diagnostic and control strategies for improved management of AT.

### 2.1.1. African animal trypanosomiasis (AAT)

Nagana, also known as African animal trypanosomiasis (AAT), is a debilitating wasting disease of livestock caused by African trypanosomes transmitted through tsetse flies. The parasites are spread by nearly 30 different species of Glossina (Diptera: *Glossinidae*) that inhabit approximately 10 million $km^2$ (Brun *et al*., 2010) and cover 37 countries in Africa. Nagana is caused by several distinct species of African trypanosomes, including *T. congolense, T. brucei brucei* and *T. vivax*. Other forms include surra disease in camels caused by *T. evansi*, dourine in

equines caused by *T. equiperdum* and *T. suis,* which causes the disease in pigs. The parasites are primarily transmitted through the bite of an infected tsetse fly during a blood meal. Because tsetse flies feed on a broad range of hosts, including wild animals, the distribution of AAT is widespread. More so, most wild animals, such as buffalo, act as reservoirs, enabling continuous transmission that significantly limits control and management strategies deployed. *T. evansi* is mechanically transmitted by insects, mainly by biting flies such as tabanus and stomoxys, on their mouthparts from one host to another during interrupted feeding. *T equiperdum* is transmitted during coitus.

The tsetse belt covers an estimated 10 million km² with nearly 60 million cattle at risk of infection (Connor & Van Den Bossche, 2004; Cecchi *et al*., 2009) (Figure 1). Therefore, mixed farming is hugely affected. Worth noting is the mechanical transmission of *T. vivax* by tabanids, namely *Atylotus agrestis* (Desquesnes & Dia, 2003), increasing the endemic region beyond Africa to also include South America (Jones & Dávila, 2001). In Africa, the AAT endemic region also represents the most fertile parts of the continent. Therefore, the disease imposes severe agricultural losses and significantly undermines the economies of the affected countries.

The inoculation of the trypanosomes after a tsetse bite causes swelling and the development of a chancre at the site of inoculation. The parasites spread to the blood and lymph nodes and localise in different sites depending on the infecting parasites. Antigenic variation, the systematic and periodic change of the pathogen surface coat by the bloodstream parasite forms, prevents parasite clearance and prolongs their survival in the host. Prolonged survival leads to antibodies building up from the immune reactions and the consequent deposition of the immune complexes resulting from the disease-forming lesions (Ponte-Sucre, 2016). These factors interfere with the normal organ function. Thus, the infected animals become weaker with time. In animals, the infection typically manifests in acute, sub-acute or chronic forms, and is characterised by anaemia, recurrent bouts of fever, rapid loss of condition and occasional diarrhea, which can terminate in death if the animals are not treated (Shaw *et al*., 2014). As the disease progresses, the animals become increasingly debilitated and ultimately, the affected animals become unfit as a source of draught power. In addition, low milk yields, low calving rate, high calf mortality rate and high treatment costs are incurred. Therefore, AAT reduces, directly and indirectly, food (both crop and animal) production and constrains optimal utilisation of fertile land (Shaw *et al*., 2014). This impact is huge, with losses in cattle production alone approximated to be USD 1.3 billion (Salah *et al*., 2015), and total agricultural loss approximated to be USD 4.75 billion annually (FAO 2012; Vreysen *et al*., 2013).

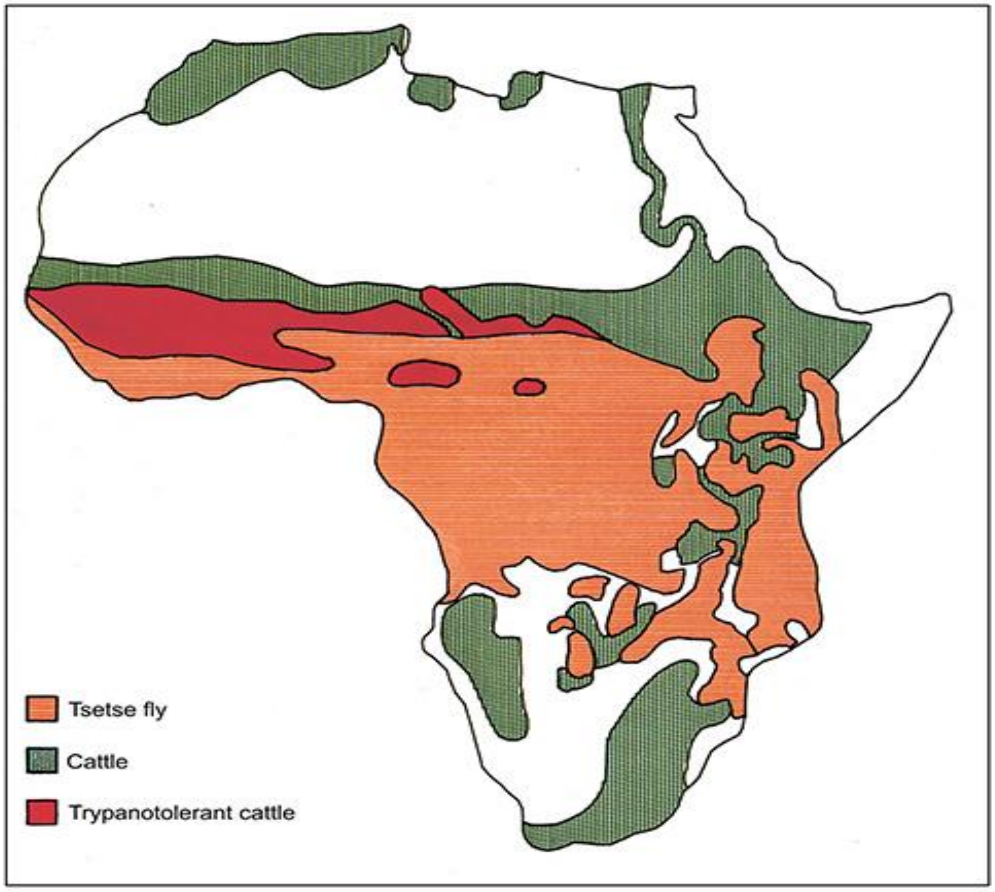



**Figure 1:** Distribution of African trypanosomiasis. Colour codes show the distribution of the tsetse fly vector, cattle and trypanotolerant cattle

Figure adapted from Connor and Van Den Bossche (2004)

### 2.1.2. Human African trypanosomiasis (HAT)

Sleeping sickness, or human African trypanosomiasis (HAT), is caused by two subspecies of *Trypanosoma brucei*: *T. b. rhodesiense* (*Tbr*) and *T. b. gambiense* (*Tbg*). The Rhodesiense form (rHAT), found in eastern and southern Africa, is typically acute, whereas the Gambiense form (gHAT), prevalent in central and western Africa, develops as a chronic disease (Figure 2). It is worth noting that the disease has been observed to present with a wide spectrum of clinical outcomes, including asymptomatic cases (Jamonneau *et al.,* 2004; Kuepfer *et al.,* 2011; Maclean *et*

*al.*, 2010). In both cases, the disease proceeds through

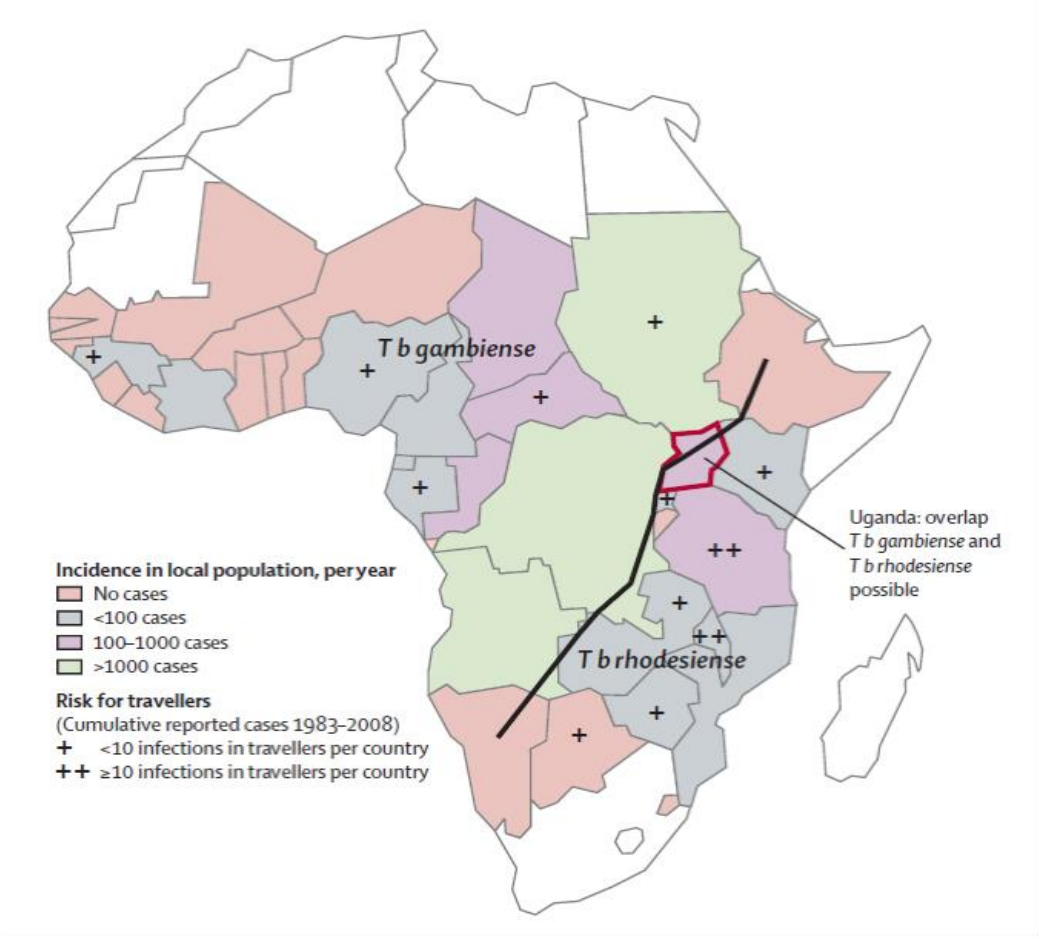



**Figure 2:** Distribution of Human African Trypanosomiasis (HAT). Colours show *T. b. gambiense* and *T. b. rhodesiense* incidence; black line marks areas of overlap (Brun *et al*., 2010)

two stages. Following the bite of an infected tsetse fly, the parasites initially colonise the blood and body tissues, leading to stage 1, also referred to as the early or haemolymphatic phase. As the infection progresses, the parasites penetrate the blood-brain barrier (BBB) or the blood cerebrospinal fluid (BCF) barrier, gaining access to the cerebrospinal fluid (CSF) and central nervous system (CNS). This marks the onset of stage 2, known as the late or meningoencephalitic stage. The second stage is characterised by neurological complications, most notably disruptions of the sleep-wake cycle, a hallmark feature that gave the disease its name. The clinical signs observed include edema, fever, fatigue, asthenia, skin rash, anorexia, joint and muscle pain (lumbalgia, arthralgia and myalgias), headaches, endocrine dysfunction, hematological alterations such as anaemia, ocular pathology such as keratitis and choroidal atrophy, psychiatric symptoms, cardiovascular alterations and impairment of motor functions (Blum *et al*., 2006; Kennedy, 2008; Kupfer *et al*., 2011; MacLean *et al*., 2010). The symptoms of the disease progress from chronic to intermittent fever, pruritus, headaches, lymphadenopathy, hepatosplenomegaly, sleep disturbance

and neuropsychiatric symptoms, to coma and death if the disease is left untreated (Brun *et al*., 2010).

The rhodesiense infection is zoonotic, with both wildlife and cattle acting as main reservoirs of *Tbr* (Ng'ayo *et al*., 2005; Simarro *et al*., 2011). However, epidemics in Eastern and Southern Africa have shown a possible and significant role of humans in the transmission of *Tbr* (Franco *et al*., 2014). gHAT, on the other hand, has humans as the main reservoir of *Tbg*. With transmission dynamics slightly varying for the two forms, various control strategies have been employed. In addition, evidence suggests a potential overlap of the two disease forms within Uganda (Brun *et al*., 2010).

HAT is a focal disease with about 360 known foci and affects 37 Sub-Saharan African countries (Franco *et al*., 2020; WHO, 2013). Out of 37 countries, the Gambian form is found in 24 countries with around 300 foci grouped depending on the disease transmission intensity of high to very high levels, moderate levels and low to very low levels (Cecchi *et al*., 2009; Franco *et al*., 2020; WHO, 2014). Rhodesiense form has about 60 foci in 13 sub-Saharan African countries, categorised according to their pattern of transmission into areas where wild animals are kept in protected areas and areas where cattle are the main reservoir (Simarro *et al*., 2013; WHO, 2020). As demonstrated by the foci, rHAT accounts for just 2% of cases, with the rest (98%) due to gHAT (Welburn *et al*., 2009). This region is extensively remote and is characterised by high poverty levels and poor and/or no health infrastructure. The impact data might not be quite accurate and are likely an underestimation (Holmes, 2015; Simarro *et al*., 2008) because non-hospitalised cases are not reported, poor roads render some regions inaccessible and reduced disease detection efforts due to reduced cases. Therefore, the burden of HAT is huge.

### 2.2. Impact of African trypanosomiasis

The estimated number of people at risk of infection and living in the Tsetse belt is about 70 million people, with the estimated mortality rate ranging between 10,000 and 40,000 per year (Franco *et al*., 2014). With the disease affecting both human and livestock, its medical, veterinary and socio-economic impact is huge. These impacts are reviewed below.

#### 2.2.1 Impact of human African trypanosomiasis

The approximate number of people living in the endemic areas and at risk of infection is about 70 million, with 300,000 infected and about 10,000-40,000 annual deaths (Franco *et al*., 2014; WHO, 2013). In 2011, the estimated number of reported cases was 6,743 people with about 960 deaths (Simarro *et al*., 2012). Together with costs incurred in seeking treatment, after death and loss of sometimes skilled and core members of the family, the costs are high. Time and resources

used in caring for the sick could be used in other economic activities that contribute to improving the lives of the already poor communities. In addition, the sick people are unable to work, limiting their contribution to economic activities, including agriculture, which is the main occupation in the affected regions.

HAT is ranked second in terms of mortality rate and fourth in disability adjusted life years (DALYs) (Matemba *et al*., 2010). It is also a neglected tropical disease (NTDs) or "disease of poverty" (neglected in terms of drug and diagnosis development due to low investment returns due to low purchasing power of the affected) with global disease burden (GDB) estimates of about 108.7 million DALYs or 4.4% of the total global burden of diseases and injury (Matemba *et al*., 2010). Currently, there has been a significant reduction of DALYs from 1.82 million to 829,000 and to 560,000 in the years 2000, 2005 and 2013, respectively (Hackett *et al*., 2014). An estimate of 1,609,041 DALYs lost was reported in 2004 (Fevre *et al*., 2008). These indicate a continued reduction in the disease burden over the years, a consequence of global, regional and national resolutions implemented under WHO Control and eradication of NTDs as of January 2012 (WHO, 2012). More so, the restoration of political and socioeconomic stability in some of the affected nations, such as the Democratic Republic of Congo (DRC), Congo Brazzaville and Angola, amongst others, contributed to this success (Büscher *et al*., 2017). The reduction of the disease burden is also contributed to by the resolutions aimed at controlling the disease.

The implementation of the resolution, which involved surveillance, disease detection and treatment, has resulted in a reduction of the reported cases, with the gHAT cases showing a 69% reduction (36,585 to 11,382) while the rhodesiense form had 21% from 1997 to 2006 (Simarro *et al*., 2008). The period between the years 1998 to 2011 registered a reduction of 82.3% of reported cases from 37,991 to 6,743 at the continental level (Simarro *et al*., 2012). In 2009, 19 countries out of the 37 countries where the disease is endemic registered no new cases (Simarro *et al*., 2011). In 2015, the total number of reported cases was reduced to 2,801 and further in 2016 to 2,164 cases (Franco *et al*., 2018). The estimated area of the region having moderate to higher risk from 2012 to 2016 was 280,000 $km^2$ (Franco *et al*., 2018). These figures show a 92% and 61% reduction in 2015 and 2016, respectively. In recent years, 992 cases were documented in 2019, followed by 663 cases in 2020 (Franco *et al*., 2022). During the 2011 to 2020 period, a total of 49 cases were identified in countries outside the endemic regions, where 71% of the cases were *Tbr* infections from travelers (Franco *et al*., 2022). Despite the efforts towards disease elimination, there are cases of reemergence of HAT infection. *Tbr* reemergence has recently been reported in Ethiopia, where two out of four patients died due to delayed treatment (Abera *et al*., 2024). While there is notable improvement, elimination and eradication will require not only better detection and drugs for treatment but also other improved and/or novel tools that disrupt transmission, implemented in

effective and efficient ways. This challenge will require our increased knowledge in various fields, including identification, validation and development of novel diagnostic and drug targets, transmission dynamics in endemic foci, and disease progression and pathology, amongst others.

### 2.2.2 Impact of animal African trypanosomiasis

The effects of Nagana on livestock production and the general health of cattle are devastating. Generally, the impact of the disease has both direct and indirect effects on livestock production. In total, the annual agricultural loss is estimated to be US$4.5 billion (FAO, 2004; Yaro *et al*., 2016). This could be an underestimate if other losses associated with the disease are included. For example, both human and animal migration and settlement are due to epidemics and disruption of other human activities, including agriculture (Swallow, 2000).

Direct loss includes losses associated with health and animal production. First, the disease affects livestock management in various ways, including livestock numbers kept, grazing and the species and breed composition of the herd (Connor, 1994). The number of animals kept by the farmer has reduced due to death. This leads to reduced herd size and consequently reduced productivity. Animal productivity is measured by reproductive performance, parasitemia status, lactation off-take and animal mortality. In general, productivity is determined by the animal output in terms of the cost of treatment, milk and meat production and animal traction (Swallow, 2000). Sick animals have low milk production and a very low calving rate, the meat production is low and the animals cannot work in the farms due to weakness, yet these regions are characterised by farming using animals. In total, an estimated loss of about US$1300 million is incurred every year in poor rural settings (Shaw, 2004; Shaw *et al*., 2015), which is significant because of the poverty levels. Secondly, the infected animals are generally weak due to infection-associated pathogenesis and pathology. These animals cannot therefore offer draught power, limiting mixed farming, which is common in the affected areas. Thirdly, infected animals are difficult to relocate when in search of pasture and water. In the tsetse fly-infested areas, pastoralism is common. Consequently, most lands are not optimally utilised and farming activities are limited (Swallow, 2000).

The indirect effects are various. First, the loss associated with infected animals limits mixed farming, more specifically, crop production. Infected animals become too weak to provide draught power. High mortality reduces manure production, which reduces crop yield (Swallow, 2000). Notably, reduced crop yield means limited plant material to be used as animal feed. Therefore, the economic benefit associated with agriculture is reduced due to the disease. Secondly, other human economic activities are affected due to the uninhabitable nature of the tsetse-infected areas. Therefore, improved control of the disease is needed to limit the losses involved.

The cost of controlling the disease is high. Chemotherapy, which is widely used by farmers in the tsetse fly-infested areas, is costly and is estimated in 2004 to be about US$30 million (Holmes *et al*., 2004). In 2015, it was estimated that the total cost of curative and prophylactic treatment per animal was US$5.69 and US$3.57 per animal annually (Mhanguzi *et al*., 2015). Together with developing drug resistance, the overall cost increases. On the other hand, the use of restricted insecticide application protocol (RAP), which involves the application of pyrethroid insecticides on the ears, legs and bellies of the animals, costs about US$0.57 per animal per single spray, which is costly in the long run (Mhanguzi *et al*., 2015). Because both humans and animals are affected, its impact is huge and consequently improved and/or novel control strategies are urgently needed. Moreover, with climate change affecting agriculture and disease transmission dynamics, novel tools and a better understanding of vector-borne infectious diseases are necessary to enable adaptation of affected local communities, who at present bear a huge burden.

### 2.3. Control of African trypanosomiasis

The main control strategies of AT include vector control and chemotherapy. The campaigns towards the eradication and elimination of the disease have utilised these two control strategies and registered some success in the past. The control strategies and the campaign by the WHO and other nongovernmental organisations (NGOs) significantly reduced case numbers to less than 3,000 in 2015, through case detection and treatment of the gHAT (Franco *et al*., 2018). In contrast, control of rHAT is made complex by the involvement of wildlife in parasite reservoirs, and hence control in the reservoirs, reduction of the vector population and medical interventions are deployed (WHO, 2020). For AAT, reduction of the vector numbers and treatment of infected animals are applied.

#### 2.3.1. Vector control

The reduction of the vector population is important in the eradication of AT and more especially for rHAT and AAT, where domestic and wild animal reservoirs play a vital role in disease transmission. This is not applicable in gHAT, where human infections can last months and even years without clinical signs (Rock *et al*., 2015), with man playing a central role in transmission. Vector control involves disruption of the transmission cycle. It is particularly important in cases where active surveillance campaigns are costly because of the low endemicity, making protection of the population against the vector a more viable option. Various approaches have been deployed and can be categorised into four, including the use of the sequential aerosol technique (SAT), the stationary attractive devices, the live bait technique and the sterile insect technique (SIT).

#### 2.3.1.1. Sequential aerosol technique (SAT)

The sequential aerosol technique (SAT) involves applying low doses of insecticides using helicopters or fixed-wing aircraft, which operate at an altitude of approximately 10-15 meters above the tree canopy. Depending on the temperatures, the spraying is done in 5-6 cycles a day separated by about 16-18 days before the next spray (Allsopp & Hursey, 2004). The success of this method is dependent on droplet sizes, which should be appropriate because small droplets remain suspended in air for longer periods, while larger droplets are denser. This method kills the adult flies when they come in contact with the insecticide mist. Subsequent spraying cycles kill the flies that are emerging before they attain their reproductive stage.

The use of modern technologies has improved the approach. For example, GPS-guided spray and navigation systems such as the SATLOCK have been deployed (Vreysen *et al*., 2013). Such improvements have made the SAT effective for covering wider areas in dense humid forests and open savannah ecosystems (Kgori *et al*., 2006). In 2001 and 2002, for example, an area of about 7180 $km^2$ and 8722 $km^{2,}$ respectively, was covered in the Okavango delta located in Botswana, utilising the same technique using an application dose rate of 0.26g/ha (Allsopp & Phillemon-Motsu, 2002). In addition, the technique is cost-effective and rapid and does not cause detrimental effects to the environment. However, the effects on organisms within the environment are a challenge, posing limitations.

Despite the improvements and wide application, a key limitation of this method is the risk of re-invasion when the treated area is not geographically isolated. The insecticide's effect on the non-targeted organisms is also detrimental. In the 1950s, there were reported severe mortality rates of wildlife from the toxic residual insecticides (Adam *et al*., 2013). Initially, the ground spraying was done using DDT and later replaced by the deltamethrin insecticide, which posed many detrimental effects to the non-target organisms in Zimbabwe (Lambert *et al*., 1991). Findings have demonstrated that the insecticide exhibits acute toxicity toward a broad spectrum of invertebrates inhabiting tree trunks, silverfish (Thysanura: Lepismatidae) and plant-hoppers (Homoptera: Fulgoroidea) with reported population reduction. The introduction of non-residual endosulfan and pyrethroids used in SAT has reduced these effects to minor levels, but does not eliminate the effects of these insecticides on non-targeted organisms (Adam *et al*., 2013). Therefore, further improvements are necessary to eliminate these limitations. Furthermore, addressing other challenges emerging due to the successful implementation of SAT is necessary within the ecosystem.

One of the interesting effects of the success of this technique is the negative interaction between humans and wildlife. In Zimbabwe, the elimination of tsetse led to an expansion of arable lands and consequently, the number of elephants was reduced (Murwira *et al*., 2010). The extensive

cattle rearing increases competition with the wild fauna for land, causing major land degradation and negative effects on the ecosystem through overgrazing (Holt *et al*., 2016). A study conducted in Senegal suggested that the successful elimination of the tsetse flies, coupled with trypanotolerant animal rearing, will result in decreased herd sizes (Bouyer *et al*., 2014).

#### 2.3.1.2. Stationary attractive devices

Stationary attractive devices involve the attraction of the flies to a device such as targets or cloth traps, which kills the flies through tarsal contact with insecticides on these devices, or starvation or heat after guiding the flies to the non-return cage (Brightwell *et al*., 1991; Vale, 1993). The method is enhanced especially in the savannah areas with oduor attractants and its efficiency is achieved if the traps or targets are impregnated with the insecticides (Rayaisse *et al*., 2010). For improved efficiency, the setting and deployment of the traps and targets are important. Maintenance of these devices is essential, with periodic replenishing of the oduors, use of cloths with the appropriate reflective patterns and use of appropriate insecticides that are not degraded by UV light from the sun (Vreysen, 2001). Thus, the success of its use requires constant improvement and modification of traps and targets.

Vector control strategies in HAT foci have been undertaken through modification of the existing insect-treated targets to make them cost-effective and sustainable. The tiny targets made up of small squares of blue cloth that are flanked by similar-sized pieces of the black net are more efficient and cost-effective in the control of Gambian vector species (Shaw *et al*., 2015). In addition, cases of decreased insect dispersal require an increase in device density to achieve high efficiency. The method is suitable for local use by the farmers to protect smaller areas, but is uneconomical for large areas (Kappmeier *et al*., 2007). However, compared to the SAT, it is much cheaper.

The method has limitations associated with its use. Though this method is important in the suppression of the insect density, it does not apply to eradication (Kagbadouno *et al*., 2011). In West Africa, for example, this method has proved effective when the blue-black target was increased but the size reduced by 1/16 of the original size (Lindh *et al*., 2009). Furthermore, it is labour-intensive and time-consuming, particularly with the need for maintenance of the traps and targets. Despite its successful use in the savannah ecosystem, the method is not efficient for the riverine ecosystem.

#### 2.3.1.3. Live bait technique

The method involves insecticide treatment of livestock to prevent and/or disrupt tsetse flies' feeding. The lethal insecticide kills the tsetse flies while they feed. The insecticide application is

easy and rapid, with the pour-on formulations requiring cheaper equipment. In addition, limited maintenance is required.

The live bait technique has limitations in its application. In a riverine ecosystem, the method was not effective because of the tendency of *G. p. gambiensis* to return to the same host of the initial blood meal (Bouyer *et al.*, 2007). Secondly, the method is limited in cases where cattle density is high within a region. In addition, high treatment frequencies, cost of insecticides, insecticide residues in the animal dung and resistance to the insecticides are major limiting factors (Torr *et al.*, 2007).

#### 2.3.1.4. Sterile insect technique (SIT)

The sterile insect technique (SIT) entails the large-scale release of sterilised male flies, which, upon mating with wild females, produce no offspring, ultimately leading to a reduction in the tsetse population (Dyck *et al.*, 2005). With the ratio of sterile to wild type increasing, the method becomes efficient in lowering the insect density populations (Vreysen & Robinson, 2011). SIT has advantages over SAT concerning effects on the environment.

SIT is environmentally friendly and poses no harmful effects on non-target organisms (Ciss *et al.*, 2019). Therefore, the method can be integrated with other biological controls. The method is economical and efficient (Shaw *et al.*, 2013). Over time, SIT proves to be economically advantageous, particularly when incorporated into area-wide integrated pest management (AW-IPM) programmes. Its effectiveness is most pronounced in areas with low target population densities and relies heavily on a comprehensive understanding of the ecology and biology of the tsetse species involved (Vreysen, 2001). Earlier applications in Zimbabwe and Burkina Faso in the 1960s and 1970s, respectively, demonstrated the potential of the technique in riverine and savannah ecosystems (Cuisance *et al.*, 1973; Dame & Schmidt, 1970; Van der Vloedt *et al.*, 1980). The method was applied successfully in Zanzibar Island and demonstrated high efficiency (Kabayo, 2002). However, challenges associated with its application are notable.

The method requires release and monitoring methods that are applied in wider areas (Vreysen, 2005). In addition, its success is also dependent on the quality of the released sterile males that should be able to adapt to the environment and compete effectively against the wild population (Vreysen, 2005). Furthermore, its application is constrained by practical challenges, including the diversity of tsetse species responsible for transmission, the high initial costs and the reliance on substantial infrastructure such as irradiation plants, large-scale insectaries and aircraft (Rogers & Randolph, 2002).

Generally, growing knowledge in population genetics of the vector is aimed at the identification of the natural barriers to fly dispersal and routes. This will enhance the field control

programs that reduce the density of the vector using traditional methods such as the SIT, aerial sprays and traps/targets (Aksoy *et al*., 2013; Solano *et al*., 2010). The limitation of the applicability of some methods in vector control, the development of insecticide resistance and the environmental disruptions associated make chemotherapy an important toolkit in the control of African trypanosomiasis.

