HAEMATOLOGICAL CHANGES, CYTOKINE AND ANTIBODY PROFILES IN GUINEA PIGS INFECTED WITH *Tunga penetrans*

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Masters of Science in Immunology of Masinde Muliro University of Science and Technology

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DEDICATION

This work is dedicated to my parents Mr. Joseph Tarus and Mrs. Sarah Tarus for their moral and financial support throughout my academic journey.I also dedicate this work to my niece Ashley Cherono Tarus. May God bless you.

ACKNOWLEDGEMENTS

Gratitude is extended to everyone who has contributed to the success of this thesis on my humble behalf. For his unwavering support, funding, and enthusiasm during my postgraduate studies, I would like to extend my heartfelt gratitude to Prof. John Omondi Muoma, former Director of Science Park, Incubation and Innovation, who also happens to be my second supervisor. All this would not have been feasible without his unwavering financial support and direction. I consider myself extremely fortunate to have had a team of mentors and supervisors that includes Dr. Mustafa Barasa, Hon. Nicholas Kitungulu, and Dr. Jackson Cheruiyot, who served as my first supervisor. I would like to thank them all for their advice and conversations, which were crucial to the development of this thesis. I thank my peers at Masinde Muliro University of Science and Technology for their encouragement and comradeship on the academic front. Godspeed and blessings. I am also grateful to my amazing parents, Mr. Joseph Tarus and Mrs. Sarah Tarus, for helping me become the well-rounded scholar I am today in addition to offering me financial and moral support throughout my Master's program. I'm grateful to my mentors and family members for their support and belief in me.

ABSTRACT

Tungiasis is a neglected tropical disease (NTDs) caused by the permanent penetration of the female sand flea into the epidermis of its host and get embedded. The disease causes swelling and intense inflammation associated with the superinfection of bacterial infections; this mechanism of inflammation still remains unknown. This study determined immunological changes; haematological changes, cytokine and antibod of experimentally raised guinea pigs during the natural cause of infection. Three to four weeks old guinea pigs in cages were exposed to *Tunga penetrans* in highly infested homes for a period four weeks. The animals were examined on a daily basis and blood samples taken before exposure to the fleas and subsequently 5 days after flea penetration. To determine the haematological changes, hematological analyzer was used and serum concentration of selected cytokine and antibody using Enzyme Linked Immunosorbent Assays (ELISA) guinea pig specific kit. Statistical analysis was done using IBM-SPSS software version 25.0 to determine descriptive statistics of means ± SD. Mann Whitney U test was performed to establish differences among the infected and non-infected groups. Post-hoc analysis was done using Wilcoxons signed rank tests to determine the haematological changes, cytokine and antibody profiles which were subjected to pairwise difference between the control and the precise days post-infestation. There was a significant decrease in erythrograms mean values; red blood cells (RBC) (5.02±0.13), haemoglobin (Hgb) (14.66±0.39), packed cell volume (PCV) (39.62±50), mean corpuscular haemoglobin (MCH) (24.27±0.18) and mean corpuscular haemoglobin concentration (MCHC) (33.77±0.60) at {Citation}($p \leq 0.05$). A considerable increase in leukograms mean values, white blood cells (WBC) (9.08±0.35), eosinophil (2.42±15), neutrophils (4.27±0.70), monocyte (0.8±0.05) and lymphocyte (5.6±0.41) as compared with control ($p \leq 0.05$). The infected subjects showed a significant elevation of total serum of pro-inflammatory cytokines both at day 10, tumor necrosis factor alpha (TNF-α) (235.39 ± 17 pg/mL), interferon gamma (IFN-γ) (425 ± 5 pg/mL) and significantly higher levels of anti-inflammatory cytokines both at day 15 of post-infestation, interleukin -10 (IL-10) (367 ±17 pg/mL) and interleukin -4 (IL-4) (356±4 pg/mL) as compared to the uninfected guinea pigs ($p \leq 0.05$). Moreover, the circulating levels of both antibodies increased significantly following natural infestation at day 15 immunoglobulin E (IgE) (231.9±13 ng/mL) and immunoglobulin G (IgG) (371.9±15 ng/mL). Tungiasis is thus characterized by changes in haematological parameters and the systemic levels of cytokine and antibody indicating an alteration in the underlying immune mechanism to the infection. Profiling of immunological responses implicated in tungiasis unravels the underlying mechanisms on inflammation and development of a diagnostic tool, treatment and control of the infection.

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DEFINATION OF TERMS

Anti-inflammatory cytokines: A series of immunoregulatory molecules that control the pro-inflammatory cytokine response.

Hemocytometer: A counting-chamber device originally designed and usually used for counting blood cells.

Hyperkeratosis: The thickening of the stratum corneum, which is frequently accompanied by a keratin quality anomaly.

Hyperplasia: Physiological proliferation of cells inside an organ or tissue.

Immunoglobulin G: is the most common antibody that is found in blood and other body fluids, and protects against parasitics, bacterial and viral infections

Immunoglubulin E: An antibody produced by the immune system that is known for its involvement in the allergic response

Interferon gamma: A pro- inflammatory cytokine that plays an important role in inducing and modulating an array of immune responses

Interleukin-10:	A cytokine with potent anti-inflammatory properties that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis
Interleukin-4:	A key regulatory cytokine in the development of allergic inflammation.
Necrosis:	Premature cell death brought on by outside events including an infection ,injury, poisoning, or inflammation that may obstruct cell blood supply.
Parakeratosis:	Incomplete keratinization of epidermal cells
Peri-domestic:	Animals living in and around human habitations.
Pro-inflammatory cytokines:	Cytokines produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions.
Tumornecrotic factor:	multifunctional cytokine that plays important roles in diverse cellular events such as cell survival, proliferation, differentiation, and death.

ABBREVIATIONS AND ACRONYMS

ANOVA:	Analysis of variance
ELISA:	Enzyme linked immunoabsorbent assay
Hb:	Hemoglobin
IFN:	Interferon
IgE:	Immunoglobulin E
IgG:	Immunoglobulin G
IL-10:	Interleukin-10
IL-4:	Interleukin-4
MCH:	Mean corpuscular concentration
MCHC:	mean corpuscular haemoglobin concentration
PCV:	Packed cell volume
RBC:	Red blood cell count
SSA:	sub-Saharan Africa
TEC:	Total erythrocyte count
TLC:	Total leukocyte count
TNF:	Tumor necrotic factor
WBC:	White blood cell

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Tungiasis is a skin neglected tropical disease (NTDs) caused by the permanent penetration of the female *Tunga penetrans* into the epidermis of its host and get embedded. The disease is zoonotic with human tungiasis being considered substantial and of a public concern, with an estimated two million people infected (*Guidelines, Standards & Policies Portal*). Sand fleas cause a Spartan morbidity in humans in close proximity with domesticated animals (Mutebi et al., 2015). The severity of the disease is caused by the intense inflammatory responses around the female sand fleas embedded in the skin (Norgan & Pritt, 2018a). Chronic illness is associated with persistent infections with large number of fleas embedded, hyperkeratosis, hypertrophy distortion and loss of nails (Gitau et al., 2021). Studies have revealed the presence of macrophages, lymphocytes, eosinophils and mast cell in the inflammatory infiltrates (Ogly, 2020). Infection in animals is less severe as compared to humans (Mutebi et al., 2023a).

Tungiasis is prevalent in the tropics of the Americas and in sub-Saharan Africa (Deka, 2020; Heukelbach et al., 2022a; Obebe & Aluko, 2020a). It is severe in countries with low economic status (Coates et al., 2020; Elson et al., 2023a; Enwemiwe et al., 2021). In Uganda, pigs are reported to be key reservoirs and major susceptible host of *T. penetrans* than the rest of the domestic pets (Mutebi et al., 2023b). The prevalence of tungiasis in pigs as well as the intensity of infection shows a strong relationship with the respective measurements in humans (Mutebi et al., 2015). Preliminary surveys in Kenya, shows the disease to be highly prevalent in parts of the Nyanza, Coast, Rift Valley, Central and

western region with Vihiga County recording high incidences (Elson et al., 2023b; Mwai et al., 2023; Nyangacha et al., 2019a; Nyangacha et al., 2019b; Wiese et al., 2017).

Guinea pig is a preffered model due to its similarity to humans with respect to parallel pathogenesis, symptomatology and immunological responses (Hunter et al., 2023; Yadavalli et al., 2023). Tumor necrotic factor (TNF) and interferon gamma (IFN) cytokines are significantly enhanced during tungiasis infection, according to immunological research (Feldmeier, Heukelbach, Ugbomoiko, Sentongo, Mbabazi, Samson-Himmelstjerna, et al., 2014). Studies have demonstrated that during tungiasis infections, the anti-inflammatory cytokine such as IL-10 are highly released concurrently with lower serum concentrations of TNF (Ashour & Othman, 2020; Palicelli et al., 2021). It is not clear if the response occurs similarly in other animals. Nevertheless, it is still unknown, precisely when cytokines are produced and released locally immediately after flea penetration. According to studies, elevated serum levels of IgE and IgG are linked to improper T-helper-2 (Th2)-type immunological reactions (Basketter & Kimber, 2011). Regarding the ability of *T. penetrans* to elicit immune responses in rodents, humoral immunity is less likely because ectoparasites are less frequently exposed to systemic circulation. As a result, little is known about the antigen-antibody reactions to *T. penetrans.* The emergimg trend in increase in tungiasis infection potrays the symptoms of secondary infections. It is not yet known whether there are new strains of microbes that could pose difficulty in treatment of the disease. Although similar studies have been done in Brazil utilizing winstar rats in profiling cytokines 20 years ago there still a neglect in Tungiasis.The present study investigated whether there is a change in haematological

parameters, pro and anti-inflammatory cytokines and antibodies profile in guinea pigs infected with *T. penetrans.*

1.2 Statement of The Problem

Tungiasis is a neglected parasitic disease, primarily affecting poor communities in tropical and subtropical regions. The pathology is caused by the *flea Tunga penetrans,* commonly known as jigger. Upon penetration into the host's skin, particularly on the feet, the flea causes intense inflammation, leading to severe pain, itching, and secondary infections such as tetanus and cellulitis. This can result in various complications, including deformation, difficulty in walking, and the inability to perform daily activities (Martins et al., 2021). Moreover, the social stigma associated with the disease can lead to low self-esteem and increased susceptibility to other infectious diseases like HIV/AIDS and hepatitis due to sharing of contaminated sharps objects and also compromised skin barrier (Feldmeier et al., 2013). The immunological response to tungiasis in humans and animal models like rats has been studied to some extent, showing involvement of Th1 and Th2 immune cells (Feldmeier, Heukelbach, et al., 2003; Feldmeier, Witt, et al., 2004). However, the immunological response in guinea pigs, which could serve as a useful model for studying this disease, remains unexplored. Understanding the pro and anti-inflammatory cytokine profile, antibody profile, and haematological changes in guinea pigs post-infection would provide insights into the host-parasite interactions and the overall immunopathology. The study determined the cytokine and antibody levels in order to analyse their expression levels at different stages of infection to understand the temporal dynamics of the immune response. The study also aimed at conducting a

complete blood count (CBC) to observe changes in red blood cells (RBC), white blood cells (WBC) to identify shifts in immune cell populations. The study is expected to elucidate the immune mechanisms underlying Tungiasis in guinea pigs, providing a comprehensive haematological changes, cytokine and antibody and haematological changes. The findings would contribute to a better understanding of the disease's immunopathology, potentially informing the development of targeted interventions and treatments for tungiasis in humans.

1.3 Justification of the Study

This study was conducted to address the significant yet undervalued health impact of Tungiasis, a neglected tropical disease caused by the flea *Tunga penetrans*. The disease inflicts severe pain, leading to irritability, deformations, and impaired mobility, which intensely affect the quality of life of those afflicted (Martins et al., 2021). Despite its severe consequences, the immunological mechanisms underlying Tungiasis are poorly understood, and there is an urgent need to develop effective therapeutic strategies. Guinea pigs were chosen as the animal model for this study because they exhibit physiological and immunological responses that are comparable to humans, making them a valuable model for studying human diseases (Padilla-Carlin et al., 2008). Additionally, guinea pigs are more practical for controlled experimental infection and can provide comprehensive data on the host immune response, including cytokine and antibody profiles and hematological changes. Understanding the immune response in guinea pigs can provide insights into the immunological processes in humans, potentially guiding the development of vaccines and treatments for tungiasis.Vihiga County in Kenya was

selected for this study because it is one of the regions heavily affected by tungiasis (Nyangacha et al., 2019c). High occurrence of the infection in this area provides a relevant context for studying the natural course of the infection and the associated immunological responses. Furthermore, insights gained from this region can inform public health strategies and policies for managing tungiasis in other endemic areas. Evaluating cytokine profiles provides insights into the host's inflammatory response and immune regulation during tungiasis. The study of antibody responses, including the identification and quantification of specific antibodies, helps to elucidate the humoral immune response. This information is crucial for understanding how the body recognizes and combats the parasite, which is fundamental for vaccine development. Hematological Changes would provide a comprehensive picture of the systemic impact of the infection. These changes can indicate the extent of immune activation and the overall health status of the host during infection. The study aims to uncover the complex interactions between the parasite and the host's immune system, ultimately contributing to the development of targeted interventions and improving health outcomes for those affected by tungiasis. Understanding the immunological response in guinea pigs provides information and reflection of what happens in humans becoming the pointer to the development of vaccines based on the immunological responses. Findings from this work would put more emphasis on the therapeutics strategies and eradication of the disease.

1.4 Objectives

1.4.1 General Objective

To evaluate the haematological changes, cytokine and antibody profile in guinea pigs infected with *Tunga penetrans*

1.4.2 Specific Objectives

1.To determine the hematological changes in guinea pigs infected with *Tunga penetrans*

2. To determine the pro-inflammatory and anti-inflammatory cytokine in guinea pigs infected with *Tunga penetrans*

3.To determine the antibody profile in guinea pigs infected with *Tunga penetrans*

1.5 Null Hypotheses

$H_0$1. There are no hematological profile in guinea pigs infected with *Tunga penetrans*

$H_0$2. There are no pro-inflammatory and anti-inflammatory cytokine profile in guinea pigs infected with *Tunga penetrans*

$H_0$3. There are no antibody profile in guinea pigs infected with *Tunga penetrans*

1.6 Significance of the Study

Tungiasis has significant implications for public health due to its morbidity and potential to cause severe secondary infections. Understanding the immunological response to *Tunga penetrans* infection is crucial for developing effective treatments and control strategies. Haematological studies in Tungiasis can reveal important insights into how the body responds to infection. Understanding these haematological changes can aid in diagnosing the severity of Tungiasis and amending appropriate therapeutic interventions. The balance between Th1-type (cell-mediated immunity) and Th2-type (humoral immunity) responses provides insights into the adaptive immune response against *Tunga penetrans*. Through the identification of the specific cytokines involved in the immune response to Tungiasis, researchers can pinpoint targets for immunomodulatory therapies and improve vaccine development strategies. Monitoring these antibodies can help in understanding the dynamics of the immune response over time. This study's findings are crucial for advancing the understanding of the immunopathogenesis of Tungiasis. By making these findings available to research and government institutions, there is potential to significantly improve the planning and development of drugs and therapeutics, as well as to enhance disease management and control strategies. The comprehensive analysis of haematological changes, cytokine, and antibody profiles in guinea pigs offers a robust model for studying tungiasis and devising effective interventions.

1.7. Limitations of the study

1. The study may not have had sufficient statistical power to identify minute changes in antibody profiles, cytokine levels, or haematological markers due to the small number of guinea pigs utilized.

2. The study's duration and infection stage surveillance time may be restricted. Since immune responses change over time, measuring them up to 20 days after infection may miss significant dynamic changes in cytokine or antibody levels

3. The study's scope of markers may have been restricted to a subset of cytokines and antibodies, possibly resulting in the exclusion of other immune mediators because of resourceconstraints.

4. The wellbeing and health of the study's guinea pigs could have an impact on the study's replication. The study may be impacted by ethical considerations and the desire to reduce suffering in terms of design and duration.

1.8. Scope of the Study

This study investigates the haematological changes cytokine and antibody profile in guinea pigs (*Cavia porcellus*) experimentally infected with *Tunga penetrans* to uncover the mechanisms behind the inflammation observed in tungiasis. The blood samples from 24 guinea pigs, control (8) and in an infected (16), the research focuses on evaluating changes in blood cell counts, redlines and whitelines changes using a full hemogram machine. Additionally, the study measured serum concentrations of guinea pigs to understand the pro-inflammatory and anti-inflammatory levels of selected cytokine, as well as the specific antibody levels using enzyme-linked immunosorbent assay (ELISA)

before and after flea penetration. Statistical methods such as descriptive statistics, Mann-Whitney U tests, and Wilcoxon signed-rank tests, the study aims to identify significant differences between infected and non-infected groups, as well as temporal changes post-infestation days. The findings are anticipated to enhance the understanding of the pathophysiological processes in tungiasis, providing valuable insights that could lead to improved therapeutic and preventive strategies for this neglected tropical disease.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tunga Penetrans Classification

Tunga penetrans also referred to as sand flea or jigger, causes a highly neglected tropical skin disease known as Tungiasis (Dos Santos et al., 2023a; Mutebi et al., 2023b; Nwalozie & Ezenwaka, 2023). The parasitic female flea lives in the soil or sand, and feeds intermittently on warm blooded host such as human beings, pigs, cats, dogs and chicken (Talhari & Gontijo, 2023; Yadavalli et al., 2023). The disease is caused by a parasitic adult female jigger or sand flea (Walle Mørkve *et al.*, 2023; Mørkve *et al*., 2023). The sand flea is classified into kingdom-Animalia, Phylum-Arthropoda, class-insecta, order-Siphonaptera, family-Hectopsyllidae, Genus-Tunga, species-penetrans; and tramamillata (Myers et al., 2006; Pampiglione et al., 2002). The genus *Tunga penetrans* has 13 subspecies which are mostly haematophagus ectopararsites (Pampiglione et al., 2002). The majority of *Tunga penetrans* are parasitic of a single host while *T. tramamillata*, *Onore, Trentini, Fiovaranti, Pampiglione* and *Rivasi,* have a range of possible hosts such as domesticated and wild mammals (Pampiglione et al., 2009a).

2.2 Sand flea Morphology and Classification

Jiggers are relatively small and have a full metamorphosis, looking either black or brown-black in color. They possess short, 3-jointed, clubbed antennae that fit into depressions on the sides of the head and robust, spiny legs that are specialized for leaping . Depending on the species, adult fleas range in size from 1 to 4 mm. In general, females are smaller than men, yet occasionally they have a larger epipharynx and maxillary palpus. The

slightly flattened head of a flea makes it easier for it to penetrate through the dermal and epidermal layers (Nagy *et al*., 2007).

Females just paw through the epidermis after attaching with their papillae since they lack sophisticated burrowing organs. Only their posterior extremities are exposed to the surroundings when they settled into the stratum granulosum after piercing through the stratum corneum.The female fleas continue feeding, and their abdomens enlarge to a length of around 1 cm. The sand flea life cycle begins once the ovulating female releases eggs into the environment, which takes place over a two-week period. Females then die. Larvae develop from eggs in around 3–4 days and eat environmental biodegradable materials. Before developing into a pupa, *Tunga penetrans* undergoes two larval forms. The pupae are enclosed in carapaces which are frequently coated by soil and other environmental waste. The larval and pupal stages last for three to four weeks. Adults emerge from pupae afterward then rifle for a warm-blooded host to feed on blood. Moreover, males and females feed on their humans frequently, but only mated females can burrows into the host epidermis (epithelium), enabling them to generate a nodular swelling that is leached off by the host's tissue (Nagy et al., 2007).

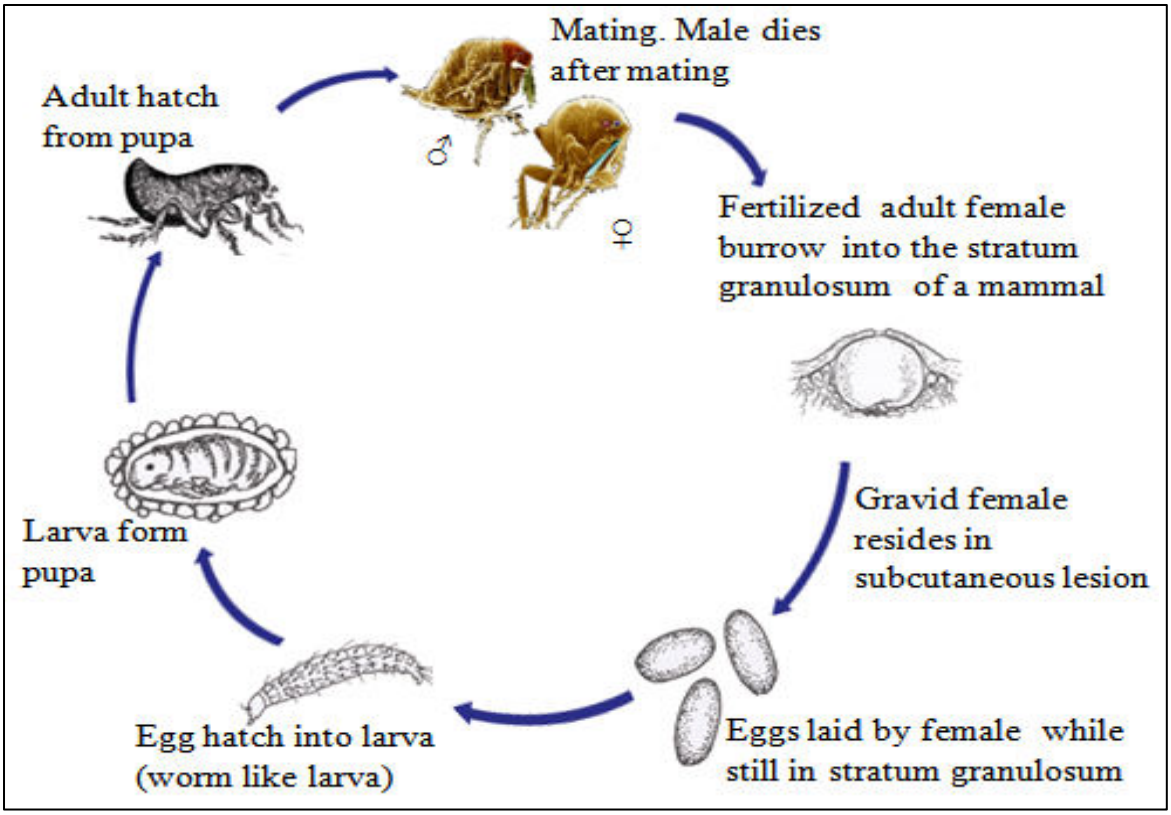


Figure 2:1.The life cycle of *T. penetrans* flea showing a complete metamorphosis cycle.