### 2.3.2. Chemotherapy

Upon infection, the only available control method is drug administration. Vaccines are not available due to the parasite's systematic change of surface coat, the variable surface glycoprotein (VSG), through a mechanism known as antigenic variation. Before drug administration, a diagnosis must be accurate. This is particularly important in HAT, where treatment is species-specific. The early-stage disease is treated using pentamidine (Pentacarinat®) and Suramin (Moranyl®) for gHAT and rHATs, respectively. For second-stage disease, melarsoprol (Arsobal®), eflornithine (DFMO or $\alpha$-difluoromethylornithine, ornidyl®), and combination therapy of Eflornithine/Nifurtimox are administered (Table 1). Some of these drugs are toxic, presenting a huge risk to infected individuals. In addition, drug resistance is also observed. Treatment efficacy varies from 70 to 95% depending on the drug (Robays *et al*., 2008). Their administration regimen is complex and thus requires close monitoring of the patients (Brun *et al*., 2010). The treatment process is further complicated by the fact that WHO supplies the drugs upon diagnosis of the disease, except for pentamidine, used to treat other infections, including leishmaniasis (Franco *et al*., 2022). Such cases have led to increased death rates, especially in acute infection (Abera *et al*., 2024). Therefore, treatment is challenging and complicated further by limited health infrastructure, specialised monitoring and long hospitalisation required.

**Table 1:** Drugs for treatment of gambiense and rhodesiense forms of HAT

| Strain | Early stage | Late Stage |
|---|---|---|
| *Tbg* | Pentamidine (Pentacarinat)<br>Fexinidazole | Eflornithine (DFMO or $\alpha$-difluoromethylornithine)<br>Melarsoprol (Arsobal)<br>Nifurtimox–eflornithine combination therapy (NECT)<br>Fexinidazole |
| *Tbr* | Suramin (Moranyl)<br>Fexinidazole | Melarsoprol (Arsobal)<br>Fexinidazole |

#### 2.3.2.1. Early-stage treatment

The early-stage disease is treated using pentamidine (Pentacarinat), fexinidazole and Suramin (Moranyl) for gambiense and rhodesiense forms. Pentamidine is a diamidine drug that is relatively well-tolerated due to low toxicity levels. However, the drug is unable to cross the BBB sufficiently to clear the parasites (Bray *et al*., 2003) and only clears the *Tbg* parasite and not *Tbr*. The drug demonstrates good safety and efficacy levels with its cure rate ranging from 93 to 98% and the lethality levels being less than 0.5% (Balasegaram *et al*., 2006). The prescribed dosage is 4 mg/kg administered daily over a period of 7 days through the intramuscular route. The most frequently incurred side effects are nausea, vomiting, hypoglycemia and pyogenic abscess. The rare side effects include diabetes, cardiac arrhythmias, allergic reactions and nephrotoxicity. To avert some of the side effects, such as hypoglycemia, the patient is encouraged to take sweets before the injection. In the prevention of hypotension, the patients are maintained in a supine position, after which they are allowed to rest for 1 to 2 hours after the injections. Monitoring of glucose, hepatic, renal, pancreatic, creatinine, potassium and calcium levels is mandatory. The limitations of the drug are the toxicity levels and its inability to cross the BBB, and it is stage and strain-specific.

The World Health Organization (WHO) recommended fexinidazole as the preferred first-line therapy for Human African trypanosomiasis (HAT) in individuals aged six years and above, weighing at least 20 kilograms, provided the cerebrospinal fluid (CSF) leukocyte count is below 100 cells/μL (WHO, 2019; WHO, 2024). The drug demonstrates efficacy in treating both stages of the disease. In cases where the leukocyte count reaches or exceeds 100 cells/μL, or when there are clinical signs suggestive of severe second-stage disease, treatment with a nifurtimox–eflornithine combination is advised. The drug has proven effective for both stages of the infection (Babokhov *et al*., 2013). The drug is orally administered at a dosage of 1800 mg daily for 4 days, followed by another dosage of 1200 mg daily over a period of 6 days, administered orally with a meal. The drug has demonstrated efficacy in the treatment of the Gambian infection (Lindner *et al*., 2020; Mesu *et al*., 2018). The drug can be used by pregnant women to prevent maternal transmission (Welburn *et al*., 2016). Notably, fexinidazole may be administered without the need for a lumbar puncture if there is no clinical indication of advanced-stage disease.

Suramin is administered in the treatment of the early stage of rHAT. The drug has a low lethal rate of less than 1% and cure rates of about 95% (Fairlamb, 2003). The regimen recommended for treating the infection is 20mg/kg of body weight administered intravenously in five doses, a single dose per week. Common side effects are conjunctivitis, hypersensitivity, asthenia, hyperpyrexia, anorexia, polyuria and arthralgias. Uncommon side effects are dermatitis, stomatitis, proteinuria, renal failure and neuropathy. Monitoring of the renal function and

erythrocyte count is recommended. The drug is limited by the toxicity levels, the treatment regimen, parasite specificity and the inability to cross the BBB.

#### 2.3.2.2. Late-stage treatment

The late stage of the disease is treated using melarsoprol (Arsobal), eflornithine, also known as DFMO ($\alpha$-difluoromethylornithine), and combination therapy of Eflornithine/Nifurtimox (Table 1). Melarsoprol is an arsenic drug combined with dimercaprol, which is an arsenic antidote. The drug was originally the first-line and sole treatment option for late-stage Rhodesiense infection; however, it is no longer recommended as the primary therapy for HAT, as the World Health Organization now endorses fexinidazole (Chappuis, 2007; WHO, 2019; WHO, 2024). Melarsoprol has a low toxicity rate of about 3 to 10% and is fatal in about 5% of patients (Kennedy, 2008). The treatment failures in Gambian infection have been reported to reach 30% (Chappuis, 2007). The recommended dosage is 2.2 mg/kg administered daily for a period of 10 days intravenously, accompanied by 1.0 mg/kg of prednisolone orally (Schmid *et al*., 2005). Occasionally, a former therapeutic protocol is used where 3.6 mg/kg of the drug is administered intravenously, separated by rest periods of 7 days (Sindato *et al*., 2008). The drug has severe side effects and is often administered under close supervision and assessment of renal, liver and blood parameters. The complication of reactive arsenical encephalopathy develops in 10% of the treatment cases, causing 50% lethality (Blum *et al*., 2001).

Eflornithine was developed as an anti-tumor drug and has proved efficient in the treatment of late-stage Gambian infection with an efficacy of about 90 to 95% (Chappuis *et al*., 2005). It is the first-line drug in the treatment of late-stage Gambian infection. Treatment using the drug is cumbersome, costly and requires venous administration for 14 days. The dosage recommended is 100 mg/kg of body weight administered every 6 hours for 14 days through intravenous perfusions every 2 hours. The drug has low bioavailability in children below the age of 12 years, requiring a higher dosage of 150 mg/kg (Chappuis *et al*., 2005). Monitoring of blood parameters is recommended after treatment.

Nifurtimox–eflornithine combination therapy (NECT) is an important treatment regimen for late-stage gHAT recommended by WHO in 2009 (WHO, 2009). Nifurtimox is administered orally and has limited efficacy when used as monotherapy (Legros *et al*., 2002). Combination therapy involves the dosage of single drugs for different durations and routes of administration (Priotto *et al*., 2009). The recommended dosage of eflornithine is 200mg/kg every 12 hours for 7 days through intravenous infusions done for 2 hours. Concurrently, 5 mg/kg of nifurtimox is orally administered every 8 hours for 10 days. The therapy has a wide range of side effects, including tremors, abdominal pains, psychotic reactions, arrhythmia and insomnia, among others. Monitoring of the

blood parameters and behaviour of the patient is highly recommended during treatment. The use of the therapy in a rural setup is difficult because it is expensive, requires good logistics and is difficult to administer (Schmid *et al*., 2005).

Generally, the main challenge in chemotherapy is drug resistance, toxicity and more often a complex treatment regimen that requires long hospitalisation and regular screening, which mainly requires lumbar puncture to check the presence of parasites in CSF and screening for organ function. Notably, the parasites are developing resistance to these drugs and recent efforts in retooling some of the trypanocidal drugs and trials of novel drugs have been done in the recent past (Mesu *et al*., 2018).

#### 2.3.2.3. New drugs for HAT treatment

Acoziborole is an oral drug under preclinical trials and has shown efficacy in the treatment of all stages of *T. b. gambiense* after administration of 960mg as a single dose (Kumeso *et al*., 2023). In a trial involving 41 patients with an early stage of *T. b. gambiense*, a success rate of 100% was achieved at 18 months, while in 167 patients with a late stage of the disease, a success rate of 95% was achieved (Kumeso *et al*., 2023; Tarral *et al*., 2023). Therefore, the drug would eliminate the need to perform a lumbar puncture, which is an invasive and painful process for patients.

Clearly, both transmission disruption through vector control and chemotherapy suffer limitations. With a disease that most likely results in death if untreated, improved and/or novel control strategies are urgently needed.

### 2.3.3. Other potential control strategies

Various alternative control approaches are being explored, with most being under investigation. The disruption of parasite establishment in tsetse is an approach that is under interrogation, with our knowledge of molecular host-pathogen interaction increasing. Here, tsetse limits trypanosome effects through their immune response (Dyer *et al*., 2013). Studies into molecular details of this complex interaction reveal insights into parasite colonisation of tsetse midgut, modulation of tsetse immune response and impact of the modulation on parasite transmission dynamics (Aksoy *et al*., 2014; Weiss & Aksoy, 2011). Alterations of such interactions could prevent the establishment of the parasite in the vector, disrupting transmission. In addition, tsetse's association with endosymbionts can allow the introduction of modified endosymbionts that disrupt trypanosome establishment and/or kill the tsetse. One of the vector's symbionts, *Sodalis*, has been identified and anti-trypanosomal molecules expressed to reduce the infection of the vector by the parasite (De Vooght *et al*., 2012; De Vooght *et al*., 2014). Insights gathered from such studies

can greatly aid the development of novel control strategies not only for AT, but other vector-borne infectious diseases.

With chemotherapy being crucial after infection, early diagnosis of the infection is paramount. More so, the most likely result of untreated infection is death. Therefore, accurate detection is important.

### 2.4. Diagnosis and staging of HAT

Diagnosis of rHAT is problematic due to the low sensitivity and specificity of most detection methods, with some suffering from high cost and difficulties in applying in remote settings with limited or no infrastructure, including electricity. Consequently, clinical diagnosis is also applied, as it can raise suspicion of an infection. Notably, other infections that occur in HAT-endemic areas have similar clinical signs. Therefore, a combination of detection methods could be applied. These methods include parasitological, serological and molecular detection.

#### 2.4.1. Parasitological detection

Parasite detection is the microscopic observation of trypanosomes in body fluids and the definitive diagnosis of HAT. It is the WHO gold standard method of detection (WHO, 1998), from which staging and appropriate treatment commence after positive detection. Microscopic examination, though highly specific, suffers from low sensitivity. Numerous techniques have been established that rely on microscopic detection of parasites in blood, lymph node aspirates and cerebrospinal fluid (CSF). In rhodesiense infection, the microscopic examination of the chancre is important in the earlier detection of trypanosomes (Lutumba *et al*., 2007). Trypanosomes are frequently identifiable in the chancre several days before their detection in the bloodstream.

Following the confirmation of the parasite in blood and/or lymph node aspirates, the next step involves disease staging, the confirmation of CNS invasion. CSF examination is only for staging and not diagnosis. In rare cases where other parasitological examinations are negative, yet there is high suspicion for HAT, CSF aspirates can be examined (Cattand *et al*., 1988; Lejon *et al*., 2003).

Staging involves assessing the presence of trypanosomes in the cerebrospinal fluid (CSF) and/or identifying a white blood cell (WBC) count exceeding 5 cells per μL (WHO, 1998), after a painful and invasive lumbar puncture. It suffers several limitations, including low specificity and sensitivity, inapplicability within rural regions where the disease is prevalent, and the need for skilled personnel and health infrastructure that are always absent (Mwanakasale *et al*., 2013). Therefore, it is essential to identify reliable diagnostic and staging biomarkers, a goal that can be pursued by investigating the molecular aspects of disease progression during infection.

Parasite detection is a labour-intensive process and is limited to suspected cases only. In cases of Gambian infection, the trypanosome load is low and in some cases below parasitological detection limits. Therefore, a negative result does not often mean that there is no infection, but requires further confirmatory tests. In most cases, a concentration technique allowing examination of larger volumes of the sample is used. The time between sampling and examination has to be minimal to reduce the chances of trypanosome lysis and immobilization. In cases of delay, the trypanosome survival can be kept longer if stored at 4°C. Various parasitological-based techniques applied in diagnosis have been developed and are explained below.

#### 2.4.1.1. Micro-haematocrit centrifugation technique

The micro-haematocrit centrifugation technique is also known as the Woo test or the capillary tube centrifugation method (Woo, 1971). In this test, blood is filled three-quarters way into anticoagulant-containing capillary tubes, then one of the ends is sealed with plasticine or through a flame with caution not to heat the blood. The blood is then centrifuged at 12000 g for 5 minutes in a haematocrit centrifuge. Trypanosomes aggregate alongside white blood cells in the layer between erythrocytes and plasma. The capillary tubes are subsequently observed under low magnification, either using a specialised holder or by positioning them between a microscope slide and cover slip, with water added between the glass surfaces to minimize light diffraction.

#### 2.4.1.2. Quantitative buffy coat test

The quantitative buffy coat (QBC) technique, initially developed for differential leukocyte counts, has been adapted for detecting haemoparasites such as trypanosomes and plasmodium (Lutumba *et al*., 2007). The method combines centrifugation with fluorescent staining of trypanosome kinetoplast and nuclear DNA using acridine orange. The capillaries are coated with acridine orange and ethylenediaminetetraacetic acid (EDTA) and the presence of trypanosomes is determined through their fluorescent kinetoplast and nuclear DNA. Ultraviolet light is generated by an LED module, which is linked via a glass fiber to the microscope objective containing a mounted filter. In this technique, the capillary tubes are expensive and the need for a dark room makes it costly and inapplicable in a field environment.

#### 2.4.1.3. Mini-anion-exchange centrifugation technique (mAECT)

Anion exchange chromatography is applied to separate the trypanosomes because, at pH 8.0, the trypanosomes remain neutral while the red blood cells (RBCs) are negatively charged (Büscher *et al*., 2009). Blood is added to a column containing diethylaminoethyl cellulose, where red blood cells are retained while trypanosomes are eluted and collected in a separate tube. Trypanosomes are

then concentrated at the bottom of the tube by centrifuging at 1,000 × g for 15 minutes. The tube tip is subsequently examined under low magnification using a specialised holder (Büscher *et al*., 2009). Buffy coat fraction can be viewed using this technique after centrifugation, with the fraction applied to the column (Camara *et al*., 2010). The sensitivity is 92.1 to 96% because it can detect fewer than 10 trypanosomes/ml (Camara *et al*., 2010; Inojosa *et al*., 2006). However, this technique is limited by cost and time. The columns are expensive and the whole process is tedious and time-consuming.

### 2.4.2. Serological detection

During an infection by trypanosomes, the parasite factors induce the production of high levels of antibodies, including IgM and IgG, that form the basis of serological tests. The sensitivity and specificity of the test depend on the particular antigens employed. For diagnosing Gambian infection, a rapid agglutination test is available and is commonly used for screening large populations. The rhodesiense infection lacks such tests and hence, surveillance is difficult to undertake. Common limitations of serological-based tests are low sensitivities and specificities that result in false positives due to cross-reactive antigens and antibodies from other pathogens (Gillet *et al*., 2013). In addition, antibodies remain present after parasite clearance, giving false-positive detection. However, these methods are very valuable in field environments, where infrastructure is limited and for surveillance.

#### 2.4.2.1. Card agglutination test for gHAT

The card agglutination test for trypanosomiasis (CATT) is a simple test used for screening gHAT by detecting specific antibodies (Camara *et al*., 2023; Magnus *et al*., 1978). The test is used in screening the population because it is simple, reliable and cost-effective. The introduction of CATT reduced the limitation of time faced by parasitological examinations. Therefore, the use of the test improved the effectiveness of screening for the infection. The antigen used is the complete bloodstream forms of *Tbg* variable antigen type LiTat 1.3. To prepare these antigens, trypanosomes are first purified from the blood of infected rats, then fixed, stained and freeze-dried. The complete test kit includes the reagent, positive and negative control sera and all necessary materials for performing the test on whole blood, such as test cards, capillary tubes, stirring rods, syringes, suction bulbs and droppers. The packaging makes the screening process easy and saves time. The test uses whole blood only, with blood drawn from the finger, which is less painful and the results are read after 5 minutes. The sensitivity of the test is 91% and the specificity of 97% (Robays *et al*., 2004). Despite its simplicity and importance in surveillance, there are limitations to the use of CATT. The reagents need to be stored at 4 °C for long-term preservation, necessitating a maintained cold chain. Additionally, each vial contains fifty test doses, and once opened and

reconstituted, the reagents should be used within one week when stored at 2-8 °C or within eight hours if maintained at 37 °C. Therefore, the use of this test is important in active screening, but not passive screening, where few clinical cases are detected.

#### 2.4.2.2. Immunological rapid diagnostic tests

Rapid individual lateral flow immunochromatographic tests are available for gHAT (Büscher *et al*., 2013). Two formats are used to detect trypanosome-specific antibodies: HAT Sero-Strip and HAT Sero-*K*-Set. In the first category, the test strip is placed in a tube containing a mixture of plasma, blood, serum and buffer. In the second case, a plastic cassette contains a test strip with a small well designed to hold a drop of blood, plasma or serum, followed by buffer. The results are read after 15 minutes of incubation, with one or two colors developing on the strip. The control line has to develop after the incubation period for the test results to be accepted. The test is effective for passive screening of Gambian infection with a sensitivity of 89 to 99% and specificity of 95 to 99% (Büscher *et al*., 2013).

#### 2.4.2.3. Indirect immunofluorescence assays

The Indirect Immunofluorescence Assay (IFA) is a sensitive serological technique used to detect antibodies against *Trypanosoma* species in host serum, indicating exposure or infection. The procedure begins by fixing *Trypanosoma* parasites onto a microscope slide. The slide is then incubated with diluted patient serum for 30 minutes, allowing any specific anti-trypanosome antibodies present to bind to the immobilized antigens. The slide is then washed with phosphate saline buffer. After washing to remove unbound antibodies, a fluorescently-labeled secondary antibody, commonly an anti-human IgG conjugated with a fluorophore such as FITC (fluorescein isothiocyanate), is added. This secondary antibody binds to the human antibodies attached to the parasite antigens. Following a final wash, the slide is examined under a fluorescence microscope. A positive result is visualized as bright fluorescence outlining the parasites, indicating the presence of specific antibodies in the serum. The immunofluorescence reagents are stable at 4 °C. This method is suitable for surveillance and laboratory diagnosis.

#### 2.4.2.4. Enzyme-linked immunosorbent assay (ELISA)

There are several methods of indirect ELISAs for both forms of HAT. The test uses different body fluids as samples to test for parasite antigens, including serum, plasma, saliva and CSF (Lejon *et al*., 1998; Lejon *et al*., 2003). Generally, the trypanosome antigen is placed in the wells of a microplate and then blocked using a protein solution, typically milk powder or bovine serum albumin, before incubation with a diluted sample. After washing the plate, an anti-human

IgG enzyme conjugate is introduced. The substrate and chromogen solution are added after another wash. A colour develops after the reaction if the trypanosome-specific antigens are present. The test is 95 to 100% sensitive and 97 to 100% specific (Lejon *et al*., 2006). Current improvements in the technique will further enhance its applicability in disease diagnosis (Geerts *et al*., 2021).

### 2.4.3. Molecular detection

The molecular tests are able to detect trypanosome nucleic acids (Deborggraeve & Büscher, 2010). The tests mainly rely on enzymatic amplification of specific DNA or RNA sequences and subsequent visualisation.

#### 2.4.3.1. Polymerase chain reaction (PCR)

PCR can amplify short fragments of DNA sequences through a series of enzymatic reactions and thermal cycling, followed by amplicon detection. Here, species and subspecies-specific primers that are specific to the target region of the DNA fragment are used to amplify the region of interest. In Gambian infection, *TgsGP* gene (Koffi *et al*., 2009) is specifically amplified to detectable levels that can be visualised by agarose gel electrophoresis after staining by ethidium bromide. Alternatively, real-time PCR assay allows high-throughput DNA detection (Becker *et al*., 2004). *TgsGP* gene is found in type 1 Gambian infection, which is more chronic than the type 2 form, which lacks the gene and is a more acute form of gHAT found within western and central Africa (Berberof *et al*., 2001; Koffi *et al*., 2009). For rhodesiense infection, the serum resistance-associated (*SRA*) gene present only in *T. b. rhodesiense* is targeted (Radwanska *et al*., 2002).

#### 2.4.3.2. Loop-mediated isothermal amplification (LAMP)

The LAMP assay enables isothermal DNA amplification and is used in HAT diagnosis by targeting specific DNA sequences with four to six primers that recognise six to eight regions of the target (Kuboki *et al*., 2003; Namangala *et al*., 2012). The technique is dependent on autocycling strand displacement DNA synthesis under isothermal conditions and requires simple heating. The LAMP product is visualised through a colour change using hydroxynaphthol blue. A lateral flow dipstick has been developed as a straightforward LAMP readout (Njiru *et al*., 2008). While suitable for field use, it remains relatively expensive.

#### 2.4.3.3. Nucleic acid sequence-based amplification

Trypanosome-specific nucleic acid sequence-based amplification assays have been employed to detect the parasite's 18S ribosomal RNA. Both lateral flow and real-time formats have been demonstrated (Mugasa *et al*., 2009). The technique has a sensitivity ranging from 90% to 97%

and specificity from 59% to 99% (Mugasa *et al*., 2012). However, the commercial kits are not available. The improvement of the diagnostic and staging tools is important, but to improve on this, the knowledge of disease pathogenesis is crucial.

Application of PCR on CSF can also be applied in staging. Also, plasma-specific enolase has been proposed as a diagnostic marker in a study that demonstrated its elevation in the late stage compared to the early stage (Sternberg & Mitchell, 2014). Cytokines such as IL-10 and IL-6 have been shown to be up-regulated during the late stage of the infection (Kato *et al*., 2015; Maranga *et al*., 2013). Therefore, there is potential in applying a combination of methods to improve staging. Based on the fact that the described methods are not 100% effective, there is a need to improve them or develop novel diagnostic tools. Thus, there is a need to identify host and pathogen-based biomarkers that can be used in diagnosis and/or staging. The knowledge of the stages of progression and factors contributing to the outcome is important in this endeavour.

### 2.5. Pathogenesis in human African trypanosomiasis (HAT)

Infection occurs through a bite by the infected tsetse fly. Upon infection, the disease progresses through two stages: stage 1 (early or hemato-lymphatic) stage, which occurs after an incubation period varying from 1 to 3 weeks after an infective bite and stage 2 (late or meningo-encephalitic), characterised by invasion of the CNS. In stage 1, the parasites proliferate in the blood, body tissues and lymphatic fluid, and if the patients are not treated, stage 2 ensues. The rate of progression from early stage to late stage varies depending on the infecting parasite. For Gambian infection, the early stage can last for months or years before progression to the late stage, while the progression of rHAT from early to late stage happens within a few weeks of infection (Brun *et al*., 2010). The first symptom that is common in the rhodesiense form of the infection is the appearance of a local skin reaction at the site of the tsetse fly's bite after a minimum of five days, called the trypanosomal chancre, which is usually followed by lymphadenopathy (Stich *et al*., 2002).

Overall, both forms present with nonspecific clinical signs resembling other common illnesses, such as malaria. Fever is an early symptom that may persist for several weeks due to successive waves of parasitaemia. In rhodesiense infection, symptoms at this stage can be severe and potentially fatal if untreated, often due to myocardial involvement (Brun *et al*., 2010). The Gambian form shows a faint rash and hepatosplenomegaly, but more specific lymphadenopathy in the posterior triangle of the neck (Winterbottom's sign) is also observed (Stich *et al*., 2002).

Invasion of the CNS results in chronic encephalopathy accompanied by headache and mental problems. The patients show mental dysfunction, difficulty concentrating, irritation and a somnolent state characterised by sleeping disorders (Stich *et al*., 2002). This stage is characterised by an alteration of circadian rhythm with sleeping hours being normal but an altered sleep-wake

cycle (Brun *et al*., 2010). This is the hallmark of the disease from which it derives its name. The second stage of the disease has varied clinical presentations (De Atouguia & Kennedy, 2000). The symptoms have been categorised into wider groups such as motor, psychiatric, sleep-wake disturbances and sensory abnormalities. The motor system involved has been linked to the tongue and limb muscle fasciculation, limb tremors, pyramidal weakness and limb hypertonia, choreiform and athetoid movements, cerebellar ataxia, dysarthria and polyneuritis (De Atouguia & Kennedy, 2000; Kristensson *et al.,* 1995). The psychiatric involvement could be subtle, including headache, overt psychiatric presentations such as hallucinations, violence, suicidal tendencies and mania, irritability, apparent personality changes and lassitude (De Atouguia & Kennedy, 2000; Kennedy, 2004). The sensory signs may manifest a puritis, deep hyperaesthesia (Kerandel's sign) and painful hyperaesthesia (Kennedy, 2004). Symptoms of sleep disturbance include distractibility, fatigue and irresistible sleepiness, along with a reversed sleep-wake cycle characterised by daytime drowsiness and nocturnal insomnia. If the patient is not treated, the progression to the final stage results in cerebral edema, double incontinence, seizures, severe somnolence, systemic organ failure, coma and inevitable death (Kennedy, 2004). There is no doubt that these symptoms are associated with the host-pathogen interaction, with the host and pathogen factors being important in the development of signs and symptoms.

### 2.6. Mammalian host-pathogen interaction

Mammalian host-trypanosome interaction is the basis of disease development and progression and these strictly extracellular parasites have developed strategies to evade clearance by the elaborate mechanisms of the host cellular and humoral immune response. Principally, the parasites change their surface coat, VSG, in a process called antigenic variation that prevents antibody clearance. To achieve this, the parasite has a repertoire of VSG genes, ~2000 in number, that are systematically expressed but also undergo recombination to have an infinite reserve of new VSGs (Li, 2015). This explains the parasite's ability to survive for long, up to many years in the mammalian hosts, increasing the window for continuous cyclic transmission. In addition, the parasites have an elegant internalisation of surface VSG-antibody complexes, destruction of the antibody and recycling back to the surface of the VSG coat (Pal *et al*., 2003). In fact, transmembrane trafficking is up-regulated in mammalian form parasites (bloodstream forms, BSF) as compared to insect stage forms, i.e., procyclic forms (PCF) (Field & Carrington, 2004). Antigenic variation has made it impossible to design vaccines.

Notably, initial inoculation of the parasites by the tsetse plays an important role in ensuring a successful infection. During a bite by tsetse, the insect saliva is suggested to play a pharmacological role (Caljon *et al.,* 2009). Tsetse saliva was demonstrated to accelerate the onset of

the infection through inhibition of local and systemic inflammatory response in mice (Caljon *et al*., 2006). In addition, saliva is highly immunogenic (Caljon *et al.*, 2006). For example, Tsetse Antigen 5 (TAg5) was demonstrated to sensitise mice and cause acute hypersensitivity reactions, allowing the efficiency of the parasite extravasation into the blood circulation (Caljon *et al.*, 2009). The immunoregulatory peptide Gloss 2 inhibits the secretion of the trypanolytic molecules by the host, such as proinflammatory cytokines, thus avoiding the initial elimination of the parasite (Bai *et al*., 2015). Therefore, vector factors contribute to successful parasite infection of the host, but the subsequent parasite survival in the host, their growth and dissemination are determined by some of the parasite factors and/or host factors and their interplay.

### 2.6.1. Pathogen factors

Parasite factors are key contributors to disease progression and pathogenesis. In particular, the serum resistance antigen (SRA) in *Tbr* and the TgsGP glycoprotein in *Tbg* help neutralise the host factor ApoL1, which normally lyses the parasites (Capewell *et al*., 2013; Stephens *et al*., 2012). The survival of the parasite in the host requires metabolic changes to uptake nutrients such as haem and glucose required for proliferation and evasion of the immune system (Stijlemans *et al*., 2015). Trypanosomes cannot synthesise haem and instead rely on the haptoglobin-haemoglobin (Hp-Hb) receptor (HpHbR) located in the flagellar pocket to acquire exogenous haem (Higgins *et al*., 2013). Haemoglobin released from lysed erythrocytes forms a complex with haptoglobin (Hp-Hb), which is recognised by the myeloid phagocyte system (MPS) through CD163 and by the trypanosome HpHbR, facilitating haem uptake (Higgins *et al*., 2013; Tripodi *et al*., 2011).