Source: The distinctive male flea (♂) with large copulatory organs dies after mating. In order to continue the cycle, the fertilized female flea (♀) infests the skin of mammals in order to nurture and mature her eggs.*(https://www.cdc.gov/dpdx/tungiasis/index.html).*

2.3 Global and Continental Occurrence of Tungiasis Disease

Globally, sub-Saharan Africa (SSA), the Caribbean islands, and Latin America are the disease's predominant distribution regions (Heukelbach et al., 2022a), Tungiasis affects primarily disadvantaged children, disabled and elderly in rural/ slum inhabitants (A Obebe & Aluko, 2020; Saboyá-Díaz *et al.,* 2023; Santana *et al*., 2023). The disease is thought to be endemic in Pakistan, the Seychelles Islands, and Bombay, In dia (Johannessen, 2022). In some communities in Brazil the incidence in individuals is as high as 50% (Trindade et al., 2023). In addition, the disease has been reported to infect

human and canine in some regions in New Zealand, Australia, and parts of North America as well as in numerous countries in Europe (Steverding, 2020). In Africa, It is estimated to infect approximately 34% of the sub-Saharan Africa (SSA) with highest occurrence being reported in Ethiopia (46.5%), Cameroon (44.9%), Tanzania (42%) and Kenya (37.2%) (Obebe & Aluko, 2020b). A third of tungiasis patients in Nigeria have mobility issues thereby influencing their daily activities (WHO, 2023).

Studies on experimental and domestic animals have demonstrated that, animals act as reservoirs (Dos Santos *et al*., 2023b ; Organization, 2022 ; Obebe & Aluko, 2020b). This has made it difficult in control and management of both the jigger and the disease.

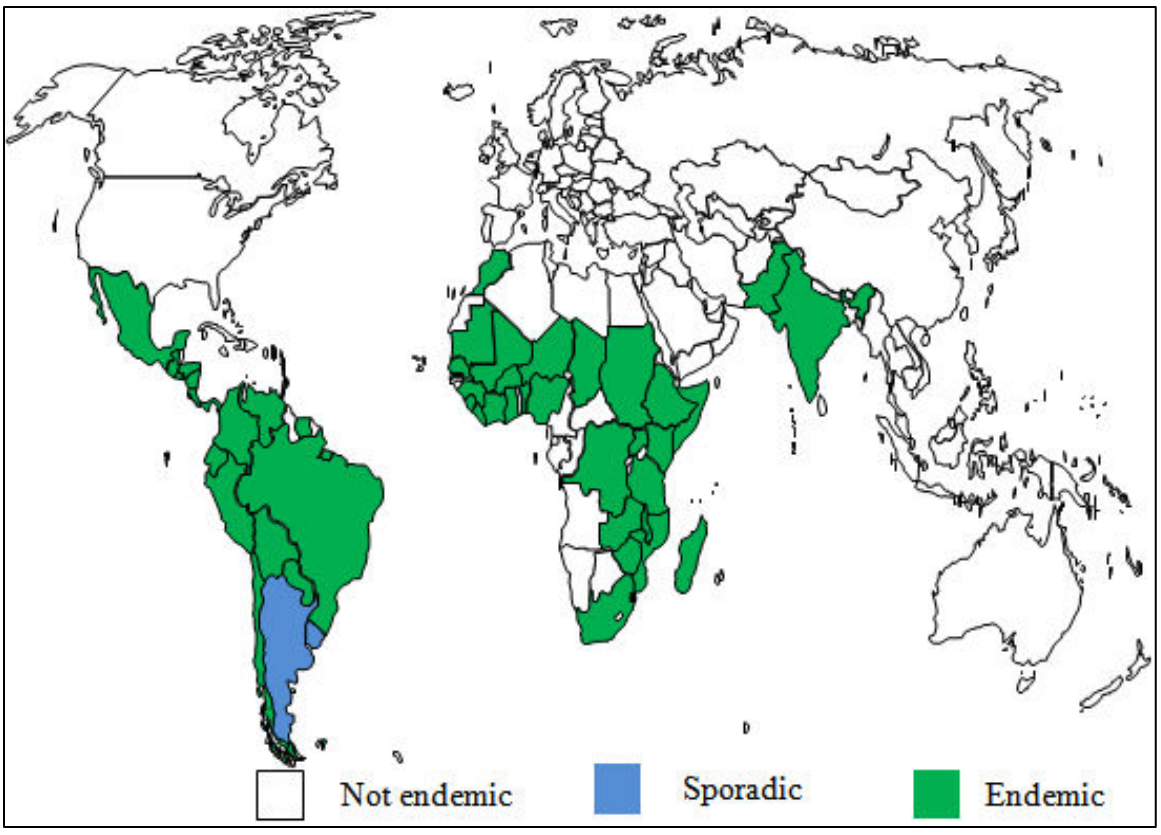


Figure 2:2. A map showing the worldwide distribution of Tungiasis

(Feldmeier, Eisele, et al., 2004)

2.4 Tungiasis Occurrence and Prevalence in Kenya

Over 2.6 million Kenyans are registered with the Ahadi Kenya Trust as having tungiasis, and many more suffer in silence because no thorough survey has been conducted, making it difficult to determine the true number of affected people (Adenkola et al., 2009). In central Kenya, studies at Kiharu constituency in primary schools shows that approximately 19% of pupils were infested with *T. penetrans* (Mwangi et al., 2015). The disease seems to affect girls more than boys with highest peak being reported in children between the ages of 10 – 12 years. In addition, the disease appears to affect more women than men in Murang'a east with majority of people infected aged between 20 to 29 years (Wambani et al., 2018).

Tungiasis is endemic at the Coastal region of the country with a prevalence ranging between 11% and 50% (Ahadi Trust, 2010). This region is ranked as the second highly infested by tungiasis in Kenya. Kilifi County has been reported to have high cases of children infested with jiggers at 48% (Elson et al., 2019). Other studies in western Kenya, have also reported high incidences of Tungiasis in Vihiga county (Nyangacha et al., 2019b). The high incidences would led to low economic burden, healthcare sytem strain, social and psychological effects due to high morbidity and mortality.

2.5. Social and Economic Impacts of Tungiasis

Tunga penetrants mostly affects children reducing their learning ability (Girma et al., 2018; Nsanzimana et al., 2019). The literature showed that school-age children diagnosed with parasitic infections had lower school attendance and cognitive and physical development (J. W. Ngunjiri, 2015). The morbidity of the disease is described when an

individual has high number of embedded female sand fleas (Feldmeier, Heukelbach, Ugbomoiko, Sentongo, Mbabazi, von Samson-Himmelstjerna, et al., 2014a). The resulting substansial morbidity has been reported to cause the absenteeism due to pains and stigmatism among the school mates, repetition and even dropouts among the children thus reducing the acquisition of basic education (J. Ngunjiri et al., 2015).

Figure 2:3 Jiggers infested toes and fingers Source (Ahadi; 2007)

The disease affects individuals leading to savere physical disability arising from pathological effects especially on limbs and also digits amputation which leads to difficulties in carrying out farming activities. This oftens leads to high poverty levels among the victims.

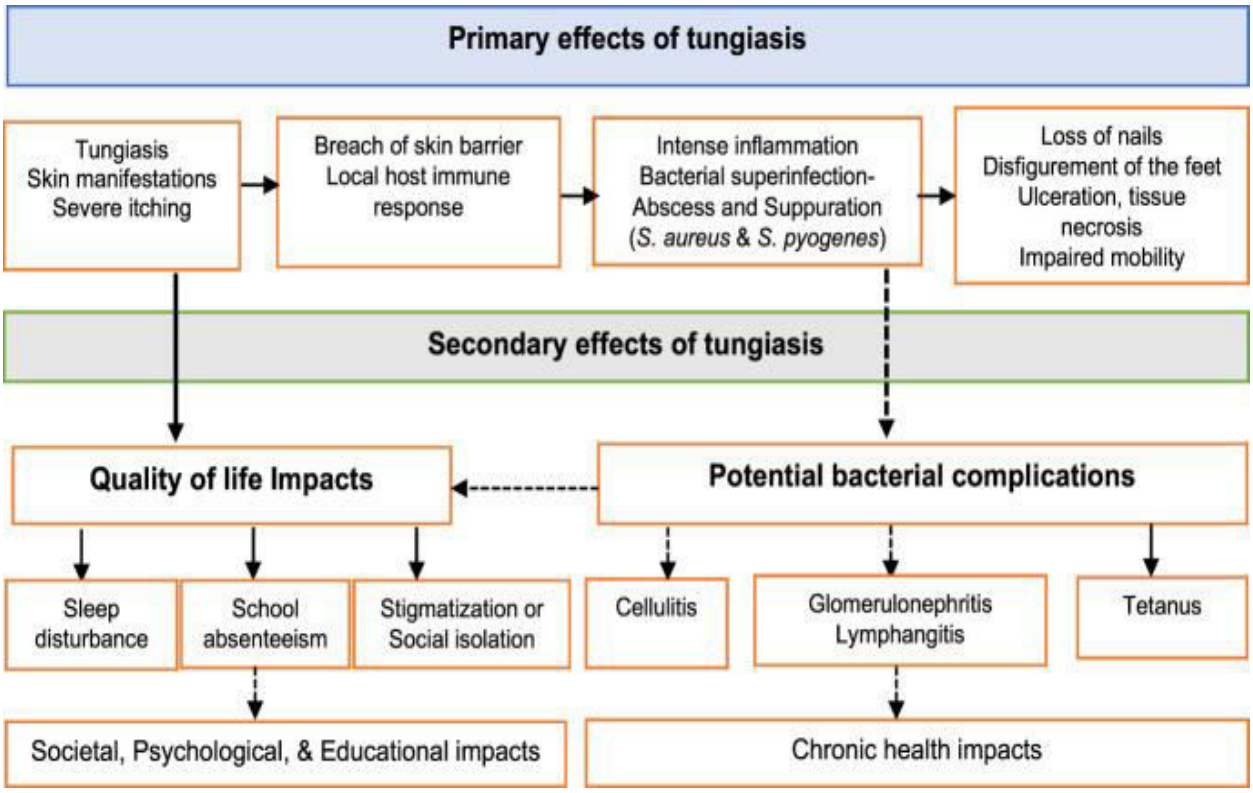


Figure 2:4. The summary of Complications of tungiasis (Solid arrows (→): direct effects and Dashed arrows (--→): potential secondary effects *(Abrha, Tesfaye, et al., 2021)*.

Studies conducted at Murandina division, central Kenya reveals that 67.35% of farmers were infected with jiggers,individuals infected with jiggers has low production units of 1.235 of annual production while unifected individuals have 2.072 units of production annually which attributes to 0.7759 unitss less than uninfected labour (Muhoro et al., 2016). Jigger flea infestation is prevalent in communities of low socioeconomic level. In Eastern Uganda, according to studies done in the Mayuge district. The majority of the household heads are artisans who engage in subsistence farming, which characterizes most of the families.

Many people araising some domestic animals such chickens, goats, calves, and pigs as well as cultivated crops, low household earnings as a result of a low level of education, which also impedes the provision of excellent health care services and housing conditions. Despite having iron roofs and brick walls, the majority of jigger-infested households' homes are in poor condition. They are typically incomplete structures with loose, dusty flooring and unplastered wall surfaces. According to the research, the bulk of the families impacted by the jigger are run by farmers (86.7%) and craftspeople (13.3%). The majority of household heads attended school up to the elementary school level (80%), with only a few completing high school (20%) (Wafula *et al.,* 2016).

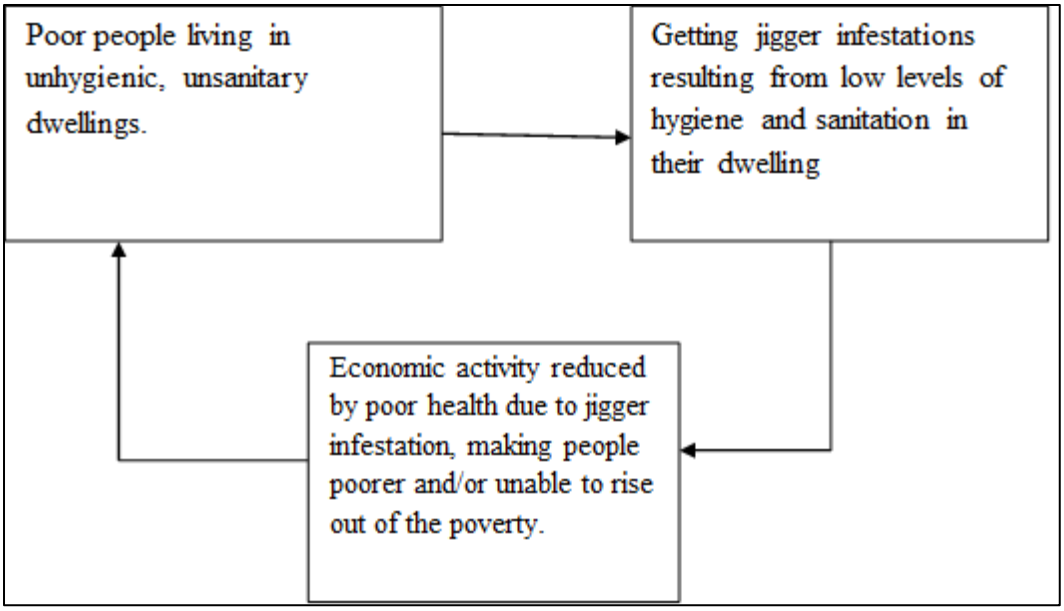


Figure 2:5.The vicious cycle of poverty on jiggers infestation .Source (Ahadi; 2007).

2.6 Tungiasis Infestation in Animals

Numerous domestic, peri-domestic, and wild mammals, including pigs, dogs, cats, goats, cattle, rats, elephants, jaguars, monkeys, and even armadillos, are susceptible to *T. penetrans* infection. From one endemic region to another, the importance of each animal species in the spread of human tungiasis differ (Harvey et al., 2021). Dogs, pigs, cats, and goats are reservoirs for *Tunga penetrans* and a major route of Tungiasis transmission (Heukelbach et al., 2022b). In Uganda, it was revealed that animals played an important role in the transmission of Tungiasis (Thielecke et al., 2023a).

Tungiasis infection in animals in Brazil has shown to increase the risks and severity of the human diseases by a factor of six (Saboyá-Díaz et al., 2023). In shanty towns of Northeast Brazil 67% of the dogs , 50% cats and 55 % of the rats are infested (Harvey et al., 2021). Close contact with infected animals increases the chance of human infection

in Brazil, where the disease is highly prevalent both individually and communally (Paranhos et al., 2022). Pigs are the animal species that are both most commonly infected and have the most severe infection (Razanakolona et al., 2022). It is obvious that pigs are the reservoir hosts that contribute the most to the incidence of tungiasis in humans based on the prevalence of tungiasis in pigs at the village and household level as well as the intensity of infection (Mutebi et al., 2015). However, it is unclear how significantly these animals contribute to the proliferation and spread of *T. penetrans* in humans. No studies in Africa have shown such relation. Among the domesticated animals, pigs have been indicated as an important animal hosts for *T. penetrans* (Dos Santos et al., 2023c). This is an indication that control strategies need to target both animals and human population in endemic communities.

2.5 Management and Treatment of Tungiasis

Treatment is the main aspect of management because the sickness is self-limiting, at least when exposure to the parasite is restricted (AO Elsaftawy et al., 2023; Zawawi & Yusof, 2023). Given that a secondary infection might result in major health problems, the suggested course of action after. A topical antibiotic is applied after the flea is surgically removed as part of the diagnostic. When extracting a flea, caution must be used to prevent ripping it, since this could cause serious irritation (Abrha, Heukelbach, et al., 2021). It is important to constantly utilize sterile equipment since contaminated devices might serve as mechanical vectors for microbes to enter the body (Thielecke et al., 2023b). There is no medication that has shown to be helpful against fleas that are

implanted. Despite the significant side effects, oral niridazole was originally thought to be a therapeutic treatment (Pampiglione et al., 2009b).

However, well-designed studies are hard to come by, so this medication is likely to do more harm than benefit. Nevertheless, there is some anecdotal evidence that it completely lyses the fleas (Gibbs, 2009). Some in endemic areas believe that oral ivermectin is a cure-all for fleas, although high-dosage trials have not supported this claim. Other medications, such metrifonate and topical ivermectin, have shown modest efficacy but not enough to be remarkable (Okoth, 2015). Although they only treat secondary infections, trimethoprim, sulfamethoxazole, metronidazole, and amoxicillin have all been used successfully for super infections (Vindenes et al., 2023).

In addition, cryotherapy and electrodesiccation of the lesion are effective topical therapies. Care should be given while managing the ensuing morbidity if formaldehyde or chloroform is applied topically. According to Deka & Heukelbach (2022), occlusive petrolatum can also be used to suffocate *Tunga penetrans* fleas. Vaseline can also kill the organism, probably through suffocation as the stigmatas would be occluded. The burrowing fleas are slated to die in five weeks, even in the absence of therapy, and would naturally shed their skin.

2.7 Immunological Responses of Animals to *Tunga Penetrans*

T. penetrans being an ectopararsites may have similar responses like any other ectopararsites such as bugs, fleas, ticks and scabies mites (Mutebi *et al*., 2021a ; Dos Santos *et al.,* 2023). The host-parasite interaction determines how differently hosts react

to ectopararsites. However, ectopararsites typically cause the host's immune system to become more active, as well as effector responses that include mast cells, complement, circulating granulocytes, T lymphocytes, B lymphocytes, antigen-presenting cells, cytokines, and other functional molecules (Elieh Ali Komi *et al.*, 2020;

2.8 Pathogenesis and Clinical Manifeststion of Tungiasis

An inflammatory response around embedded female sand fleas causes the disease's severe persistent morbidity, which is aggravated by bacterial superinfection. Erythema,oedema, desquamation, discomfort, and itching are persistent during the acute phase.Intense scratching caused by the lesion's itchiness stimulates bacterial infection (Feldmeier, Eisele, et al., 2004). Fissures, ulcers, lymphangitis, lymphoedema, ascending neuritis, deformation and loss of nails, and tissue necrosis are chronic complications (*Tungiasis*, n.d.) Studies on histopathology shows that when the parasite is about to to die or dead , the eosinophils cell and bacteria are seen on the surface of of the parasite and later on neutrophils causing microabcesses (Eisele et al., 2003a). The skin shows hyperplasia and hyperkeratosis with a high inflammatory insinuates with neutrophils, lymphocytes, and eosinophils mostly around the dilated blood vessels (Eisele et al., 2003b).

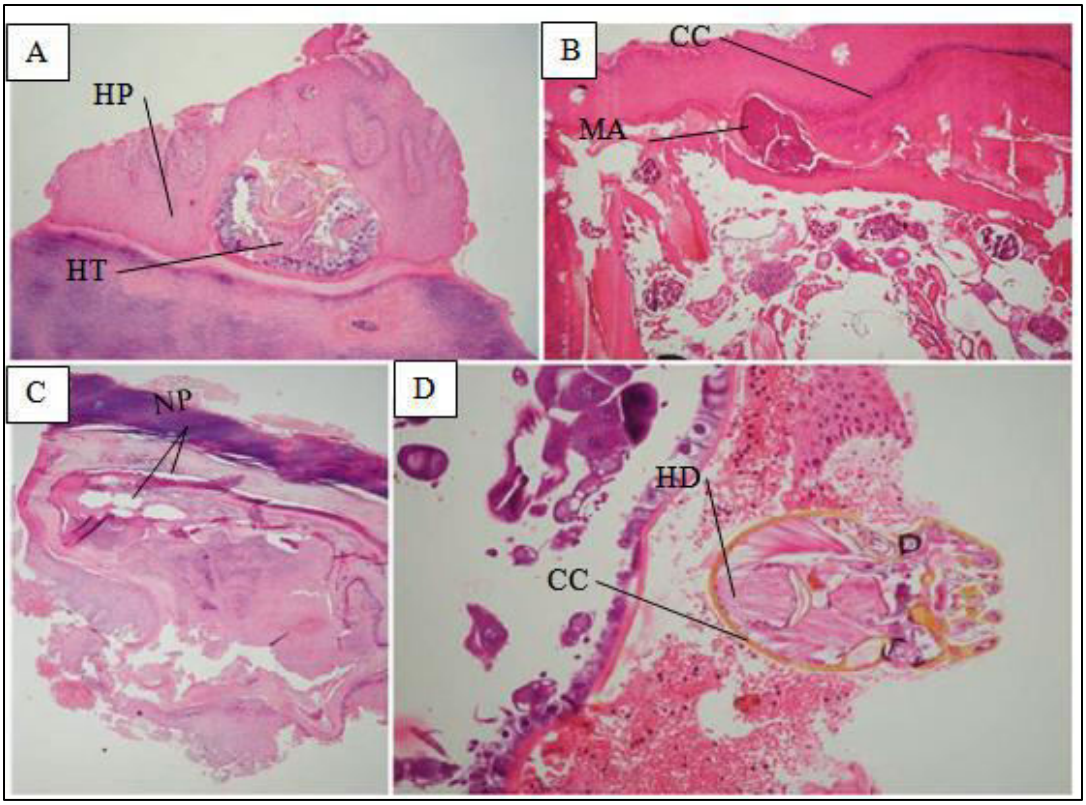


Figure 2:6. The hypathological findings on tungiasis *(Scott & Stockham, 2013)*

2.8.1 The Hypathological Findings on Tungiasis

The hypertrophy anterior abdominal segments enfold the flea's head and thorax (HT). In addition to papillomatosis, parakeratosis, and hyperkeratosis, the epidermis is hyperplastic (HP). B. Tangential cut across a sand flea's posterior abdominal segments. A microabscess (MA) develops next to the chitinous cuticle (CC). C. Dead parasite; the posterior abdominal fragment's exoskeleton is still intact; the cuticle at the epidermal-dermal junction has decomposed. Neutrophils has invade the tissue, and pus has developed (NP). D. The flea's head (HD), which has pierce the basal membrane and is surrounded by several erythrocytes that most likely leak from a blood vessel, is situated

at the epidermal-dermal interface (Scott & Stockham, 2013) as shown in figure 2.5 above.