Human serum contains trypanosome lytic factor 1 (TLF-1), a 500 kDa complex associated with high-density lipoprotein (HDL), as well as TLF-2, which includes apolipoprotein A-1 (ApoA1), apolipoprotein L1 (ApoL1) and haptoglobin-related protein (Hpr) (Namangala *et al*., 2012; Shiflett *et al*., 2005). The Hpr gene is homologous to Hp and interacts with Hb to form the Hpr-Hb complex on the TLF-1 (Maeda *et al*., 1985; Widener *et al*., 2007). The binding of the TLF-1 on the HpHbR causes its endocytosis and targets the lysosome for lysis. The ApoL1 forms pores on the endolysosomal membrane, triggering lysosomal swelling and subsequent release of lethal lysosome content into the parasite (Perez-Morga *et al*., 2005). Furthermore, it has been documented that the C-terminal kinesin *TbKIFC1* also plays a role in ApoL1-mediated lysis through transportation of ApoL1 from the endolysosomal membrane to mitochondrion, resulting in depolarisation of mitochondrial membrane and subsequent lysis (Vanwalleghem *et al*., 2015). However, TLF-2 has been proposed to use a different mode of internalisation compared to TLF-1 (Molina-Portela *et al*., 2008). The human infective strains, *T. b. rhodesiense* and *T. b. gambiense*, express the serum resistance antigen (SRA) and a specific glycoprotein (TgsGP), respectively,

which counteract the activity of ApoL1 (Capewell *et al*., 2013; Stephens *et al*., 2012). *T. b. gambiense* has been shown to have decreased HpHbR expression, along with an L210S amino acid substitution in HpHbR, which leads to reduced uptake of TLF-1 (DeJesus *et al*., 2013; Vanhamme *et al*., 2003). Earlier research has demonstrated that SRA can be transmitted to *T. b. brucei* via membranous nanotubes when the flagellar membrane breaks down into free extracellular vesicles (Szempruch *et al*., 2016). *Tbr* SRA and *Tbg* TgsGP are the principal distinctions of human-infective and non-human-infective species; a defective human trypanolytic machinery permits human infection by all African trypanosome species (Capewell *et al*., 2013; Radwanska *et al*., 2002).

Secondly, certain parasite molecules have been found to suppress proinflammatory responses mediated by classically activated macrophages (M1), which are essential for early parasite control (De Muylder *et al*., 2013). The *T. brucei* kinesin heavy chain 1 (TbKHC1) protein released by the parasite sustains the first peak of parasitemia and the control by the host (De Muylder *et al*., 2013). The binding of TbKHC1 to the surface C-type lectin SIGN-R1 molecule results in the arginine/NO metabolism modulation in favour of the arginase activity through an IL-10-dependent induction of arginase-1 and down-regulation of iNOS activities (Willert & Phillips, 2012). This causes the subsequent stimulation of the production of l-ornithine and synthesis of polyamines essential in the growth of trypanosomes (Stijlemans *et al*., 2018). As a result, immune cells that produce IL-10 and arginase-1 show reduced ability to destroy parasites, allowing parasite numbers to increase. Additionally, *T. brucei* adenylate cyclase (TbAdC), a transmembrane receptor-like enzyme that converts ATP into cyclic adenosine monophosphate (cAMP), also contributes to parasite proliferation (Salmon *et al*., 2012). In cases of stress induced by the phagocytosis by M1 cells, the TbAdC levels can be elevated up to 250-fold, causing the cytoplasmic cAMP levels to increase within the phagocytes and the activation of the protein kinase A (Rolin *et al*., 1996; Salmon *et al*., 2012). This results in inhibition of the synthesis of trypanolytic cytokine tumor necrosis factor (TNF) (Vanwalleghem *et al*., 2017), disrupting parasite clearance.

Thirdly, the trypanosomes are extracellular parasites, yet they are not cleared by the host immune system because of the surface coat remodeling process that prevents their elimination by both mammalian and insect immune responses (Shimogawa *et al*., 2015). The long slender bloodstream forms (BSFs) are covered with a VSG coat composed of approximately $5\times10^6$ homodimers, each made of 50–60 kDa subunits, which are anchored to the parasite's plasma membrane via glycosylphosphatidylinositol (GPI) (Mehlert *et al*., 2002). This VSG coat shields invariant proteins from detection by the host's innate and adaptive immune responses and protects the parasite from complement-mediated lysis. The rapid VSG recycling plays a role in the removal of surface-bound VSG-IgG complexes, aided by the rapid hydrodynamic motility (Engstler *et al*., 2007). More so, the trypanosomes release vast amounts of the soluble VSG (sVSG), especially

during peak parasitaemia, to scavenge the complement factors which are important in the destruction of the parasites, inducing the hypocomplementemia state (Balber *et al*., 1979). The binding of the antibodies on the surface coat normally results in the aggregation of the parasites. The trypanosomes use a protein kinase-C to disaggregate (Stijlemans *et al*., 2011). Hence, the parasite's survival in the bloodstream and its growth are enhanced. In PCFs, the surface procyclin is protease resistant (Vassella *et al*., 2004), preventing degradation by tsetse proteases.

Additionally, VSG is recognized by specific antibodies that bind to the entire trypanosome surface. However, the parasite has developed a mechanism of evading immune clearance through endocytosis with the help of hydrodynamic flow (Engstler *et al*., 2007). Endocytosis occurs in the flagellar pocket, which is posterior. The trypanosome flagellum is a structure used for movement and runs along the body, propelling away from the flagellar pocket. Consequently, flagellar movement creates a hydrodynamic drag force on the immune complexes, causing their movement toward the flagellar pocket for internalization. This process of endocytosis has been described previously to be rapid (Engstler *et al*., 2007). Therefore, trypanosome parasites can survive longer in the bloodstream through rapid endocytosis, whose end products are recycled VSG and lysosomal degradation of antibodies.

Finally, the trypanosome-derived lymphocyte-triggering factor (TLTF) is a parasite-derived molecule that stimulates $CD8^+$ T cells to produce IFN-γ (Abdulla *et al*., 2013; Nishimura *et al*., 2004; Olsson *et al*., 1991). The IFN-γ further activates the phagocytic M1 cells. In a study by Hamadien *et al*. (2000), IFN-γ has been shown to trigger TLTF secretion *in vitro* parasite cultures in a tyrosine protein kinase and dose-dependent manner and stimulates parasite growth. This suggests that these molecules exert bidirectional activation between the $CD8^+$ cells and the parasites. Therefore, there is a possibility of involvement in the host-parasite interaction that needs to be unraveled. These pathogen factors support parasite survival and colonisation, consequently contributing to disease progression and their interaction with host factors, dictating infection outcome.

### 2.6.2. Host factors

The host immune system, comprising both innate and adaptive components, is vital in countering parasite factors. The innate immune system serves as the first line of defense, whereas the adaptive immune system provides long-term protection. Following pathogen entry, innate immune cells such as macrophages mount the initial response and facilitate activation of adaptive immunity. Macrophages express pattern recognition receptors (PRRs), including toll-like receptors (TLRs), which detect pathogen-associated molecular patterns (PAMPs) (Janeway, 2002). This activation causes the macrophages to produce reactive oxygen and nitrogen species, which have a

direct killing effect on the pathogen (Garzon *et al*., 2013). Moreover, macrophages have other roles, including enhancing the adaptive response by antigen presentation with the help of dendritic cells, coupled to major histocompatibility complexes (MHCs), to T helper cells. This leads to the production of soluble factors such as cytokines and chemokines, which are immunomodulatory factors. T cell proliferation and differentiation trigger a cellular response through cytotoxic lymphocytes (CTLs) or a humoral response via antibody production.

The introduction of trypanosomes into the mammalian host via the bite of an infected tsetse fly causes activation of the innate cells, such as macrophages. Many trypanosome factors have been shown to be involved in this activation; the interaction with VSG, free DNA, the VSG-GPI and soluble VSG with GPI anchor glycosylinositol phosphate (GIP-sVSG) core directly activates the macrophages (Sternberg, 2004). The soluble VSG with GIP-sVSG core has also been shown to activate the macrophages and induce TNF-α and nitric oxide (NO) production (Drennan *et al*., 2005). These trypanosome-derived factors are also proposed to induce IFN-γ production by $CD8^+$ T cells, which subsequently activate macrophages to release trypanolytic mediators such as nitric oxide (NO) and reactive oxygen species (ROS) (Salerno *et al*., 2016). These factors also enhance immunosuppression and consequent alteration of the BBB (Masocha & Kristensson, 2012). The presence of free *Tbr* DNA in the infected wild-type C57BL/6 and C57BL/6-*scid* mice blood suggests its involvement as the ligand for the macrophage receptor, activating the involvement of the innate immune system (Harris *et al*., 2006). The activation of macrophages leads to activation of adaptive response via antigen presentation, and results in the production of immunomodulatory molecules that may also play an important role in the polarization of Th-1. The Th-1 polarisation has been shown to aid in the production of the initial high pulse of IFN-γ levels (Salerno *et al*., 2016). IFN-γ production is crucial in resistance to infection and in inducing IL-12 production, which further contributes to Th-1 polarisation (Drennan *et al*., 2005; Shi *et al*., 2008). The production of pro-inflammatory cytokines is therefore linked to reduced parasite numbers. Counter-inflammatory cytokines, on the other hand, have been shown to extend the survival of the parasite in the host and the change to the Th-2 pattern (Namangala *et al*., 2001). The production of IL-10 has been suggested to control the extent of immunopathology and plays a role in its limitation, especially during the late stage (MacLean *et al*., 2001). These various factors play a crucial role in disease progression and pathogenesis.

High levels of IFN-γ in the early stage have been associated with clinical signs such as elevated body temperatures and coma (Bosschaerts *et al*., 2010; Liu *et al*., 2015). The episodes of fever lasting for about 6 days occurring together with generalised lymphadenopathy are associated with such elevation (Kennedy, 2004). The early symptoms, such as malaise, headache, arthralgia, general weakness, and weight loss, result from the inflammatory response (Bosschaerts *et al*.,

2010). TNF-α plays a role in immune system dysfunction, neuropathogenesis and BBB dysfunction, enabling the parasite to enter the CNS (MacLean *et al*., 2004). The immune system dysfunction is associated with multiple organ infections such as the spleen, liver, skin, cardiovascular system, endocrine system, and the eyes (Kennedy, 2004). Elevated TNF-α levels are associated with rapid disease progression, as was demonstrated in Ugandan patients with *Tbr* infection as compared to chronic infections in Malawi patients (MacLean *et al*., 2004).

The crossing of the BBB has been associated with both the host and parasite factors. Parasite proteases such as cathepsin-L-like proteases have been proposed to be involved in *Tbr* crossing to the CNS (Masocha & Kristensson, 2012). It has also been proposed that the crossing is associated with the host immune system activation (Silva *et al*., 2010). The host's innate immune system is activated during parasite infection, leading to the production of cytokines such as TNF-α and IFN-γ (Janeway, 2002). TNF-α promotes increased expression of ICAM and VCAM on cerebral endothelial cells, facilitating T cell adhesion (Mulenga *et al*., 2001). IFN-γ induces limited production of CXCL-10 by endothelial cells or astrocytes, which is enough for the penetration of the T cells, accompanied by some trypanosomes into the perivascular space (Masocha & Kristensson, 2012). Eventually, the parasites cross the BBB and reach the brain, ushering in the onset of the late stage.

The second stage of the disease is characterised by a shift toward a counter-inflammatory response that facilitates parasite persistence within the host (Kato *et al.,* 2016). Increased levels of anti-inflammatory cytokines, such as IL-10 and IL-6, have been associated with reduced neuropathology, underscoring their protective role (Kato *et al*., 2015). The resulting symptoms associated with the invasion of the CNS are categorised into general groups such as psychiatric, motor, sensory abnormalities and sleep disturbances as elaborated by Brun and colleagues (2010). Psychiatric symptoms can include irritability, headaches and more severe manifestations such as suicidal tendencies, aggression, hallucinations and mania. Motor system abnormalities may involve limb tremors, hypertonia, pyramidal weakness, choreiform and athetoid movements, dysarthria, cerebellar ataxia and polyneuritis. Sleep disturbances have been shown to be characterised by fatigue, distractibility, uncontrollable sleep urges and disruptions of the normal sleep-wake cycle (Kennedy, 2004). The infection is associated with sleeping disorders, where the presence of the parasites in the perivascular space results in circadian rhythm disorder (Lundkvist *et al*., 2004). Despite the similarity of the disease presentation at the two stages, the period of disease progression to the late stage varies between the Gambian form and the rhodesiense form of the disease. These differences suggest possible variation(s) in host-pathogen interactions, which is principally driven by the pathogen. However, varying clinical outcomes complicate the assessment of this. Notably,

little is known about this, and insight gathered could help resolve clinical diagnosis, identifying diagnostic and staging biomarkers.

### 2.7. Varying disease outcomes in HAT

The interspecific variation could be responsible for varying disease outcomes in the two forms of HAT. In addition, intra-subspecies variations also occur, with the same strain responsible for acute or chronic infection (MacLean *et al.,* 2007; Truc *et al*., 1997), or asymptomatic cases (Jamonneau *et al*., 2004). *T. b. rhodesiense* infection is typically regarded as the acute form of human African trypanosomiasis, characterised by rapid disease progression (Barrett *et al.,* 2003). Virulence has been shown to increase from the southern to the northern regions of the rHAT belt, reflecting the genetic diversity of infecting strains both across and within endemic foci (MacLean *et al.,* 2004; MacLean *et al.,* 2010). These variations have been linked to parasite-related factors that influence disease outcome (MacLean *et al.,* 2007). The role of ethnicity of the inhabitants of the regions can also not be ruled out (Rutto *et al*., 2013).

Unlike in rhodesiense infection, where chancre is used to estimate the time of infection, this phenomenon is lacking in Gambian infection (Gelfand, 1966). Therefore, the onset of the infection is often a challenge (Cecchi *et al*., 2008). However, there has been reported variability with some of the individuals depicting an acute form of the disease (Garcia *et al*., 2000; Truc *et al*., 1997). A follow-up on the patient's refusing treatment in Cote d'Ivoire was able to identify some of the patients who were initially diagnosed to be at the first stage of the disease, with no detectable symptoms (Jamonneau *et al.,* 2000; Jamonneau *et al.,* 2004). These patients depicted a drop in the antibody to the seronegative level, showing a clear indication of self-cure, while others maintained the same levels of parasitaemia undetectable by microscopy (Jamonneau *et al*., 2004).

Generally, the difference in disease progression in the two strains is notable. These differences are influenced by host and parasite genetics, environmental factors and their interactions. Host genetic factors are thought to regulate immune responses, thereby influencing infection levels and mortality rates. For example, a study on *T. congolense* identified resistance loci on chromosomes 1, 5 and 17 (Iraqi *et al*., 2000). Notably, the interleukin-10 (IL-10) and TNF-α genes are located within two of these regions (chromosomes 1 and 17) (Courtin *et al*., 2006). These genes have been suggested to influence the control of parasite infection, with elevated pro-inflammatory cytokine levels linked to rapid progression of the disease to CNS involvement (MacLean *et al*., 2012). Other cytokines, such as IL-6, IL-8 and IL-10 levels in blood and CSF have been shown to be altered by HAT treatment, where there is a significant reduction in levels to normal levels upon treatment of second-stage disease (Lejon *et al*., 2002). This suggests that these cytokines play a role in late-stage pathogenicity. Cytokine gene polymorphisms have been

implicated in host-parasite interactions, contributing to differences in disease outcomes among individuals (MacLean *et al*., 2004; Ofon *et al*., 2019). Investigations into single-nucleotide polymorphisms (SNPs) in genes encoding TNF-α, IL-10, IL-1α, IL-4, IL-6, IL-8 and IFN-γ suggest that variability in host responses to trypanosome infections may be influenced by immune response gene polymorphisms (Ahouty *et al*., 2017; MacLean *et al*., 2004). Studies conducted in southern Cameroon, Guinea and the Democratic Republic of Congo demonstrated that specific IL-6 and IL-10 variants were significantly associated with reduced risk of human African trypanosomiasis (HAT) (Courtin *et al*., 2007; Kaboré *et al*., 2017; Ofon *et al*., 2019). Conversely, polymorphisms in IL-1α and TNF-α genes were found to be significantly associated with increased susceptibility to disease development.

An elegant example of host genetics can be demonstrated in cases of defective trypanolytic machinery. In a study to determine the roles of components of TLF in trypanolysis, human serum lacking Hp and Hpr that was obtained from anaphthoglobulinemic patients lacking both Hpr and Hp resulting from homozygous gene deletion (20 kb) from the *Hp* promoter region to exon 5 of Hpr (Koda *et al*., 1998). To analyze this genetic alteration, the researchers employed Southern blotting and polymerase chain reaction (PCR) techniques. These methods allowed them to detect and characterise the deletion within the Hp-Hpr gene cluster. The study's findings highlighted that individuals with the Hp(del)/Hp(del) genotype exhibited anhaptoglobinemia, while those with the Hp2/Hp(del) genotype had significantly reduced Hp levels, a condition known as hypohaptoglobinaemia. In a different study, the researchers investigated the role of apolipoprotein L1 (ApoL1) in the innate immune defense against trypanosome infections. They focused on a human patient who had contracted *Trypanosoma evansi*, a parasite typically non-infective to humans due to the protective trypanolytic factors present in human serum (Joshi *et al*., 2006). Upon analysis, it was found that the patient's serum lacked ApoL1, a critical component responsible for the lysis of trypanosomes. This deficiency was attributed to frameshift mutations in both alleles of the APOL1 gene, leading to the production of truncated, non-functional proteins (Vanhollebeke *et al*., 2006). Consequently, the patient's serum exhibited no trypanolytic activity. These studies demonstrate the importance of host genetics in HAT.

*Tbr* virulence is proposed to increase towards the north of the affected region (MacLean *et al*., 2004). This virulence is also thought to be linked to variations in host inflammatory cytokine responses and differences in parasite serum resistance-associated (SRA) gene polymorphisms (MacLean *et al*., 2010). The SRA gene is unstable and has been shown to undergo allelic polymorphisms in *Tbr* isolates obtained from East and South African countries (Gibson & Ferris, 2003; MacLean *et al.,* 2004). Notably, there is a significant difference between the patients from the northern and southern regions of East Africa, with patients from the northern part displaying a

typical acute form of the disease while the southern region displayed characteristics similar to the chronic Gambian infection (Kato *et al*., 2016). Clinical diversity has also been reported between and within the foci (MacLean *et al*., 2004). The clinical diversity is attributed partially to the trypanosome strain variation and highly to the genetic variation in the trypanosome strains (MacLean *et al.*, 2007). This means that the diversity is partly because of the difference between *Tbg* and *Tbr* strains, but even though the same strain could be involved, say *Tbr*, the genetic diversity of the parasite contributes largely to the difference in the outcome. In Uganda, the polymorphic microsatellite markers were compared from Tororo and Soroti foci, and the results showed two distinct genotype clusters showing varied responses (MacLean *et al.*, 2007). However, the parasite genetic diversity is not associated with the clinical diversity within the foci and could be associated with environmental factors and host genetics (Kato e*t al.,* 2017).

The human data referred to in most studies suffer major limitations, including unknown time of infection, which is equally quite difficult to determine based on varying disease progression, outcomes and interplays involved. Therefore, the individual data are difficult to compare and consenting study participants is low (MacLean *et al*., 2004). To address this, non-human primate (NHP) models with radically similar disease profiles have been developed, the vervet monkey (*Chlorocebus aethiops*, African Green Monkey) (Schmidt & Sayer, 1982). Previously, a tsetse-mediated infection of this model has also been demonstrated by Thuita *et al*. (2008). Together with the ability to accurately determine the time of infection, this model can allow elaborate interrogation of host-pathogen interaction during disease progression. Using samples from such previous studies, this work sought to investigate immune modulation associated with varying disease outcomes after infection by two strains of *Tbr*, KETRI 3801 and 3928, that are responsible for acute and chronic infection (Thuita *et al*., 2008).

### 2.8. Summary

The rhodesiense infection is an acute disease that could kill patients within days or weeks if left untreated. Current microscopic diagnosis, which is the main diagnostic tool, is limited by sensitivity, especially in cases of very low parasitemia in the early few days post-infection. Treatment is complicated by the timely availability of drugs, where the WHO is the main distributor, causing delays and the death of diagnosed patients. The infection with *Tbr* strain results in varying disease outcomes, with the patients showing acute or chronic infection. The role of host immune factors in the varying outcomes and, by extension, disease progression remains unknown or unclear and is an important question that unravels the dynamic host-pathogen interactions. In this study, the levels of host cytokines in vervet monkeys infected separately with two strains of *Tbr* that cause acute and chronic infections were determined. This allowed interrogation of the host immune

response associated with progression and varying outcomes. The increased levels of specific cytokines during the early stage and their lower levels at the late stage, and vice versa, were notable. Therefore, this information provides insight into the role of the host immune response to disease outcomes and can be exploited in the identification of potential diagnostic and staging biomarkers.

# CHAPTER THREE
# MATERIALS AND METHODS

## 3.1 Study design

Part of the data and material used in this study were secondary, i.e., they were not generated during this study. These data were obtained from the original monkey experiment where the samples were retrieved (Thuita & Masiga, 2016). Therefore, the accuracy and integrity of these data remain the responsibility of the sources. This includes clinical data of the experimental monkeys, including parasitaemia, packed cell volume (PCV), weights, temperatures, survival times and feed consumption that were collected and analysed. The data were used solely for analysis and interpretation within the scope of this research.

Archived vervet monkey plasma and CSF samples from a previous study were used. Briefly, two groups of animals (N = 4) were infected with *T. b. rhodesiense* parasites, namely KETRI 3801 and KETRI 3928, which cause acute and chronic infections, respectively. The third group (N = 4 per group) was uninfected and termed as control. The animals infected with KETRI 3801 include 708 (F), 701 (F), 709 (M) and 704 (M); KETRI 3928 were 710 (F), 719 (F), 699 (M) and 715 (M), and uninfected were 703 (F), 717 (F), 705 (M) and 706 (M). M and F represent male and female, respectively. Including both sexes ensures that results are not biased toward one sex, making findings generalised and applicable to a broader population. The monkeys were handled according to the prescribed protocol by the AICUC (Thuita *et al*., 2008). The infection and sample collection were done as per the protocol approved by the same committee.

On account of resource limitations, plasma samples from three (3) animals per cohort were retrieved and analysed for cytokines. The animals infected with KETRI 3801 selected include 708 (F), 709 (M) and 704 (M); KETRI 3928 infected monkeys were 710 (F), 699 (M) and 715 (M), and uninfected monkeys were 703 (F), 705 (M) and 706 (M). On the other hand, CSF samples from two animals per cohort were also selected. In KETRI 3801, the animals included 708 (F) and 709 (M); KETRI 3928, 710 (F) and 715 (M), and uninfected were 703 (F) and 705 (M).

## 3.2 Immune factors

### 3.2.1 Cytokines assayed

A thorough literature review was conducted to identify cytokines relevant to the study. The search was carried out using scientific databases, including PubMed and Google Scholar. The search strategy involved the use of specific keywords, including cytokines, Human African Trypanosomiasis, immune response, *Trypanosoma brucei*, pro-inflammatory cytokines, anti-inflammatory cytokines, sleeping sickness and cytokine biomarkers. Filters were applied to limit results to peer-reviewed articles and full-text availability and publications from the past 15 years to

ensure relevance and up-to-date evidence. Titles and abstracts were screened for relevance and full texts of potentially eligible studies were reviewed. Key cytokines repeatedly reported in association with HAT pathogenesis, particularly those implicated in the early and late stages of the disease, were selected for further evaluation and quantification in the current study.

Eight immune factors linked to different outcomes of African trypanosome infection in various HAT models and human cases (Kato *et al*., 2015; Kato *et al*., 2016; MacLean *et al*., 2001; Maina *et al*., 2004; Namangala *et al*., 2001; Ngotho *et al.*, 2006; Sternberg *et al*., 2005) were considered. These include INF-γ, TGF-β, TNF-α, IL-1β, IL-6, IL-10, IL-13 and IL-12. S100B was also included, as it is a serum biomarker of brain injury and may indicate neuronal and blood-brain barrier (BBB) damage, making it a potential biomarker for HAT staging (Bloomfield *et al*., 2007; Marchi *et al*., 2004; Rothermundt *et al*., 2003).

#### 3.2.1.1 Sample preparation and standards

Cytokine levels in monkey plasma and CSF were measured using ELISA kits from U-Cytech Biosciences (Utrecht, Netherlands) for TNF-α, IFN-γ, IL-10, IL-6, IL-12, IL-13 and IL-1β. CSF levels of TGF-1β and S100β were assessed using ABclonal Technology kits (Massachusetts, USA). Samples (500 µL) were aliquoted into eppendorf tubes to prevent repeated freeze-thaw cycles. For U-Cytech kits, $^{1}/_{20}$ volume of cytokine stabilization buffer (CSB) was added before dilution, while for ABclonal kits, 10 µL of balance solution was added to every 100 µL of diluted sample following the manufacturer's instructions.

To generate a standard curve, Serial two-fold dilutions were prepared to generate a standard curve ranging from the highest concentration specified in the kit data sheet, down to the assay's detection limit. This was achieved by transferring 400 µl of the reconstituted standard into a tube containing 400 µl of dilution buffer to create the first standard (Std 1), followed by sequential dilution through seven additional tubes (Std 2 to Std 7). A blank, containing only the dilution buffer, was included as the negative control. All dilutions were thoroughly mixed before use. Each standard concentration was tested in triplicate to ensure accuracy and reproducibility. After completing the ELISA assay, the optical densities (ODs) of the standards were measured at 450 nm using a microplate reader. These OD values were plotted against their corresponding known concentrations using curve-fitting software (Microsoft Excel). The best-fit standard curve was generated, and the resulting equation of the curve was applied to interpolate the cytokine concentrations of the unknown samples.

### 3.2.2 Cytokine assays

The solid-phase sandwich ELISA was performed following the protocols provided by the manufacturers (U-Cytech Biosciences and ABclonal Technology). Briefly, according to the U-Cytech Biosciences protocol, each well was coated with 50 µL of reconstituted coating antibody. The volume was then adjusted to 100 µL with PBS, sealed, and left to incubate overnight at 4 °C in an ALB refrigerator (ALB Service Pty Ltd, West Burleigh, Australia). Plates were then brought to room temperature and the coating solution was discarded, followed by washing, which was done six times using wash buffer. Subsequently, each well was blocked with 200 µL of blocking buffer and incubated at 37 °C for 1 hour. After removing the blocking buffer, 100 µl per well of blank, standard or sample was added as appropriate. The plates were covered and incubated at 37 °C for 2 hours. After incubation, blanks, standards and samples were discarded, and the wells were washed six times with washing buffer. Subsequently, 100 µL of biotinylated anti-cytokine detection antibody was added to each well, followed by sealing and incubation at 37 °C for 1 hour. The wells were then washed six times before adding 100 µL of diluted SPP conjugate, which was incubated for 1 hour at 37 °C under sealed conditions, followed by another six washes. Thereafter, 100 µL of TMB substrate was dispensed into each well and incubated at room temperature for 20 minutes. The reaction was terminated with 100 µL of stop solution and optical density (OD) values were measured at 450 nm using a Stat Fax 3200 Microplate Reader (GMI, Minnesota, USA), with each sample measured in triplicate. Incubation at 37 °C was performed using an MB100-4A microplate shaker incubator (MRC Ltd, Holon, Israel).

According to the ABclonal Technology protocol, all reagents, standards and samples were equilibrated to room temperature before use. The 96-well plate supplied in the kit was pre-coated with a specific monoclonal antibody of interest. Initially, the plates were washed three times with 350 µL of diluted wash buffer, with a 40-second pause between washes to ensure removal of unbound material. Standards and diluted serum samples (100 µL per well) were then added in duplicate and incubated at 37 °C for 2 hours to allow antigen binding. After incubation, the wells were washed three times before the addition of a biotinylated detection antibody, which was then followed by another 1-hour incubation at 37 °C. The plates were washed again, after which 100 µL of streptavidin-HRP conjugate was added to each well and incubated for 30 minutes at 37 °C. Following another wash, 100 µL of TMB substrate (prepared by mixing equal volumes of substrate solutions A and B) was added to each well and incubated at room temperature in the dark for 20 minutes. The reaction was terminated with 50 µL of stop solution per well and absorbance was recorded at 450 nm using a Stat Fax 3200 Microplate Reader (Awareness Technology Inc., Palm City, Florida, USA).