T. penetrans infestation in the vertebrate's host is a self-limiting process, although there is a clear risk of complications from secondary infections (Eisele et al., 2003b). The cause of bacterial super infection in many cases is improper patient handling of the lesion with non-sterile equipment. Around the advanced lesion, there is notable desquamation of the skin. This has a clinical and pathological relationship to the stratum corneum's erythematous and metaplasia (Heukelbach et al., 2002). Another frequent complication of severe infestation and superinfection is necrosis (Pilger et al., 2008). Death from secondary Clostridium tetani infection is frequently seen in infected individuals (Eisele et al., 2003a). However, 10% of tetanus victims in Brazil die from infection with C. tetani, which is fatal (Feldmeier, Eisele, et al., 2003).

Tetanus is frequently a problem if the patient is not immunized due mainly to potential pathogens. The ovaries of *T. penetrans* has recently been found to contain wolbachia species of bacteria. In several intestinal parasites disorders like filariasis, antigens of these bacteria's endosymbiont have been linked to the pathologic immune response. Wolbachia antigens produced from dead fleas may possibly contribute to the heightened immune response seen in tungiasis. Along with other diverse infections like bacillus species, the chigoe flea can also transmit Wolbachia endobacteria and Staphylococcus aureus (Eisele et al., 2003a).

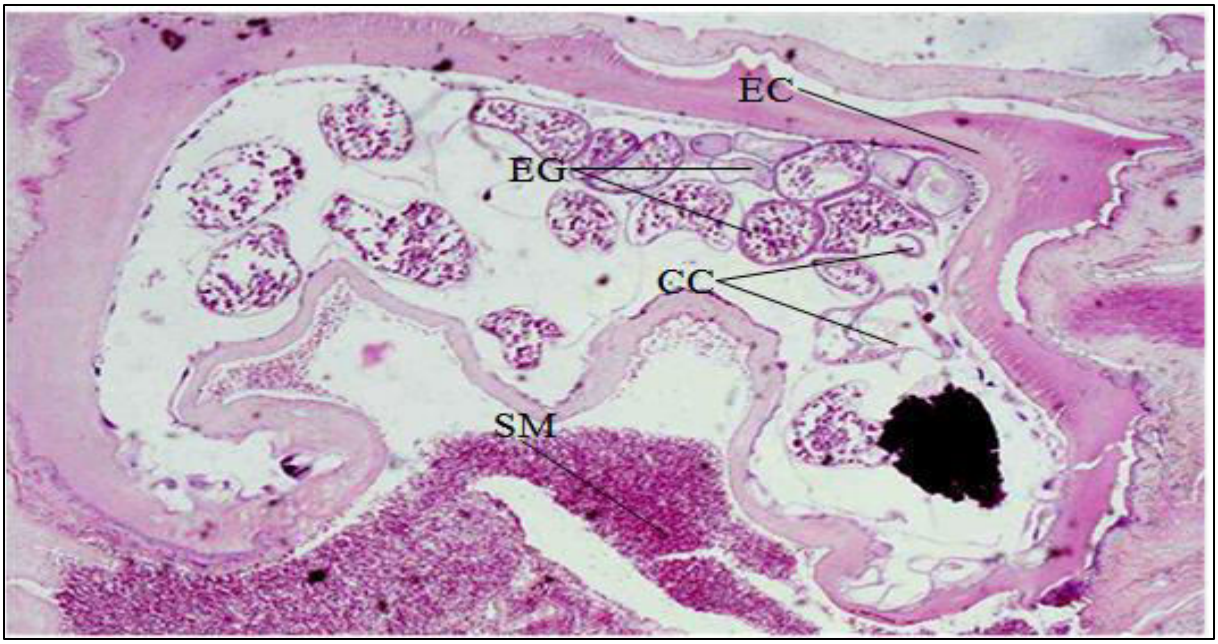


Figure 2:7. Histopathological examination of engorged *T. penetrans* *(Scott & Stockham, 2013)*.

2.5.2 Histopathological Examinations of Engorged *T.penetrans*

The intra-epidermal cavity shown by EC- corresponds to the flea's body and is lined with an eosinophilic cuticle. There are round to oval eggs in the cavity (EG). The digestive tract and its components is depicted in CC as hollow rings. From the head to the terminal orifice, a substantial band of striated muscle runs (SM). Usually, the adjacent dermis has an inflammatory infiltration (Scott & Stockham, 2013).

2.9 Relevance of Hematological profiles

Hematological parameters are widely used as clinical indicators of health and disease. The existence of surface of the skin along with the specialized appendageal cells and immune cells serve as the protective measure against the ectopararsites and other

invading microorganisms (Adenkola et al., 2009). The disease cause pain and distress to humans and animals thus adversely affecting the homeostatic mechanism rendering the body dysfunctional (Eze et al., 2010).

The blood consisting of immune cells and plasma serve the role of regulation, protection and homeostatic functions (Nasyrova et al., 2006). If the pre-infestation is treated, a primary infestation in dogs, pigs, rabbits, and people results in partial or whole immunity to subsequent infection (Allen, 2005; Bhat et al., 2017a; Chen et al., 2021). Inflammatory and immunological responses of the host is coupled to clinical symptoms during both primary and secondary infestations. Substantial efflux of neutrophils, plasma cells, CD4 and CD8 T lymphocytes, and weaker infilltrates of macrophages, eosinophils, and mast cells are characteristics of scabietic lesions which has similarities with tungiasis lesion being an ectoparasitic (Huang et al., 2023; Taylor et al., 2023). Studies have revealed that stress also as a result of sucking by ectopararsites e.g. jiggers can cause decrease in thenumber of lymphocytes with simultaneous increase in the number of neutrophils (Satyaningtijas et al., 2020). Haematological profiles are key gages of health and disease for diagnosis, control, treatment and management of diseases (Eze et al., 2010). This study determined the role of hematological parameters of guinea pigs infected with *T. penetran*.

2.10 Cytokine Responses

Immunological responses demonstrated that tumour necrotic factor (TNF) and interferon (IFN) cytokines such as IFN-Y are highly elevated during Tungiasis infections in rats revealed that, innate arm of immunity play a major role in protecting rats during tungiasis

infection (Feldmeier, Heukelbach, Ugbomoiko, Sentongo, Mbabazi, von Samson-Himmelstjerna, et al., 2014b; Muehlen et al., 2003). It was Studies have shown that anti-inflammatory IL-10 cytokines are highly secreted with reduced serum concentrations of TNF-α during tungiasis infections (Feldmeier, Eisele, et al., 2004). Whether the response is similar in other animals is unknown. The study evaluated the immunological response of *T. penetrans* infection in guinea pigs. The findings from this study shades more light on cytokines and antibodies interactions.

In models for myiasis caused by Lucilia cuprina and scabies, two ectoparasitic diseases with symptoms comparable to tungiasis, release of IL-1 and IL-8 within a few hours after penetration (Bhat et al., 2017a; Eisele et al., 2003a). Cells from the epithelial layer are assumed to be the sources of these proinflammatory cytokines in the case of s.scabiei (Arlian et al., 2004). Research on histological analysis indicates the presence of mast cells, eosinophils, lymphocytes, and macrophages in the inflammatory infiltrates within 24 hours of penetration (Eisele et al., 2003a). The activation of lymphocytes or mast cells may be the cause of the existence of IL-4 (Xue & Falcon, 2019). It has been demonstrated that tungiasis in humans results in a mixed Th1 and Th2 immune response with elevated levels of the pro-inflammatory cytokines TNF-α and IFN-γ, as well as a slight rise in IL-4 concentrations (Eisele et al., 2003c; Ruttoh et al., 2012). According to studies, tungiasis patients have both activated Th1 and Th2 immune responses. IFN-Y and IL-4 levels increase noticeably in the first five days following flea penetration (Eisele et al., 2003c), according to research on two emigrants whose exact timing of flea penetration is known, who had no additional ectoparasites and were not afflicted with

intestinal helminthes (Muehlen et al., 2003). However, it is still unclear exactly when cytokines are generated and released locally soon after flea penetrantion. Furthermore, there are indications that immune cells become activated and begin to release cytokines very quickly when an ectoparasite enters the epidermis. The stsudy investigated the role of cytokines during tungiasis infections.

2.11 Role of Antibodies

Adaptive immunity plays a role in controlling and regulating the host immune reactions which is elicited by the ectopararsites. The responses are mediated by the immune mechanism facilitated by the mast cells and IgE antibodies to reduce the severity of the parasitemia (Wilson, 2014). Studies have revealed that the presence of increased serum levels of IgE and IgG is associated with inappropriate T-helper-2 (Th2)-type immune reactions (Roberts et al., 2005). Though *T. penetrans* can induce immunological responses in rodents, there is reduced chances of humoral immunity as ectopararsites, i.e ,jiggers are not largely exposed to systemic circulation. There is a dearth of information on *T. penetrans* antigen-antibody responses as a result. However, in Brazil, the genetic diversity of the Tunga species has been studied at the molecular level. Jigger mitochondrial DNA isolates from cats, dogs, and humans discovers to have identical sequences, indicating that the same species of Tunga may be responsible for the infestation. However, variations in sequence of up to 49% was identified when these isolates was compared to those of fleas from pigs and rats, leading to the assumption that one or more new species of Tunga may exist (Vobis et al., 2005). The study evaluated the role of IgG and IgE as a protective measure against *T. penetrans* infestation in guinea pig.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The research was carried out in homes that were severely sand flea infested in Kegondi village, West-Sabatia sub-county, Vihiga County. At a height of 1300 to 1500 meters above sea level, Vihiga County is known for its undulating terrain (Mathenge et al., 2020). Rainfall occurs twice a year in the County: long rains from March to May and brief rains from August to October. The annual mean rainfall varies from 1500 mm to 1750 mm. The temperature fluctuates between 17.0 °C at minimum and 30.0 °C at highest. The loose, iron-rich red volcanic soils provide the perfect environment for jigger infestation, which leads to a high infestation and an increase in the number of jigger occurrences (Nyangacha et al., 2019b).The figure 3.1 below shows the homes which were higly infested with jiggers.

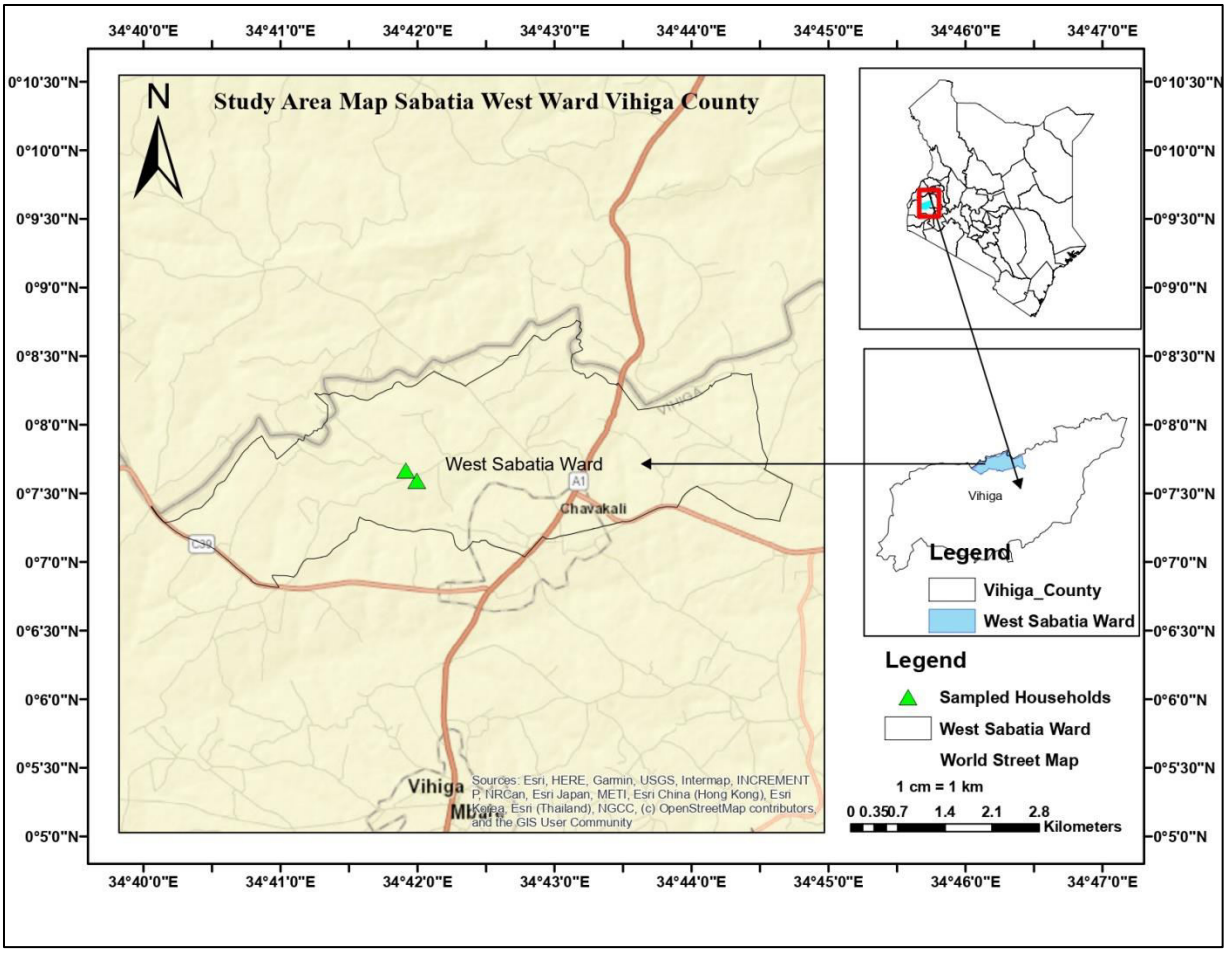


Figure 3:1. Map of study area .

Source: Author, 2024.(ArcGIS Software version 10.5)

3.2 Experimental animals and sample size calculation.

The study entailed field (natural infection) and Laboratory bioassays. A total of 24 guinea pigs weighing 200-280g were used in the study. The animals were purchased and confirmed to be free of infection and any other parasites from a commercial farmer carrying out pure line breeding of American calico guniea pigs and taken to Masinde Muliro University of Science and Technology where they were kept in wire-mesh cages in a clean and well-ventilated animal house. They were routinely fed with standard feed

pellets from Royal feeds company fibre 18%, protein 13%, oil 3.5%, ash 5% ,calcium 0.7% , phosphorus 0.5% , fat 2.6% and vitamin D3 1000IU/kg), and water then left to acclimatize to the laboratory conditions for two weeks prior to commencement of the experiment.

The number of guinea pigs required for the study was determined using Snedecor (1956) formula:

$N = 1+2\mathbf{C}(s/d)^2$

Where;

- N, is the sample size
- s, is the standard deviation set at 1
- d, is the Cohen's effect size (set at 1)
- **C**, is a constant value dependent on the alpha and beta selected

Using the C table (Snedecor and cochran, 1989) at alpha 0.05 and beta at 90%, where C is 10.51, the number of animals required was;

$$\mathbf{N = 1 + 2 \{10.51\} \times [1.0 \times 1]^2 = 24}$$

Determination of the sample size was critical for determining the statistical power and clinically significant differences. While choosing the sample size, ethical considerations were also taken into account.

3.3 Experimental design and Natural infestation

The experimental procedure was done in accordance with regulations of the Institutional Scientific and Ethical Review Committee (ISERC) of Masinde Muliro University of Science and Technology and the National Commission for Science Technology and Innovation (NACOSTI). Guinea pigs were divided into infected and non-infected groups, 16 animals were infected with sand fleas, 8 uninfected (control) for cytokines, antibodies and haematological examination. After marking and identifying the animals, at day Zero, all the animals were bled for cytokines, antibodies, haematology to establish a baseline data. The animals were examined daily and, as soon as they became infected, they were relocated to the laboratory, where up to 6 guinea pigs were kept in an ordinary cage. To

confirm the presence of fleas, a small white lump was observed, redness and inflammation followed by itching and discomfort of the animal. Atleast three fleas got in the animal's limbs pads and ear lobes.

Figure 3:2. Uninfected guinea pigs (Controls)

Figure 3:3. Infected guinea pigs in the cages under inspection

Figure 3:4.The infected Limb pads and earlobes of the guinea pigs

3.4 Sample Collections and Processing.

A sample of approximately 1.5 mL of peripheral blood was drawn from infested and controls guinea pigs at an interval of 5 days (0, 5, 10, 15, and 20 days after flea penetration) using a 25-gauge needle and 2 ml syringe from the lateral saphenous vein. The blood was transferred into tubes containing EDTA anticoagulant. The blood sample (1mL) was centrifuged and sera aliquots (500 µL) were stored at –20 °C until the immunoassays were performed for cytokines and antibodies. Whole blood sample (0.5 mL) was used to perform heamatogical analysis.

3.5 Determination of Hematological Profiles

Haematology processing and analysis were done within 24 hours of specimen collection.Determination of hematological profiles was done by full haemogram using vet ACT5 Diff Haematology Analyzer (Beckman Coulter Inc, Miami, Florida, USA) as per local standard operating procedures (SOPs). Routine Quality Assurance checks were performed basing on the guidelines of the machine; commercial controls were used in accordance with manufacturer's references. The haematological analyzer provided data on selected erythrograms and leukograms profiles, automatically bearing absolute numbers of red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), total white blood cell count (WBC), basophil, monocyte, lymphocyte, eosinophil and neutrophil.

3.6 Determination of the Cytokine profile of Guinea pigs

The positive serum sample and a negative control sample including the standards were used to optimize the enzyme-linked immunosorbent test (ELISA) through the use of checkboard titrations using different dilution rates to achieve the most effective optimal concentration and signal. Serum samples, standards and blanks were assayed in duplicates. The concentration of tumor necrotic factor alpha (TNF α), Interleukin -10 (IL -10) and Interleukin -4 (IL -4) were determined using Guinea Pig Interleukins ELISA Kit (96 wells) (Wuhan Fine Biotech Co. Ltd, China). Samples, standards and reagents were prepared according to manufactures instructions. Briefly, the samples (100 µl each) 2-fold diluted were pipetted into sample test wells followed by incubation for 90 minutes at 37 ºC to allow the sample (antigen) to be immobilized to the surface of the microtiterplate. The plate contents were discarded and washed twice with wash buffer to remove the unbound serum. 100 µl of Biotin–labeled antibody (Wuhan Fine Biotech Co. Ltd, China) at a dilution of 1:100 was pipetted into the bottom wells containing test sample, standards and blank wells to allow binding of antibody and antigen followed by incubation for 60 minutes at 37 ºC to enable the antigen-antibody complex formation and for easy detection. The cover was removed and the plate was washed three times with wash buffer, allowing the buffer to stay for two minutes in every wash to remove the unbound antibodies. 100 µl of HRP –Streptavidin Conjugate (SABC) was added into each well, covered and incubated at 37 ºC for 30 minutes which allows specific binding and easy detection. The plate was then washed five times allowing the wash buffer to stay for 2 minutes for every wash. 90µl of TMB substrate was pipetted into each well and incubated in a dark room for 20 minutes to allow chromogenic reaction followed by

addition of 50µl of stop solutions (2M H_2SO_4) into each well to stabilize signal for measurement at specific time point and avoid overproduction of signal which can lead to inaccurate results. The optical density was measured at absorbance of 450nm in a Microplate reader (RT-9600) (Rayto Life and Analytical Sciences Co., Ltd) immediately after addition of the stop solution.