### 3.3 Statistical analyses

To assess differences in cytokine levels with disease progression between monkeys infected with the two *T. b. rhodesiense* strains, a generalised additive mixed model (GAMM) (Wood, 2006) was applied, incorporating individual monkeys as a random effect. The model included strain (group) as a fixed effect, time (days post-infection) as a nonparametric smooth function and a time-by-strain interaction term to allow each strain to vary differently over time. Uninfected monkeys served as the reference group. GAMMs were chosen to accommodate highly nonlinear trends in cytokine data (Figure 9), with nonlinearity quantified using the effective degrees of freedom (edf) of the smoothing term, where an edf of 1 indicated a linear relationship, while higher values reflected greater nonlinearity. Models were fitted using the 'gamm' function from the 'mgcv' R package (Wood, 2004), separately for each cytokine using plasma data. Six plasma cytokines were analysed, which included IFN-γ, TNF-α, IL-10, IL-6, IL-12 and IL-1β. Cytokines showing no significant changes, which were CSF cytokines and IL-13 (both plasma and CSF), were excluded from further analysis.

Median survival times, along with their interquartile ranges (IQR), were estimated using Kaplan-Meier curves (Kirkwood & Sterne, 2010), and differences between strains were assessed with the Log-rank test. Generalized additive mixed model (GAMM) analyses were conducted in R version 3.6.1 (R Core Team, 2019), while survival analyses were carried out using Stata v15.1 (StataCorp, College Station, TX). The survival analysis approach allowed assessment of whether variations in cytokine levels were associated with differences in survival outcomes, which serve as an indicator of disease severity and pathogenicity. While this method does not directly evaluate correlations between continuous cytokine concentrations and clinical severity scores, it provides an indirect means of testing the hypothesis by examining the impact of cytokine expression on disease progression and outcome. All tests were performed at a 5% significance level.

# CHAPTER FOUR

# RESULTS

## 4.1 Pathogenicity of *T. b. rhodesiense* strains in vervet monkey

### 4.1.1 Changes in parasitaemia levels with disease progression

Rhodesiense infection has been linked previously to the formation of a chancre at the site of infection. In this study, a trypanosome-induced chancre was also noted in 1 out of 4 monkeys (708) infected with KETRI 3801, and in 2 out of 4 monkeys (699 and 715) infected with KETRI 3928. Following infection, there was a recorded pre-patent period during which parasites were not detectable in blood. The median pre-patent period was 6.0 days (range: 6 - 6 dpi) for KETRI 3801 and 6.3 days (range: 6 - 7 dpi) for KETRI 3928 (Table 3).

After the pre-patent period, parasitaemia levels differed between KETRI 3801 and KETRI 3928 infections as the disease progressed. In monkeys infected with KETRI 3801, parasitaemia reached a peak of antilog 7.8 parasites/mL by 12 dpi, followed by a decline, with second and third peaks occurring before termination of the experiment (Figure 3). In contrast, KETRI 3928 infection showed a lower peak of antilog 7.3 parasites/mL by 8 dpi. Overall, parasitaemia fluctuated between antilog 6.1 and 7.8 parasites/mL during the course of infection, with higher levels consistently observed in monkeys infected with KETRI 3801 compared to those infected with KETRI 3928.

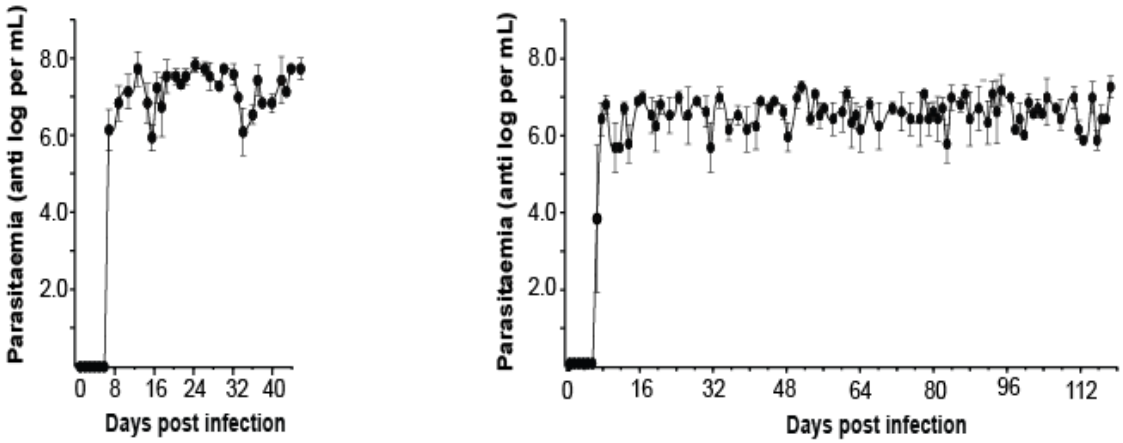



**Figure 3:** Changes in parasitaemia levels in monkeys infected with two strains of *T. b. rhodesiense*

*The graph was plotted from the number of parasites per ml (y-axis) against time post-infection (x-axis). Error bars indicate the standard error of the mean (±SEM) for parasitaemia, calculated as the standard deviation (SD) divided by the square root of the sample size (n). During African trypanosomiasis, the host immune system is subjected to persistent pressure due to fluctuations in Variant Surface Glycoprotein (VSG) expression and parasite counts, resulting in periodic cycles of parasitemia (Mugnier *et al.*, 2016). The causative parasite, mainly *Trypanosoma*, avoids immune detection by altering its surface VSG proteins. As the parasite multiplies and shifts to new VSG

variants, the host generates antibodies against these new forms, which leads to a reduction in parasite numbers and a temporary decline in parasitemia.

**Table 2:** Clinical and parasitological effects of *T. b. rhodesiense* parasites in monkeys

| **Parameter** | **Control** | **KETRI 3801** | **KETRI 3928** |
|---|---|---|---|
| Proportion of monkeys with chancre | N/A | $^{1}/_{4}$ | $^{2}/_{4}$ |
| Chancre observation (dpi) | N/A | 4 | 6,10 |
| Pre-patent period (range of days) | N/A | 6.0 (6-6) | 6.3 (6-7) |
| First peak parasitemia in dpi (parasite load/mL) | N/A | 12(antilog 7.8/mL) | 8(antilog 7.3/mL) |
| PCV at baseline (0 dpi) | 49.3 ± 2.6 | 50.3 ± 5.2 | 51.6 ± 7.5 |
| % PCV change at day 12/extremis[‡] | -2.69/-3.23 | -13.27/-44.90 | -12.63/-59.60 |
| Time to first peak temperature (dpi) | N/A | 12 | 8 |
| Highest temperature increase (℃) | 0.68 | 1.80 (day 12) | 1.78 (day 8) |
| First detection of parasites in CSF (dpi) | 0 | 12/16/16/24 | 8/12/16/28 |
| Day of first detection of CSF white cells numbers ≥ 5cells/uL | -/-/16/24 | 0/8/16/28 | 12/16/16/28 |
| Baseline weight at 0 dpi (Kg) | 4.68 ± 0.77 | 4.50 ± 0.60 | 4.12 ± 0.60 |
| Weight loss at extremis (Kg) | -0.6 | -0.8 | -1.2 |
| Median (range) survival time (dpi) | N/A | 28 (23 - 34) | 95 (57 - 115) |

[‡]Values are from the last surviving animal in each infected cohort at extremis. For CSF parasites and white cell count, the dpi for individual animals per cohort are shown and in animals where white blood cells did not reach ≥ 5cells/μL cut off, a "-" is used; b, survival time of control animals was censored data since all were euthanised at the end of the study without attaining an extremis condition. N/A represents not applicable.

### 4.1.2 Monkey survival times with disease progression

This study found a varied disease progression period to the extremis condition in both infected groups as compared to uninfected controls. Animals infected with KETRI 3801 survived for shorter times compared to those infected with KETRI 3928. The median survival time was 28 (IQR 23 – 34) days for KETRI 3801, 95 (IQR 57 – 115) days for KETRI 3928, and 120 (IQR 120 – 120) days for uninfected monkeys (Table 3; Figure 5). Clinical monitoring of uninfected monkeys was terminated at the termination of the study (120 days post-infection) without a requirement for them to attain the extremis condition. Therefore, this figure was used for statistical comparison despite uninfected animals surviving longer.

Generally, monkeys infected with the KETRI 3801 strain died faster compared to those infected with KETRI 3928 (Figure 4). Compared to uninfected animals and KETRI 3928 infected monkeys, time to death was about three times shorter in KETRI 3801 infections (HR=2.48, 95%CI: 1.42-4.30). However, between KETRI 3928 infections and uninfected controls the difference was not statistically significant (p=0.0704) (HR=0.66, 95%CI: 0.42-1.00) (Figure 5 B) but individual animals infected with KETRI 3928 attained extremis condition at different days post infection including 95, 115 and 119 while in uninfected control cohort, the experiment was terminated at 120 days without attaining extremis condition. As in previous studies described by Limo *et al*. (2021) and Thuita *et al*. (2008), these data indicate that KETRI 3801 causes an acute infection compared to the chronic KETRI 3928 infections.

A

| Strain ID | Year of isolation | Isolate type | Region of isolation | Lab derivative ID | Derivative type | Passages |
|---|---|---|---|---|---|---|
| KETRI 3199 | 1989 | Pleomorphic | Busia, Kenya | KETRI 3801 | Clone | 1 |
| KETRI 3928* | 2003 | Clone | Tororo, Uganda | KETRI 3928 | Clone | 2 |

B

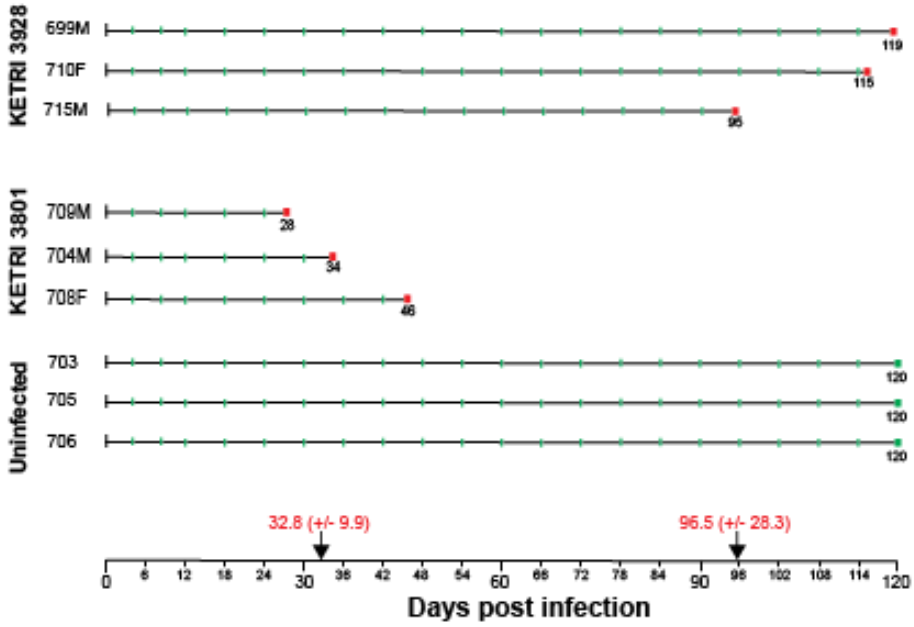


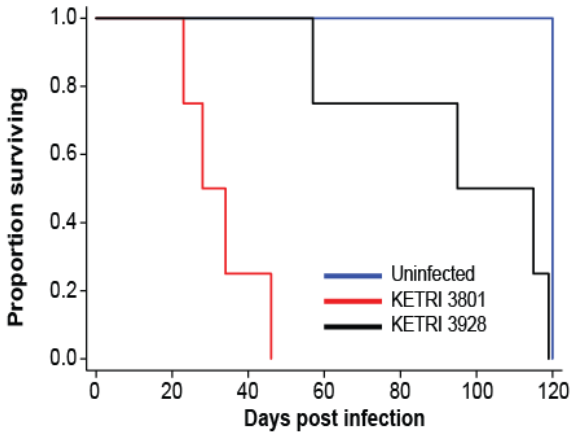

**Figure 4:** Strain biodata and sampling regime obtained from secondary data. **A**. Biological and historical data. **B**. Monkeys infected with two strains of *T. b. rhodesiense*

Plasma and CSF samples were taken as marked on different days post-infection. Animals were sacrificed at extremis, indicated with red boxes, due to the emergence of clear late-stage symptoms. The mean survival time (and the SD) is shown for the infected cohorts.

F = female, M = male.

*A cloned strain was provided to the KALRO BioRI through donation by the National Livestock Resources Research Institute (NaLIRRI), Uganda.

**A. Survival curves**




**B. Estimated risk of dying**

| Strain | Hazard ratio (95% CI) | p-value |
|---|---|---|
| Control | | |
| KETRI 3801 | 2.48 (1.42 - 4.3) | 0.001 |
| KETRI 3928 | 0.66 (0.42 - 1.0) | 0.070 |

**Figure 5:** Survival times of infected vervet monkeys. **A**. Kaplan-Meier (survival) curves. **B**. Estimated risk of dying using the Cox proportional hazard model

### 4.1.3 PCV changes with disease progression

Packed cell volume (PCV) was determined as the proportion (%) of red blood cells in whole blood samples. A reduced PCV level is an indicator of anaemia. In this study, all infected animals exhibited progressive reductions in PCV during the course of infection, while uninfected animals showed insignificant fluctuations (Figure 6A). With reference to 0 dpi, the highest reductions were observed at the end of the experiment in both infections. The average reduction of 11% for KETRI 3801 and 9% for KETRI 3928 infections was observed at day 12 dpi. A higher reduction rate is observed when the two infections are compared, when the animals attained extremis condition with an average of 34% for KETRI 3801 and 55% for KETRI 3928 infection (Figure 6 B). The level of reduction rate is higher in the KETRI 3801 infected cohort compared to the KETRI 3928 cohort.

The initial drastic reduction was observed around 8 - 12 dpi, which coincides with the time of the first peak parasitaemia (Figure 3; Figure 6B). A comparison in percentage change at this time was done at 12 dpi to assess the difference between the two infections (Figure 6B). The trend was followed by a slight recovery, and thereafter a slow and progressive reduction. Notably, the overall magnitude of PCV reduction correlates with survival time, with the highest reduction observed at the extremis in monkeys infected with KETRI 3928, which survived the longest. In addition, a higher rate of PCV reduction could be associated with acute infections, as shown in Figure 6.

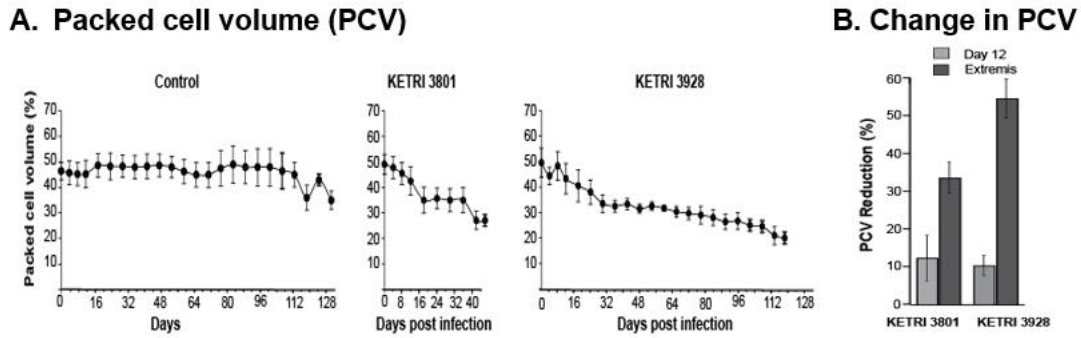



**Figure 6:** Anaemia in *T. b. rhodesiense* infection of vervet monkeys. **A**. PCV change in different cohorts with disease progression. **B**. Change in PCV levels at 12 dpi and extremis

*The graph was plotted from the values of PCV read (y-axis) against time post-infection (x-axis). Error bars indicate the standard error of the mean (±SEM) for PCV, calculated as the standard deviation (SD) divided by the square root of the sample size (n).

### 4.1.4 Changes in body temperature with disease progression

Body temperature is used as an indicator of fever when there is a temporary rise above 37 ℃ (Balli *et al*., 2023). All infected animals showed increased body temperature as compared to the controls, an indication of infection-induced fever (Figure 7A). The highest average temperatures in KETRI 3801 (40.20 ℃) and KETRI 3928 (40.03 ℃) infections were observed at 12 and 8 dpi, respectively, coinciding with the time of the first peak parasitaemia (Figure 7 B; Figure 3).

In the course of infection, there were fluctuations of average body temperature with higher fluctuations in infected animals compared to controls. These fluctuations ranged from 38.30 – 39.13 ℃ (Δ0.833) in control, 38.40 - 40.20 ℃ (Δ1.80) in KETRI 3801 and 38.25- 40.03 ℃ (Δ 1.78) in KETRI 3928 infections. This suggests larger temperature fluctuations associated with infections. Overall, temperature increases in the monkeys were intermittent and consistent with the undulating fever of trypanosomiasis.

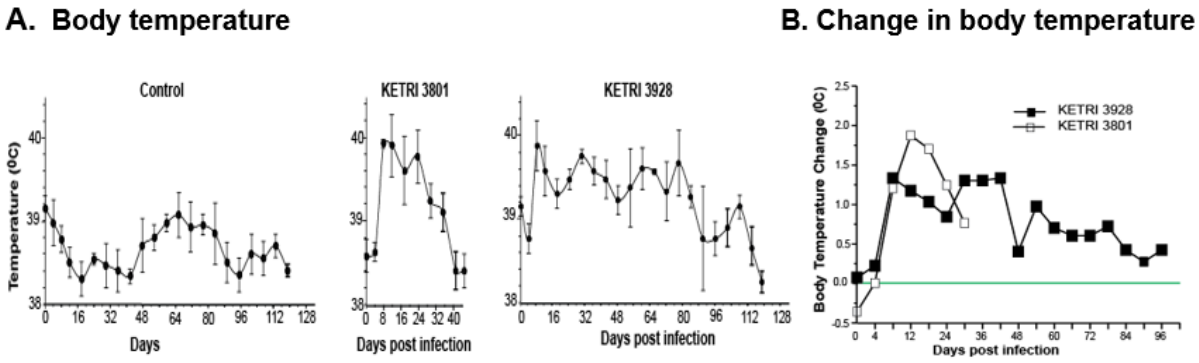



**Figure 7:** Effect of *T. b. rhodesiense* strains on body temperature. **A**. Body temperature changes with disease progression. **B**. Change in body temperature in infected monkeys compared to control

*The green line is the reference point representing no change relative to the control. These fluctuations appear to a lesser extent in uninfected control animals due to physiological or environmental factors. The routine monitoring, including the use of anesthesia, could transiently elevate temperature. More so, ambient temperature or humidity shifts in the animal facility could influence thermoregulation.

### 4.1.5 Changes in body weight with disease progression

All infected animals registered weight loss, which increased with disease progression (Figure 8A). Mean losses of 1.2 kg and 0.8 kg in KETRI 3928 and KETRI 3801 infections, respectively, were observed (Table 3). A higher reduction rate in KETRI 3801 infected cohort than KETRI 3928 infected cohort was observed, with a loss of about 30% observed at the extremis in both infections (Figure 8 B).

Weight loss also coincides with the parasitaemia trend, where a progressive decrease in weight occurs at 8 dpi in both infection cohorts. This is followed by a short recovery in body weight, and thereafter a steady and drastic decrease was observed in KETRI 3801 infection to the extremis. However, a similar case was observed in KETRI 3928 infection, but the decrease was slower till extremis. With a standard feed ration and water provided ad libitum to all the monkeys, the infection altered feeding and decreased feed intake, as shown in Figure S1, could in part contribute to the observed weight loss. The varying rate of loss was strain-associated, with the highest total loss observed at extremis in all infected animals (Figure 8 B).

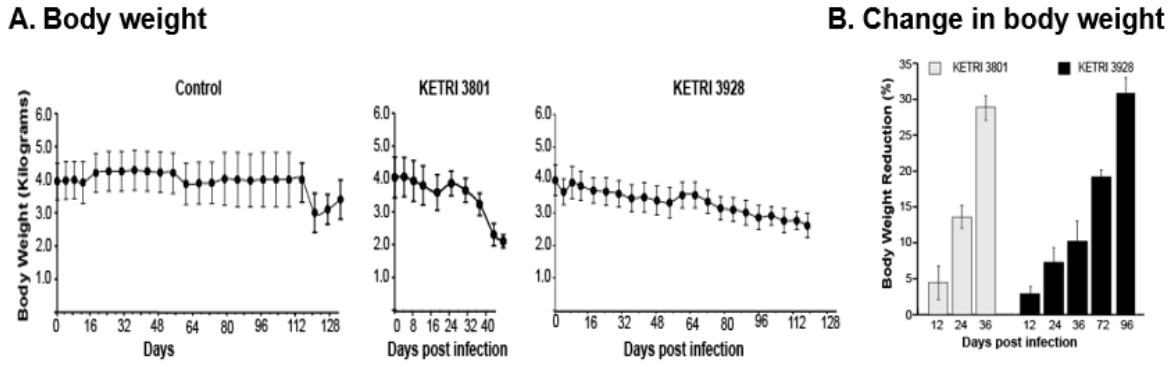



**Figure 8:** Effect of *T. b. rhodesiense* strains on body weight. **A**. Changes in body weight with disease progression. **B**. Average reduction in body weight compared to controls

*The graph was plotted from the recorded body weight in kilograms (y-axis) against time post-infection (x-axis). Error bars represent the standard error of the mean (±SEM) for body weight, calculated as the standard deviation (SD) divided by the square root of the sample size (n).

Together, PCV and body weight reduction were observed in the course of infection, and were highest at the extremis. Since water was provided ad libitum, and all monkeys were provided a standard feed ration, the observed weight reduction can be associated with infection. Further, infection-mediated fever was initially observed at the first peak parasitaemia, and remained until the extremis, but with some minor fluctuations. Comparatively, observations on PCV, weight loss, infection-mediated fever, median survival time and risk of dying indicate that KETRI 3801 is acute, while KETRI 3928 infection is chronic. A similar observation has been made in a mouse model (Limo *et al.*, 2021).

**4.2 Cytokine changes with disease progression in plasma**

The outcome of an infection is the consequence of host and pathogen factors, their interactions with the host environment, e.g., co-infection, and nutritional status, among many other factors. An important host contribution is the immune response against the infectious agent(s), which can also contribute to immunopathology. Consequently, investigation of immune factor alterations in the course of the two varying infection outcomes was sought, acute and chronic, to attempt to understand which, if any, factors may have roles in modulating the course of infection.

TNF-α is a broadly influential regulator of immunity and is produced by many immune cells. TNF-α plasma levels showed no variation with the baseline levels with disease progression in all infected monkeys. In uninfected and infected monkeys, TNF-α levels remained stable throughout the infection. However, the trend in KETRI 3928 infection was highly non-linear with higher estimated degrees of freedom (Table 4).

IL-12 was assessed due to its key role in T cell differentiation, particularly in promoting Th1 and NK cell responses, as well as in stimulating TNF-α production. At baseline, levels of IL-12 ranged between 0.8 to 1.0 ng/mL of plasma. Following infection, IL-12 levels increased to a peak by 8 dpi in both groups of infected monkeys. In the monkeys infected with KETRI 3801, the IL-12 levels at 8 dpi were three times the baseline level. Thereafter, IL-12 levels declined but then rose again to a second peak at 24 dpi, which coincides with another parasitaemia peak (Figure 9 B; Figure 3). In the monkeys infected with the chronic strain KETRI 3928, the first peak of IL-12 was twice the baseline and was observed at 8 dpi. Thereafter, IL-12 remained elevated above baseline with another peak at 60 dpi. Toward the end of the experiment, IL-12 levels fell to baseline. The first peak of IL-12 was significantly greater in monkeys infected with the KETRI 3801 strain compared to those infected with KETRI 3928.

IFN-γ production is also influenced by TNF-α, and is important in responses to a wide range of infections, inducing macrophage activation, and is produced by Th1 cells, amongst others. A single spike of IFN-γ was observed at 8 dpi in both groups of infected monkeys (Figure 9C). No

other peaks were observed thereafter until the extremis. In controls, the IFN-γ levels remained steady at baseline levels throughout the study.

IL-1β levels were analysed, a cytokine involved in the inflammatory response and mainly produced by macrophages and monocytes. No notable peaks were observed in controls or monkeys infected with KETRI 3801. However, in monkeys infected with KETRI 3928, IL-1β levels in monkey 710 were significantly elevated from 16 dpi, peaking at 40 dpi, and showing wide variations between individuals of the cohort (wide error bars in Figure 9D). Thereafter, IL-1β levels subsided to be similar to the other animals by 64 dpi.

IL-6 is associated with microbial infections and specifically pathways mediated by Toll-like receptors. A first IL-6 peak was detected at 12 dpi in both groups of infected monkeys. In monkeys infected with the acute strain, mean IL-6 levels were significantly greater than in those infected with the chronic isolate $p<0.05$ (Figure 9 E; Table 3). A second IL-6 peak was evident terminally, at 42 and 95 dpi for the acute and chronic strain infections, respectively.

Finally, IL-10 was considered, which has an anti-inflammatory function, downregulating Th1 cytokine synthesis and antigen presentation. An increase in IL-10 levels was observed at 8 dpi in both groups of infected monkeys, with those infected with the acute strain showing higher levels of the cytokine (Figure 9F). At the extremis, both infected cohorts had levels that were nearly 1.5 times their baseline levels.

In summary, IFN-γ and IL-12 had similar profiles in both infections. IL-6 concentrations were elevated in acute infections relative to chronic infections, with a peak recorded at 12 dpi. Notably, the levels of IL-6, IL-12, IL-10 and IFN-γ were significantly elevated in early-stage disease of both groups of infected monkeys at 4 -8 dpi ($p<0.05$), suggesting that these can be considered as markers of early-stage disease.

**Table 3:** Estimates from generalised additive mixed model comparing average cytokine levels (ng/mL) across two strains of T. b. rhodesiense

| **Cytokine** | **Strain** | **Estimate** | **Std. Error** | ***t*-value** | ***p*-value** |
|---|---|---|---|---|---|
| TNF-α | KETRI 3801 | 0.036 | 0.008 | 4.4 | < 0.001 |
| | KETRI 3928 | 0.073 | 0.008 | 9.7 | < 0.001 |
| IFN-γ | KETRI 3801 | 1.216 | 0.377 | 3.2 | 0.001 |
| | KETRI 3928 | 1.223 | 0.313 | 3.9 | < 0.001 |
| IL-12 | KETRI 3801 | 0.850 | 0.105 | 8.1 | < 0.001 |
| | KETRI 3928 | 0.957 | 0.088 | 10.8 | < 0.001 |
| IL-1β | KETRI 3801 | 0.134 | 0.010 | 13.4 | < 0.001 |
| | KETRI 3928 | 0.095 | 0.009 | 10.4 | < 0.001 |
| IL-6 | KETRI 3801 | 0.436 | 0.061 | 7.2 | < 0.001 |
| | KETRI 3928 | 0.121 | 0.051 | 2.4 | 0.018 |
| IL-10 | KETRI 3801 | 0.026 | 0.002 | 11.2 | < 0.001 |
| | KETRI 3928 | 0.026 | 0.002 | 12.5 | < 0.001 |

**Table 4:** Estimated degrees of freedom showing trends of cytokines with disease progression

| **Cytokine** | **Strain** | ***E* df** | ***Ref* df** | ***F*-value** | ***p*-value** |
|---|---|---|---|---|---|
| TNF-α | KETRI 3801 | 0.000 | 7 | 0.000 | 0.999 |
| | KETRI 3928 | 5.498 | 8 | 3.229 | < 0.001 |
| IFN-γ | KETRI 3801 | 2.567 | 8 | 1.375 | 0.004 |
| | KETRI 3928 | 4.970 | 8 | 2.548 | < 0.001 |
| IL-12 | KETRI 3801 | 3.313 | 8 | 4.821 | < 0.001 |
| | KETRI 3928 | 6.436 | 8 | 8.660 | < 0.001 |
| IL-1β | KETRI 3801 | 0.000 | 8 | 0.000 | 0.554 |
| | KETRI 3928 | 7.307 | 8 | 27.100 | < 0.001 |
| IL-6 | KETRI 3801 | 3.591 | 8 | 9.089 | < 0.001 |
| | KETRI 3928 | 0.000 | 8 | 0.000 | 0.385 |
| IL-10 | KETRI 3801 | 3.724 | 8 | 55.170 | < 0.001 |
| | KETRI 3928 | 7.589 | 8 | 155.700 | < 0.001 |

*E.df refers to the estimated degrees of freedom associated with the error term. Ref.df refers to the denominator degrees of freedom. An E.df equal to 0 implies a linear function and the higher the E.df, the highly nonlinear the function is.