Interferon concentration was determined using Guinea pig Interferon Gamma (IFNg) ELISA Kit 96T (Wuxi Donglin Sci & Tech Develop Co., Ltd, China). Samples, reagents and standards for this experiment were prepared according to manufactures instructions. Seven wells were prepared for standards and two wells for blank in duplicates. Standards, blank and sample (100 µl) was pipetted into the respective wells, covered and incubated for 2 hours at 37 ºC. The respective liquids were discarded carefully. Detection reagent A (100 µl) was added to each well, covered with plate sealer and incubated for 1 hour at 37 ºC to allow binding of antigen and antibody. The solution was then aspirated and wells were washed three times with wash buffer using multichannel pipette and allowed to stay for 2 minutes in every wash to remove the unbound antigen-antibody complex. The buffer was removed from wells by inverting and tapping the plate on an absorbent paper to completely dry it. Detection reagent B (100 µL) was added to each well, covered and incubated for 1 hour at 37 ºC to allow specific binding followed by washing five times. TMB Substrate solution (90 µl) was added to each well and incubated for 25 minutes at 37 ºC in a dark room to allow chromogenic formation for easy detection and signal amplification. 50 µl of stop solution (1.5M H_2SO_4) (Wuxi Donglin Sci & Tech Develop Co., Ltd, China) was added and mixed gently by tapping the plate to stop the antigen-

antibody reaction and allow results to be read at optimal time. The optical density was measured at absorbance 450 nm in a Microplate reader (Rayto Life and Analytical Sciences Co., Ltd) immediately after addition of the stop solution.

3.7 Determination of Antibody Profile

Immunoglobulin G and Immunoglobulin E antibodies detection was performed using Guinea Pig specific IgG and IgE pre-coated ELISA Assay Kit (Eagle Biosciences, USA). 100µl of serum sample in duplicate were pipetted into pre-designated wells and incubated at room temperature for 20 minutes to allow immobilization of serum (antigen) to the wells. After incubation, the contents were aspirated. The wells were then completely filled with wash buffer and washed three times. 100 µL of enzyme antibody conjugated with horseradish peroxidase at a dilution of 1:1000 was pipetted to each well and incubated at room temperature for 20 minutes. After incubation the wells were washed with washing buffer three times. 100µL of TMB substrate solution at 1:100 dilution was pipetted of into each well and incubated in the dark at room temperature for 10 minutes. 100µL of 1:100 dilution Stop Solution (2.0M H_2SO_4) was then added to each well followed by Optical density measurement in a plate reader at absorbance at 450 nm (Rayto Life and Analytical Sciences Co., Ltd) to obtain optical readings.

3.8 Data Analysis

Baseline, control and weekly data were entered into Microsoft Excel 2019. Statistical analysis was done by IBM SPSS software version 25.0. All the results were tested for normality using Shapiro-Wilk test and expressed as mean ± SD using descriptive

statistics. Data not normally distributed was subjected to the Mann-Whitney U test for independent samples to test the hypothesis and significance differences between the infected and control groups at each day. Friedman ANOVA was used to test the significance difference across the post-infection days. Wilcoxon signed rank test was used to test pairwise differences between the precise time points. The relationship of cytokines and antibodies was determined by computing a pearsons correlation. The test data were compared with that of the control to determine statistically significant differences at $p \leq 0.05$.

3.9 Ethical Consideration

Experimental animals were taken care to ensure that the procedures are appropriate and humane. Care and ethics were being considered through the following principles: Reducing the numbers of animals used for experimentation, avoiding unnecessary duplication of experiments, and minimizing pain and distress. For this study 24 animals were used. Unnecessary duplication was avoided to minimize the bleeding times of animals. The vein was punctured using a 25G needle and enough volume of blood was collected with a syringe.The punctured site was compressed to stop the bleeding .During the collection of blood the local anaesthetic injection (propofol) was applied on the collection site, no more than three attempts was made, continuous sampling was avoided and collecting more than three samples per day was avoided. Aseptic techniqsues were involved on laboratory animals whenever possible. All surgical procedures and anesthetization were conducted under the direct supervision of a person who was competent in the use of the phlebotomy procedures. The Masinde Muliro University of

Science and Technology ethical review committee provided ethical approval for this study and NACOSTI provided permit.

CHAPTER FOUR

RESULTS

4.1 Natural infestation of guinea pigs with *Tunga penetrans*

After controlled exposure, each animal became infested with a minimum of two and a maximum of five sand fleas on the foot pad and inside the earlobe. The most rapid penetration took place within 48 hours. Mean characteristics of experimental animals are shown in the Table 4.1 below.

Table 4:1. Demographic characterictics of experimental guinea pigs

Parameter	Infected (n=16)	Control (n=8)
Age in Days (mean and range)	111 (96-128)	108 (90-126)
Number of Male subjects	7	4
Number of Female subjects	9	4
Number of Embedded fleas (range)	2-5	0

4.2. Determination of Hematological Changes

4.2.1 Erythrograms Profile

The study revealed significant decrease in red blood cell (RBC) levels, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) during the post-infestation period. RBC levels declined consistently from day 5 to day 20 ($p= 0.0001$, $p= 0.002$, $p= 0.0.044$, $p= 0.003$) respectively. Hb levels notably decreased at day 5 ($p= 0.001$) and 10 ($p= 0.0001$). PCV showed substantial reductions at day 5 ($p= 0.039$), 15 ($p= 0.0001$), and 20 ($p= 0.001$).

MCHC decreased significant at day 5 (p= 0.004). MCH showed a significant decrease from day 10 to 20 (p= 0.005, p= 0.000, p= 0.0001) , onwards as shown in (Table 4.2) (Figure 4.1).

Table 4:2 Erythron changes in guinea pigs with tungiasis at post-infestation days.

Parameter	Baseline	Control	Day 5	Day 10	Day 15	Day 20
RBC (10^8 /µL)	5.25±0.23	5.26±0.22	5.02±0.22 **p = 0.0001**	4.86±0.10 **p = 0.002**	4.79±0.13 **p = 0.044**	4.68±0.13 **p = 0.003**
Hb (g/dl)	15.42±0.46	15.41±0.42	15.41±0.4 **p = 0.001**	14.66±0.3 **p = 0.0001**	13.99±0.3 **ns**	14.08±0.5 **ns**
PCV(%)	40.14±1.6	40.11±1.1	39.59±0.5 **p = 0.039**	38.55±0.5 **ns**	38.35±0.5 **p= 0.0001**	37.84±0.5 **p= 0.001**
MCHC (%)	34.70±0.78	34.70±0.79	33.77±0.6 **p=0.004**	33.50±0.6 **ns**	33.03±0.4 **ns**	32.78±0.6 **ns**
MCH (pg)	25.21±0.18	25.07 ±0.14	24.95±0.3 **ns**	24.59±0.2 **p= 0.005**	24.27 ±0.1 **p= 0.0001**	24.01±0.2 **p= 0.0001**

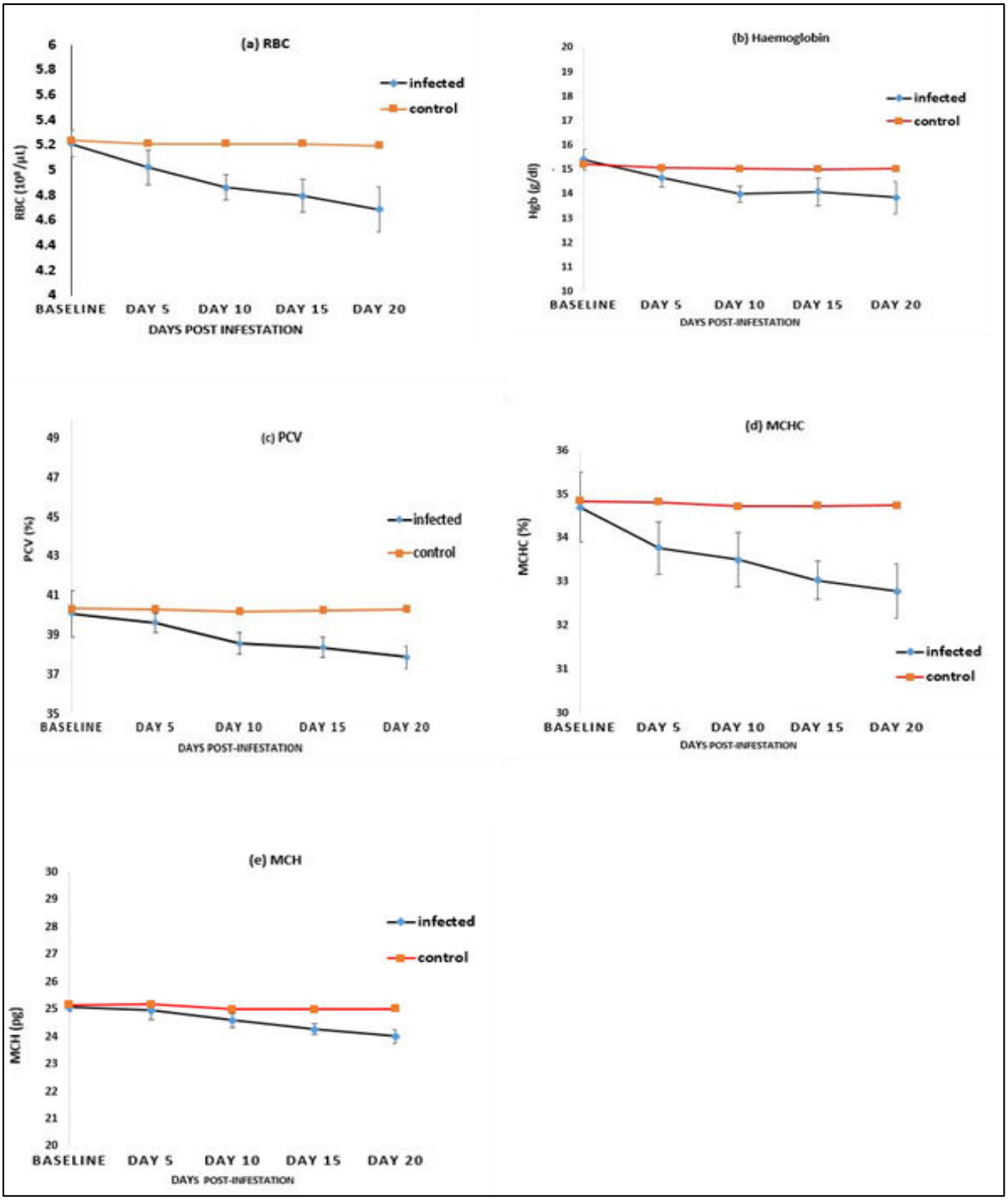


Figure 4:1.Erythrograms changes of guinea pigs infected with *T. penetrans* and the control (non-infected).

a.Red blood cells (RBC); b.hemoglobin (Hb) ; c.Packed Cell Volume (PCV) ; d.Mean Corpuscular Hemoglobin concentration (MCHC), e.Mean Corpuscular Hemoglobin Concentration MCH).

4.2.2 Leukograms Profile

White blood cells levels peaked significantly on day 10 ($p = 0.026$) and 15($p = 0.0001$), while neutrophils peaked at day 5($p = 0.001$), 10 ($p = 0.002$), and 20 ($p = 0.005$). Eosinophils elevated from day 5 to 20 ($p = 0.0001$, $p= 0.006$, $p = 0.0001$, $p = 0.03$) monocytes increased significantly from day 5 ($p = 0.002$),10 ($p = 0.005$) and 15 ($p = 0.007$), lymphocytes also exhibited increased levels at day 5 ($p = 0.001$) and day 10 ($p = 0.007$) significantly during the infection, indicating dynamic immune responses over the course of the study as shown in (Table 4.3) (Figure 4.2).

Table 4:3. Leukons changes in guinea pigs infected with *T. penetrans*

Parameter	Baseline	Control	Day 5	Day 10	Day 15	Day 20
WBC(10/µL)	8.85±0.42	8.86 ±0.44	8.90±0.42 **ns**	9.08 ±0.35 **P = 0.026**	9.52±0.19 **P = 0.0001**	9.39±0.33 **ns**
Neutrophils /Heterophils (10/µL)	3.07±0.16	3.07±0.14	3.42±0.4 **P = 0.001**	3.81±0.26 **P = 0.002**	3.93±0.3 **ns**	4.27 ±0.07 **P = 0.005**
Eosinophils (10/µL)	0.57±0.06	0.58 ±0.07	1.29±0.15 **P = 0.0001**	1.5±0.15 **ns**	2.32±0.15 **P = 0.0001**	2.43 ±0.26 **P = 0.032**
Monocytes (10/µl)	0.71±0.05	0.70 ±0.04	0.75±0.02 **P = 0.002**	0.78±0.03 **P = 0.005**	0.81±0.05 **ns**	0.72±0.03 **ns**
Lymphocytes (10/µl)	5.25±0.06	5.24 ±0.04	5.51±0.16 **P = 0.001**	5.65±0.09 **p = 0.007**	5.60 ±0.4 **ns**	5.53±0.3 **ns**

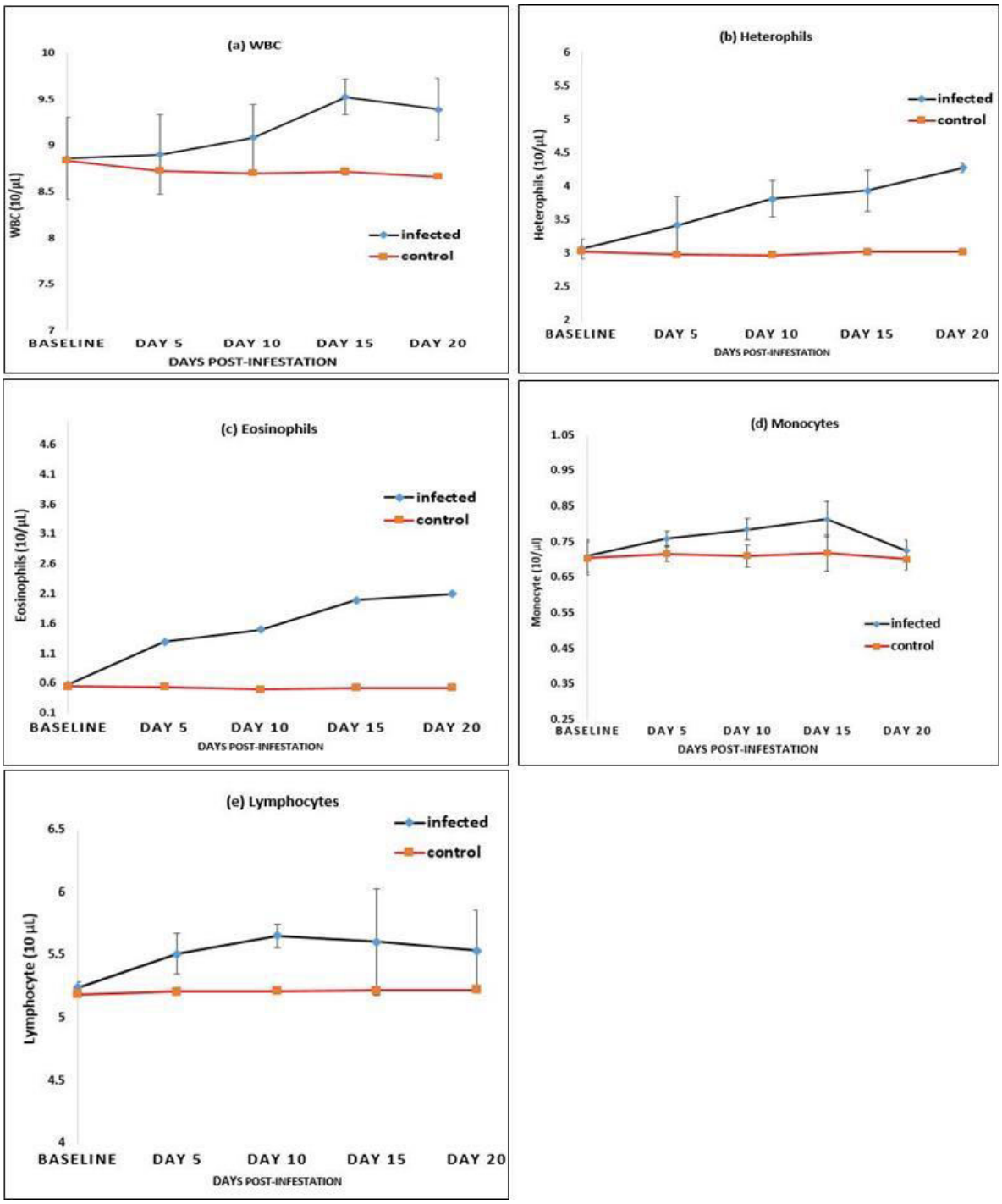


Figure 4:2 Leukograms changes of guinea pigs infected with *T. penetrans* and and the control (non-infected).

a.White blood cells (WBC) ; b.Neutrophils (Heterophils); c.Eosinophils ; d.Monocytes and e.Lymphocytes

4.3 Systemic levels of pro-inflammatory and anti-inflammatory cytokines

The systemic levels of TNF-α and IFN-γ increased significantly during the first two weeks of infection compared to the controls. TNF-α level was 109.88 ±11 pg/mL at baseline day zero (0) and this drastically increased at day (five) 5 (155.96 ±20 pg/mL; p = 0.000643) and peaked at day 10 (235.39±17 pg/mL; p = 0.000438) and declined steadily from day 15 (225.1±9 pg/mL; p = 0.0878) and 20 (155.263±9 pg/mL; p = 0.000436) respectively as shown in Figure 4.3.

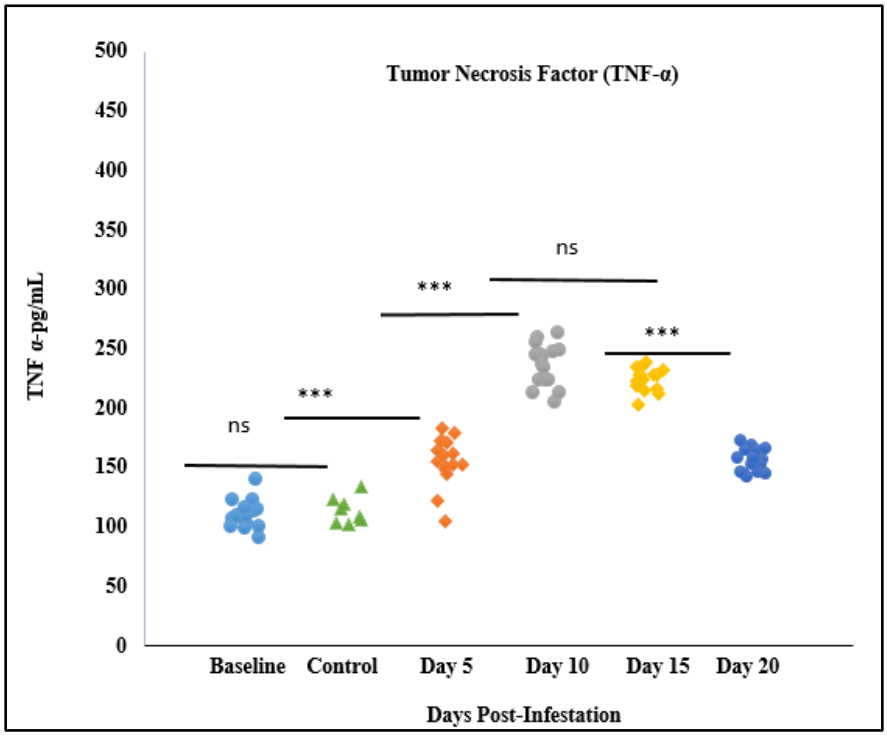


Figure 4:3 TNF-α concentrations following infestation with *T. penetrans*

*Asterisk indicates the significant effect, ***p≤ 0.0001, **p≤ 0.01, *p≤0.05. Friedman ANOVA analysis was used to (n=8) and infected (n=16). calculate p-value and Wilcoxon pairwise tests for multiple comparisons between the control*

IFN-γ, had 342.92±7 at day zero (0) and rose at day 5 (388.23±8 pg/mL; p = 0.00043) and 10 (425±5 pg/mL; p = 0.00044). The levels declined steadily on day 15 (383.53±7 pg/mL; p = 0.00043) and day 20 360.94±9 pg/mL; p = 0.00042) as shown in Figure 4.4.

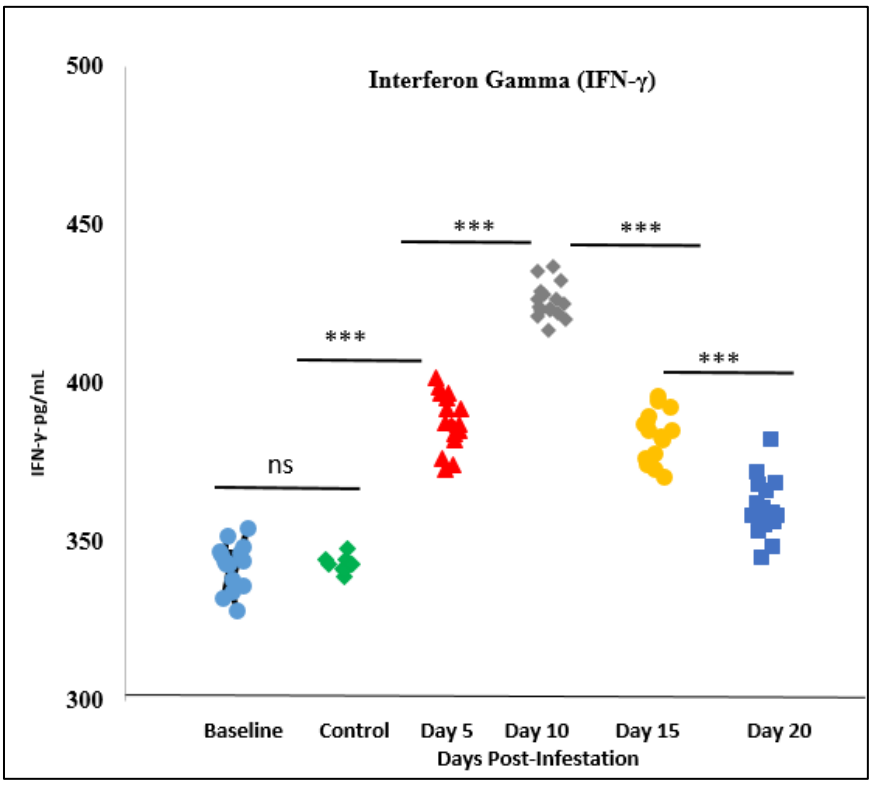


Figure 4:4. IFN-Y concentrations after infestation with *T. penetrans.*

*Asterisk shows significant effect, ****p≤ 0.0001, **p≤ 0.01, *p≤0.05. Friedman ANOVA analysis was used to calculate p-value and Wilcoxon pairwise tests for multiple comparisons between the control (n=8) and infected (n=16).*

IL-4 and IL-10 elevated during the course of infection. At day zero the levels of IL- 4 was 224.57±7 and this was elevated to (267.03±7 pg/mL; p = 0.00043) at day 5 IL-4 started to elevate significantly at day 5) , increased considerably at day 10 and 15 (305.45±5 pg/mL; p = 0.00044), (356.7±6 pg/mL; p = 0.000437) respectively and reduced at day 20 significantly (307.08±8 pg/mL, p = 0.00134) as shown in Figure 4.5.

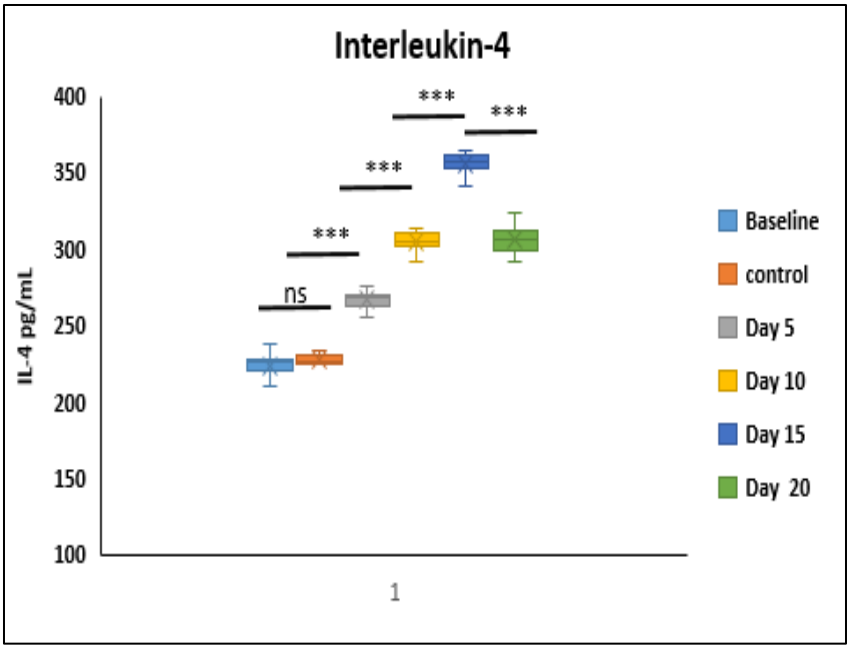


Figure 4:5. IL-4 concentrations after infestation with *T. penetrans*

*Asterisk shows significant effect, ****p≤ 0.0001, **p≤ 0.01, *p≤0.05. Friedman ANOVA analysis was used to calculate p-value and Wilcoxon pairwise tests for multiple comparisons between the control (n=8) and infected (n=16).*

IL-10 at day zero (0) was 230.12±5 pg /mL and started to elevate considerebly at day 5 upto day 15 (293.41±6 pg/mL; p = 0.00043), (324.41±4 pg/mL; p = 0.000436) (367.17±4 pg/mL; p = 0.000437) respectively and decrease significantly at day 20 (358.48±6 pg/mL; p = 0.00134) as shown in Figure 4.6.

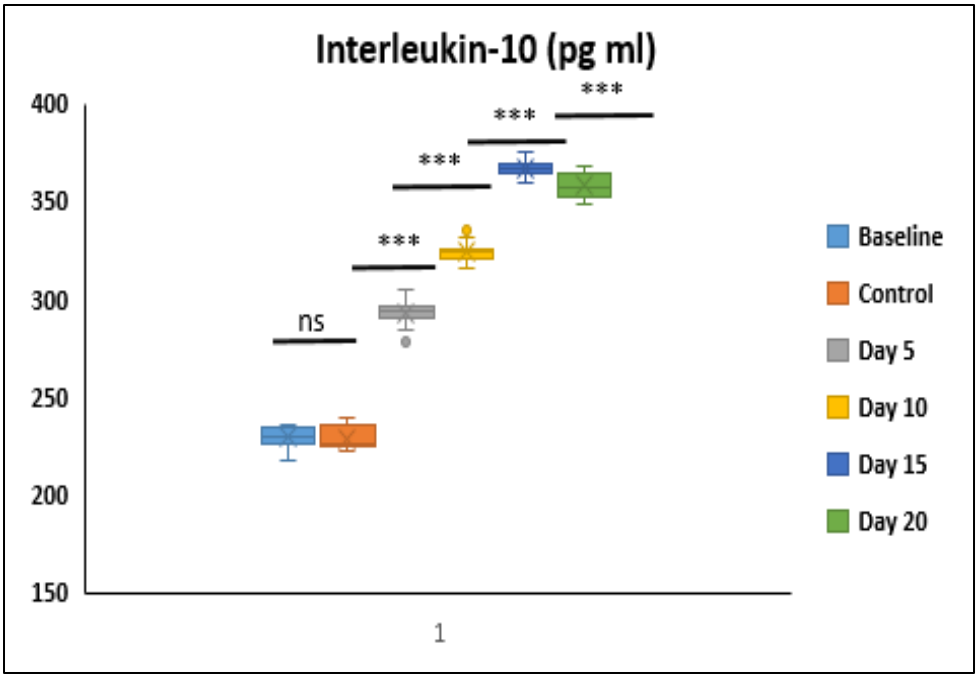


Figure 4:6. IL-10 concentrations following infestation with *T. penetrans.*

*Asterisk indicates significant effect, ****p≤ 0.0001, **p≤ 0.01, *p≤0.05. Friedman ANOVA analysis was used to calculate p-value and Wilcoxon pairwise tests for multiple comparisons between the control (n= 8) and infected (n= 16).*

4.3.1 Determination of Systemic Levels of IgE and IgG

IgE showed a significance increase in levels of serum concentrations at day 5 (100.70±3 ng/mL; p = 0.00043), 10 (167.81±5 ng/mL; p = 0.00043), 15 (231.9±13 ng/mL; p= 0.00044) and decreases significantly at day 20 (117.66±7 ng/mL; p = 0.00065) as compared with control as shown Figure 4.7.

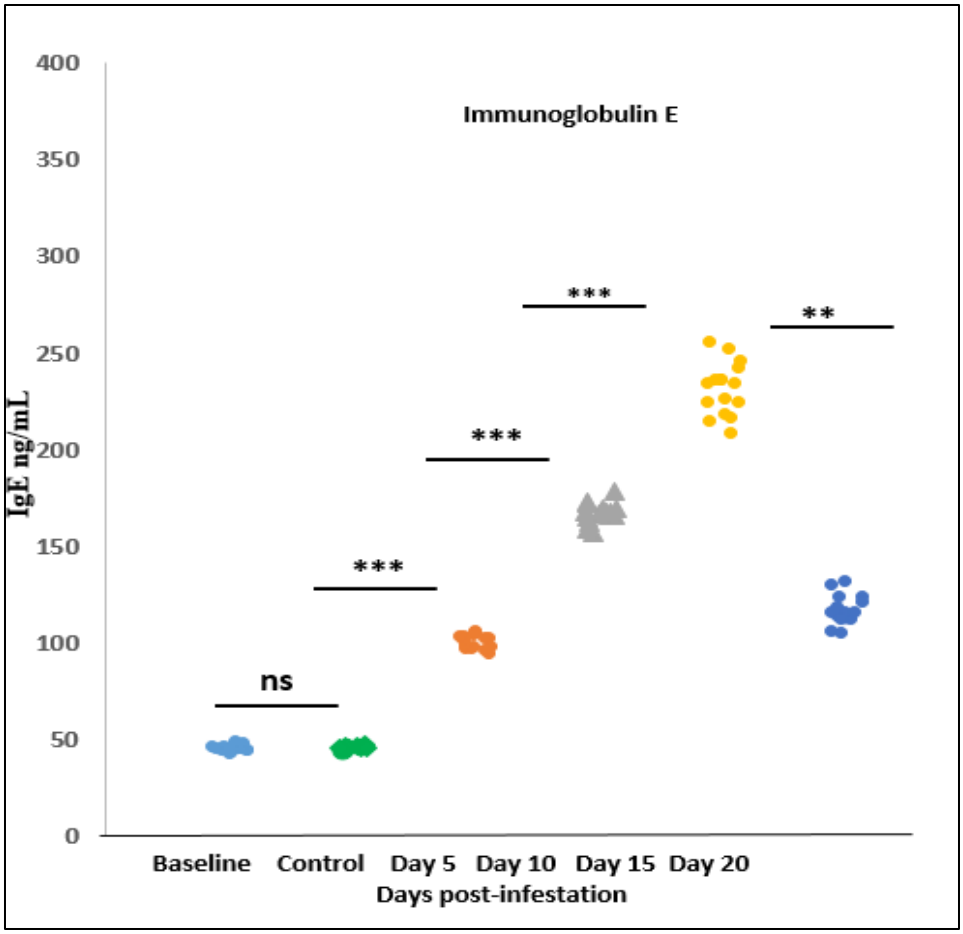


Figure 4:7.Concentrations of IgE following infestation with *T. penetrans*.

*Asterisk demonstrates significant effect, ***p≤ 0.0001, **p≤ 0.01, *p≤0.05. Friedman ANOVA analysis was used to calculate p-value and Wilcoxon pairwise tests for multiple comparisons between the control (n=8) and infected (n=16).*

IgG increases significantly from day 5 (196.1 ± 9 ng/mL; p = 0.00043), 10 (253.91±13 ng/mL; p = 0.00044), 15 (371.61±15 ng/mL; p = 0.00043) and decreases considerably at day 20 (261.95±7 ng/mL; p = 0.00049) after flea penetration as compared with control as shown in Figure 4.8.

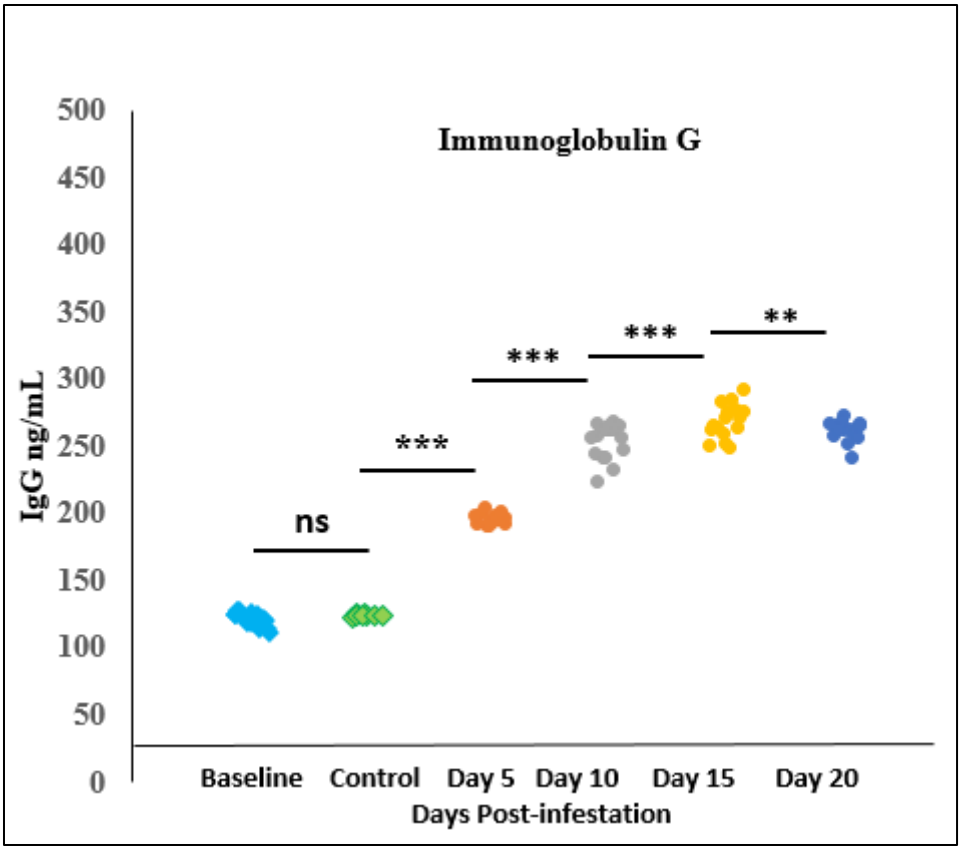


Figure 4:8. Concentrations of IgG following infestation with *T. penetrans*.

*Asterisk shows the significance, ***p≤ 0.0001, **p≤ 0.01, *p≤0.05. Friedman ANOVA analysis was used to calculate p-value and Wilcoxon pairwise tests for multiple comparisons between the control (n=8) and infected (n=16)*

4.4 Determining the Relationship between Cytokines and Antibodies

A strong positive correlation between TNF -α and IgE, IFN-Y and TNF-α, IgE and IL-4 ;IgG and IL-4 (r = 0.774, r = 0.636, r = 0.89, r = 0.791) was observed (Table 4.4). Negative correlation between IFN-γ and IL -10 (r= -0.518) and a moderate negative correlation between TNF-α and IL -10 (r = -0.369) (Table 4.4). A positive significance correlation was observed between TNF-α and Monocytes (r = 0.249) (Figure 4.9) TNF-α and lymphocytes (r = 0.036) with no significance (Figure 4.10).

Table 4:4 Pearsons correlation of cytokines and antibodies at post-infestation.

	N	Mean	1	2	3	4	5	6
TNF	16	192.931	1					
IFN	16	389.6611	0.636**	1				
IL-4	16	309.0694	0.548**	-0.100	1			
IL-`10	16	340.2647	-0.369	-0.518**	0.739**	1		
IgE	16	154.469	0.774**	0.246	0.896**	0.444**	1	.
IgG	16	245.295	0.479**	-0.094	0.791**	0.241	0.626**	1

The spearman correlation analysis of cytokines and antibodies in tungiasis infection.

*The key pro-inflammatory (TNF-α and IFN-γ) and the master regulatory (IL-10) anti-inflammatory cytokine (IL-4) and antibodies (IgE and IgG).**.Correlation is significant at the 0.01 level (2-tailed*).

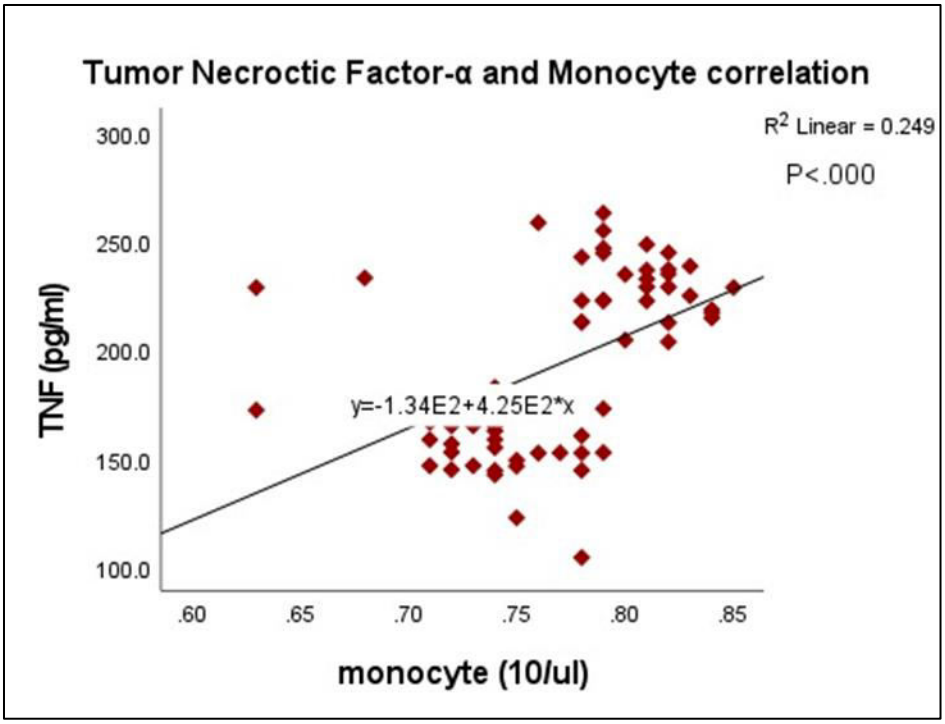


Figure 4:9. Pearson correlation was performed between TNF –α and Monocytes during disease progression (n=16).

Relationship between TNF –α expression and production as result of monocytes stimulation .The solid line represent the linear regression curve of the best fit.Correlation significant at the 0.01 level (2-tailed).

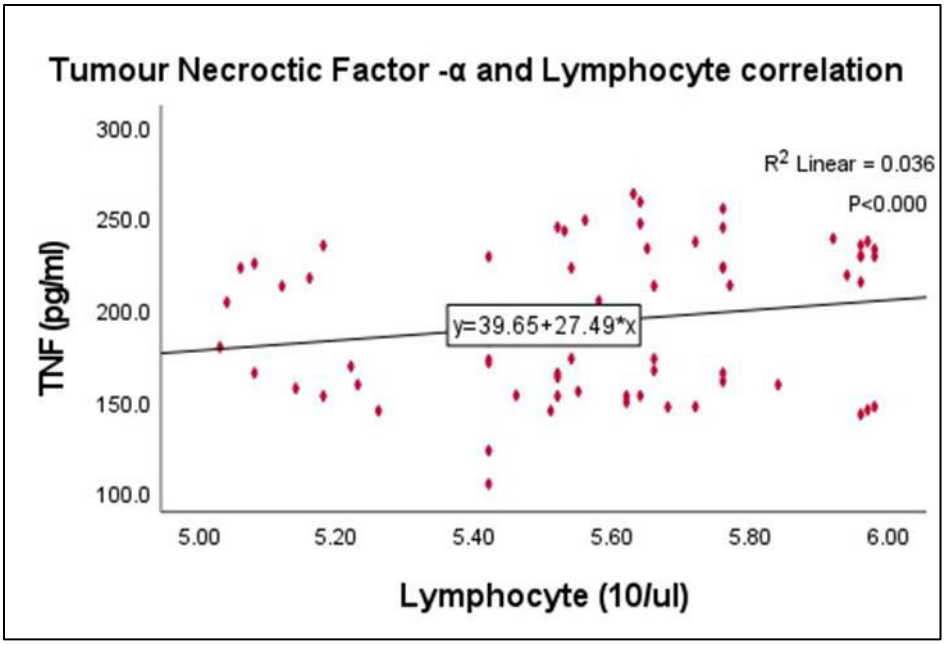


Figure 4:10 .Pearson correlation was performed between TNF –α and Monocytes during disease progression (n=16).

Relationship between TNF –α expression and production as result of monocytes stimulation .The solid line represent the linear regression curve of the best fit.Correlation is significant at the 0.01 level (2-tailed).

CHAPTER FIVE

DISCUSSIONS

5.1 Decrease in Erythrograms and increase in leukograms in guniea pigs infected with *Tunga penetrans*

Studies have shown that during ectoparasitic infections, there is a decline in erythrograns as well as an elevetion in leukograms (Nogueira et al., 2023). In the current study, a substantial decrease in values of RBC, Hb, PCV, MCH and MCHC post-infection days as compared to the controls was observed. These findings could be due to localized hemorrhaging, immune-mediated red blood cell execution and a slight decrease in RBC count in acute tungiasis. This is defined by a significant reduction in hemoglobin levels and red blood cell count as a result of persistent inflammation, and perhaps subsequent infections .This study showed similar results with the preliminary review on ecto-parasites done in invivo models which shows a decrease in total red blood cell count and its parameters (Nogueira et al., 2023). Simillarly, in intestinal parasites, a decrease in total RBC, PCV and haemoglobin was observed (Qadir et al., 2011). The low PCV found in guinea pigs with the infection may be related to destruction of red blood cells due to blood-feeding, which lead to development of lesions and ulcers, also as a result of immune response during infection (Almendros et al., 2023; Beus et al., 2024; Giadinis et al., 2012).