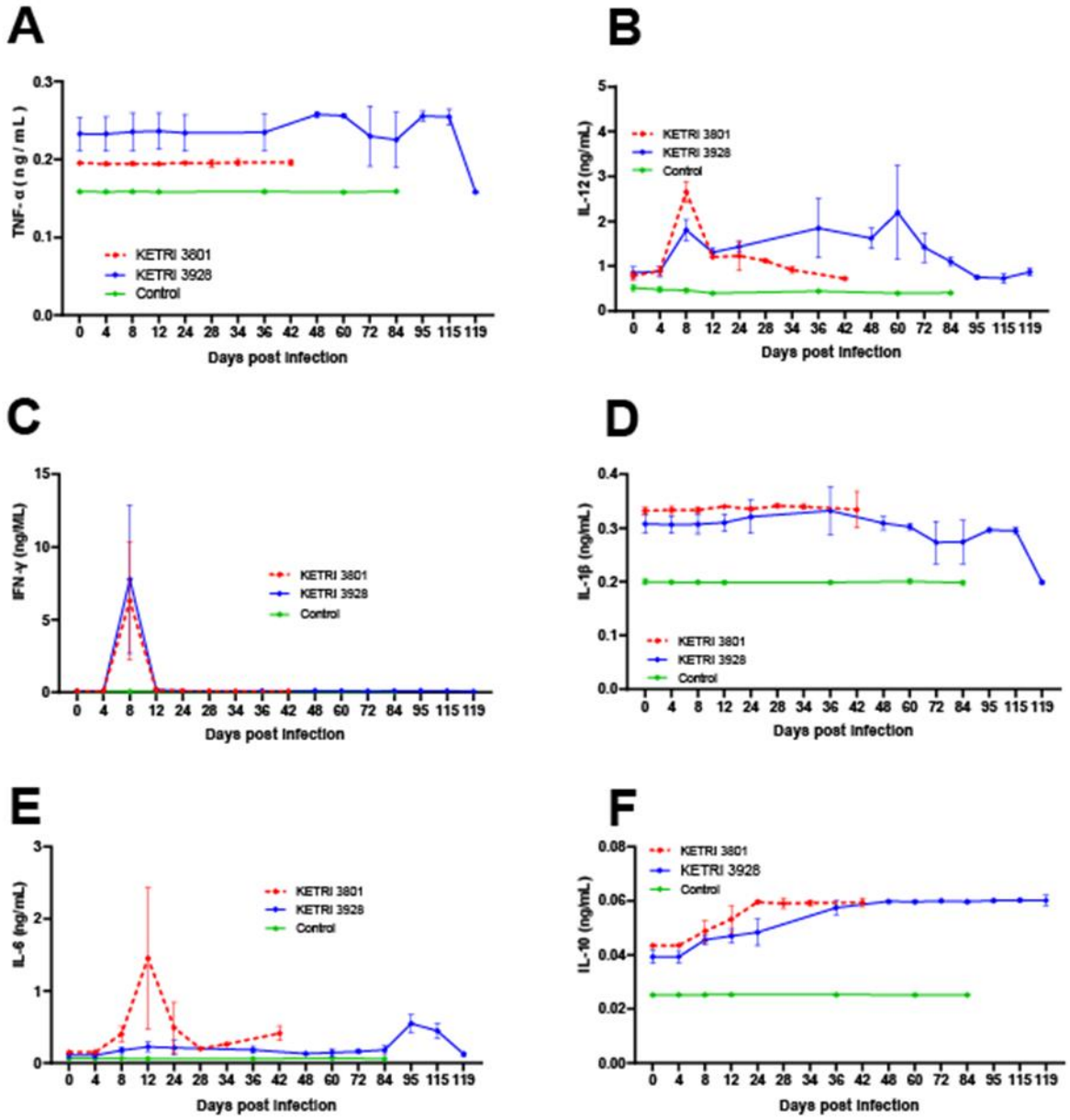



**Figure 9:** Plasma cytokine alterations in the course of *T. b. rhodesiense* infection of vervet monkey

*Error bars were calculated as the standard error of the mean (SEM) calculated from standard deviation (SD) and sample size (n). The respective host immune factors were monitored till the extremis and termination of the experiment.

### 4.3 Cytokine changes with disease progression in the cerebrospinal fluid (CSF)

To stage sleeping sickness, either the presence of parasites and/or > 5 WBC/µl in the CSF is considered an indicator of late or stage two disease (Brun *et al*., 2010). Parasites were detected in the CSF between 12 - 24 dpi and 8 - 28 dpi in KETRI 3801 and KETRI 3928 in all cohort animals, respectively (Figure 11A). Notably, CNS invasion cannot be excluded due to the interval of lumbar puncture for the determination of parasites and WBC. Similarly, CSF WBC counts > 5 cells/µl were noted at 0 – 28 dpi and 12 – 28 dpi in KETRI 3801 and KETRI 3928 infected animals, respectively.

For the uninfected cohort, a WBC >5 cells/µl was detected in two animals on days 16 and 24 (Figure 11 B). Generally, CSF parasite and WBC levels increased more rapidly in KETRI 3801 infection than in KETRI 3928 infection. This is indicative of more rapid parasite replication and/or invasion, progression and severity of late-stage disease in KETRI 3801 infections as compared to KETRI 3928 infections.

Elevated plasma IL-6 and IL-12 levels were observed in monkeys with late-stage disease, indicating potential utility as diagnostic agents/molecules even after the onset of late-stage disease. However, none of the cytokines were exclusively detected in CSF late-stage disease and therefore are, at this level, unreliable biomarkers of late-stage disease except when considering levels, which are highly variable.

Notably, the levels of TNF-α, IL-1β and IL-10 in plasma were higher in infected animals than in controls at day 0 of infection; varying baseline is a normal phenomenon and is a consequence of various factors (Tarantula *et al*., 2022). In all cases, the levels in control remained unchanged. The estimated effective degrees of freedom for cytokines indicated strong evidence of highly nonlinear additive effect over time except for TNF-α (KETRI 3801), IL-1β (KETRI 3801) and IL-6 (KETRI 3928) (Table 4). In the CSF, the levels of the six (6) cytokines were not detected except for IL-12 (in monkeys 715 and 709, Figure S 2b), TGF-β and S100B.

The levels of TGF-β and S100B in CSF were also analysed. TGF-β has multiple roles in inflammation, mainly acting to suppress B cell and macrophage activity as well as promoting differentiation of CD4 T cells. A variable TGF-β response was observed in the monkey cohorts. In monkeys infected with KETRI 3801, two animals, 708 and 704 showed an increasing trend with reference to baseline; a peak at 4 dpi and 24 dpi (Figure 10). In monkeys infected with KETRI 3928, the levels in monkey 699 were elevated throughout the infection, with variable responses in the remaining two monkeys.

S100B is secreted by mature astrocytes and is a marker of CNS injury and blood-brain barrier permeability. Wide variations in S100B levels were observed. In KETRI 3801 infected monkeys, two individuals, 704 and 708 showed an increase in levels with disease progression, whereas in KETRI 3928 infected monkeys, a variable response with no clearly defined trend was observed.

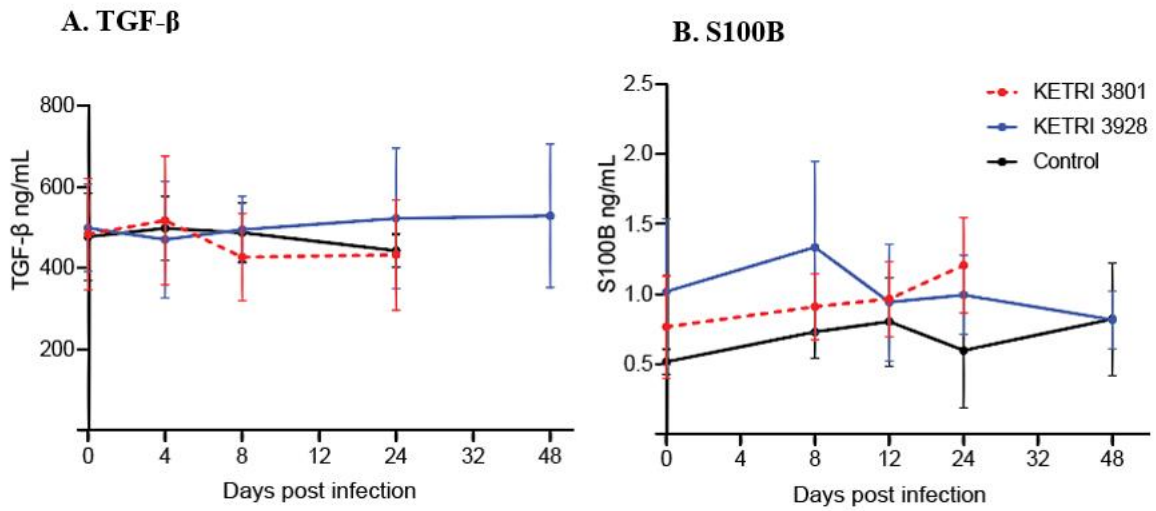



**Figure 10:** Cerebrospinal fluid (CSF) cytokine levels. **A**. TGF-β with disease progression. **B**. S100B levels in the course of *T. b. rhodesiense* infection of vervet monkey

*Error bars were calculated as the standard error of the mean cytokines (SEM), calculated from standard deviation (SD) and sample size (n).

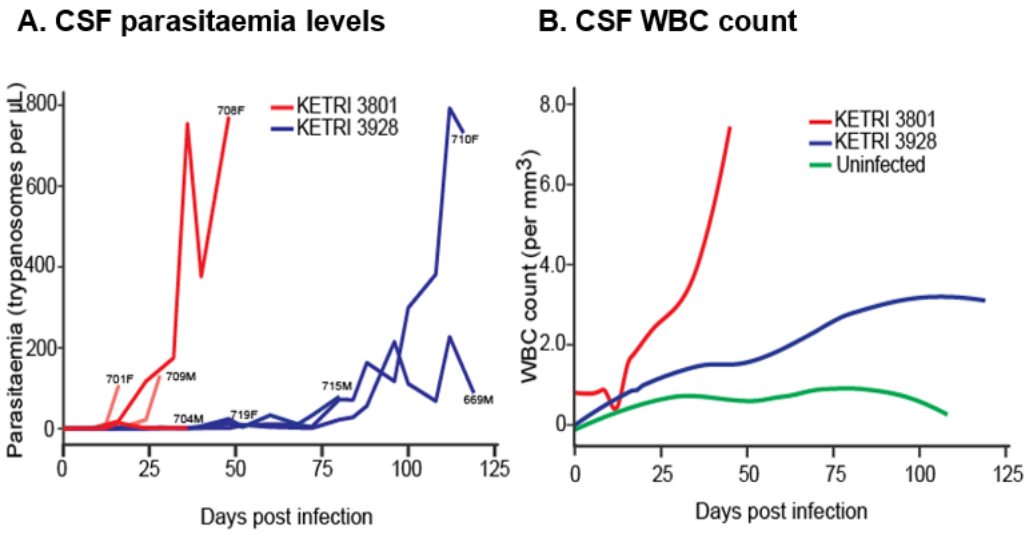



**Figure 11:** Cerebrospinal fluid (CSF) trypanosome invasion and associated white blood cells (WBC) levels in the course of infection

*The graphs were plotted from the number of trypanosome parasites per microlitre and the number of WBC per cubic millimeter on the y-axis, respectively, against time (days post-infection) on the x-axis.

In summary, the majority of CSF-assayed immune factors remain unchanged throughout the course of infection. In general, plasma immune factors are higher in infected animals as compared to controls, with significant differences in specific factors between acute and chronic infection

noted. In addition, higher CSF parasite and WBC counts are observed earlier in the second stage disease of KETRI 3801(acute) as compared to KETRI 3928 (chronic) infection.

# CHAPTER FIVE

# DISCUSSIONS

The immunopathogenesis in HAT is characterised by a complex interplay between parasite evasion strategies and host immune responses. During infection, parasite-derived factors enable immune evasion through mechanisms such as antigenic variation and immunomodulation. Disease progression and severity are further influenced by host-related factors, involving complex interactions between immune cells, their secreted products and parasite-derived molecules. The activation of both innate and adaptive immune pathways is critical in determining disease outcome, with cytokines acting as central signalling molecules that modulate the magnitude and duration of immune responses, thereby influencing the balance between parasite control and immunopathology. Previous studies have suggested that cytokines influence infection outcome. However, their specific role remains unclear. This study focused on the role of different individual cytokines in modulating disease outcomes in rHAT using cryopreserved samples from uninfected controls and infected monkeys who were infected through a single bite from an infected tsetse fly (Thuita & Masiga, 2016).

The early stage of infection is marked by the development of a trypanosomal chancre at the bite site in some cases. Chancre results from a localised inflammatory response to the inoculated parasites (McGovern *et al*., 1995; Sekhar *et al*., 2014), tsetse salivary components (Alfituri *et al*., 2020; Caljon *et al*., 2006) and possibly laceration caused by probing for hemorrhagic pool feeding. This response is the first host attempt to contain and prevent initial parasite colonisation, and is crucial in infection outcomes. However, neutrophils recruited at the site of infection contribute to parasite colonisation, facilitated by the disruption of extracellular DNA traps mediated by TatD, a cytoplasmic protein with DNase activity observed in mice (Caljon *et al*., 2018; Mabille & Caljon, 2020; Zhang *et al*., 2021). Chancre is not observed in all bites and is suggested to vary depending on the infecting species and host (Crilly & Munger, 2021; Urech *et al*., 2011). Similar to these previous observations, 1 of 4 monkeys and 2 of 4 infected with KETRI 3801 and KETRI 3928 developed a chancre, respectively. The host also elicits an inflammatory response through neutrophils and NK cells in response to trypanosome-specific pathogen-associated molecular patterns (PAMPs), including CpG DNA and VSG. Previous studies have linked neutrophils to the production of pro-inflammatory cytokines, such as IL-6 and IL-1β, during the early stage of infection (Maina *et al*., 2004). Similarly, the first peak of IL-6 was recorded at day 12 dpi in both groups of infected monkeys. However, a second IL-6 peak was evident towards the end of the experiment, at 42 and 95 dpi for the acute and chronic strain infections, respectively, suggesting the action of neutrophils during late-stage infection. Involvement of IL-1β in early or late-stage infection was not evident since no peaks were recorded except in one animal infected with a chronic

strain, whose peak was recorded at the late stage. Therefore, IL-1β would not be a good biomarker for rHAT staging.

Parasite antigenic variation of their VSG coat is central to disease progression and virulence. Trypanosomes are extracellular parasites covered entirely by a monolayer of surface antigen, VSG, which is highly variable with high antigenicity (Pinger *et al*., 2017). VSG protects the parasite by creating a physical barrier that protects it against the host's immune system. Additionally, the highly variable nature makes vaccine development a challenge, rendering chemotherapy the main disease management method involving the host. The parasite's capacity to switch VSG variants determines the outcome as acute or chronic infection due to the elimination of the parasite by the host immune system after recognition and clearance of expressed surface antigen (Jayaraman *et al*., 2019). VSGs are held by glycosylphosphatidylinositol (GPI) anchors to the surface of trypanosomes. Previous studies involving *T. brucei*-resistant mice reported the protein to elicit a polarised Th-1 immune response (Caljon *et al*., 2018; Grob *et al*., 2020; Hertz *et al*., 1998). This resistance was linked to IFN-γ cytokine due to the inability of susceptible mice to elicit a Th-2 response in the absence of a Th-1 cytokine response. Similar to these reports, this study demonstrates an initial increase in IFN-γ cytokine levels in all infected animals, a typical activation of Th-1 response by *T. b. rhodesiense* during early-stage infection.

Additionally, GPI interacts with macrophages, leading to their direct activation. Previous studies have also demonstrated that the interaction of VSG with macrophages leads to macrophage activation due to the presence of GPI anchors (Borges *et al*., 2021; Magez *et al*., 2002). Furthermore, macrophages are classically activated by IFN-γ and TNF-α secreted by NK cells, leading to a pro-inflammatory response characterised by the production of reactive oxygen intermediates (ROIs), nitric oxide (NO), reactive nitrogen intermediates (RNIs) and additional TNF-α (Stijlemans *et al*., 2010). TNF-α further promotes the recruitment and activation of T cells, which are maintained through IL-12 secretion. Activated T cells, in turn, stimulate B cell responses via IL-4 production and enhance macrophage activity through the release of additional IFN-γ (Hertz *et al*., 1998). Further activation of B cells is through dendritic cells (DCs) via IFN-γ, IL-6 and IL-12, targeting clearance of the trypanosome parasite (Kupani *et al*., 2021). However, resistance to African trypanosomiasis (AT) in mice that is associated with VSG cytokine response is specific to the disease stage, with a typical type-I response in the early stage and type-II during late-stage disease. Additionally, the ability of the murine model to launch a specific VSG B-cell response also differs depending on the susceptibility of the specific strain to infection. With antigenic variation, a type-II response is mounted through alternative activation of macrophages, where cytokines such as TGF-β and IL-10 promote arginase macrophage activation. This activation results in the production of counter-inflammatory cytokine IL-10 and subsequent suppression of IFN-γ and NO. Similarly,

this study demonstrated an impulse of IFN-γ production during early-stage infection. However, during the same time of disease progression, TNF-α plasma levels were not elevated in infected monkeys and remained stable throughout the infection without any peaks (Figure 10A). On the other hand, secretion of IL-12 was higher with the first peak at 8 dpi in all infected animals, suggesting similar involvement at the early stage. With disease progression, an increase in IL-10 levels was observed after 4 dpi in both groups of infected monkeys, with those infected with the acute strain showing higher levels of the cytokine (Figure 10F).

VSG coat is made up of up to $10^7$ identical homodimers forming a dense layer of glycoprotein on the trypanosome surface. Variant Surface Glycoproteins (VSGs) activate the host immune system, inducing IgM antibody production through B cell activation (Dubois *et al*., 2005). However, antigenic variation through the expression of different VSG coats enables the parasite to evade immune clearance. Studies in mouse models have highlighted the role of IgM in *T. b. brucei* infection, particularly during peak parasitaemia (Baral *et al*., 2007; Magez *et al*., 2006; Magez *et al*., 2008). These studies demonstrate that VSG expression elicits specific IgM antibodies that mediate trypanosome clearance. However, the switch to a new variant of VSG causes an increase in parasitemia as the B cells are activated. Apart from B cell activation, a previous study demonstrated the involvement of pro-inflammatory cytokines IFN-γ and TNF-α in parasitemia control (Magez *et al*., 2006). In the study, IFN-γ receptor-deficient mice were used to show that IFN-γ–driven immune activation plays a critical role in controlling parasitaemia. Furthermore, the MHC class II-deficient mice demonstrated the importance of the molecule in IFN-γ initiation, leading to the production of TNF-α. Similar to these studies, the strain KETRI 3801 exhibits higher primary peak parasitaemia, growth rate and parasitaemia as compared to KETRI 3928 (Figure 3). A similar trend was observed in the mouse model (Limo *et al*., 2021); thus, this study suggested differences due to strain-dependent factors on replication (proliferation and differentiation) and host-mediated clearance. A lower parasitaemia is observed in chronic infection in trypanosomes (Turner *et al*., 1995), consistent with chronic KETRI 3928 infection outcomes in vervet monkeys and mice. This indicates that KETRI 3928 maintains sub-lethal levels by balancing host clearance and differentiation into nondividing stumpy forms, and proliferation, without having adverse effects on the hosts, hence longer host survival and extended transmission window. Observations by Murray and Morrison (1979) suggest that higher parasitaemia, which is associated with a higher degree of pathogenesis, can, in part, explain the varying pathologies observed here.

Parasitaemia waves have also been linked with complement lysis. However, in *T. b. rhodesiense* studies using mice, it was demonstrated that complement-mediated lysis was not important in parasitaemia clearance or peak parasite control during infection (Magez *et al*., 2020). Studies involving *T. congolense* using C5-deficient mice reported similar arguments (Tabel *et al*.,

2000). A peak trypanosome clearance without involvement of C5 in *T. vivax* and *T. b. rhodesiense* also demonstrated a lack of complement-mediated lysis during infection (La Greca *et al*., 2012; Seed & Sechelski, 1988). However, the generation of the C3b component of complement during the initial stages is important because it is a powerful opsonin that has the ability to bind covalently to its target (Law & Levine, 2019). C3b is converted to iC3bi through the interaction of Factor H and I in plasma, which is a target for the CR3 receptor that is involved in the phagocytosis of pathogens (Lukácsi *et al*., 2017). CR3 phagocytosis has been linked to TNF-α production by macrophages and which has a negative effect on parasite fitness (Acharya *et al*., 2020; Stevens & Moulton, 1978). In this study, TNF-α plasma levels were not elevated in infected monkeys. These findings suggest low CR3 phagocytosis during infection.

Humans can resist infection by other strains of *Trypanosoma* due to the presence of Trypanolytic factor (TLF 1 and TLF 2) in their serum. However, human-infective strains such as *T. b. gambiense* and *T. b. rhodesiense* have evolved mechanisms to resist the lytic activity of serum factors. The TLFs contain two important compounds: haptoglobin-related protein (Hpr) and apolipoprotein L1 (APOL1) (Bullard *et al*., 2012; De Simone *et al*., 2022; Shiflett *et al*., 2005). Hpr component is required for recognition by the receptor, while APOL1 is the lytic compound. The haptoglobin-haemoglobin receptor (HpHbR), expressed on the trypanosome surface, mediates the uptake of haptoglobin-haemoglobin complexes (Bullard *et al*., 2012). *T. b. rhodesiense* infection occurs due to the presence of SRA (serum resistance antigen), which forms the larger part of the trypanosome VSG coat, having a neutralising effect on lysis (Pays & Nolan, 2021). Notably, during infection, APOL1 neutralisation by SRA occurs through the endocytosis process in trypanosomes. Clearance of VSG-antibody complexes is an essential process during VSG switching. In *T. brucei* previous studies have shown the process to rely on high endocytosis efficiency exhibited by the trypanosome flagellar pocket (Manna *et al*., 2017). Trypanosomes have clathrin-associated proteins mediating the endocytosis process (Adung'a *et al*., 2013). Additionally, trypanosome motility with the aid of the flagellum results in fluid flow on the trypanosome surface that drags VSG-antibody complexes to the flagellar pocket for internalisation (Engstler *et al*., 2007). Thereafter, the VSG is recycled rapidly to the trypanosome surface while antibodies are transported for degradation in the lysosome. *T. brucei* has endogenous phospholipase C (PLC) called GPI-PLC, which is activated during antigenic variation (Garrison *et al*., 2021). The GIP-VSG moiety, a product of rapid endocytosis, initiates expression of pro-inflammatory genes and activation of MAPK and NF-κB pathways (Leppert *et al*., 2007). Pro-inflammatory cytokines, including TNF-α, IL-6 and IL-12, are activated in the process, which together with the activated pathways are enhanced by myeloid cells primed with T-cells from IFN-γ (Cnops *et al*., 2015). Similarly, this study reported high levels of these pro-inflammatory cytokines during early-stage infection of both chronic and acute infection,

suggesting an involvement of rapid endocytosis in cytokine production, which further modulates disease progression in *T. b. rhodesiense* infection.

Efforts towards identifying biomarkers for CNS invasion, allowing easy staging and eliminating the invasive and risky lumbar puncture, are being made. Correlation of plasma and CSF levels with CNS invasion was done, potentially identifying staging biomarkers. However, CSF levels of most cytokines were low and unchanged between infected and controls. This was contrary to other observations. For example, Maranga *et al*. (2013) observed unchanged and elevated serum and CSF IL-6 levels, respectively, while this study shows elevated plasma IL-6 levels. Only CSF TGF-β and S100B (a suggested biomarker of BBB damage) (Bloomfield *et al*., 2007; Marchi *et al*., 2004; Rothermundt *et al*., 2003) were measurable (Figure 12), though not informative. The reasons for unchanging CSF cytokine levels are unknown but could be due to loss of sample integrity due to cryopreservation and/or levels not within the dynamic range of our detection method, among other possible reasons. The application of a more sensitive multi-cytokine assay with a short turnaround time is recommended.

This study used two *T. b. rhodesiense* strains, KETRI 3801 and KETRI 3928, that are responsible for acute and chronic infection, respectively. The basis of such outcomes is driven by pathogen and host genetics and their interactions, indicative of a consequential dynamic and varying host-pathogen crosstalk. Here, host-dependent immunomodulation is suggested as the basis of the different infection outcomes. This will need further qualification by establishing individual roles of the immune factors and integrating them into their complex network. Insight gathered will be important in understanding HAT progression, which can help identify staging biomarkers and improve clinical management of cases. Further, clinical overlap in rHAT and gHAT could become clear. In the context of vector-transmitted pathogens, maladaptation in the strain causing acute infections marked by short host survival and hence a narrow transmission window could be suggested. A significant limitation of this study is the reliance on biological samples obtained from a prior experiment. Consequently, there was no control over critical aspects of the original experimental design, such as infection protocols, environmental conditions and sample collection timelines. This lack of control may introduce variables that could influence the interpretation of the results. Despite this limitation, the samples used in this study were obtained from a well-documented and ethically approved experiment. This ensured the maintenance of relevance and reliability of the findings.

# CHAPTER SIX

# SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Summary

In summary, both IFN-γ and IL-12 displayed comparable patterns in acute and chronic infections, while IL-6 was more elevated in acute cases, peaking at 12 dpi. Elevated concentrations of IL-6, IL-12, IL-10 and IFN-γ were detected between 4 and 8 dpi in both groups, suggesting their usefulness as indicators of early-stage disease. For most immune factors measured in CSF, levels remained stable throughout infection, whereas plasma immune factors were generally higher with disease progression in relation to 0 dpi, with notable differences between acute and chronic forms. Additionally, second-stage disease in KETRI 3801 (acute) was characterised by earlier increases in CSF parasite load and white blood cell counts compared with KETRI 3928 (chronic).

## 6.2 Conclusions

This study demonstrates the immunological and clinical impacts of two *Tbr* strains responsible for varied outcomes inferred from anaemic states, weight loss, risk of death and survival time.

i. INF-γ, TNF-α, IL-1β, IL-6, IL-10 and IL-12 cytokines were identified to mediate both protective and pathological processes during infection. In the early (haemolymphatic) stage of HAT, pro-inflammatory cytokines such as IL-6, IL-12 and IFN-γ are critical for controlling parasite replication and activating innate immune responses. However, as the disease advances to the late meningoencephalitic stage, persistent cytokine production drives neuroinflammation and compromises the blood-brain barrier, resulting in the neurological manifestations characteristic of HAT. The regulatory balance provided by the anti-inflammatory cytokine IL-10 is therefore critical, as it influences whether the host effectively controls infection or progresses to severe pathology.

ii. The findings suggest strain-directed and host-dependent immunomodulation as the basis of the different infection outcomes. The strain infecting the animals results in them dying faster or living longer. Furthermore, this study has provided valuable insights into the multifaceted role of cytokines in varying disease outcomes in rHAT. Through a comprehensive analysis of cytokine responses during *Tbr* infection, pro-inflammatory cytokines, including IL-12, IFN-γ and IL-6, act as key mediators of host defense by coordinating immune responses against the parasite. Their interaction with IL-10 reflects the characteristic balance between pro- and anti-inflammatory signals observed during trypanosome infection.

iii. Monkeys that were infected with *Tbr* KETRI 3801 experienced more severe disease pathogenesis and rapid disease progression than monkeys that were infected with KETRI

3928. The disease was characterised by variation in survival times, where monkeys that were infected with KETRI 3801 had three times higher risk of dying from the disease, shorter pre-patent period, higher fever, faster-progressing anaemia and higher peak parasitaemia compared to monkeys infected with KETRI 3928. This confirms previous findings that KETRI 3801 causes a more acute disease compared to KETRI 3928, which causes a relatively more chronic infection. Importantly, since these parasites were initially recovered from clinical cases of HAT, our findings confirm that both acute and chronic *Tbr* isolates are in circulation in the endemic foci, information that is important for public health personnel in charge of controlling the disease.