There is little information known on how leucocyte counts serve as identification of parasite clearing and healing.Therefore, the observations require more in-depth studies. However, according to research done in Brazil 20 years ago, there was a significant neutrophil-based inflammatory infiltrate (Eisele et al., 2003a; Muehlen et al., 2003) .The

current study showed significantly elevated total WBC, lymphocyte,monocyte, eosinophils and neutrophils in infected guinea pigs as compared to uninfected animals. The haematological changes typically as a result of tungiasis infection which could be due to host immune responses triggered by pathogens

The increased total white blood cell count observed in the current study might be as a result of inflammation as the immune system tries to fight off the parasitic infection by the sand flea and cellular and hormonal immune response in dermatitis (Ajith et al., 2017). Ectoparasites release toxins that cause local inflammation and necrosis thus predispose the animal to secondary bacterial infection (Sharma et al., n.d.). The eosinophilia reported in this study could be as a result of a hypersensitivity reaction caused by sand fleas as well as an increase in histamine levels that trigger the release of eosinophils into the bloodstream (Streicher, 2019). Several reports in helminthic and ecto-parasitic infections support the present findings (Milstein *et al.*, 2022; Norgan & Pritt, 2018b). Neutrophilia was observed to increase during post-infestation days and this can be attributed to secondary bacterial infections that result from ulcerated wounds induced by sand fleas (Mutebi et al., 2021c; Nyangacha et al., 2017). Similar observation was also reported by Shameena *et al* (2021)

Lymphocytosis observed in the study could be as a result of immune response and inflammatory responses that lead to migration of cells to the infection site thus the increase in lymphocyte in blood. Similar findings was reported by Udeh,Akhtar and Beyzay (Akhtar et al., 2023, 2023; Beyzay et al., 2023). Monocytosis was observed in the present findings; which may be as a result of cells recruitment at the site of infection due to feeding mechhanism and embedding of sandfleas that causes ulceration thus

inflammation as reported by Osorio,Ehsan and cunningham (Cunningham & Mills, 2023; Ehsan et al., 2024; Osorio et al., 2023). These findings therefore showed that haematological alterations are hallmark for identification of tungiasis infections. The haematological values reported can guide veterinarians in clinical diagnosis of tungiasis and other ectoparasitic blood parasitic infections and how it is involved in parasite clearing.

5.2 Upregulation of pro and anti-inflammatory cytokines in guniea pigs infected with *Tunga penetrans* .

The results indicated that the infected animals had elevated serum concentrations of TNF-α during the first two weeks of infestation as compared to the controls. Penetration of *Tunga penetrans* into the skin, triggers immune response that involved both Th1 and Th2 pathways. Early phase of infection, particularly day 5 and 10, which is marked by a pronounced increase in Th1 cytokines that is IFN-γ and TNF-α (Feldmeier, Eisele, et al., 2004; Feldmeier, Witt, et al., 2004). The cytokines play crucial role in immediate inflammatory response in ectoparasites (Huang et al., 2023; Palavecino et al., 2023). This significant change is attributed to inflammatory responses where TNF-α is released after stimulation of mast cells, lymphocytes and macrophages. TNF-α is typically involved with the immunity to intracellular pathogens and inflammation (Baeten & Kuchroo, 2013). The findings also indicated that guinea pigs with infection had elevated serum concentrations of IFN-γ. This could be as a result of inflammation after flea penetration. Previous study indicated that after a day of penetration, lymphocytes, eosinophil, macrophages and mast cells were observed in the inflammatory infiltrates in human tissue sections which prompt the activation and release of the cytokines (Eisele et al.,

2003d). As the infection progress to the later stages, specifically on day 15 and 20, a significant decline in the levels of TNF-α and IFN-γ is observed. The shift from the acute inflammatory phase to a resolution phase. The reduction in these pro-inflammatory cytokines is crucial to prevent chronic inflammation and potential tissue damage that could result from prolonged immune activation. This findings could be attributed to the lymphocyte activation effect which causes the release of IFN-γ cytokine in serum during the first two weeks of infestation.

The current study observed elevated expression of anti-inflammatory cytokines. Regulatory cytokine IL-10 and IL-4 concentration was significantly higher in animals with infection than in the control group. The serum concentration of IL-4 elevated during the first week of infection at day 5 which could indicate early switch of TH-2 helper cells. The prompt increase in IL-4 in the current research is attributed to the fact that Interleukin-4 play a central role in humoral immune defense against parasite during early phase of infection leading to precursor helper cells differentiation into TH2 subsets (Nono et al., 2017; Vacca & Le Gros, 2022). Modulatory cytokine IL-10 also got elevated during the tenth day of infection. This scenario is similar to previous studies where the up-regulation of type-2 helper T cells was observed in scabies after mites infestation and helminthic disease caused by *Strongyloides stercolaris* in humans (Anuradha et al., 2016; Bhat et al., 2017b). The relative elevated IL-10 detected in systemic circulation could define the release of immunosuppressive molecules released by the ectoparasite and host defense mechanisms due to invasive Th1 cytokines, TNF-α and IFN-γ in regulating inflammation thus preventing tissue damage (Richmond & Harris, 2014) . The release of parasitic infections triggers immune responses composed of

type-2 cytokines, which contributes to general protective immune response to infections as observed in intestinal helminths (Mishra et al., 2014). A negative correlation between TNF-α, IFN-Y and IL-10,as the pro-inflammatory cytokines is elevated the modulatory cytokine (IL-10) declines and conversely , this was seen also in patients with *schistosoma mansoni* infections (Gomides et al., 2024) . This mechanism of inverse correlation indicates the modulatory nature of IL-10 in regulateting the immune response so as to protect the host from tissue damage as result of inflammatory cytokines production.

5.3 Elevation of antibody levels in guinea pigs infected with *Tunga penetrans*

There were elevated levels of IgE during the first week of *T. penetrans* infestation and with a peak at day 10 as compared to the controls. This could be attributed to inflammation and allergies during the first days of infection as humoral immune response confers protective immunity (Baeten & Kuchroo, 2013). Consequently, studies done on scabies, an ecto-parasitic disease shows an increased circulating IgE levels in humans which is similar to the findings of the current study (Bhat et al., 2017c; Leiferman & Peters, 2018). Similarly, as observed in helminths, enzymes have also been found to be powerful inducers of type 2 immunity and IgE responses, since various skin, connective tissue, basement membranes and extracellular matrix structures are digested during penetration within the host (d'Alessandro et al., 2021; Male et al., 2020).

As the immune response evolves, there was a significant increase in IgG levels, typically observed after the initial weeks of infection. This significant increase in total serum

concentrations occurred since IgG antibodies are produced after IgM and thus take at least two weeks to reach significant levels (Bhat et al., 2017a; Huang et al., 2023).

IgG antibodies are essential for long-term immunological responses in neutralizing parasite through preventing further infection. This pattern of antibody response, with early IgE followed by sustained IgG, is not unique to tungiasis but is also seen in other ectoparasitic infections, in infested humans, myasis a self limited infection that has similar manifestation to tungiasis resulted in an increase in IgG levels in serum as compared to non-infected patients as described by Walton (Walton, 2010).This observation is also consistent with the serological studies with increased IgG1 and IgG2 responses in mites infected pigs and sheep during primary infestation

CHAPTER SIX

CONCLUSION AND RECCOMENDATIONS

6.1 Conclusions

Following review of literature, methodology and results of this study, the following conclusions were drawn:

1. Tungiasis infection induces elevation of pro-inflammatory and anti-inflammatory cytokines. The potential pro-inflammatory cytokines, the TNF-α and IFN-γ significantly elevated at day 5 to 15 of disease progression as high potential indicators for inflammation responses following sand flea penetration to enable parasite clearance. The master regulatory cytokine IL-10 an anti-inflammatory cytokine peaked at day 15 -20 to curb the inflammatory effects of invasive pro-inflammatory cytokines.
2. Tungiasis results to elevation of IgE and IgG antibodies, with peaks occurring 10 to 15 days post infection followed by a subsequent decline.
3. Tungiasis results to a change in haematological parameters with a significant decrease in Erythrograms indices and elevation of leukograms.

6.2 Recommendations from the Current Study

1. The findings elucidated consequent anemia and increase in leukocytes by day 15 post-infestation day. We recommend total blood cell count as an early indicator of infection.

2. There is need to explore the potential role of pro-inflammatory and anti-inflammatory cytokines for diagnostic and treatment purposes as they serve as early indicators of infection.
3. There is necessity to discover the potential therapeutic role of antibody for diagnostic and treatment purposes as they serve as early indicators of infection.

6.3 Recommendations for Future Studies

1. Limited number of cytokines was used in this study. Future studies can consider the whole range of cytokines to get a comprehensive picture
2. There is need to examine other aspects of the immune system, such as T cell responses, antibody subclasses, on the development and resolution of tungiasis. This may reveal new therapeutic targets and offer greater understanding into the host-parasite relationship.
3. To further our understanding of the disease, future research on the hematological profiling of tungiasis should employ molecular analysis in addition to a multifaceted approach. Integrating therapeutic approaches would improve our capacity to properly control and mitigate the impact of tungiasis.
4. This study was done using animal models. It would be imperative to have these studies in humans in order to see whether similar responses would be seen.
5. There is need to unravel mechanisms involved in regulation of the disease through understanding interplay of various immunological mediators.

REFERENCES

Abrha, S., Heukelbach, J., Peterson, G. M., Christenson, J. K., Carroll, S., Kosari, S., Bartholomeus, A., Feldmeier, H., & Thomas, J. (2021). Clinical interventions for tungiasis (sand flea disease): A systematic review. *The Lancet Infectious Diseases*, *21*(8), e234–e245. https://doi.org/10.1016/S1473-3099(20)30853-7

Abrha, S., Tesfaye, W., & Thomas, J. (2021). Therapeutic Potential of Tea Tree Oil for Tungiasis. *The American Journal of Tropical Medicine and Hygiene*, *105*(5), 1157–1162. https://doi.org/10.4269/ajtmh.21-0427

Adenkola, A. Y., Ayo, J. O., Sackey, A. K. B., & Adelaiye, A. B. (2009). Haematological and serum biochemical changes in pigs administered with ascorbic acid and transported by road for four hours during the harmattan season. *J. Cell Anim. Biol*, *3*(2), 21–28. https://doi.org/10.5897/jcab.9000086

Ade-Serrano, M. A., & Ejezie, G. C. (1981). Prevalence of tungiasis in Oto-Ijanikin village, Badagry, Lagos State, Nigeria. *Annals of Tropical Medicine and Parasitology*, *75*(4), 471–472. https://doi.org/10.1080/00034983.1981.11687469

Ahmed, S. M., & Ali, A. H. (2013). Serum proteins and leucocytes differential count in the common carp (Cyprinus carpio L.) infested with ectoparasites. *Mesopotamian Journal of Marine Sciences*, *28*(2), 151–162. https://doi.org/10.58629/mjms.v28i2.148

Ajith, Y., Dimri, U., Singh, S. K., Gopalakrishnan, A., Devi, G., Verma, M. R., Joshi, V., & Alam, S. (2017). Lice induced immuno-oxidative wreckage of goats. *Veterinary Parasitology*, *242*, 24–30. https://doi.org/10.1016/j.vetpar.2017.05.019

Akhtar, T., Amanat, M. U., Wazir, N., Naeem, M. I., Ammar, M., Naeem, M. A., Idrees, A., Ahmad, W., Tyagi, R., & Slack, V. (2023). Pathology of Parasitic Infections. In *Parasitism and Parasitic Control in Animals: Strategies for the Developing World* (pp. 21–39). CABI GB. https://doi.org/10.1079/9781800621893.0002

Allen, K. E. (2005). *Improved transmission of Ehrlichia canis to dogs by sequential tick infestations using field-representative numbers of naturally infected Rhipicephalus sanguineus*. University of Georgia. https://doi.org/10.1016/j.vetpar.2013.07.026

Almendros, A., Choi, Y. R., Bęczkowski, P. M., Baiker, K., Barrs, V. R., & Beatty, J. A. (2023). Babesia gibsoni Infection in a Cat with Immune-Mediated Haemolytic Anaemia and Thrombocytopenia. *Animals*, *13*(13), 2128. https://doi.org/10.3390/ani13132128

Anuradha, R., Munisankar, S., Bhootra, Y., Jagannathan, J., Dolla, C., Kumaran, P., Nutman, T. B., & Babu, S. (2016). IL-10-and TGFβ-mediated Th9 responses in a human helminth infection. *PLoS Neglected Tropical Diseases*, *10*(1), e0004317. https://doi.org/10.1371/journal.pntd.0004317

AO Elsaftawy, E., Samanoudy, S., M Wassef, R., & H Morsy, S. (2023). Zoonotic parasites with significant importance for tropical dermatology. *Parasitologists United Journal*, *16*(2), 100–113. https://doi.org/10.21608/puj.2023.228039.1218

Ariza, L., Seidenschwang, M., Buckendahl, J., Gomide, M., Feldmeier, H., & Heukelbach, J. (2007). Tungiasis: A neglected disease causing severe morbidity in a shantytown in Fortaleza, State of Ceará. *Revista Da Sociedade Brasileira de Medicina Tropical*, *40*(1), 63–67. https://doi.org/10.1590/s0037-86822007000100013

Arlian, L. G., Morgan, M. S., Estes, S. A., Walton, S. F., Kemp, D. J., & Currie, B. J. (2004). Circulating IgE in patients with ordinary and crusted scabies. *Journal of Medical Entomology*, *41*(1), 74–77. https://doi.org/10.1603/0022-2585-41.1.74.

Ashour, D. S., & Othman, A. A. (2020). Parasite–bacteria interrelationship. *Parasitology Research*, *119*(10), 3145–3164. https://doi.org/10.1007/s00436-020-06804-2

Baeten, D. L., & Kuchroo, V. K. (2013). How Cytokine networks fuel inflammation: Interleukin-17 and a tale of two autoimmune diseases. *Nature Medicine*, *19*(7), 824–825. https://doi.org/10.1038/nm.3260

Basketter, D. A., & Kimber, I. (2011). Assessing the potency of respiratory allergens: Uncertainties and challenges. *Regulatory Toxicology and Pharmacology*, *61*(3), 365–372. https://doi.org/10.1016/j.yrtph.2011.10.001

Beus, K., Goudarztalejerdi, A., & Sazmand, A. (2024). Molecular detection and identification of hemotropic Mycoplasma species in dogs and their ectoparasites in Iran. *Scientific Reports*, *14*(1), 580. https://doi.org/10.1038/s41598-024-51173-w

Beyzay, F., Hosseini, A. Z., Hazrati, A., Karimi, M., & Soudi, S. (2023). Autophagy induced macrophages by α-alumina (α-AL2O3) conjugated cysteine peptidase, enhances the cytotoxic activity of CD8+ T lymphocytes against Leishmania major. *BioImpacts*. https://doi.org/10.34172/bi.2023.25282

Bhat, S. A., Mounsey, K. E., Liu, X., & Walton, S. F. (2017a). Host immune responses to the itch mite, Sarcoptes scabiei, in humans. *Parasites & Vectors*, *10*(1), 385. https://doi.org/10.1186/s13071-017-2320-4

Bhat, S. A., Mounsey, K. E., Liu, X., & Walton, S. F. (2017b). Host immune responses to the itch mite, Sarcoptes scabiei, in humans. *Parasites & Vectors*, *10*, 385. https://doi.org/10.1186/s13071-017-2320-4

Chen, Y., Cui, W., Li, X., & Yang, H. (2021). Interaction Between Commensal Bacteria, Immune Response and the Intestinal Barrier in Inflammatory Bowel Disease. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.761981

Coates, S. J., Thomas, C., Chosidow, O., Engelman, D., & Chang, A. Y. (2020). Ectoparasites: Pediculosis and tungiasis. *Journal of the American Academy of Dermatology*, *82*(3), 551–569. https://doi.org/10.1016/j.jaad.2019.05.110

Cockerell, C., Mihm Jr, M. C., Hall, B. J., Chisholm, C., Jessup, C., & Merola, M. (2013). *Dermatopathology: Clinicopathological Correlations*. Springer Science & Business Media. https://doi.org/10.46889/jdr.2022.3210

Cunningham, K. T., & Mills, K. H. (2023). Modulation of haematopoiesis by protozoal and helminth parasites. *Parasite Immunology*, e12975. https://doi.org/10.1111/pim.12975

d'Alessandro, M., Bergantini, L., Perrone, A., Cameli, P., Beltrami, V., Alderighi, L., Pini, L., Bargagli, E., & Saletti, M. (2021). House Dust Mite Allergy and the Der p1 Conundrum: A Literature Review and Case Series. *Allergies*, *1*(2), 108–114. https://doi.org/10.3390/allergies1020008

Deka, M. A. (2020). Mapping the geographic distribution of tungiasis in Sub-Saharan Africa. *Tropical Medicine and Infectious Disease*, *5*(3), 122. https://doi.org/10.3390/tropicalmed5030122

Deka, M. A., & Heukelbach, J. (2022). Distribution of tungiasis in Latin America: Identification of areas for potential disease transmission using an ecological niche model. *The Lancet Regional Health–Americas*, *5*. https://doi.org/10.1016/j.lana.2021.100080

Dos Santos, K. C., Brandão Guedes, P. E., Teixeira, J. B. de C., Harvey, T. V., & Carlos, R. S. A. (2023c). Treatment of Animal Tungiasis: What's New? *Tropical Medicine and Infectious Disease*, *8*(3), 142. https://doi.org/10.3390/tropicalmed8030142

Ehsan, M., Hu, R.-S., Wang, M., Hou, J.-L., Rashid, M., & Malik, M. I. (2024). Immune modulation of goat monocytes by Fasciola gigantica Legumain-1 protein (Fg-LGMN-1). *Experimental Parasitology*, *256*, 108671. https://doi.org/10.1016/j.exppara.2023.108671

Eisele, M., Heukelbach, J., Van Marck, E., Mehlhorn, H., Meckes, O., Franck, S., & Feldmeier, H. (2003a). Investigations on the biology, epidemiology, pathology and control of Tunga penetrans in Brazil: I. Natural history of tungiasis in man. *Parasitology Research*, *90*(2), 87–99. . https://doi.org/10.1007/s00436-002-0817-y

Elieh Ali Komi, D., Wöhrl, S., & Bielory, L. (2020). Mast cell biology at molecular level: A comprehensive review. *Clinical Reviews in Allergy & Immunology*, *58*, 342–365. https://doi.org/10.1007/s12016-019-08769-2

Elson, L., Kamau, C., Koech, S., Muthama, C., Gachomba, G., Sinoti, E., Chondo, E., Mburu, E., Wakio, M., Lore, J., Maia, M., Adetifa, I., Orindi, B., Bejon, P., & Fillinger, U. (2023a). National prevalence and risk factors for tungiasis in Kenya. *Infectious Diseases of Poverty*, *12*(1), 85. https://doi.org/10.1186/s40249-023-01131-x

Elson, L., Wiese, S., Feldmeier, H., & Fillinger, U. (2019). Prevalence, intensity and risk factors of tungiasis in Kilifi County, Kenya II: Results from a school-based observational study. *PLoS Neglected Tropical Diseases*, *13*(5), e0007326. https://doi.org/10.1371/journal.pntd.0007326

Enwemiwe, V. N., Ojianwuna, C. C., & Anyaele, O. O. (2021). Intensity and clinical morbidities of s*and Control*, *14*, e00215. https://doi.org/10.1016/j.parepi.2021.e00215

Eze, J. I., Onunkwo, J. I., Shoyinka, S. V. O., Chah, F. K., Ngene, A. A., Okolinta, N., Nwanta, J. A., & Onyenwe, I. W. (2010). Haematological profiles of pigs raised under intensive management system in South-Eastern Nigeria. *Nigerian Veterinary Journal*, *31*(2).

Feldmeier, H., Eisele, M., Sabóia-Moura, R. C., & Heukelbach, J. (2003). : Severe Tungiasis in Underprivileged Communities: Case Series from Brazil. *Emerging Infectious Diseases*, *9*(8), 949. https://doi.org/10.3201/eid0908.030041

Feldmeier, H., Eisele, M., Van Marck, E., Mehlhorn, H., Ribeiro, R., & Heukelbach, J. (2004). Investigations on the biology, epidemiology, pathology and control of Tunga penetrans in Brazil: IV. Clinical and histopathology. *Parasitology Research*, *94*(4), 275–282. https://doi.org/10.1007/s00436-004-1219-0

Feldmeier, H., Heukelbach, J., Eisele, M., Ribeiro, R., Harms, G., Mehlhorn, H., & Liesenfeld, O. (2003). Investigations on the biology, epidemiology, pathology and control of Tunga penetrans in Brazil: III. Cytokine levels in peripheral blood of infected humans. *Parasitology Research*, *91*(4), 298–303.

Feldmeier, H., Heukelbach, J., Ugbomoiko, U. S., Sentongo, E., Mbabazi, P., Samson-Himmelstjerna, G. von, Krantz, I., & Tungiasis, T. I. E. G. for. (2014). Tungiasis—A Neglected Disease with Many Challenges for Global Public Health. *PLOS Neglected Tropical Diseases*, *8*(10), e3133. https://doi.org/10.1371/journal.pntd.0003133

Feldmeier, H., Heukelbach, J., Ugbomoiko, U. S., Sentongo, E., Mbabazi, P., von Samson-Himmelstjerna, G., Krantz, I., & The International Expert Group for Tungiasis. (2014a).

Tungiasis—A Neglected Disease with Many Challenges for Global Public Health. *PLoS Negl Trop Dis*, *8*(10), e3133. https://doi.org/10.1371/journal.pntd.0003133

Feldmeier, H., Heukelbach, J., Ugbomoiko, U. S., Sentongo, E., Mbabazi, P., von Samson-Himmelstjerna, G., Krantz, I., & The International Expert Group for Tungiasis. (2014b). Tungiasis—A Neglected Disease with Many Challenges for Global Public Health. *PLoS Negl Trop Dis*, *8*(10), e3133. https://doi.org/10.1371/journal.pntd.0003133

Feldmeier, H., Sentongo, E., & Krantz, I. (2013). Tungiasis (sand flea disease): A parasitic disease with particular challenges for public health. *European Journal of Clinical Microbiology & Infectious Diseases*, *32*(1), 19–26.

Feldmeier, H., Witt, L.-H., Schwalfenberg, S., Albuquerque Ribeiro, R., Queiroz Cunha, F., Harms, G., Mehlhorn, H., Liesenfeld, O., & Heukelbach, J. (2004). Investigations on the biology, epidemiology, pathology and control of Tunga penetrans in Brazil. V. Cytokine concentrations in experimentally infected Wistar rats. *Parasitology Research*, *94*(5), 371–376.