### 6.3 Recommendations

Our study has uncovered critical insights into the role of cytokines in determining disease outcomes. It is therefore imperative to build on the findings to enhance the understanding and prospects of research on rHAT treatment strategies despite the complex interplay between the cytokines and varying clinical manifestations observed in rHAT. Thus, the following recommendations will deepen the understanding of the cytokine-mediated mechanisms in rHAT, improving therapeutic and diagnostic approaches, and better rHAT management and outcomes.

i. Further qualification of cytokines by establishing individual roles of the immune factors and integrating them into their complex network.
ii. Further research is to facilitate the development of parasite strains that do not adapt well, leading to infections characterised by shorter survival in the vector and thus a narrow transmission window.
iii. Implementation of strain-specific diagnostic and treatment approaches is crucial. Given the observed differences in disease progression between acute (KETRI 3801) and chronic (KETRI 3928) infections, diagnostic tools should incorporate strain-specific biomarkers to predict disease course.

### 6.4 Suggestions for further study

Future research should focus on integrating detailed cytokine profiling with strain-specific parasite characterisation to map the complex host–parasite–immune network. Such studies should aim to identify the precise roles of individual cytokines in disease progression, develop biomarkers that can differentiate between acute and chronic *Tbr* infections and explore parasite genetic traits that limit vector adaptation, thereby narrowing the transmission window.

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## REFERENCES


Abdulla, M. H., Bakhiet, M., Lejon, V., Andersson, J., McKerrow, J., Al-Obeed, O., & Harris, R. A. (2013). TLTF in cerebrospinal fluid for detection and staging of *T. b. gambiense* infection. *PloS one*, ***8***(11), e79281.

Abera, A., Mamecha, T., Abose, E., Bokicho, B., Ashole, A., Bishaw, T., Mariyo, A., Bogale, B., Terefe, H., Tadesse, H., Belachew, M., Difabachew, H., Eukubay, A., Kinde, S., Ali, A., Regasa, F., Seife, F., Kebede, Z., Wossen, M., … & Tasew, G. (2024). Reemergence of Human African Trypanosomiasis Caused by *Trypanosoma brucei rhodesiense*, Ethiopia. *Emerging Infectious Diseases*, ***30***(1), 125.

Acharya, D., Li, X. R., Heineman, R. E. S., & Harrison, R. E. (2020). Complement receptor-mediated phagocytosis induces proinflammatory cytokine production in murine macrophages. *Frontiers in Immunology*, ***10***, 3049.

Adam, Y., Cecchi, G., Kgori, P. M., Marcotty, T., Mahama, C. I., Abavana, M., Anderson, B., Paone, M., Mattioli, R., & Bouyer, J. (2013). The sequential aerosol technique: a major component in an integrated strategy of intervention against riverine tsetse in Ghana. *PLoS Negl Trop Dis*, ***7***(3), e2135.

Adung'a, V. O., Gadelha, C., & Field, M. C. (2013). Proteomic analysis of clathrin interactions in trypanosomes reveals dynamic evolution of endocytosis. *Traffic*, ***14***(4), 440-457.

Ahouty, B., Koffi, M., Ilboudo, H., Simo, G., Matovu, E., Mulindwa, J., Hertz-Fowler, C., Bucheton, B., Sidibe´, I., Jamonneau, V., MacLeod, A., Noyes, H., N'Guetta, S., & TrypanoGEN Research Group as members of The H3Africa Consortium. (2017). Candidate genes-based investigation of susceptibility to Human African Trypanosomiasis in Côte d'Ivoire. *PLoS neglected tropical diseases*, ***11***(10), e0005992.

Aksoy, S., Attardo, G., Berriman, M., Christoffels, A., Lehane, M., Masiga, D., & Toure, Y. (2014). Human African trypanosomiasis research gets a boost: unraveling the tsetse genome. *PLoS Negl Trop Dis*, ***8***(4), e2624.

Aksoy, S., Caccone, A., Galvani, A. P., & Okedi, L. M. (2013). Glossina fuscipes populations provide insights for human African trypanosomiasis transmission in Uganda. *Trends in Parasitology*, ***29***(8), 394-406.

Alfituri OA., Quintana JF., MacLeod A., Garside P., Benson RA., James M. Brewer JM., Mabbott NA., Morrison LJ., & Capewell P. (2020). To the skin and beyond: the immune response of African trypanosomes as they enter and exit the vertebrate host. *Frontier in Immunology*. **11**: 1250.

Allsopp, R. and Hursey, B. S. (2004). The economics of African trypanosomiasis. In Maudlin, I., Holmes, P. H. & Miles, M. A (Eds.), *The Trypanosomiases* (pp. 491–507). Wallingford, UK: CABI International.

Allsopp, R., & Phillemon-Motsu, T. K. (2002). Tsetse control in Botswana-a reversal in strategy. *Pesticide Outlook*, ***13***(2), 73-76.

Amin, D. N., Vodnala, S. K., Masocha, W., Sun, B., Kristensson, K., & Rottenberg, M. E. (2012). Distinct Toll-like Receptor Signals Regulate Cerebral Parasite Load and Interferon α/β and Tumor Necrosis Factor α–Dependent T-Cell Infiltration in the Brains of Trypanosoma brucei–Infected Mice. *Journal of Infectious Diseases*, ***205***(2), 320-332.

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.

Bai, X., Yao, H., Du, C., Chen, Y., Lai, R., & Rong, M. (2015). An immunoregulatory peptide from tsetse fly salivary glands of Glossina morsitans morsitans. *Biochimie*, ***118***, 123-128.

Balasegaram, M., Harris, S., Checchi, F., Hamel, C., & Karunakara, U. (2006). Treatment outcomes and risk factors for relapse in patients with early-stage human African trypanosomiasis (HAT) in the Republic of the Congo. *Bulletin of the World Health Organization*, ***84***, 777-782.

Balber, A. E., Bangs, J. D., Jones, S. M., & Proia, R. L. (1979). Inactivation or elimination of potentially trypanolytic, complement-activating immune complexes by pathogenic trypanosomes. *Infection and Immunity*, ***24***(3), 617-627.

Balli, S., Shumway, K. R., & Sharan, S. (2023). Physiology, fever. *In StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing. PMID: 32966005.

Baral, T. N., De Baetselier, P., Brombacher, F., & Magez, S. (2007). Control of Trypanosoma evansi infection is IgM mediated and does not require a type I inflammatory response. *The Journal of Infectious Diseases*, ***195***(10), 1513-1520.

Barrett, M. P., Burchmore, R. J., Stich, A., Lazzari, J. O., Frasch, A. C., Cazzulo, J. J., & Krishna, S. (2003). The trypanosomiases. *The Lancet*, ***362***(9394), 1469-1480.

Becker, S., Franco, J. R., Simarro, P. P., Stich, A., Abel, P. M., & Steverding, D. (2004). Real-time PCR for detection of Trypanosoma brucei in human blood samples. *Diagnostic Microbiology and Infectious Disease*, ***50***(3), 193-199.

Berberof, M., Pérez-Morga, D., & Pays, E. (2001). A receptor-like flagellar pocket glycoprotein specific to Trypanosoma brucei gambiense. *Mol Biochem Parasitol*, ***113***, 127–138.

Bloomfield, S. M., McKinney, J., Smith, L., & Brisman, J. (2007). Reliability of S100B in predicting severity of central nervous system injury. *Neurocritical Care*, ***6***, 121-138.

Blum, J., Nkunku, S., & Burri, C. (2001). Clinical description of encephalopathic syndromes and risk factors for their occurrence and outcome during melarsoprol treatment of human African trypanosomiasis. *Tropical Medicine & International Health*, ***6***(5), 390-400.

Blum, J., Schmid, C., & Burri, C. (2006). Clinical aspects of 2541 patients with second stage human African trypanosomiasis. *Acta Tropica*, ***97***(1), 55-64.

Borges, A. R., Link, F., Engstler, M., & Jones, N. G. (2021). The glycosylphosphatidylinositol anchor: a linchpin for cell surface versatility of trypanosomatids. *Frontiers in Cell and Developmental Biology*, ***9***, 720536.

Bosschaerts, T., Guilliams, M., Stijlemans, B., Morias, Y., Engel, D., Tacke, F., Hérin, M., De Baetselier, P., & Beschin, A. (2010). Tip-DC development during parasitic infection is regulated by IL-10 and requires CCL2/CCR2, IFN-γ and MyD88 signaling. *PLoS Pathog*, ***6***(8), e1001045.

Bouyer, F., Seck, M. T., Dicko, A. H., Sall, B., Lo, M., Vreysen, M. J., Chia, E., Bouyer, J., & Wane, A. (2014). Ex-ante benefit-cost analysis of the elimination of a Glossina palpalis gambiensis population in the Niayes of Senegal. *PLoS Negl Trop Dis*, ***8***(8), e3112.

Bouyer, J., Stachurski, F., Kaboré, I., Bauer, B., & Lancelot, R. (2007). Tsetse control in cattle from pyrethroid footbaths. *Preventive Veterinary Medicine*, ***78***(3-4), 223-238.

Bray, P. G., Barrett, M. P., Ward, S. A., & de Koning, H. P. (2003). Pentamidine uptake and resistance in pathogenic protozoa: past, present and future. *Trends in Parasitology*, ***19***(5), 232-239.

Brightwell, R., Dransfield, R. D., & Kyorku, C. (1991). Development of a low-cost tsetse trap and odour baits for Glossina pallidipes and G. longipennis in Kenya. *Medical and Veterinary Entomology*, ***5***(2), 153-164.

Brun, R., Blum, J., Chappuis, F., & Burri, C. (2010). Human African trypanosomiasis. *Lancet*, ***375***, 148-159.

Brun, R., Schumacher, R., Schmid, C., Kunz, C., & Burri, C. (2001). The phenomenon of treatment failures in Human African Trypanosomiasis. *Tropical Medicine & International Health*, ***6***(11), 906-914.

Bullard, W., Kieft, R., Capewell, P., Veitch, N. J., Macleod, A., & Hajduk, S. (2012). Haptoglobin-haemoglobin receptor independent killing of African trypanosomes by human serum and trypanosome lytic factors. *Virulence*, ***3***(1), 72-76.

Büscher, P., Gilleman, Q., & Lejon, V. (2013). Rapid diagnostic test for sleeping sickness. *The New England Journal of Medicine*, ***368***(11), 1069-1070.

Büscher, P., Ngoyi, D. M., Kaboré, J., Lejon, V., Robays, J., Jamonneau, V., Bebronne, N., Van der Veken, W., & Biéler, S. (2009). Improved models of mini anion exchange centrifugation

technique (mAECT) and modified single centrifugation (MSC) for sleeping sickness diagnosis and staging. *PLoS Negl Trop Dis*, ***3***(11), e471.

Büscher, P., Cecchi, G., Jamonneau, V., & Priotto, G. (2017). Human african trypanosomiasis. *The Lancet*, ***390***(10110), 2397-2409.

Caljon G., Abbeele JVD., Stijlemans B., Marc Coosemans M., Baetselier PD., & Magez S. (2006). Tsetse fly saliva accelerates the onset of *Trypanosoma brucei* Infection in a mouse model associated with a reduced host inflammatory response. *Infect Immun*. **74** (11), 6324 - 6330.

Caljon, G., Broos, K., De Goeyse, I., De Ridder, K., Sternberg, J. M., Coosemans, M., De Baetselier, P., Guisez, Y., & Van Den Abbeele, J. (2009). Identification of a functional Antigen5-related allergen in the saliva of a blood feeding insect, the tsetse fly. *Insect Biochemistry and Molecular Biology*, ***39***(5-6), 332-341.

Caljon, G., Mabille, D., Stijlemans, B., De Trez, C., Mazzone, M., Tacchini-Cottier, F., Malissen, M., Van Ginderachter, J., Magez, S., De Baetselier, P., & Van Den Abbeele, J. (2018). Neutrophils enhance early Trypanosoma brucei infection onset. *Scientific Reports*, ***8***(1), 11203.

Camara, M., Camara, O., Ilboudo, H., Sakande, H., Kaboré, J., N'Dri, L., Jamonneau, V., & Bucheton, B. (2010). Sleeping sickness diagnosis: use of buffy coats improves the sensitivity of the mini anion exchange centrifugation test. *Tropical Medicine & International Health*, ***15***(7), 796-799.

Camara, O., Camara, M., Falzon, L. C., Ilboudo, H., Kaboré, J., Compaoré, C. F. A., Fèvre, E. M., Büscher, P., Bucheton, B., & Lejon, V. (2023). Performance of clinical signs and symptoms, rapid and reference laboratory diagnostic tests for diagnosis of human African trypanosomiasis by passive screening in Guinea: a prospective diagnostic accuracy study. *Infectious Diseases of Poverty*, ***12***(1), 22.

Capewell, P., Clucas, C., DeJesus, E., Kieft, R., Hajduk, S., Veitch, N., Steketee, P., Cooper, A., Weir, W., & MacLeod, A. (2013). The TgsGP gene is essential for resistance to human serum in Trypanosoma brucei gambiense. *PLoS Pathog*, ***9***(10), e1003686.

Cattand, P., Miezan, B. T., & De Raadt, P. (1988). Human African trypanosomiasis: use of double centrifugation of cerebrospinal fluid to detect trypanosomes. *Bulletin of the World Health Organization*, ***66***(1), 83.

Cecchi, G., Mattioli, R. C., Slingenbergh, J., & De La Rocque, S. (2008). Land cover and tsetse fly distributions in sub-Saharan Africa. *Medical and Veterinary Entomology*, ***22***(4), 364-373.

Cecchi, G., Paone, M., Franco, J. R., Fèvre, E. M., Diarra, A., Ruiz, J. A., Mattioli, R., & Simarro, P. P. (2009). Towards the Atlas of human African trypanosomiasis. *International Journal of Health Geographics*, ***8***(1), 1-12.

Chappuis, F. (2007). Melarsoprol-free drug combinations for second-stage Gambian sleeping sickness: the way to go. *Clinical Infectious Diseases*, ***45***(11), 1443-1445.

Chappuis, F., Stietenroth, K., Meussen, A., & Bovier, P. A. (2005). Eflornithine is safer than melarsoprol for the treatment of second-stage Trypanosoma brucei gambiense human African trypanosomiasis. *Clinical Infectious Diseases*, ***41***(5), 748-751.

Ciss, M., Bassène, M. D., Seck, M. T., Mbaye, A. G., Sall, B., Fall, A. G., Vreysen, M. J. B., & Bouyer, J. (2019). Environmental impact of tsetse eradication in Senegal. *Scientific Reports*, ***9***(1), 20313.

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), e1004964.

Connor R.J. & Van den Bossche P. (2004). African animal trypanosomes.In Coetzer, J. A. W, & Tustin, R. C. (Eds.), *Infectious Diseases of Livestock.* (pp 251–96). Oxford University Press

Connor, R. J. (1994). The impact of nagana. *Onderstepoort J Vet Res*, ***61***(4):379-83.

Courtin, D., & Garcia, A. (2007). Human African Trypanosomiasis: Involvement of host genetics. *Med Trop (Mars)*, ***67***(2), 131-133.

Courtin, D., Argiro, L., Jamonneau, V., N'dri, L., N'guessan, P., Abel, L., Dessein, A., Cot, M., Laveissiere, C., & Garcia, A. (2006). Interest of tumor necrosis factor-alpha− 308 G/A and interleukin-10− 592 C/A polymorphisms in human African trypanosomiasis. *Infection, Genetics and Evolution*, ***6***(2), 123-129.

Courtin, D., Milet, J., Jamonneau, V., Yeminanga, C. S., Kumeso, V. K., Bilengue, C. M., Betarde, C., & Garcia, A. (2007). Association between human African trypanosomiasis and the IL6 gene in a Congolese population. *Infection, Genetics and Evolution*, ***7***, 60-68.

Crilly, N. P., & Mugnier, M. R. (2021). Thinking outside the blood: Perspectives on tissue-resident Trypanosoma brucei. *PLoS Pathog*, ***17***(9): e1009866.

Cuisance, D., Itard, J., & Boreham, P. F. (1973). Behavior of sterile males of Glossina tachinoides West. released in their natural habitat. Fort Lamy (Chad) area. 3. Resting sites and altitude. Feeding behavior. *Revue d'Élevage et de Medecine Veterinaire des Pays Tropicaux*, ***26***(3), 323-338.

Dame, D. A., & Schmidt, C. H. (1970). The sterile-male technique against tsetse flies, Glossina spp. *Bulletin of the ESA*, ***16***(1), 24-30.

De Atouguia, J. L., & Kennedy, P. G. (2000). Neurological aspects of human African trypanosomiasis. *Advances in Parasitology*, **47**, 321-372

De Muylder, G., Daulouède, S., Lecordier, L., Uzureau, P., Morias, Y., Van Den Abbeele, J., Caljon, G., Hérin, M., Holzmuller, P., Semballa, S., Courtois, P., Vanhamme, L., Stijlemans, B., De Baetselier, B., Barrett, M. P., Barlow, J. L., McKenzie, A. N. J., Barron, L., Wynn, T. A., ... & Pays, E. (2013). A Trypanosoma brucei kinesin heavy chain promotes parasite growth by triggering host arginase activity. *PLoS Pathog*, ***9***(10), e1003731.

De Simone, G., Pasquadibisceglie, A., Polticelli, F., di Masi, A., & Ascenzi, P. (2022). Haptoglobin and the related haptoglobin protein: the N-terminus makes the difference. *Journal of Biomolecular Structure and Dynamics*, ***40***(5), 2244-2253.

De Vooght, L., Caljon, G., De Ridder, K., & Van Den Abbeele, J. (2014). Delivery of a functional anti-trypanosome Nanobody in different tsetse fly tissues via a bacterial symbiont, Sodalis glossinidius. *Microbial Cell Factories*, ***13***(1), 1-10.

De Vooght, L., Caljon, G., Stijlemans, B., De Baetselier, P., Coosemans, M., & Van Den Abbeele, J. (2012). Expression and extracellular release of a functional anti-trypanosome Nanobody® in Sodalis glossinidius, a bacterial symbiont of the tsetse fly. *Microbial Cell Factories*, ***11***(1), 1-11.

Deborggraeve, S., & Büscher, P. (2010). Molecular diagnostics for sleeping sickness: what is the benefit for the patient? *The Lancet Infectious Diseases*, ***10***(6), 433-439.

DeJesus, E., Kieft, R., Albright, B., Stephens, N. A., & Hajduk, S. L. (2013). A single amino acid substitution in the group 1 Trypanosoma brucei gambiense haptoglobin-haemoglobin receptor abolishes TLF-1 binding. *PLoS Pathog*, ***9***(4), e1003317.

Desquesnes, M., & Dia, M. L. (2003). Mechanical transmission of Trypanosoma congolense in cattle by the African tabanid Atylotus agrestis. *Experimental Parasitology*, *105*(3-4), 226-231.

Drennan, M. B., Stijlemans, B., Van Den Abbeele, J., Quesniaux, V. J., Barkhuizen, M., Brombacher, F., De Baetselier, P., Ryffel, B., & Magez, S. (2005). The induction of a type 1 immune response following a Trypanosoma brucei infection is MyD88 dependent. *The Journal of Immunology*, ***175***(4), 2501-2509.

Dubois, M. E., Demick, K. P., & Mansfield, J. M. (2005). Trypanosomes expressing a mosaic variant surface glycoprotein coat escape early detection by the immune system. *Infection and Immunity*, ***73***(5), 2690-2697.

Dyck, V. A., Hendrichs, J., & Robinson, A. S. (2005). *Sterile insect technique: principles and practice in area-wide integrated pest management* (p. 1216). Taylor & Francis.

Dyer, N. A., Rose, C., Ejeh, N. O., & Acosta-Serrano, A. (2013). Flying tryps: survival and maturation of trypanosomes in tsetse flies. *Trends in Parasitology*, ***29***(4), 188-196.

Engstler, M., Pfohl, T., Herminghaus, S., Boshart, M., Wiegertjes, G., Heddergott, N., & Overath, P. (2007). Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes. *Cell*, ***131***(3), 505-515.

Fairlamb, A. H. (2003). Chemotherapy of human African trypanosomiasis: current and future prospects. *Trends in Parasitology*, ***19***(11), 488-494.

FAO. (2004). *The State of Food Insecurity in the World (SOFI)*. Food and Agriculture Organization of the United Nations. Viale delle Terme di Caracalla, 00100 Rome, Italy.

FAO. (2012). *FAO helps Somali to cash in on livestock*. Food and Agriculture Organization of the United Nations.

Fevre, E. M., Wissmann, B. V., Welburn, S. C., & Lutumba, P. (2008). The burden of human African trypanosomiasis. *PLoS Negl Trop Dis*, ***2***(12), e333.

Field, M. C., & Carrington, M. (2004). Intracellular membrane transport systems in Trypanosoma brucei. *Traffic*, ***5***(12), 905-913.

Franco, J. R., Cecchi, G., Paone, M., Diarra, A., Grout, L., Kadima Ebeja, A., Simarro, P. P., Zhao, W., & Argaw, D. (2022). The elimination of human African trypanosomiasis: Achievements in relation to WHO road map targets for 2020. *PLoS Neglected Tropical Diseases*, ***16***(1), e0010047.

Franco, J. R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Simarro, P., Zhao, W., & Argaw, D. (2020). Monitoring the elimination of human African trypanosomiasis at continental and country level: Update to 2018. *PLoS Neglected Tropical Diseases*, ***14***(5), e0008261.

Franco, J. R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Simarro, P., Zhao, W., & Argaw, D. (2018). Monitoring the elimination of human African trypanosomiasis: Update to 2016. *PLoS Neglected Tropical Diseases*, ***12***(12): e0006890.

Franco, J. R., Cecchi, G., Priotto, G., Paone, M., Kadima Ebeja, A., Simarro, P. P., Diarra, A., Sankara, D., Zhao, W., & Dagne, D. A. (2022). Human African trypanosomiasis cases diagnosed in non-endemic countries (2011–2020). *PLOS Neglected Tropical Diseases*, **16**(11), e0010885.

Franco, J. R., Simarro, P. P., Diarra, A., & Jannin, J. G. (2014). Epidemiology of human African trypanosomiasis. *Clinical Epidemiology*, ***6***, 257–275.

Garcia, A., Jamonneau, V., Magnus, E., Laveissière, C., Lejon, V., N'guessan, P., N'Dri, L., Van Meirvenne, N., & Büscher, P. (2000). Follow-up of Card Agglutination Trypanosomiasis Test (CATT) positive but apparently aparasitaemic individuals in Côte d'Ivoire: evidence for a complex and heterogeneous population. *Tropical Medicine & International Health*, ***5***(11), 786-793.

Garrison, P., Umaer, K., & Bangs, J. D. (2021). The role of glycosylphosphatidylinositol phospholipase C in membrane trafficking in Trypanosoma brucei. *Molecular and Biochemical Parasitology*, ***245***, 111409.

Garzón, E., Holzmuller, P., Bras-Gonçalves, R., Vincendeau, P., Cuny, G., Lemesre, J. L., & Geiger, A. (2013). The Trypanosoma brucei gambiense Secretome Impairs Lipopolysaccharide-Induced Maturation, Cytokine Production, and Allostimulatory Capacity of Dendritic Cells. *Infection and Immunity*, ***81***(9), 3300–3308.

Geerts, M., Van Reet, N., Leyten, S., Berghmans, R., Rock, K. S., Coetzer, T. H., Eyssen, L., & Büscher, P. (2021). *Trypanosoma brucei gambiense*-iELISA: a promising new test for the post-elimination monitoring of human African trypanosomiasis. *Clinical Infectious Diseases*, ***73***(9), e2477-e2483.

Gelfand, M. (1966). The early clinical features of Rhodesian trypanosomiasis with special reference to the" chancre"(local reaction). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, ***60***(3), 376-9.

Gibson, W. C. (2005). The SRA gene: the key to understanding the nature of *Trypanosoma brucei rhodesiense*. *Parasitology*, ***131***(2), 143-150.

Gibson, W., & Ferris, V. (2003). Conservation of the genomic location of the human serum resistance associated gene in Trypanosoma brucei rhodesiense. *Mol Biochem Parasitol*, ***130***, 159-162.

Gillet, P., Ngoyi, D. M., Lukuka, A., Kande, V., Atua, B., van Griensven, J., Muyembe, J., Jacobs, J., & Lejon, V. (2013). False positivity of non-targeted infections in malaria rapid diagnostic tests: the case of human african trypanosomiasis. *PLoS Negl Trop Dis*, ***7***(4), e2180.

Grob, D., Conejeros, I., Velásquez, Z. D., Preußer, C., Gärtner, U., Alarcón, P., Burgos, R. A., Hermosilla, C., & Taubert, A. (2020). Trypanosoma brucei brucei induces polymorphonuclear neutrophil activation and neutrophil extracellular traps release. *Frontiers in Immunology*, ***11***, 559561.

Hackett, F., Ford, L. B., Fevre, E., & Simarro, P. (2014). Incorporating scale dependence in disease burden estimates: the case of human African trypanosomiasis in Uganda. *PLoS Negl Trop Dis*, ***8***(2), e2704.

Hamadien, M., Bakhiet, M., & Harris, R. A. (2000). Interferon-γ induces secretion of trypanosome lymphocyte triggering factor via tyrosine protein kinases. *Parasitology*, ***120***(3), 281-287.

Harris, T. H., Cooney, N. M., Mansfield, J. M., & Paulnock, D. M. (2006). Signal Transduction, Gene Transcription, and Cytokine Production Triggered in Macrophages by Exposure to Trypanosome DNA. *Infection and Immunity*, ***74***(8), 4530–4537.

Hertz, C. J., Filutowicz, H., & Mansfield, J. M. (1998). Resistance to the African trypanosomes is IFN-γ dependent. *The Journal of Immunology*, ***161***(12), 6775-6783.

Higgins, M. K., Tkachenko, O., Brown, A., Reed, J., Raper, J., & Carrington, M. (2013). Structure of the trypanosome haptoglobin–haemoglobin receptor and implications for nutrient uptake and innate immunity. *PNAS*, ***110***(5), 1905–1910.

Holmes, P. (2015). On the road to elimination of rhodesiense human African trypanosomiasis: first WHO meeting of stakeholders. *PLoS Negl Trop Dis*, ***9***(4), e0003571.

Holmes, P. H., Eisler, M. C., & Geerts, S. (2004). 23 Current Chemotherapy of Animal Trypanosomiasis. In Maudlin, I., Holmes, P. H., & Miles, M. A. *The Trypanosomiases* (pp 431-444). CABI Publishing.

Holt, H. R., Selby, R., Mumba, C., Napier, G. B., & Guitian, J. (2016). Assessment of animal African trypanosomiasis (AAT) vulnerability in cattle-owning communities of sub-Saharan Africa. *Parasites & Vectors*, ***9***, 1-12.

Inojosa, W. O., Augusto, I., Bisoffi, Z., Josenado, T., Abel, P. M., Stich, A., & Whitty, C. J. (2006). Diagnosing human African trypanosomiasis in Angola using a card agglutination test: observational study of active and passive case finding strategies. *BMJ*, ***332***(7556), 1479.

Iraqi, F., Clapcott, S. J., Kumari, P., Haley, C. S., Kemp, S. J., & Teale, A. J. (2000). Fine mapping of trypanosomiasis resistance loci in murine advanced intercross lines. *Mammalian Genome*, ***11***, 645–648.

Jamonneau, V., Ravel, S., Koffi, M., Kaba, D., Zeze, D. G., Ndri, L., Sane, B., Coulibaly, B., Cuny, G., & Solano, P. (2004). Mixed infections of trypanosomes in tsetse and pigs and their epidemiological significance in a sleeping sickness focus of Cote d'Ivoire. *Parasitology*, ***129***(6), 693.

Jamonneau, V., Truc, P., Garcia, A., Magnus, E., & Büscher, P. (2000). Preliminary evaluation of LATEX/T. b. gambiense and alternative versions of CATT/T. b. gambiense for the serodiagnosis of human african trypanosomiasis of a population at risk in Cote d'Ivoire: considerations for mass-screening. *Acta Tropica*, ***76***(2), 175-183.

Janeway Jr, C. A., & Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology*, ***20***(1), 197-216.

Jayaraman, S., Harris, C., Paxton, E., Donachie, A. M., Vaikkinen, H., McCulloch, R., Hall, J. P. J., Kenny, J., Lenzi, L., Hertz-Fowler, C., Cobbold, C., Reeve, R., Michoel, T., & Morrison, L. J. (2019). Application of long read sequencing to determine expressed antigen diversity in Trypanosoma brucei infections. *PLoS Neglected Tropical Diseases*, ***13***(4), e0007262.

Jones, T. W., & Dávila, A. M. (2001). Trypanosoma vivax–out of Africa. *TRENDS in Parasitology*, ***17***(2), 99-101.

Joshi, P. P., Chaudari, A., Shegokar, V., Powar, S., Dani, V., Somalwar, M., Jannin, J., & Truc, P. (2006). Treatment and follow-up of the first case of human trypanosomiasis caused by Trypanosoma evansi in India. *Trans R Soc Trop Med Hyg*, ***100***(10), 989–991.