Francesconi, F., & Lupi, O. (2012). Myiasis. *Clinical Microbiology Reviews*, *25*(1), 79–105.

Giadinis, N. D., Chochlakis, D., Kritsepi-Konstantinou, M., Makridaki, E., Tselentis, Y., Kostopoulou, D., Karatzias, H., & Psaroulaki, A. (2012). Haemolytic disease in sheep attributed to a Babesia lengau-like organism. *Veterinary Record-English Edition*, *170*(6), 155.

Gibbs, N. F. (2009). *Tungiasis. eMedicine. WedMD*.

Girma, M., Astatkie, A., & Asnake, S. (2018). Prevalence and risk factors of tungiasis among children of Wensho district, southern Ethiopia. *BMC Infectious Diseases*, *18*, 456. https://doi.org/10.1186/s12879-018-3373-5

Gitau, A. K., Oyieke, F. O., & Richard, W. (2021). *Assessment of tungiasis management knowledge in Kandara sub county, Kenya*.

Gomides, T. A. R., de Souza, M. L. M., de Figueiredo, A. B., Lima, M. R., Silveira, A. M. S., de Assis, G. F. M., Fraga, L. A. O., Silveira-Nunes, G., Martucci, L., & Garcia, J. D. (2024). Expression of SmATPDases 1 and 2 in Schistosoma mansoni eggs favours IL-10 production in infected individuals. *Parasite Immunology*, *46*(1), e13017.

Guidelines, Standards & Policies Portal. (n.d.). Retrieved May 20, 2023, from http://guidelines.health.go.ke/#/category/12/95/meta

Harvanová, G., Duranková, S., & Bernasovská, J. (2023). The role of cytokines and chemokines in the inflammatory response. *Alergologia Polska-Polish Journal of Allergology*, *10*(3), 210–219.

Harvey, T. V., Linardi, P. M., Carlos, R. S. A., & Heukelbach, J. (2021). Tungiasis in domestic, wild, and synanthropic animals in Brazil. *Acta Tropica*, *222*, 106068.

Heukelbach, J., Harvey, T. V., & Calheiros, C. M. L. (2022a). Tunga Spp. And Tungiasis in Latin America. In *Infectious Tropical Diseases and One Health in Latin America* (pp. 151–168). Springer.

Heukelbach, J., Harvey, T. V., & Calheiros, C. M. L. (2022b). Tunga Spp. And Tungiasis in Latin America. In *Infectious Tropical Diseases and One Health in Latin America* (pp. 151–168). Springer.

Heukelbach, J., Wilcke, T., Eisele, M., & Feldmeier, H. (2002). Ectopic localization of tungiasis. *The American Journal of Tropical Medicine and Hygiene*, *67*(2), 214–216.

Huang, K., Jiang, L., Huang, W., Li, X., Yuan, L., Jiang, J., Zhou, S., Wang, Y., & Xie, J. (2023). Investigating the interplay between *Cryptocaryon irritans* ectoparasite infection and the

immune responses of the head kidney in silver pomfret (*Pampus argenteus*). *Aquaculture*, *575*, 739780. https://doi.org/10.1016/j.aquaculture.2023.739780

Hunter, L., Ruedas-Torres, I., Agulló-Ros, I., Rayner, E., & Salguero, F. J. (2023). Comparative pathology of experimental pulmonary tuberculosis in animal models. *Frontiers in Veterinary Science*, *10*.

Johannessen, S. F. (2022). The Chagos Islands and Indian Ocean Geopolitics. In *Oxford Research Encyclopedia of Asian History*.

Leiferman, K. M., & Peters, M. S. (2018). Eosinophil-related disease and the skin. *The Journal of Allergy and Clinical Immunology: In Practice*, *6*(5), 1462-1482. e6.

Male, D., Male, V., & Peebles, R. S. (2020). *Immunology E-Book*. Elsevier Health Sciences.

Martins, A. C. T., Brito, A. R. de, Kurizky, P. S., Gonçalves, R. G., Santana, Y. R. T., Carvalho, F. C. A. de, & Gomes, C. M. (2021). The efficacy of topical, oral and surgical interventions for the treatment of tungiasis: A systematic review of the literature. *PLOS Neglected Tropical Diseases*, *15*(8), e0009722. https://doi.org/10.1371/journal.pntd.0009722

Mathenge, M., Sonneveld, B. G. J. S., & Broerse, J. E. W. (2020). A Spatially Explicit Approach for Targeting Resource-Poor Smallholders to Improve Their Participation in Agribusiness: A Case of Nyando and Vihiga County in Western Kenya. *ISPRS International Journal of Geo-Information*, *9*(10), Article 10. https://doi.org/10.3390/ijgi9100612

McNair, C. M. (2015). Ectoparasites of medical and veterinary importance: Drug resistance and the need for alternative control methods. *Journal of Pharmacy and Pharmacology*, *67*(3), 351–363.

Milstein, M. S., Shaffer, C. A., Suse, P., Marawanaru, A., Heinrich, D. A., Larsen, P. A., & Wolf, T. M. (2022). A mixed-methods approach to understanding domestic dog health and

disease transmission risk in an indigenous reserve in Guyana, South America. *PLoS Neglected Tropical Diseases*, *16*(6), e0010469.

Mishra, P. K., Palma, M., Bleich, D., Loke, P., & Gause, W. C. (2014). Systemic impact of intestinal helminth infections. *Mucosal Immunology*, *7*(4), 753–762.

Mørkve, Å. W., Sitienei, J., & Van den Bergh, G. (2023). A qualitative case study of community experiences with Tungiasis in high prevalence villages of Bungoma County, Kenya:"The whole body aches and the jiggers are torturing me!" *PLOS Neglected Tropical Diseases*, *17*(4), e0011304.

Muehlen, M., Heukelbach, J., Wilcke, T., Winter, B., Mehlhorn, H., & Feldmeier, H. (2003). Investigations on the biology, epidemiology, pathology and control of Tunga penetrans in Brazil. *Parasitology Research*, *90*(6), 449–455.

Muhoro, B., Mbithi, D. M., & Sule, D. O. (2016). Effect of Jigger Infestation on Agricultural Productivity: A Case Study of Murarandia Division. *International Journal of Economics*, *1*(1), Article 1. https://www.iprjb.org/journals/index.php/IJECON/article/view/7

Mutebi, F., Krücken, J., Feldmeier, H., & von Samsom-Himmelstjerna, G. (2021a). Clinical implications and treatment options of tungiasis in domestic animals. *Parasitology Research*, 1–11.

Mutebi, F., Krücken, J., Feldmeier, H., & von Samsom-Himmelstjerna, G. (2021b). Clinical implications and treatment options of tungiasis in domestic animals. *Parasitology Research*, 1–11.

Mutebi, F., Krücken, J., Feldmeier, H., & von Samsom-Himmelstjerna, G. (2021c). Clinical implications and treatment options of tungiasis in domestic animals. *Parasitology Research*, *120*(12), 4113–4123. https://doi.org/10.1007/s00436-021-07121-y

Mutebi, F., Krücken, J., Feldmeier, H., Waiswa, C., Mencke, N., Sentongo, E., & von Samson-Himmelstjerna, G. (2015). Animal reservoirs of zoonotic tungiasis in endemic rural villages of Uganda. *PLoS Neglected Tropical Diseases*, *9*(10), e0004126.

Mutebi, F., McNeilly, H., Thielecke, M., Reichert, F., Wiese, S., Mukone, G., & Feldmeier, H. (2023a). Prevalence and Infection Intensity of Human and Animal Tungiasis in Napak District, Karamoja, Northeastern Uganda. *Tropical Medicine and Infectious Disease*, *8*(2), 111.

Mutebi, F., McNeilly, H., Thielecke, M., Reichert, F., Wiese, S., Mukone, G., & Feldmeier, H. (2023b). Prevalence and Infection Intensity of Human and Animal Tungiasis in Napak District, Karamoja, Northeastern Uganda. *Tropical Medicine and Infectious Disease*, *8*(2), 111.

Mwai, J., Nyole, D., Abdi, M. H., & Omogi, J. (2023). Factors associated with tungiasis among school-age children in Kwale County, rural Kenya. *International Health*, *15*(1), 85–92.

Mwangi, J. N., Ozwara, H. S., & Gicheru, M. M. (2015). Epidemiology of tunga penetrans infestation in selected areas in Kiharu constituency, Murang'a County, Kenya. *Tropical Diseases, Travel Medicine and Vaccines*, *1*(1), 1–6.

Myers, P., Espinosa, R., Parr, C. S., Jones, T., Hammond, G. S., & Dewey, T. A. (2006). The animal diversity web. *Accessed October*, *12*(2006), 2.

Nagy, N., Abari, E., D'Haese, J., Calheiros, C., Heukelbach, J., Mencke, N., Feldmeier, H., & Mehlhorn, H. (2007). Investigations on the life cycle and morphology of Tunga penetrans in Brazil. *Parasitology Research*, *101*(2), 233–242.

Nasyrova, D. I., Sapronova, A. Y., Nigmatullina, R. R., & Ugrumov, M. V. (2006). Changes in blood plasma volume in rats during ontogenesis. *Russian Journal of Developmental Biology*, *37*(5), 301–305.

Ngunjiri, J., Keiyoro, P. N., & Mwanda, W. (2015). Quantifying burden of disease caused by Tungiasis using disability adjusted life years metric among the children aged 5–14 years in Murang' a County, Kenya. *Int Res J Public Environ Heal*, *2*(October), 151–158.

Ngunjiri, J. W. (2015). *Impact of Tungiasis on school age children in Muranga county, Kenya.* University of Nairobi.

Nogueira, B. C. F., da Silva Soares, E., Orozco, A. M. O., da Fonseca, L. A., & Campos, A. K. (2023). Evidence that ectoparasites influence the hematological parameters of the host: A systematic review. *Animal Health Research Reviews*, 1–12.

Nono, J. K., Ndlovu, H., Aziz, N. A., Mpotje, T., Hlaka, L., & Brombacher, F. (2017). Interleukin-4 receptor alpha is still required after Th2 polarization for the maintenance and the recall of protective immunity to Nematode infection. *PLOS Neglected Tropical Diseases*, *11*(6), e0005675. https://doi.org/10.1371/journal.pntd.0005675

Norgan, A. P., & Pritt, B. S. (2018a). Parasitic infections of the skin and subcutaneous tissues. *Advances in Anatomic Pathology*, *25*(2), 106–123.

Norgan, A. P., & Pritt, B. S. (2018b). Parasitic infections of the skin and subcutaneous tissues. *Advances in Anatomic Pathology*, *25*(2), 106–123.

Nsanzimana, J., Karanja, S., Kayongo, M., Nyirimanzi, N., Umuhoza, H., Murangwa, A., Muganga, R., & Musafili, A. (2019). Factors associated with tungiasis among primary school children: A cross-sectional study in a rural district in Rwanda. *BMC Public Health*, *19*(1), 1192. https://doi.org/10.1186/s12889-019-7481-y

Nwalozie, R., & Ezenwaka, C. O. (2023). Tungiasis: Biology, Life Cycle, Epidemiology, Diagnosis, Prevention, and Treatment. *South Asian Journal of Parasitology*, *6*(2), 83–93.

Nyangacha, R. M., Odongo, D., Oyieke, F., Bii, C., Muniu, E., Chasia, S., & Ochwoto, M. (2019a). Spatial distribution, prevalence and potential risk factors of Tungiasis in Vihiga County,

Kenya. *PLOS Neglected Tropical Diseases*, *13*(3), e0007244. https://doi.org/10.1371/journal.pntd.0007244

Nyangacha, R. M., Odongo, D., Oyieke, F., Bii, C., Muniu, E., Chasia, S., & Ochwoto, M. (2019b). Spatial distribution, prevalence and potential risk factors of Tungiasis in Vihiga County, Kenya. *PLoS Neglected Tropical Diseases*, *13*(3), e0007244.

Nyangacha, R. M., Odongo, D., Oyieke, F., Bii, C., Muniu, E., Chasia, S., & Ochwoto, M. (2019c). Spatial distribution, prevalence and potential risk factors of Tungiasis in Vihiga County, Kenya. *PLoS Neglected Tropical Diseases*, *13*(3), e0007244.

Nyangacha, R. M., Odongo, D., Oyieke, F., Ochwoto, M., Korir, R., Ngetich, R. K., Nginya, G., Makwaga, O., Bii, C., & Mwitari, P. (2017). Secondary bacterial infections and antibiotic resistance among tungiasis patients in Western, Kenya. *PLoS Neglected Tropical Diseases*, *11*(9), e0005901.

Obebe, O. O., & Aluko, O. O. (2020a). Epidemiology of tungiasis in sub-saharan Africa: A systematic review and meta-analysis. *Pathogens and Global Health*, *114*(7), 360–369.

Obebe, O. O., & Aluko, O. O. (2020b). Epidemiology of tungiasis in sub-saharan Africa: A systematic review and meta-analysis. *Pathogens and Global Health*, *114*(7), 360–369.

Ogly, S. R. F. (2020). Infiltration of lymphocytes and mast cells to bladder tissues in experimental models of interstitial cystitis/bladder pain syndrome. *Nephro-Urology Monthly*, *12*(1).

Okoth, A. A. (2015). Morbidity, Risk Factors, and flea species responsible for Tungiasis in selected villages in Kisumu County, Kenya. *Nairobi: Kenya: Kenyatta University*.

Organization, W. H. (2022). *Report of a WHO informal meeting on the development of a conceptual framework for tungiasis control: Virtual meeting, 11-13 January 2021*.

Organization, W. H. (2023). *Global report on neglected tropical diseases 2023*. World Health Organization.

Osorio, E. Y., Uscanga-Palomeque, A., Patterson, G. T., Cordova, E., Travi, B. L., Soong, L., & Melby, P. C. (2023). Malnutrition-related parasite dissemination from the skin in visceral leishmaniasis is driven by PGE2-mediated amplification of CCR7-related trafficking of infected inflammatory monocytes. *PLoS Neglected Tropical Diseases*, *17*(1), e0011040.

Padilla-Carlin, D. J., McMurray, D. N., & Hickey, A. J. (2008). The Guinea Pig as a Model of Infectious Diseases. *Comparative Medicine*, *58*(4), 324–340.

Palavecino, C. C., Fantozzi, M. C., Cuervo, P. F., Antoniazzi, L. R., Beldomenico, P. M., & Racca, A. L. (2023). Pro- and anti-inflammatory cytokine expression in wild Akodon azarae: Individual variability due to host factors, seasonality and parasite infections. *Mammalian Biology*, *103*(4), 405–417. https://doi.org/10.1007/s42991-023-00363-6

Palicelli, A., Croci, S., Bisagni, A., Zanetti, E., De Biase, D., Melli, B., Sanguedolce, F., Ragazzi, M., Zanelli, M., & Chaux, A. (2021). What do we have to know about PD-L1 expression in prostate cancer? A systematic literature review. Part 3: PD-L1, intracellular signaling pathways and tumor microenvironment. *International Journal of Molecular Sciences*, *22*(22), 12330.

Pampiglione, S., Fioravanti, M. L., Gustinelli, A., Onore, G., Mantovani, B., Luchetti, A., & Trentini, M. (2009a). Sand flea (Tunga spp.) infections in humans and domestic animals: State of the art. *Medical and Veterinary Entomology*, *23*(3), 172–186.

Pampiglione, S., Fioravanti, M. L., Gustinelli, A., Onore, G., Mantovani, B., Luchetti, A., & Trentini, M. (2009b). Sand flea (Tunga spp.) infections in humans and domestic animals: State of the art. *Medical and Veterinary Entomology*, *23*(3), 172–186.

Pampiglione, S., Trentini, M., Fioravanti, M. L., Onore, G., & Rivasi, F. (2002). A new species of Tunga (Insecta, Siphonaptera) in Ecuador. *Parassitologia*, *44*, 127–127.

Paranhos, L. S., de Castro, P. A. S. V., de Araújo, G. R., do Carmo Magalhães, F., & Bezerra, J. M. T. (2022). Prevalence of tungiasis in humans in Brazil and in its federative units: A systematic review. *Revista de Patologia Tropical/Journal of Tropical Pathology*, *51*(1).

Pilger, D., Schwalfenberg, S., Heukelbach, J., Witt, L., Mehlhorn, H., Mencke, N., Khakban, A., & Feldmeier, H. (2008). Investigations on the biology, epidemiology, pathology, and control of Tunga penetrans in Brazil: VII. The importance of animal reservoirs for human infestation. *Parasitology Research*, *102*(5), 875–880.

Qadir, S., Dixit, A. K., Dixit, P., & Sharma, R. L. (2011). Intestinal helminths induce haematological changes in dogs from Jabalpur, India. *Journal of Helminthology*, *85*(4), 401–403.

Razanakolona, L. R.-S., Raharisoa, A., Soankasina, A. H., Jaonasoa, J. D. L. C., Nicolas, P., Antilahy, J. A., Chebbah, D., Akhoundi, M., & Izri, A. (2022). Clinical and epidemiological survey of tungiasis in Madagascar. *Travel Medicine and Infectious Disease*, *50*, 102449.

Richmond, J. M., & Harris, J. E. (2014). Immunology and skin in health and disease. *Cold Spring Harbor Perspectives in Medicine*, *4*(12).

Roberts, L. J., Huffam, S. E., Walton, S. F., & Currie, B. J. (2005). Crusted scabies: Clinical and immunological findings in seventy-eight patients and a review of the literature. *Journal of Infection*, *50*(5), 375–381.

Ruttoh, S. K., Ochieng, K., Omondi, D., Inviolata, N., & Wanyama, M. (2012). Tunga penetrans—A Silent Setback to Development in Kenya. *J. Environ. Sci. Eng. B*, *1*(1).

Saboyá-Díaz, M. I., Nicholls, R. S., Castellanos, L. G., & Feldmeier, H. (2023). Current status of the knowledge on the epidemiology of tungiasis in the Americas. *Revista Panamericana de Salud Pública*, *46*, e124.

Santana, Y. R. T., Oliveira, L. F. C., Lima, G. M., Timbó, R. V., Pires, E. M., de Brito, A. R., Martins, A. C. T., Magalhães, V. S., de Faria, A. C. M., & Urdapilleta, A. A. A. (2023). Tungiasis in

the Sanumas amerindians in the amazon rainforest, Brazil: Prevalence, intensity and morbidity. *Tropical Medicine and Infectious Disease*, *8*(8), 386.

Satyaningtijas, A. S., INDRAWATI, A., SYARAFINA, R. F., MILANI, T. F., SURYAPUTRA, M., SALEEMA, A. K., & HANADHITA, D. (2020). Erythrocytes and leukocytes profiles of bottlenose dolphins (Tursiops aduncus) at conservation site. *Biodiversitas Journal of Biological Diversity*, *21*(7).

Scott, M. A., & Stockham, S. L. (2013). *Fundamentals of veterinary clinical pathology*. John Wiley & Sons.

Shameena, S. S., Kumar, K., Kumar, S., Kumari, P., Krishnan, R., Karmakar, S., Kumar, H. S., Rajendran, K. V., & Raman, R. P. (2021). Dose-dependent co-infection of Argulus sp. And Aeromonas hydrophila in goldfish (Carassius auratus) modulates innate immune response and antioxidative stress enzymes. *Fish & Shellfish Immunology*, *114*, 199–206.

Sharma, S., Sharma, D., Pathak, V., & Singh, E. (n.d.). *Ectoparasites of cattle and their control strategies*. *8*.

Steen, C. J., Carbonaro, P. A., & Schwartz, R. A. (2004). Arthropods in dermatology. *Journal of the American Academy of Dermatology*, *50*(6), 819–842l.

Steverding, D. (2020). The spreading of parasites by human migratory activities. *Virulence*, *11*(1), 1177–1191.

Streicher, M. (2019). Flea Allergy Dermatitis. In *Small Animal Dermatology for Technicians and Nurses* (pp. 85–101). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119108641.ch6

Talhari, C., & Gontijo, B. (2023). Parasitic and Protozoal Infections. In *Dermatology in Public Health Environments: A Comprehensive Textbook* (pp. 339–360). Springer.

Taylor, S., Hales, B. J., & Thomas, W. R. (2023). Host Immune Response to Scabies. In *Scabies* (pp. 45–73). Springer.

Thielecke, M., McNeilly, H., Mutebi, F., Banalyaki, M. B., Arono, R., Wiese, S., Reichert, F., Mukone, G., & Feldmeier, H. (2023a). High Level of Knowledge about Tungiasis but Little Translation into Control Practices in Karamoja, Northeastern Uganda. *Tropical Medicine and Infectious Disease*, *8*(9), 425.

Thielecke, M., McNeilly, H., Mutebi, F., Banalyaki, M. B., Arono, R., Wiese, S., Reichert, F., Mukone, G., & Feldmeier, H. (2023b). High Level of Knowledge about Tungiasis but Little Translation into Control Practices in Karamoja, Northeastern Uganda. *Tropical Medicine and Infectious Disease*, *8*(9), 425.

Trindade, D., Carvalho, R., Machado, V., Chambrone, L., Mendes, J. J., & Botelho, J. (2023). Prevalence of periodontitis in dentate people between 2011 and 2020: A systematic review and meta-analysis of epidemiological studies. *Journal of Clinical Periodontology*, *50*(5), 604–626.