Kabayo, J. P. (2002). Aiming to eliminate tsetse from Africa. *Trends in Parasitology*, ***18***(11), 473-475.

Kaboré, J. W., Ilboudo, H., Noyes, H., Camara, O., Kaboré, J., Camara, M., Kaboré, J., Camara, M., Koffi, M., Lejon, V., Jamonneau, V., MacLeod, A., Hertz-Fowler, C., Belem, A. M., Matovu, E., Bucheton, B., Sidibe, I., & TrypanoGEN Research Group as members of The H3Africa Consortium. (2017). Candidate gene polymorphisms study between human African trypanosomiasis clinical phenotypes in Guinea. *PLoS Neglected Tropical Diseases*, ***11***(8), e0005833.

Kagbadouno, M. S., Camara, M., Bouyer, J., Courtin, F., Onikoyamou, M. F., Schofield, C. J., & Solano, P. (2011). Progress towards the eradication of Tsetse from the Loos islands, Guinea. *Parasites & Vectors*, ***4***(1), 1-9.

Kappmeier Green, K., Potgieter, F. T., & Vreysen, M. J. B. (2007). A strategy for an area-wide control campaign with an SIT (Glossina austeni and Glossina brevipalpis) free South Africa. *Area-wide Control of Insect Pests*, ***308***, 23.

Kato, C. D., Alibu, V. P., Nanteza, A., Mugasa, C. M., & Matovu, E. (2015). Interleukin (IL)-6 and IL-10 Are Up Regulated in Late-Stage Trypanosoma brucei rhodesiense Sleeping Sickness. *PLoS Negl Trop Dis*, ***9***(6), e0003835.

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 Clin Immunol*, ***12***(4), 1-10.

Kato, C. D., Mugasa, C. M., Nanteza, A., Matovu, E., & Alibu, V. P. (2017). Relationship between Trypanosoma brucei rhodesiense genetic diversity and clinical spectrum among sleeping sickness patients in Uganda. *BMC Research Notes*, ***10***(1), 1-7.

Kennedy, G. (2004). Human African trypanosomiasis of the CNS: current issues and challenges. *Journal of Clinical Investigation*, ***113***(4), 496-504.

Kennedy, P. (2008). The continuing problem of human African trypanosomiasis (sleeping sickness). *Ann Neurol*, ***64***, 116-126.

Kgori, P. M., Modo, S., & Torr, S. J. (2006). The use of aerial spraying to eliminate tsetse from the Okavango Delta of Botswana. *Acta Tropica*, ***99***(2-3), 184-199.

Kirkwood, B. R., & Sterne, J. A. (2010). *Essential medical statistics*. John Wiley & Sons.

Koda, Y., Soejima, M., Yoshioka, N., & Kimura, H. (1998). The Haptoglobin-Gene Deletion Responsible for Anhaptoglobinemia. *Am. J. Hum. Genet*, ***62***, 245–252.

Koffi, M., De Meeûs, T., Bucheton, B., Solano, P., Camara, M., Kaba, D., Cuny, G., Ayala, F., & Jamonneau, V. (2009). Population genetics of Trypanosoma brucei gambiense, the agent of sleeping sickness in Western Africa. *Proceedings of the National Academy of Sciences*, ***106***(1), 209-214.

Kristensson, K., Grassi-Zucconi, G., & Bentivoglio, M. (1995). Nervous system dysfunctions in African trypanosomiasis. *Recent Advances in Tropical Neurology*, ***2***, 165-174.

Kuboki, N., Inoue, N., Sakurai, T., Di Cello, F., Grab, D. J., Suzuki, H., Sugimoto, C., & Igarashi, I. (2003). Loop-mediated isothermal amplification for detection of African trypanosomes. *Journal of Clinical Microbiology*, ***41***(12), 5517-5524.

Kuepfer, I., Hhary, E. P., Allan, M., Edielu, A., Burri, C., & Blum, J. A. (2011). Clinical presentation of Tb rhodesiense sleeping sickness in second stage patients from Tanzania and Uganda. *PLoS Negl Trop Dis*, ***5***(3), e968.

Kumeso, V. K. B., Kalonji, W. M., Rembry, S., Mordt, O. V., Tete, D. N., Prêtre, A., Delhomme, S., Ilunga, M., Camara, M., Catusse, J., Schneitter, S., Nusbaumer, M., Mwamba, E. M., Mahenzi, H. M., Makaya, J. M., Layba, M. C., Akwaso, F. M., Kaninda, L. B., Kasongo, A. B., ... & Tarral, A. (2023). Efficacy and safety of acoziborole in patients with human African trypanosomiasis caused by Trypanosoma brucei gambiense: a multicentre, open-label, single-arm, phase 2/3 trial. *The Lancet Infectious Diseases*, ***23***(4), 463-470.

Kupani, M., Pandey, R. K., & Mehrotra, S. (2021). Neutrophils and Visceral Leishmaniasis: Impact on innate immune response and cross-talks with macrophages and dendritic cells. *Journal of Cellular Physiology*, ***236***(4), 2255-2267.

La Greca, F., Haynes, C., Stijlemans, B., De Trez, C., & Magez, S. (2014). Antibody-mediated control of T rypanosoma vivax infection fails in the absence of tumour necrosis factor. *Parasite Immunology*, ***36***(6), 271-276.

Lambert, M. R. K., Grant, I. F., Smith, C. L., Tingle, C. C. D., & Douthwaite, R. J. (1991). *Effects of deltamethrin ground-spraying on non-target wildlife* (Technical Report No. 1). Department of Veterinary Services, Harare.

Law, S. A., & Levine, R. P. (2019). The covalent binding story of the complement proteins C3 and C4 (I) 1972-1981. *Immunobiology*, ***224***(6), 827-833.

Legros, D., Ollivier, G., Gastellu-Etchegorry, M., Paquet, C., Burri, C., Jannin, J., & Büscher, P. (2002). Treatment of human African trypanosomiasis—present situation and needs for research and development. *The Lancet Infectious Diseases*, ***2***(7), 437-440.

Lejon, V., Büscher, P., Magnus, E., Moons, A., Wouters, I., & Van Meirvenne, N. (1998). A semi-quantitative ELISA for detection of Trypanosoma brucei gambiense specific antibodies in serum and cerebrospinal fluid of sleeping sickness patients. *Acta Tropica*, ***69***(2), 151-164.

Lejon, V., Jamonneau, V., Solano, P., Atchade, P., Mumba, D., Nkoy, N., Bébronne, N., Kibonja, T., Balharbi, F., Wierckx, A., Boelaert, M., & Büscher, P. (2006). Detection of trypanosome-specific antibodies in saliva, towards non-invasive serological diagnosis of sleeping sickness. *Tropical Medicine & International Health*, ***11***(5), 620-627.

Lejon, V., Lardon, J., Kenis, G., Pinoges, L., Legros, D., & Bisser, S. (2002). Interleukin (IL)-6, IL-8 and IL-10 in serum and CSF of trypanosoma brucei gambiense sleeping sickness patients before and after treatment. *Trans R SOC Trop Med Hyg*, ***187***, 329-333.

Lejon, V., Reiber, H., Legros, D., Djé, N., Magnus, E., Wouters, I., Sindic, C., & Büscher, P. (2003). Intrathecal immune response pattern for improved diagnosis of central nervous system involvement in trypanosomiasis. *The Journal of Infectious Diseases*, ***187***(9), 1475-1483.

Leppert, B. J., Mansfield, J. M., & Paulnock, D. M. (2007). The soluble variant surface glycoprotein of African trypanosomes is recognized by a macrophage scavenger receptor and induces IκBα degradation independently of TRAF6-mediated TLR signaling. *The Journal of Immunology*, ***179***(1), 548-556.

Li, B. (2015). DNA double-strand breaks and telomeres play important roles in Trypanosoma brucei antigenic variation. *Eukaryotic Cell*, ***14***(3), 196-205.

Limo, K. W., John, T. K., Owino, O. B., John, O., Robert, S., & Daniel, M. (2021). Mouse experiments demonstrate differential pathogenicity and virulence of Trypanosoma brucei rhodesiense strains. *Experimental Parasitology*, ***228***, 108135.

Lindh, J. M., Torr, S. J., Vale, G. A., & Lehane, M. J. (2009). Improving the cost-effectiveness of artificial visual baits for controlling the tsetse fly Glossina fuscipes fuscipes. *PLoS Negl Trop Dis*, ***3***(7), e474.

Lindner, A. K., Lejon, V., Chappuis, F., Seixas, J., Kazumba, L., Barrett, M. P., Mwamba, E., Erphas, O., Akl, E., Villanueva, G., Bergman, H., Simarro, P., Ebeja, A., Priotto, G., & Franco, J. R. (2020). New WHO guidelines for treatment of gambiense human African trypanosomiasis including fexinidazole: substantial changes for clinical practice. *The Lancet Infectious Diseases*, ***20***(2), e38-e46.

Liu, G., Sun, D., Wu, H., Zhang, M., Huan, H., Xu, J., Zhang, X., Zhou, H., & Shi, M. (2015). Distinct contributions of CD4+ and CD8+ T cells to pathogenesis of Trypanosoma brucei infection in the context of gamma interferon and interleukin-10. *Infection and Immunity*, ***83***(7), 2785-2795.

Lukácsi, S., Nagy-Baló, Z., Erdei, A., Sándor, N., & Bajtay, Z. (2017). The role of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in complement-mediated phagocytosis and podosome formation by human phagocytes. *Immunology Letters*, ***189***, 64-72.

Lundkvist, G. B., Kristensson, K., & Bentivoglio, M. (2004). Why Trypanosomes Cause Sleeping. *Physiology*, ***19***, 198–206.

Lutumba, P., Meheus, F., Robays, J., Miaka, C., Kande, V., Büscher, P., Dujardin, B., & Boelaert, M. (2007). Cost-effectiveness of Algorithms for Confi rmation Test of Human African Trypanosomiasis. *Tropical Medicine & International Health*, ***13***(10), 1484-1490.

Mabille, D., & Caljon, G. (2020). Inflammation following trypanosome infection and persistence in the skin. *Current Opinion in Immunology*, ***66***, 65-73.

MacLean, L. M., Odiit, M., Chisi, J. E., Kennedy, P. G., & Sternberg, J. M. (2010). Focus–Specific Clinical Profiles in Human African Trypanosomiasis Caused by Trypanosoma brucei rhodesiense. *PLoS Negl Trop Dis*, ***4***(12), e906.

MacLean, L., Chisi, J. E., Odiit, M., Gibson, W. C., Ferris, V., Picozzi, K., & Sternberg, J. M. (2004). Severity of human African trypanosomiasis in East Africa is associated with geographic location, parasite genotype, and host inflammatory cytokine response profile. *Infection and Immunity*, ***72***(12), 7040-7044.

MacLean, L., Odiit, M., & Sternberg, J. (2001). Nitric Oxide and Cytokine Synthesis in Human African Trypanosomiasis. *Journal of Infectious Diseases*, 1086–1090.

MacLean, L., Odiit, M., MacLeod, A., Morrison, L., Sweeney, L., Cooper, A., Kennedy, P., & Sternberg, J. M. (2007). Spatially and genetically distinct African trypanosome virulence variance defined by host interferon-gamma response. *J Infect Dis*, ***196***(11), 1620–1628.

MacLean, L., Reiber, H., Kennedy, P. G., & Sternberg, J. M. (2012). Stage progression and neurological symptoms in Trypanosoma brucei rhodesiense sleeping sickness: role of the CNS inflammatory response. *PLoS Negl Trop Dis*, ***6***(10), e1857.

Maeda, N. (1985). Nucleotide sequence of the haptoglobin and haptoglobin-related gene pair. The haptoglobin-related gene contains a retrovirus-like element. *Journal of Biological Chemistry*, ***260***(11), 6698-6709.

Magez, S., Radwanska, M., Drennan, M., Fick, L., Baral, T. N., Brombacher, F., & Baetselier, P. D. (2006). Interferon-γ and nitric oxide in combination with antibodies are key protective host immune factors during Trypanosoma congolense Tc13 infections. *The Journal of Infectious diseases*, ***193***(11), 1575-1583.

Magez, S., Schwegmann, A., Atkinson, R., Claes, F., Drennan, M., De Baetselier, P., & Brombacher, F. (2008). The role of B-cells and IgM antibodies in parasitaemia, anemia, and VSG switching in trypanosoma brucei–infected mice. *PLoS Pathogens*, ***4***(8), e1000122.

Magez, S., Stijlemans, B., Baral, T., & De Baetselier, P. (2002). VSG-GPI anchors of African trypanosomes: their role in macrophage activation and induction of infection-associated immunopathology. *Microbes and Infection*, ***4***(9), 999-1006.

Magez, S., Pinto Torres, J. E., Obishakin, E., & Radwanska, M. (2020). Infections with extracellular trypanosomes require control by efficient innate immune mechanisms and can result in the destruction of the mammalian humoral immune system. *Frontiers in Immunology*, ***11***, 382.

Magnus, E., Vervoort, T., & Van Meirvenne, N. (1978). A card-agglutination test with stained trypanosomes (CATT) for the serological diagnosis of *T. b. gambiense* tripanosomiasis. *In Annales de la Societe Belge de Medecine Tropicale*, ***58***(3), 169-176.

Maina, N., Ngotho, J. M., Were, T., Thuita, J. K., Mwangangi, M. D., Kagira, J. M., Ndung'u, J. M., & Sternberg, J. (2004). Proinflammatory Cytokine Expression in the Early Phase of Trypanosoma brucei rhodesiense Infection in Vervet Monkeys (Cercopithecus aethiops). *Infection and Immunity*, ***72***(5), 3063–3065.

Manna, P. T., Obado, S. O., Boehm, C., Gadelha, C., Sali, A., Chait, B. T., Rout, M. P., & Field, M. C. (2017). Lineage-specific proteins essential for endocytosis in trypanosomes. *Journal of Cell Science*, ***130***(8), 1379-1392.

Maranga, D. N., Kagira, J. M., Kinyanjui, C. K., Karanja, S. M., Maina, N. W., & Ngotho, M. (2013). IL-6 is Upregulated in Late-Stage Disease in Monkeys Experimentally Infected with Trypanosoma brucei rhodesiense. *Clinical and Developmental Immunology*, ***2013***, 1-6.

Marchi, N., Cavaglia, M., Fazio, V., Bhudia, S., Hallene, K., & Janigro, D. (2004). Peripheral markers of blood–brain barrier damage. *Clinica Chimica Acta*, ***342***(1-2), 1-12.

Masocha, W., & Kristensson, K. (2012). Passage of parasites across the blood-brain barrier. *Landes Bioscience*, ***3***(2), 202-212.

Masocha, W., Robertson, B., Rottenberg, M. E., Mhlanga, J., Sorokin, L., & Kristensson, K. (2004). Cerebral vessel laminins and IFN-γ define Trypanosoma brucei brucei penetration of the blood-brain barrier. *The Journal of Clinical Investigation*, ***114***(5), 689-694.

Matemba, L. E., Fevre, E. M., Kibona, S. N., Picozzi, K., Cleaveland, S., Shaw, A. P., & Welburn, S. C. (2010). Quantifying the Burden of Rhodesiense Sleeping Sickness in Urambo District, Tanzania. *PLoS Negl Trop Dis*, ***4***(11), 1-10.

McGovern, T. W., Williams, W., Fitzpatrick, J. E., Cetron, M. S., Hepburn, B. C., & Gentry, R. H. (1995). Cutaneous manifestations of African trypanosomiasis. *Archives of Dermatology*, ***131***(10), 1178-1182.

Mehlert, A., Bond, C. S., & Ferguson, M. A. (2002). The glycoforms of a Trypanosoma brucei variant surface glycoprotein and molecular modeling of a glycosylated surface coat. *Glycobiology*, ***12***(10), 607-612.

Mesu, V. K. B. K., Kalonji, W. M., Bardonneau, C., Mordt, O. V., Blesson, S., Simon, F., Delhomme, S., Bernhard, S., Kuziena, W., Lubaki, J. F., Vuvu, S. L., Ngima, P. N.,

Mbembo, H. M., Ilunga, M., Bonama, A. K., Heradi, J. A., Solomo, J. L., Mandula, G., Badibabi, L. K., Dama, F. R., Lukula, P. K., Tete, D. N., Lumbala, C., Scherrer, B., Strub-Wourgaft, N., & Tarral, A. (2018). Oral fexinidazole for late-stage African Trypanosoma brucei gambiense trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. *The Lancet*, ***391***(10116), 144-154.

Molina-Portela, M. P., Samanovic, M., & Raper, J. (2008). Distinct roles of apolipoprotein components within the trypanosome lytic factor complex revealed in a novel transgenic mouse model. *The Journal of Experimental Medicine*, ***205***(8), 1721-1728.

Mpanya, A., Hendrickx, D., Vuna, M., Kanyinda, A., Lumbala, C., Tshilombo, V., Mitashi, P., Luboya, O., Kande, V., Boelaert, M., Lefèvre, P., & Lutumba, P. (2012). Should I get screened for sleeping sickness? A qualitative study in Kasai province, Democratic Republic of Congo. *PLoS Neglected Tropical Diseases*, ***6***(1), e1467.

Mugasa, C. M., Adams, E. R., Boer, K. R., Dyserinck, H. C., Büscher, P., Schallig, H. D., & Leeflang, M. M. (2012). Diagnostic accuracy of molecular amplification tests for human African trypanosomiasis—systematic review. *PLoS Negl Trop Dis*, ***6***(1), e1438.

Mugasa, C. M., Laurent, T., Schoone, G. J., Kager, P. A., Lubega, G. W., & Schallig, H. D. (2009). Nucleic acid sequence-based amplification with oligochromatography for detection of Trypanosoma brucei in clinical samples. *Journal of Clinical Microbiology*, ***47***(3), 630-635.

Mugnier, M. R., Stebbins, C. E., & Papavasiliou, F. N. (2016). Masters of disguise: antigenic variation and the VSG coat in Trypanosoma brucei. *PLoS pathogens*, ***12***(9), e1005784.

Muhanguzi, D., Okello, W. O., Kabasa, J. D., Waiswa, C., Welburn, S. C., & Shaw, A. P. (2015). Cost analysis of options for management of African Animal Trypanosomiasis using interventions targeted at cattle in Tororo District; south-eastern Uganda. *Parasites & Vectors*, ***8***(1), 1-9.

Mulenga, C., Mhlanga, J., Kristensson, K., & Robertson, B. (2001). Trypanosoma brucei brucei crosses the blood-brain barrier while tight junction proteins are preserved in a rat chronic disease model. *Neuropathology and Applied Neurobiology*, ***8***(433), 77-85.

Murray, M. & Morrison, w. I. (1979). Parasitaemia and host susceptibility to African trypanosomiasis. In *Pathogenicity of Trypanosomes* (ed. Losos, G. & Chouinard, A.), pp. 71-81. IDRC No. 132e

Murwira, A., Skidmore, A. K., Huizing, H. J. G., & Prins, H. H. (2010). Remote sensing of the link between arable field and elephant (Loxodonta africana) distribution change along a tsetse eradication gradient in the Zambezi valley, *Zimbabwe. International Journal of Applied Earth Observation and Geoinformation*, *12*, S123-S130.

Mwanakasale, V., Songolo, P., & Daka, V. (2013). Challenges in the control of Human African Trypanosomiasis in the Mpika district of Zambia. *BMC Research Notes*, ***6***(180), 1-7.

Namangala, B., Hachaambwa, L., Kajino, K., Mweene, A. S., Hayashida, K., Simuunza, M., & Sugimoto, C. (2012). The use of Loop-mediated Isothermal Amplification (LAMP) to detect the re-emerging Human African Trypanosomiasis (HAT) in the Luangwa and Zambezi valleys. *Parasites & Vectors*, ***5***(1), 1-5.

Namangala, B., Noe, W., De Baetselier, P., Brys, L., & Beschin, A. (2001). Relative Contribution of Interferon-g and Interleukin-10 to Resistance to Murine African Trypanosomosis. *Journal of Infectious Diseases*, ***183***, 1794–1800.

Ndung'u, K., Murilla, G. A., Thuita, J. K., Ngae, G. N., Auma, J. E., Gitonga, P. K., Thungu, D. K., Kurgat, R. K., Chemuliti, J. K., & Mdachi, R. E. (2020). Differential virulence of Trypanosoma brucei rhodesiense isolates does not influence the outcome of treatment with anti-trypanosomal drugs in the mouse model. *PLoS One*, ***15***(11), e0229060.

Ng'ayo, M. O., Njiru, Z. K., Kenya, E. U., Muluvi, G. M., Osir, E. O., & Masiga, D. K. (2005). Detection of trypanosomes in small ruminants and pigs in western Kenya: important reservoirs in the epidemiology of sleeping sickness? *Kinetoplastid Biology and Disease*, ***4***(1), 1-7.

Ngotho, M., Maina, N., Kagira, J., Royo, F., Farah, I. O., & Hau, J. (2006). IL-10 is up regulated in early and transitional stages in vervet monkeys experimentally infected with Trypanosoma brucei rhodesiense. *Parasitol Int.* ***55***(4): 243 - 248.

Nishimura, K., Hamashita, K., Okamoto, Y., Kawahara, F., Ihara, H., Kozaki, S., Ohnishi, Y., & Yamasaki, S. (2004). Differential effects of interferon-γ on production of trypanosome-derived lymphocyte-triggering factor by Trypanosoma brucei gambiense and Trypanosoma brucei brucei. *Journal of Parasitology*, ***90***(4), 740-745.

Njiru, Z. K., Mikosza, A. S. J., Armstrong, T., Enyaru, J. C., Ndung'u, J. M., & Thompson, A. R. C. (2008). Loop-mediated isothermal amplification (LAMP) method for rapid detection of Trypanosoma brucei rhodesiense. *PLoS Neglected Tropical Diseases*, ***2***(2), e147.

Ofon, E., Noyes, H., Eyanga, V. E. O., Njiokou, F., Koffi, M., Fogue, P., Hertz-Fowler, C., MacLeod, A., Matovu, E., Simo, G., & as members of The H3Africa Consortium. (2019). Association between IL1 gene polymorphism and human African trypanosomiasis in populations of sleeping sickness foci of southern Cameroon. *PLoS Neglected Tropical Diseases*, ***13***(3), e0007283.

Olsson, T., Bakhiet, M., Edlund, C., Höjeberg, B., van der Meide, P. H., & Kristensson, K. (1991). Bidirectional activating signals between Trypanosoma brucei and CD8+ T cells: a

trypanosome-released factor triggers interferon-γ production that stimulates parasite growth. *European Journal of Immunology*, ***21***(10), 2447-2454.

Oluwafemi, R. A., Ilemobade, A. A., & Laseinde, E. A. (2007). The impact of African animal trypanosomosis and tsetse on the livelihood and well-being of cattle and their owners in the BICOT study area of Nigeria. *Scientific Research and Essay*, ***2***(9), 380-383.

Ormerod, W. E. (1967). Taxonomy of the sleeping sickness trypanosomes. *The Journal of Parasitology*, 824-830.

Pal, A., Hall, B. S., Jeffries, T. R., & Field, M. C. (2003). Rab5 and Rab11 mediate transferrin and anti-variant surface glycoprotein antibody recycling in Trypanosoma brucei. *Biochemical Journal*, ***374***(2), 443-451.

Pays, E., & Nolan, D. P. (2021). Genetic and immunological basis of human African trypanosomiasis. *Current Opinion in Immunology*, ***72***, 13-20.

Pérez-Morga, D., Vanhollebeke, B., Paturiaux-Hanocq, F., Nolan, D. P., Lins, L., Homblé, F., Vanhamme, L., Tebabi, P., Pays, A., Poelvoorde, P., Jacquet, A., Brasseur, R., & Pays, E. (2005). Apolipoprotein LI promotes trypanosome lysis by forming pores in lysosomal membranes. *Science*, ***309***(5733), 469-472.

Pinger, J., Chowdhury, S., & Papavasiliou, F. N. (2017). Variant surface glycoprotein density defines an immune evasion threshold for African trypanosomes undergoing antigenic variation. *Nature Communications*, ***8***(1), 828.

Ponte-Sucre, A. (2016). An overview of Trypanosoma brucei infections: an intense host–parasite interaction. *Frontiers in Microbiology*, ***7***, 2126.

Priotto, G., Kasparian, S., Mutombo, W., Ngouama, D., Ghorashian, S., Arnold, U., Ghabri, S., Baudin, E., Buard, V., Kazadi-Kyanza, S., Ilunga, M., Mutangala, W., Pohlig,G., Schmid, C., Karunakara, U., Torreele, E., & Kande, V. (2009). Nifurtimox-eflornithine combination therapy for second-stage African Trypanosoma brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. *The Lancet*, ***374***(9683), 56-64.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Radwanska, M., Chamekh, M., Vanhamme, L., Claes, F., Magez, S., Magnus, E., De Baetselier, P., Bu¨ scher, P., & Pays, E. (2002). The serum resistance-associated gene as a diagnostic tool for the detection of Trypanosoma brucei rhodesiense. *Am J Trop Med Hyg*, ***67***(6), 684–690.

Rayaisse, J. B., Tirados, I., Kaba, D., Dewhirst, S., Logan, J., Diarrassouba, M., & Esterhuizen, J. (2010). Prospects for odour bait development to control vectors of Trypanosomiasis in West Africa, the tsetse flies Glossina tachinoides and G. palpalis sl. *PLoS Negl Trop Dis*, ***4***, e632.

Robays, J., Ebeja Kadima, A., Lutumba, P., Miaka mia Bilenge, C., Kande Betu Ku Mesu, V., De Deken, R., Makabuza, J., Deguerry, M., Van der Stuyft, P., & Boelaert, M. (2004). Human African trypanosomiasis amongst urban residents in Kinshasa: a case-control study. *Tropical Medicine & International Health*, ***9***(8), 869-875.

Robays, J., Nyamowala, G., Sese, C., Kande, V. B. K. M., Lutumba, P., Van der Veken, W., & Boelaert, M. (2008). High failure rates of melarsoprol for sleeping sickness, Democratic Republic of Congo. *Emerging Infectious Diseases*, ***14***(6), 966.

Rock, K. S., Torr, S. J., Lumbala, C., & Keeling, M. J. (2015). Quantitative evaluation of the strategy to eliminate human African trypanosomiasis in the Democratic Republic of Congo. *Parasites & Vectors*, ***8***(1), 1-13.

Rogers, D. J., & Randolph, S. E. (2002). A response to the aim of eradicating tsetse from Africa. *Trends in Parasitology*, ***18***(12), 534-536.

Rolin, S., Hanocq-Quertier, J., Paturiaux-Hanocq, F., Nolan, D., Salmon, D., Webb, H., Carrington, M., Voorheis, P., & Pays, E. (1996). Simultaneous but independent activation of adenylate cyclase and glycosylphosphatidylinositol-phospholipase C under stress conditions in Trypanosoma brucei. *Journal of Biological Chemistry*, ***271***(18), 10844-10852.

Rothermundt, M., Peters, M., Prehn, J. H., & Arolt, V. (2003). S100B in brain damage and neurodegeneration. *Microscopy Research and Technique*, ***60***(6), 614-632.

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), e2186.

Salah, A. A., Robertson, I., & Mohamed, A. (2015). Estimating the economic impact of Trypanosoma evansi infection on production of camel herds in Somaliland. *Tropical Animal Health and Production*, ***47***(4), 707-714.

Salerno, F., Guislain, A., Cansever, D., & Wolkers, M. (2016). TLR-Mediated Innate Production of IFN-g by CD8 + T Cells Is Independent of Glycolysis. *J Immunol*, ***196***, 1-12.

Salmon, D., Vanwalleghem, G., Morias, Y., Denoeud, J., Krumbholz, C., Lhommé, F., Bachmaier, S., Kador, M., Gossmann, J., Dias, F., De Muylder, G., Uzureau, P., Magez, S., Moser, M., De Baetselier, P., Van Den Abbeele, J., Beschin, A., Boshart, M., & Pays, E. (2012). Adenylate cyclases of Trypanosoma brucei inhibit the innate immune response of the host. *Science*, ***337***(6093), 463-466.

Schmid, C., Richer, M., Bilenge, C. M. M., Josenando, T., Chappuis, F., Manthelot, C. R., Nangouma, A., Doua, F., Asumu, P., Simarro, P., & Burri, C. (2005). Effectiveness of a 10-day melarsoprol schedule for the treatment of late-stage human African trypanosomiasis:

confirmation from a multinational study (IMPAMEL II). *The Journal of Infectious Diseases*, ***191***(11), 1922-1931.

Schmidt, H., & Sayer, P. (1982). Trypanosoma brucei rhodesiense infection in vervet monkeys. II. Provocation of the encephalitic late phase by treatment of infected monkeys. *Tropenmedizin und Parasitologie*, ***33***(4), 255-259.

Seed, J. R., & Sechelski, J. (1988). Growth of pleomorphic Trypanosoma brucei rhodesiense in irradiated inbred mice. *The Journal of Parasitology*, **74**(5), 781-789.

Sekhar, G. N., Watson, C. P., Fidanboylu, M., Sanderson, L., & Thomas, S. A. (2014). Delivery of antihuman African trypanosomiasis drugs across the blood–brain and blood–CSF barriers. *Advances in Pharmacology*, ***71***, 245-275.

Shaw, A. P. M., Torr, S. J., Waiswa, C., Cecchi, G., Wint, G. R. W., Mattioli, R. C., & Robinson, T. P. (2013). Estimating the costs of tsetse control options: an example for Uganda. *Preventive Veterinary Medicine*, ***110***(3-4), 290-303.

Shaw, A. P. M., Inaki, T., Mangwiro Clement, T. N., Johan, E., & Lehane Michael, J. (2015). Costs of a field trial using 'tiny targets' to control Glossina fuscipes fuscipes, a vector of Gambiense sleeping sickness in Arua District of Uganda. *PLoS Negl Trop Dis*, ***26***(9), 3.

Shaw, A. P. (2004). 20 Economics of African Trypanosomiasis. *The Trypanosomiases*, 369.

Shaw, A. P. M., Cecchi, G., Wint, G. R. W., Mattioli, R. C., & Robinson, T. P. (2014). Mapping the economic benefits to livestock keepers from intervening against bovine trypanosomosis in Eastern Africa. *Preventive Veterinary Medicine*, ***113***(2), 197-210.

Shaw, A. P., Tirados, I., Mangwiro, C. T., Esterhuizen, J., Lehane, M. J., Torr, S. J., & Kovacic, V. (2015). Costs of using "tiny targets" to control Glossina fuscipes fuscipes, a vector of gambiense sleeping sickness in Arua District of Uganda. *PLoS Negl Trop Dis*, ***9***(3), e0003624.

Shaw, A., Robays, J., Fevre, E. M., Lutumba, P., & Boelaert, M. (2010). The Burden of Human African Trypanosomiasis. *Springer Science*, ***83***, 1434-1441.

Shi, M., Lin, T. H., Appell, K. C., & Berg, L. J. (2008). Janus-kinase-3-dependent signals induce chromatin remodeling at the Ifng locus during T helper 1 cell differentiation. *Immunity*, ***28***(6), 763-773.

Shiflett, A. M., Bishop, J. R., Pahwa, A., & Hajduk, S. L. (2005). Human high-density lipoproteins are platforms for the assembly of multi-component innate immune complexes. *Journal of Biological Chemistry*, ***280***(38), 32578-32585.

Shimogawa, M. M., Saada, E. A., Vashisht, A. A., Barshop, W. D., Wohlschlegel, J. A., & Hill, K. L. (2015). Cell surface proteomics provides insight into stage-specific remodeling of the

host-parasite interface in Trypanosoma brucei. *Molecular & Cellular Proteomics*, ***14***(7), 1977-1988.

Silva, N. M., Manzan, R. M., Carneiro, W. P., Milanezi, C. M., Silva, J. S., Ferro, E. A. V., & Mineo, J. R. (2010). Toxoplasma gondii: the severity of toxoplasmic encephalitis in C57BL/6 mice is associated with increased ALCAM and VCAM-1 expression in the central nervous system and higher blood–brain barrier permeability. *Experimental Parasitology*, ***126***(2), 167-177.

Simarro, P. P., Cecchi, G., Franco, J. R., Paone, M., Diarra, A., Ruiz-Postigo, J. A., Fe`vre, E. M., Mattioli, R. C., & Jannin, J. G. (2012). Estimating and Mapping the Population at Risk of Sleeping Sickness. *PLoS Negl Trop Dis*, ***6***(10), e1859.

Simarro, P. P., Diarra, A., Ruiz Postigo, J. A., Franco, J. R., & Jannin, J. G. (2011). The Human African Trypanosomiasis Control and Surveillance Programme of the World Health Organization 2000–2009: The Way Forward. *PLoS Negl Trop Dis*, ***5***(2), e1007.

Simarro, P. P., Jannin, J., & Cattand, P. (2008). Eliminating human African trypanosomiasis: Where do we stand and what comes next? *PLoS Med*, ***5***(2), 174-180.

Simarro, P., Franco, J., Diarra, A., Ruiz Postigo, J., & Jannin, J. (2013). Diversity of human African trypanosomiasis epidemiological settings requires fine-tuning control strategies to facilitate disease elimination. *Res Rep Trop Med*, ***4***, 1-6.

Sindato, C., Kibona, S., Nkya, G., Mbilu, T., Manga, C., Kaboya, J., & Rawille, F. (2008). Challenges in the diagnosis and management of sleeping sickness in Tanzania: a case report. *Tanzania Journal of Health Research*, ***10***(3), 177-181.

Solano, P., Ravel, S., & De Meeûs, T. (2010). How can tsetse population genetics contribute to African trypanosomiasis control? *Trends in Parasitology*, ***26***(5), 255-263.

Stephens, N. A., Kieft, R., MacLeod, A., & Hajduk, S. L. (2012). Trypanosome resistance to human innate immunity: targeting Achilles heel. *Trends Parasitol*, ***28***(12), 539–545.

Sternberg, J. M. (2004). Human African trypanosomiasis: clinical presentation and immune response. *Parasite Immunology*, ***26***, 469–476.

Sternberg, J. M., & Mitchell, J. A. (2014). Plasma neuronal specific enolase: a potential stage diagnostic marker in human African trypanosomiasis. *Trans R Soc Trop Med Hyg*, ***108***, 449–452.

Sternberg, J. M., Rodgers, J., Bradley, B., MacLean, L., Murray, M., & Kennedy, P. G. (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.

Stevens, D. R., & Moulton, J. E. (1978). Ultrastructural and immunological aspects of the phagocytosis of Trypanosoma brucei by mouse peritoneal macrophages. *Infection and Immunity*, ***19***(3), 972-982.

Stich, A., M Abel, P., & Krishna, S. (2002). Human African trypanosomiasis. *BMJ*, ***235***, 203-206.

Stijlemans, B., Beschin, A., Magez, S., Van Ginderachter, J. A., & De Baetselier, P. (2015). Iron Homeostasis and Trypanosoma brucei associated immunopathogenicity development: a battle/quest for iron. *BioMed Research International*, *2015*, Article 906098.

Stijlemans, B., Caljon, G., Natesan, S. K. A., Saerens, D., Conrath, K., Perez-Morga, D., Skepper, J., Nikolaou, A., Brys, L., Pays, E., Magez, S., Field, M., De Baetselier, P., & Muyldermans, S. (2011). High affinity nanobodies against the Trypanosome brucei VSG are potent trypanolytic agents that block endocytosis. *PLoS Pathog*, ***7***(6), e1002072.

Stijlemans, B., Vankrunkelsven, A., Caljon, G., Bockstal, V., Guilliams, M., Bosschaerts, T., Beschin, A., Raes, G., Magez, S., & Baetselier, P. D. (2010). The central role of macrophages in trypanosomiasis-associated anemia: rationale for therapeutical approaches. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders)*, ***10***(1), 71-82.

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***, 218.

Swallow, B. M. (2000). *Impacts of trypanosomiasis on African agriculture* (Vol. **2**, pp. 1-52). Food and Agriculture Organization of the United Nations.

Szempruch, A. J., Sykes, S. E., Kieft, R., Dennison, L., Becker, A. C., Gartrell, A., Martin, W., Nakayasu, E., Almeida, I., Hajduk, S., & Harrington, J. M. (2016). Extracellular vesicles from Trypanosoma brucei mediate virulence factor transfer and cause host anemia. *Cell*, ***164***(1-2), 246-257.

Tabel, H., Kaushik, R. S., & Uzonna, J. E. (2000). Susceptibility and resistance to Trypanosoma congolense infections. *Microbes and Infection*, ***2***(13), 1619-1629.

Tarantula AF., Doctor SC, & Hartigan-O'Connor DJ. (2022) Nonhuman primates in translational research. *Ann Rev Anim Biosci.* ***10***:441 - 468.

Tarral, A., Hovsepian, L., Duvauchelle, T., Donazzolo, Y., Latreille, M., Felices, M., Gualano, V., Delhomme, S., Mordt, O., Blesson, S., Voiriot, P., & Strub-Wourgaft, N. (2023). Determination of the Optimal Single Dose Treatment for Acoziborole, a Novel Drug for the Treatment of Human African Trypanosomiasis: First-in-Human Study. *Clinical Pharmacokinetics*, ***62***(3), 481-491.

Thuita, J. K., Kagira, J. M., Mwangangi, D., Matovu, E., Turner, C. M., & Masiga, D. (2008). Trypanosoma brucei rhodesiense Transmitted by a Single Tsetse Fly Bite in Vervet Monkeys as a Model of Human African Trypanosomiasis. *PLoS Negl Trop Dis*, ***2***(5), e238.

Thuita, J. K & Masiga, D. (2016). *Experimental Profiling of African Trypanosomes in Mouse and Primate Hosts* [Unpublished data]. Kenya Agricultural and Livestock Research organization, Biotechnology Research Institute (KALRO-BioRI).

Thumbi, S. M., Jung'a, J. O., Mosi, R. O., & Mc Odimba, F. A. (2010). Spatial distribution of African Animal Trypanosomiasis in Suba and Teso districts in Western Kenya. *BMC Research Notes*, ***3***(6), 1-6.

Torr, S. J., Maudlin, I., & Vale, G. A. (2007). Less is more: restricted application of insecticide to cattle to improve the cost and efficacy of tsetse control. *Medical and Veterinary Entomology*, ***21***(1), 53-64.

Tripodi, K. E., Menendez Bravo, S. M., & Cricco, J. A. (2011). Role of heme and heme-proteins in trypanosomatid essential metabolic pathways. *Enzyme Research, 2011*, Article 873230.

Truc, P., Formenty, P., Diallo, P. B., Komoin-Oka, C., & Lauginie, F. (1997). Confirmation of two distinct classes of zymodemes of Trypanosoma brucei infecting man and wild mammals in Cote d'Ivoire: suspected difference in pathogenicity. *Annals of Tropical Medicine & Parasitology*, ***91***(8), 951-956.

Turner, C. M. R., Aslam, N., & Dye, C. (1995). Replication, differentiation, growth and the virulence of Trypanosoma brucei infections. *Parasitology*, ***111***(3), 289-300.

Urech, K., Neumayr, A., & Blum, J. (2011). Sleeping Sickness in Travelers - Do They Really Sleep? *PLoS Negl Trop Dis* ***5***(11): e1358.

Vale, G. A. (1993). Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. *Journal of Medical Entomology*, ***30***(5), 831-842.

Van der Vloedt, A. M. V., Baldry, D. A. T., Politzar, H., Kulzer, H., & Cuisance, D. (1980). Experimental helicopter applications of decamethrin followed by release of sterile males for the control of riverine vectors of trypanosomiasis in Upper Volta. *International Journal of Tropical Insect Science*, ***1***(1), 105-112.

Vanhamme, L., Paturiaux-Hanocq, F., Poelvoorde, P., Nolan, D. P., Lins, L., Van Den Abbeele, J., Pays, A., Tebabi, P., Van Xong, H., Jacquet, A., Moguilevsky, N., Dieu, M., Kane, J., De Baetselier, P., Brasseur, R., & Pays, E. (2003). Apolipoprotein LI is the trypanosome lytic factor of human serum. *Nature*, ***422***(6927), 83-87.

Vanhollebeke, B., Truc, P., Poelvoorde, P., Pays, A., Joshi, P. P., Katti, R., Jannin, J., & Pays, E. (2006). Human Trypanosoma evansi infection linked to a lack of apolipoprotein LI. *New England Journal of Medicine*, ***355***(26), 2752-2756.

Vanwalleghem, G., Fontaine, F., Lecordier, L., Tebabi, P., Klewe, K., Nolan, D. P., Yamaryo-Botté, Y., Botté, C., Kremer, A., Burkard, G., Rassow, J., Roditi, I., Pérez-Morga, D., & Pays, E. (2015). Coupling of lysosomal and mitochondrial membrane permeabilization in trypanolysis by APOL1. *Nature Communications*, ***6***(1), 1-10.

Vanwalleghem, G., Morias, Y., Beschin, A., Szymkowski, D. E., & Pays, E. (2017). Trypanosoma brucei growth control by TNF in mammalian host is independent of the soluble form of the cytokine. *Scientific Reports*, ***7***(1), 1-7.

Vassella, E., Probst, M., Schneider, A., Studer, E., Renggli, C. K., & Roditi, I. (2004). Expression of a major surface protein of Trypanosoma brucei insect forms is controlled by the activity of mitochondrial enzymes. *Molecular Biology of the Cell*, ***15***(9), 3986-3993.

Von Wissmann, B., Machila, N., Picozzi, K., Fèvre, E. M., Barend, M., Handel, I. G., & Welburn, S. C. (2011). Factors associated with acquisition of human infective and animal infective trypanosome infections in domestic livestock in western Kenya. *PLoS Negl Trop Dis*, ***5***(1), e941.

Vreysen, M. J. B. (2001). Principles of area-wide integrated tsetse fly control using the sterile insect technique. *Médecine Tropicale*, ***61***(4-5), 397-410.

Vreysen, M. J. B. (2005). Monitoring sterile and wild insects in area-wide integrated pest management programmes. *Sterile Insect*, 485.

Vreysen, M. J., & Robinson, A. S. (2011). Ionising radiation and area-wide management of insect pests to promote sustainable agriculture. *Sustainable Agriculture*, ***2***, 671-692.

Vreysen, M. J., Seck, M. T., Sall, B., & Bouyer, J. (2013). Tsetse flies: their biology and control using area-wide integrated pest management approaches. *Journal of Invertebrate Pathology*, ***112***, S15-S25.

Weiss, B., & Aksoy, S. (2011). Microbiome influences on insect host vector competence. *Trends in Parasitology*, ***27***(11), 514-522.

Welburn, S. C., Maudlin, I., & Simarro, P. P. (2009). Controlling sleeping sickness-a review. *Parasitology*, ***136***(14), 1943-1949.

Welburn, S. C., Molyneux, D. H., & Maudlin, I. (2016). Beyond tsetse–implications for research and control of human African trypanosomiasis epidemics. *Trends in Parasitology*, ***32***(3), 230-241.

WHO. (1998). Expert Committee on the Control, Surveillance of African Trypanosomiasis, & World Health Organization. Control and surveillance of African trypanosomiasis: report of a WHO expert committee (No. 881-884). World Health Organization.

Widener, J., Nielsen, M. J., Shiflett, A., Moestrup, S. K., & Hajduk, S. (2007). Haemoglobin is a co-factor of human trypanosome lytic factor. *PLoS Pathog*, ***3***(9), e129.

Willert, E., & Phillips, M. A. (2012). Regulation and function of polyamines in African trypanosomes. *Trends in Parasitology*, ***28***(2), 66-72.

Wilson, E. H., Wille-Reece, U., Dzierszinski, F., & Hunter, C. A. (2005). A critical role for IL-10 in limiting inflammation during toxoplasmic encephalitis. *Journal of Neuroimmunology*, ***165***(1-2), 63-74.

Winton EF, Srinivasiah J, Kim BK, Hillyer CD, Strobert EA, Orkin JL, Swenson RB, McClure HM., Mylers LA., & Saral R. (1994). Effect of recombinant human interleukin-6 (rhIL-6) and rhIL-3 on hematopoietic regeneration as demon- strated in a nonhuman primate chemotherapy model. *Blood.* ***84***:65-73.

Woo, P. T. K. (1971). Evaluation of the haematocrit centrifuge and other techniques for the field diagnosis of human trypanosomiasis and filariasis. *Acta Tropica*, ***28***(3), 298-303.

Wood, S. N. (2004). Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association*, ***99***(467), 673-686.

Wood, S. N. (2006). *Generalized additive models: an introduction with R*. chapman and hall/CRC.

World Health Organization. (2009). *WHO includes combination of eflornithine and nifurtimox in its Essential List of Medicines for the treatment of human African trypanosomiasis*. World Health Organization

World Health Organization. (2012). *Accelerating work to overcome the global impact of neglected tropical diseases: a roadmap for implementation* (No. WHO/HTM/NTD/2012.5). World Health Organization.

World Health Organization. (2013). *Report of a WHO meeting on elimination of human African trypanosomiasis (trypanosoma brucei gambiense)*: *Geneva, 3-5 December 2012* (No. WHO/HTM/NTD/IDM/2013.4). World Health Organization.

World Health Organization. (2014). *Report of the first stakeholders meeting on gambiense human African trypanosomiasis elimination, Geneva, 25-27 March 2014* (No. 9789241508070). World Health Organization.

World Health Organization. (2019). *WHO interim guidelines for the treatment of gambiense human African trypanosomiasis (2019)*. World Health Organization.

World Health Organization. (2020). *Report of the third WHO stakeholders meeting on rhodesiense human African trypanosomiasis: Geneva, Switzerland, 10-11 April 2019*. World Health Organization.

World Health Organization. (2022). *Trypanosomiasis, human African (sleeping sickness)*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness).

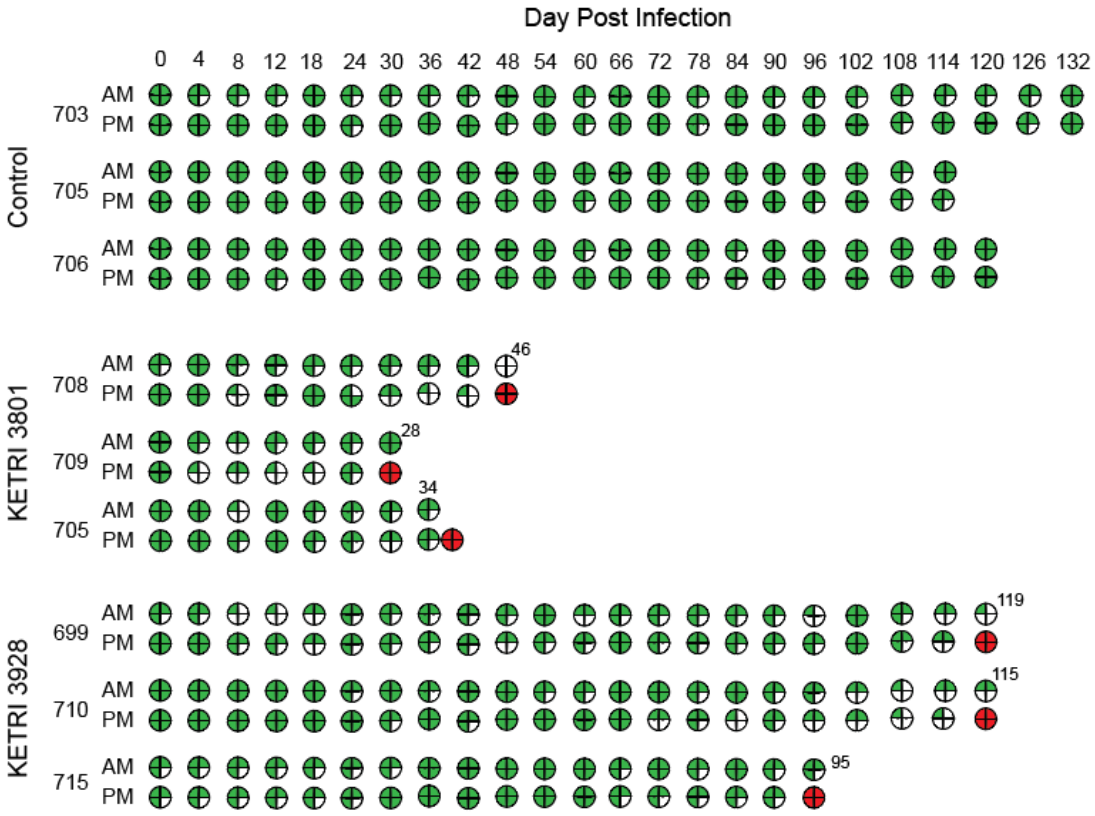
Yaro, M., Munyard, K. A., Stear, M. J., & Groth, D. M. (2016). Combatting African animal trypanosomiasis (AAT) in livestock: the potential role of trypanotolerance. *Veterinary Parasitology*, ***225***, 43-52.

Zhang, K., Jiang, N., Chen, H., Zhang, N., Sang, X., Feng, Y., Feng, Y., Chen, R., & Chen, Q. (2021). TatD DNases of African trypanosomes confer resistance to host neutrophil extracellular traps. *Science China Life Sciences*, ***64***, 621-632.

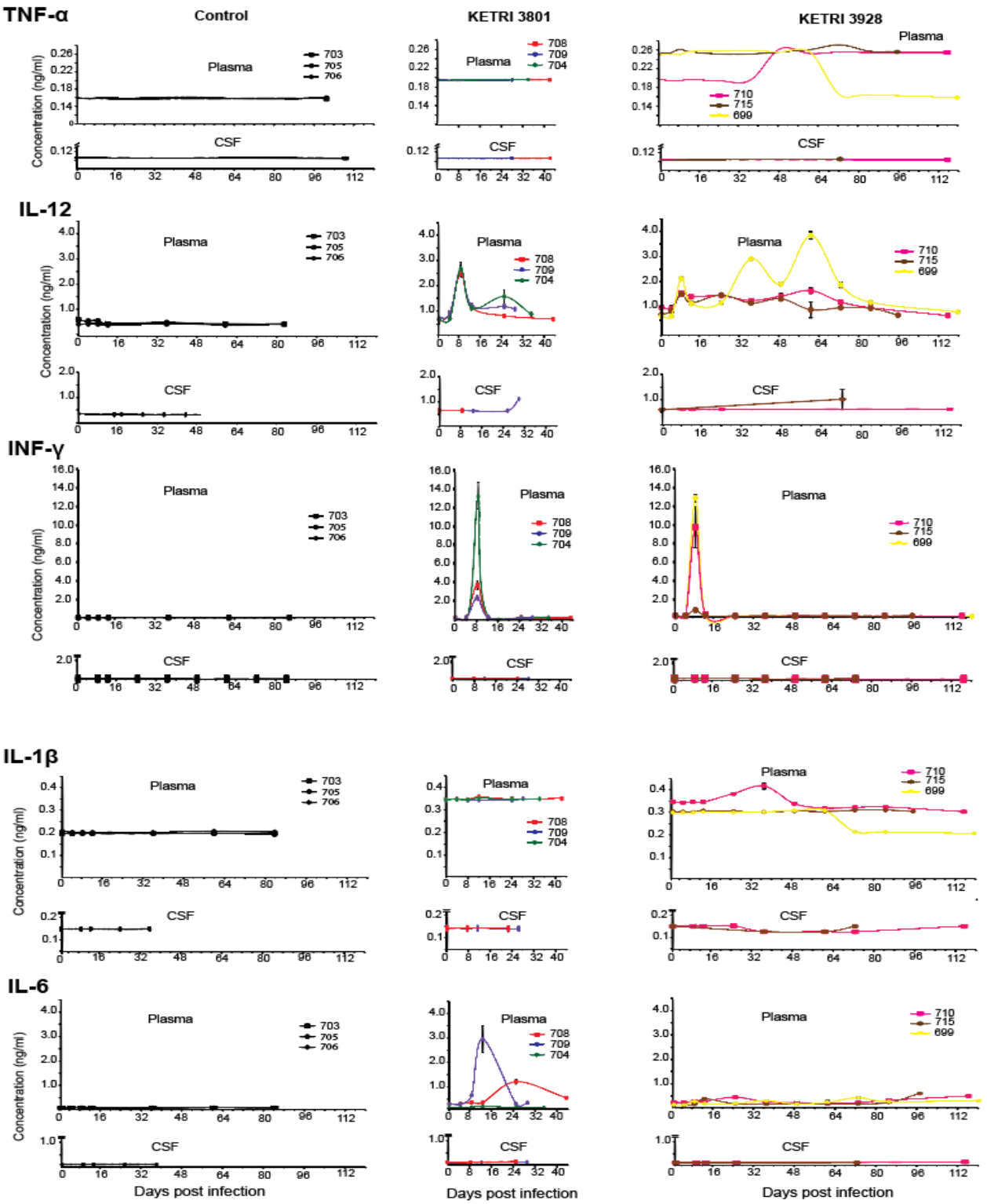
## APPENDICES

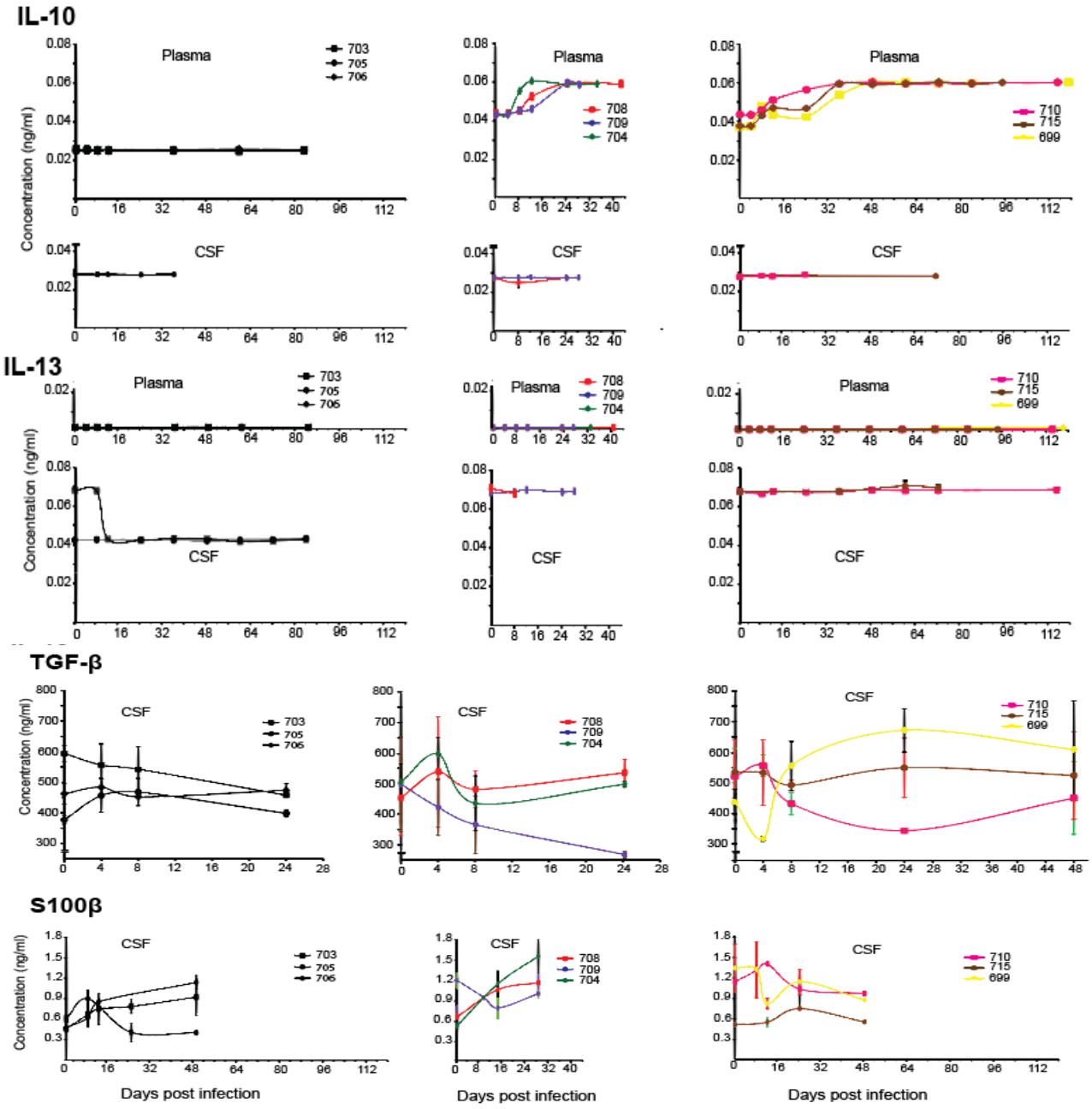
**Appendix I:** Monkey food intake.

Fed on all food items provided; three quarter consumed; half consumed; a quarter consumed and finally animal did not feed. Represents extremis, with the day post-infection indicated as a superscript for each infected animal. Animals eating just a quarter and below were clearly anorexic.



**Appendix II:** Cytokine levels in plasma and CSF at different days post infection.




IL-10
Plasma
CSF
703
705
706
708
709
704
710
715
699
Concentration (ng/ml)
IL-13
TGF-β
S100β
Days post infection

**Appendix III:** NACOSTI research permit.

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