Tungiasis. (n.d.). Retrieved August 2, 2024, from https://www.who.int/news-room/fact-sheets/detail/tungiasis

Vacca, F., & Le Gros, G. (2022). Tissue-specific immunity in helminth infections. *Mucosal Immunology*, *15*(6), Article 6. https://doi.org/10.1038/s41385-022-00531-w

Vindenes, T., Melinscak, H., Linder, K., & Alsoubani, M. (2023). Antimicrobial Stewardship in Immunocompromised Hosts. In *Antimicrobial Stewardship in Non-Traditional Settings: A Practical Guide* (pp. 123–159). Springer.

Vobis, M., D'Haese, J., Mehlhorn, H., Heukelbach, J., Mencke, N., & Feldmeier, H. (2005). Molecular biological investigations of Brazilian Tunga sp. Isolates from man, dogs, cats, pigs and rats. *Parasitology Research*, *96*(2), 107–112. https://doi.org/10.1007/s00436-005-1320-z

Walle Mørkve, Å., Jackline, S., & Van den Bergh, G. (2023). *A qualitative case study of community experiences with Tungiasis in high prevalence villages of Bungoma County, Kenya:"The whole body aches and the jiggers are torturing me!".* *https://doi.org/10.1371/journal.pntd.0011304*

Walton, S. F. (2010). The immunology of susceptibility and resistance to scabies. *Parasite Immunology*, *32*(8), 532–540. https://doi.org/10.1111/j.1365-3024.2010.01218.x

Wambani, Z., Nyamari, J., & Kimani, H. (2018). ENVIRONMENTAL FACTORS PREDISPOSING RURAL COMMUNITY MEMBERS TO TUNGIASIS IN MURANG'A EAST SUB COUNTY, MURANG'A COUNTY. *Journal of Health, Medicine and Nursing*, *3*(2), 90–97. https://www.iprjb.org/journals/index.php/JHMN/article/view/714

Weise, P., Czirják, G. A., Lindecke, O., Bumrungsri, S., & Voigt, C. C. (2017). Simulated bacterial infection disrupts the circadian fluctuation of immune cells in wrinkle-lipped bats (Chaerephon plicatus). *PeerJ*, *5*, e3570. https://doi.org/10.7717/peerj.3570

Wiese, S., Elson, L., Reichert, F., Mambo, B., & Feldmeier, H. (2017). Prevalence, intensity and risk factors of tungiasis in Kilifi County, Kenya: I. Results from a community-based study. *PLoS Neglected Tropical Diseases*, *11*(10), e0005925. https://doi.org/10.1371/journal.pntd.0005925

Wilson, A. D. (2014). Immune responses to ectoparasites of horses, with a focus on insect bite hypersensitivity. *Parasite Immunology*, *36*(11), 560–572. https://doi.org/10.1111/pim.12142

Xue, X., & Falcon, D. M. (2019). The role of immune cells and cytokines in intestinal wound healing. *International Journal of Molecular Sciences*, *20*(23), 6097. https://doi.org/10.3390/ijms20236097

Yadavalli, T., Patil, C., Sharma, P., Volety, I., Borase, H., Kapoor, D., & Shukla, D. (2023). Unique Attributes of Guinea Pigs as New Models to Study Ocular Herpes Pathophysiology and Recurrence. *Investigative Ophthalmology & Visual Science*, *64*(14), 41–41. . https://doi.org/10.1167/iovs.64.14.41

Zawawi, N. S., & Yusof, Y. A. M. (2023). PET-ASSOCIATED ZOONOTIC DISEASES IN CAT: PREVENTION AND MANAGEMENT FOR PET OWNERS. *Zulfaqar Journal of Defence Science, Engineering & Technology*, *6*(3).https://zulfaqarjdset.upnm.edu.my/index.php/zjdset/article/view/71

APPENDIX

Appendix I. Blood Sample Collection Procedures.

Collection tubes

1.Purple Top tube- The anticoagulant ethylene-diamine tetra-acetic acid (EDTA) are contained in the tubes, used for collecting whole blood. A differential count and full blood count are the most often performed tests.

2.Red Top tube- There is no anticoagulant in the tube. Employed in serum samples collection for Immunological and diagnostic tests, as well as serological tests.

Order of Draw

I. **First blood withdrawal –** Red-topped tubes without additives.

II. **Second blood withdrawal –** Tubes with purple tops

Preparation

- ✓ Assemble all the materials and equipments .
- ✓ Ascertain the animal. Ensure that the laboratory labels for identification is for the respective animal.
- ✓ Select the proper tube.
- ✓ If there are additive-containing tubes, tap them lightly to release suspended particles surrounding the cap.

- ✓ Habituate the guinea pigs to the sound of the electric shaver to minimise additional stress.
- ✓ Anaesthesized the guinea pig and restrain for the minimum duration necessary. Guinea pigs should be habituated to restrain; this would improve the experience
- ✓ Do not re-palpate disinfected site.
- ✓ of the animal and handler thus obtaining a better-quality blood sample.
- ✓ Disininfect and clean the area with 70% isopropyl alcohol, starting in the middle then spreading outward in a circular motion. Based on the width of the chosen vein, use a suitable needle.

The Vacuitainer Method

- Avoid taking away the needle shield; instead, unzip your needle packaging. Once firmly in place, insert the needle inside the holder. Use touriquiet. Refrain from touching the cleaned area again.
- Applying light downward pressure slightly above the kneecap point and immobilize the hindleg in an extended position. To make it easier to shave off hair and stop the saphenous vein, stretch the skin above the ankle.
- To make sure the needle is undamaged, take off the needle cap and examine it.
- The needle should be positioned above the top of the vein, horizontal over it, and bevel up. Swiftly insert the needle beneath the skin.
- Once the needle has been inserted into the vein, press it into the holder and let the blood fill it.

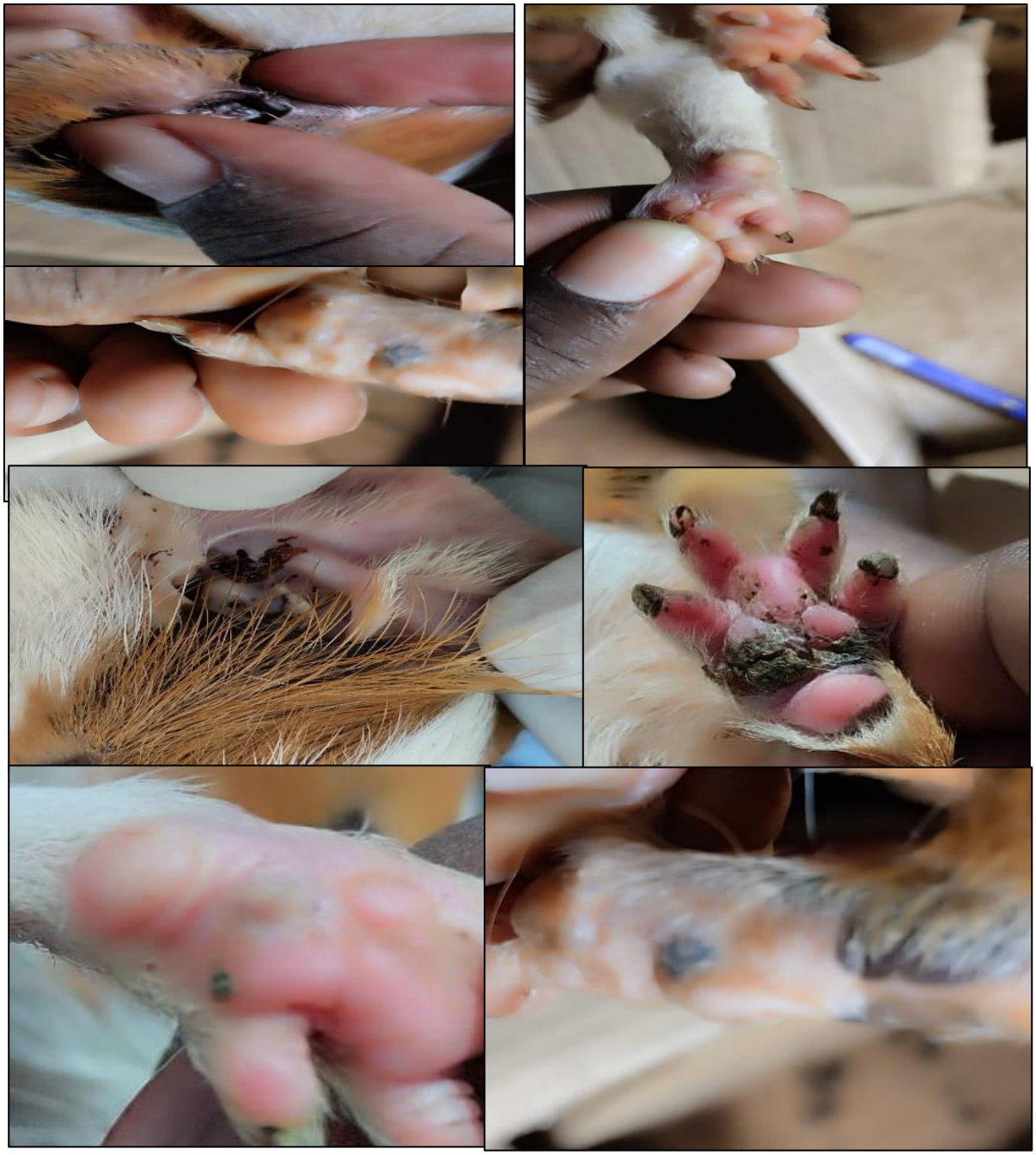
- Relax the tourniquet as the blood begins to flow into the tube, and then, once the first tube has been collected, have the animal release it. Remove the filled tube and insert new tubes to continue filling the remaining tubes until the required number of tubes is reached. As said previously, adhere to the draw order. To ensure a proper specimen collection, the following actions are advised if blood does not flow into the tube or stops flowing before a sufficient amount is obtained.

    - Till the tube stopper has been penetrated, push the tube forward. Maintain position if needed to provide a full vacuum suction.
    - Check if the needle is inserted into the vein correctly.
    - For the Vacuum-filled tubes, exlude them then substitute with a new one.
    - Remove the needle, discard it, and repeat the process if the second tube fails to be withdraw.
    - After the venipuncture completed, pull the needle out of the saphenous vein and put pressure on the area with a sanitized piece of cotton. For two to three minutes, keep the hindleg up and apply pressure. Blood flow can be halted by lightly applying pressure to the point of puncture with a swab or by releasing the phlebotomist's grip on the animal leg. Do not put the animal back to its cage before the blood flow ceased.

- Should more than one sample be needed, legs can be switched around. It is recommended to take no more than four blood samples in a 24-hour period. Temporary or surgical cannulation should be taken into consideration if additional samples are required.

- Sharps container should be used to dispose of needles .Inform the laboratory supervisor right away if there is an incidental needles stick.For the next round of treatment, clean the surface with water and detergent.
- Label every tube and arrange them in the proper rack for testing in the laboratory and transporting them. Experimental animal identification number, draw date and time, and any other necessary information should all be written on labels.

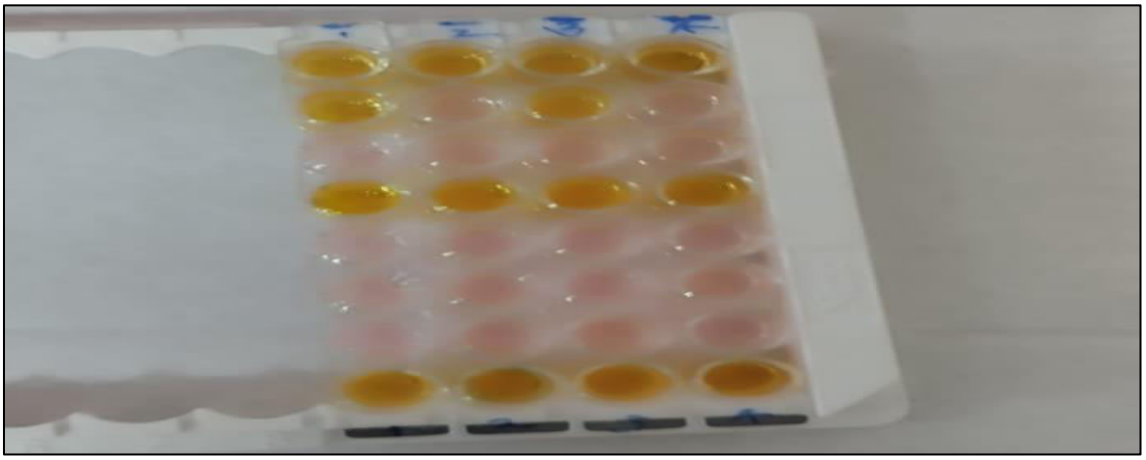
Appendix II. Infected Guniea Pig Foot Pad and earlopes

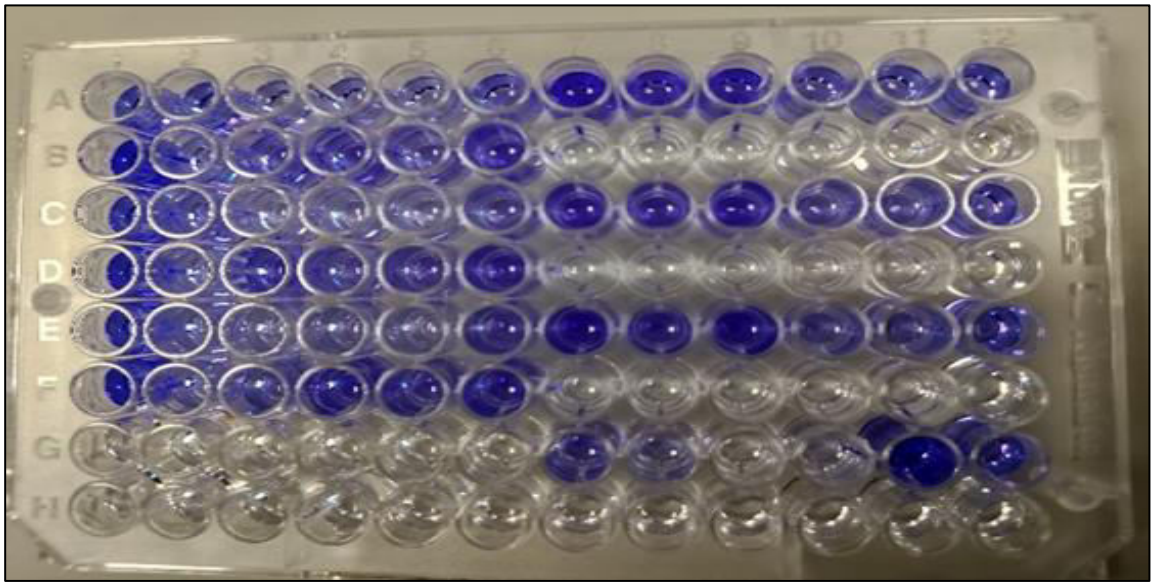


Appendix III. Treatment of Infested human with tungiasis

Appendix IV. Withdrawal of Blood Samples

Appendix V. ELISA Positive Plates





Appendix VI. TNF-α,IL-10 and IL-4, IgE and IgG ELISA TECHNIQUE.

a) Maintain and calibrate equipment as needed in accordance with manufacturer's instructions

b) Duplicate analyses are performed on all samples, standards, and blanks. Using guinea Pig Interleukins ELISA Kit (96 wells) (Wuhan Fine Biotech Co. Ltd, China), the concentrations of tumor necrotic factor alpha (TNF α), interleukin -10 (IL -10), and interleukin -4 (IL -4) are measured.

c) Samples, standards and reagents are prepared according to manufactures instructions.

d) Briefly, add (100 µl each) of 2-fold diluted samples was pipetted into sample test wells followed by incubation for 90 minutes at 37 ºC.

e) Discard and wash the wells two times with wash buffer provided in the kit.

f) Add 100 µl of Biotin –labeled antibody (Wuhan Fine Biotech Co. Ltd, China) at a dilution of 1: 100 into the bottom wells containing test sample, standards and blank wells and incubate for 60 minutes at 37 ºC.

g) Remove the cover and wash the plate three times with wash buffer and allow the buffer to stay for two minutes in every wash.

h) Add 100 µl of HRP –Streptavidin Conjugate (SABC) into each well, cover and incubate at 37 ºC for 30 minutes.

i) Wash the plate five times allowing the wash buffer to stay for 2 minutes for every wash.

j) Fill each well with 90 µl of TMB substrate, cover the microwell plates with fresh plate sealer to reduce evaporation, and incubate for 20 minutes in a darkened room.

k) Gently take off the cover plate and fill each well with 50 µL of stop solution 2M H2SO4 to put a stop to the reaction.Gently tap the plate to ensure proper mixing.

l) Before reading, make sure that each strip has been firmly put into place. Immediately after adding the stopping solution, measure the absorbance using a 450 nm microplate reader (RT-9600) and record the results within 30 minutes.

IFN-γ ELISA PROCEDURE

a) Interferon concentration procedures using guinea pig Interferon Gamma (IFNg) ELISA Kit 96T (Wuxi Donglin Sci & Tech Develop Co., Ltd, China).

b) Samples, reagents and standards are prepared according to manufactures instructions.

c) prepare seven wells for standards and two wells for blank in duplicates and the rest samples. Add 100 µL of each dilution of standards, blank and sample into the respective wells, cover and incubate for 2 hours at 37 ºC and thereafter discard the liquids carefully.

d) Add 100 µL of detection reagent A to each well, cover with plate sealer and incubate for 1 hour at 37 ºC.

e) Discard the solution and wash the wells three times with wash buffer using the multichannel pipette and allow to stay for 2 minutes in every wash.

f) Invert and tap the plate on the absorbent paper to completely dry.

g) Add 100 µL of detection reagent B to each well of the control and sample, cover and incubate for 1 hour at 37 ºC .Wash the wells five times

h) Add 90 µL of TMB Substrate solution to each well and incubate for 25 minutes at 37 ºC in a dark room.

i) Add 50 µL of stop solution (1.5M H_2SO_4) to each well of controls and samples and mixe gently by tapping the plate.

j) Use a microplate reader and a 450 nm filter with a reference wavelength of 615 nm to 630 nm to measure the absorbance within 20 minutes offter adding the stop solution.Before taking readings, make sure all of the strips are securely in place.

IgE and IgG ELISA PROCEDURE

k) IgG and IgE antibodies concentration procedures using guinea pig pre-coated ELISA Assay Kit (Eagle Biosciences, USA).

l) Samples, reagents and standards are prepared according to manufactures instructions.

m) prepare seven wells for standards and two wells for blank in duplicates and the rest samples. Add 100 µL of each dilution of standards, blank and sample into the respective wells, cover and incubate for 2 hours at 37 ºC and thereafter discard the liquids carefully.

n) Add 100 µL of detection reagent A to each well, cover with plate sealer and incubate for 1 hour at 37 ºC.

o) Discard the solution and wash the wells three times with wash buffer using the multichannel pipette and allow to stay for 2 minutes in every wash.

p) Invert and tap the plate on the absorbent paper to completely dry.

q) Add 100 µL of of Enzyme- Antibody Conjugated with Horseradish Peroxidase at a dilution of 1:1000 to each well of the control and sample, cover and incubate for 1 hour at 37 ºC .Wash the wells five times

r) Add 100 µL of TMB Substrate Solution at 1:100 dilution into each well and incubated in the dark at room temperature for 10 minutes.

s) Add 100 µL of 1:100 dilution Stop Solution 2.0M H_2SO_4 to each well of controls and samples and mixe gently by tapping the plate.

t) After adding the stopping solution, measure the absorbance in 20 minutes using a microplate reader at 450 nm wavelength.

Appendix VII. NACOSTI Research Licence

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 104929

Date of Issue: 30/June/2023

RESEARCH LICENSE

This is to Certify that Miss.. Janet Tarus Jepkurui of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Vihiga on the topic: CYTOKINES, ANTIBODIES AND HEMATOLOGICAL PROFILE IN GUINEA PIGS INFECTED WITH TUNGA PENETRANS (JIGGERS) for the period ending : 30/June/2024.

License No: NACOSTI/P/23/26624

104929

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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See overleaf for conditions

Appendix VIII. Institutional Ethical Approval Letter

File Copy.

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke
P. O. Box 190,
50100.
Kakamega,
KENYA

Institutional Scientific and Ethics Review Committee (ISERC)

REF: MMU/COR: 403012 Vol 6 (01) Date: June 20th, 2023

To: Ms. Janet J. Tarus

Dear Ms.

RE: CYTOKINES, ANTIBODIES AND HEMATOLOGIAL PROFILE IN GUINEA PIGS INFECTED WITH TUNGA PENETRANS.

This is to inform you that the ***Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMUST-ISERC)*** has reviewed and approved your above research proposal. Your application approval number is **MMUST/IERC/172/2023.** The approval covers for the period ***June 20th, 2023 to June20th, 2024.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-ISERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-ISERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MMUST-ISERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MMUST-ISERC.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed

Yours Sincerely,

Prof. Gordon Nguka (PhD)
Chairperson, Institutional Scientific and Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix IX. Letter of Approval of Proposal

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: dpgstudies@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

15th March 2023

Janet J. Taras
SIM/G/01-70225/2021
P.O. Box 190-50100
KAKAMEGA

Dear Ms. Taras,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Masters proposal entitled: '***Cytokines, Antibodies and Hematological Profile in Guinea Pigs Infected with Tunga penetrans***' and appointed the following as supervisors:

1. Dr. Jackson Cheruiyot - MMUST
2. Prof. John Maima - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director of Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Natural Sciences Graduate Studies Committee; Chairman, Department of Biological sciences & Departmental Graduate Studies Committee. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of two years from the date of registration to complete your Master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
DIRECTORATE OF POSTGRADUATE STUDIES
P.O. BOX 190 - 50100, KAKAMEGA (K)

Prof. Stephen O. Odeny, PhD, FIEP
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES