

**EPIDEMIOLOGY OF *TUNGA PENETRANS* INFESTATION AND ANTIGENS
CHARACTERIZATION IN SELECTED LOCATIONS IN KIHARU
CONSTITUENCY, MURANG'A COUNTY, KENYA**

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REG. NO. I56/CE/10952/2006

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF
SCIENCE (IMMUNOLOGY) IN THE SCHOOL OF PURE AND APPLIED
SCIENCES OF KENYATTA UNIVERSITY**

MARCH 2015

DECLARATION

I hereby declare that this thesis is my original work and has not been presented for degree or other awards in any other university.

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DEDICATION

I dedicate this work to all the scientists who devote their life in active research in an endeavor to come up with long term solutions to neglected tropical diseases affecting the developing countries.

ACKNOWLEDGEMENTS

I express my appreciation to my supervisors, Dr. Michael Gicheru of Kenyatta University and Dr. Hastings Ozwara of Institute of Primate Research for their support and very useful advice that shaped up my thesis. My regards goes to Kenyatta University, Department of Zoological sciences for training me and allowing me to use their laboratory facilities and resources during sample analysis. I also appreciate the Graduate School Kenyatta University for guiding me throughout the MSc program.

I am grateful to Murang'a County Hospital and several other health centers in Kahuro and Murang'a East sub Counties that permitted me and facilitated collection of samples in the field. My regards also goes to the Public Health Officers, their assistants and Community Health workers who accompanied me to the field during sample collection. I am grateful to District Education Officers in the two regions for their permission to access various primary schools within their jurisdiction for my research activities; the head teachers and their staff for their support when I visited their primary schools for data collection. The study subjects that include pupils, parents and guardians are highly appreciated for their co-operation during the field study.

I am grateful to laboratory technicians, Mr. John Macharia of Institute of Primate Research and Henry Ondieki of Kenyatta University for their assistance in sample analysis in the laboratory. I am especially grateful to NACOSTI for evaluating this research, approving and funding it. I appreciate my family for its emotional support during this study. Most of all, I thank God for the strength he gave me and his guidance throughout my study.

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ACRONYMS AND ABBREVIATIONS

APS	Ammonium persulfate
BPB	Bromophenol Blue
BSA	Bovine Serum Albumen
CBB	Commasie Brilliant Blue
CDF	Constituency Development Fund
HRP	Horse Radish Peroxidase
IFN- γ	Interferon gamma
IL	Interleukin (cytokines)
IPR	Institute of Primate Research
kDa	Kilo Daltons
NACOSTI	National Commission for Science, Technology and Innovation
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate Buffered Saline
SDS	Sodium Dodecyl Sulfate (a detergent)
TEMED	N, N, N, N-Tetramethylenethylene Diamine
TNF- α	Tumor Necrosis Factor alpha

DEFINITION OF TERMS

- a) Chigoe flea - Jigger. (*Tunga penetrans*)
- b) Erythema - The redness of the skin as a result of capillary congestion, caused by an infection.
- c) Hematophagous - Feeding on blood. A characteristic of many parasites.
- d) Hyperkeratosis - The thickening of the stratum corneum often associated with a qualitative abnormality of the keratin.
- e) Hyperplasia - A general term referring to the physiological proliferation of cells within an organ or tissue such as constantly dividing cells which result in organ enlargement or formation of a benign tumor.
- f) Necrosis - Premature cell death due to external factors such as infection, injury, poisoning or inflammation which may hinder blood flow to the cells.
- g) Parakeratosis - Incomplete keratinization of epidermal cells
- h) Reservoir host - A host that serves as a source of infection and potential reinfection of humans and as a means of sustaining a parasite when it is not infecting humans.
- i) Sequelae - Pathological condition resulting from a disease, injury or other trauma.
- j) Spongiosis - Intracellular edema (increased intracellular fluid in the epidermis) between the keratinocytes in the epidermis, manifested clinically by vesicles, 'juicy' papules and lichenification.
- k) Stratum corneum - Horny outer layer of the skin.
- l) Venipuncture - (venopuncture or venepuncture). The process of obtaining intravenous access for the purpose of intravenous therapy or for blood sampling of venous blood.

ABSTRACT

Tungiasis is the condition brought about by *Tunga penetrans* fleas when they burrow into the skin of their hosts. It is a medical condition that has largely been ignored. The distribution, prevalence, pathological effects, causes and means by which it can be treated or prevented have not been widely studied in Kenya hence there is scarce epidemiological data. Moreover, the parasite antigens have not been characterized and this could seriously affect its intervention. This study therefore sought to investigate the epidemiology of tungiasis in selected locations in Kiharu constituency, Murang'a County and characterize immunodominant antigens of *T. penetrans*. This research comprised of field study as well as laboratory procedures. The study population mainly comprised of primary school children (n=508), but also included their relatives at home (n=43). Purposive sampling was used to select the four study locations namely: Gaturi, Kimathi, Kahuhia and Mugoiri. In each of the locations, three primary schools and their pupils were randomly sampled. Through questionnaires, interviews guides, focused group discussions and observations, data was collected. Jigger and blood samples were collected from participants for protein characterization. Crude jigger proteins were used to immunize five albino laboratory rats (*Rattus norvegicus*) to raise sera. Antigens were analyzed in gel electrophoresis, immunodiffusion and western blot. The mean prevalence of jiggers in pupils in the study locations was 19.1% while that of adults was 14%. In multivariate logistic regression analysis some independent factors were identified to be associated with tungiasis; lack of regular use of closed foot ware (OR=10.45; 95% CI=1.49-73.23), living in earthen mud walled houses (OR=13.78; 95% CI=3.127-60.69) sharing living quarters with domestic animals (OR=0.11; 95% CI=0.003-.046), and learning in classrooms with dusty floors (OR=14.657; 95% CI=2.262-94.95). The level of income of the parents was observed to be associated with some risk factors suggesting that poverty is an important factor in jigger infestation. Three major immunodominant antigens in *T. penetrans* reactive to human immune system during infestation were identified. Their molecular weight ranges between 15.38 and 51.795 kDa. This study shows that tungiasis is an emerging neglected disease in Kiharu constituency with a significant health concern. Modifiable risk factors were identified that should be the focus of sustainable and effective control measures.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Tungiasis is the condition caused by jigger infestation. It is endemic in many countries in Latin America, the Caribbean and sub-Saharan Africa (Heukelbach *et al.*, 2001). In Kenya, it is a neglected serious health problem that demands attention by health officers (Ahadi, 2008). It has led to severe morbidity to its victims especially those in economically disadvantaged rural communities (Eisele *et al.*, 2003). Moreover its incapacitating nature due to severe physical disability emanating from its pathological effects contributes to high poverty levels to its victims. In Nigeria, one third of the jigger infested finds it difficult to walk (Ugbomoiko *et al.*, 2007). Tungiasis is characterized by high transmission rate due to poor housing conditions, social neglect and inadequate health care (Heukelbach *et al.*, 2001). In Erekit, a rural community in Lagos state of Nigeria, sand or clay floors inside houses, presence of pigs in the compound, having a common resting place outside the house, and lack of regular use of closed foot ware are important risk factors for tungiasis (Ugbomoiko *et al.*, 2007). In a traditional fishing community North East Brazil, households with infested pets show that intensity of infestation in animals correlate to intensity of infestation in humans (Pilger *et al.*, 2008).

A relationship between jigger infestation and age bracket exist. The prevalence is high between 5-14 years, decrease in adults and increases again in the elderly (Ugbomoiko *et al.*, 2007). The handicapped such as physically handicapped who are always in contact with soil, including the mentally handicapped, who are unable to maintain proper hygiene are likely to have high incidence of tungiasis (Ariza *et al.*, 2007). In Kenya,

estimated 1.6 million Kenyans are infested and 10 million at risk (Ministry of Public Health and Sanitation 2009). A survey by Ahadi Kenya (2008) suggests that prevalence rates in different counties are not very clear, Murang'a being suggested to have high prevalence of tungiasis.

1.2 Problem statement

Tungiasis is a serious medical issue that has been neglected for quite a long time by researchers and medical practitioners. Little work has been done concerning the parasite's epidemiology in Kenya. The prevalence of jiggers in Murang'a south was suggested to be 57% in children of 5-12 yrs (Ngomi, 2010). Moreover, poor hygiene has been identified as a major cause of jigger infestation in Kenya (Ruttoh *et al.*, 2012). In addition weather conditions such as high temperatures and low precipitation increases the prevalence of tungiasis (Heukelbach *et al.*, 2005). Moreover soil factors such as soil moisture, organic matter content, soil pH, soil texture and soil colour influences the prevalence of tungiasis by up to 33% and the *T. penetrans* population by up to 39.7 % in Murang'a County (Ngunjiri *et al.*, 2011). Though such information is already available, more work on epidemiology on a wider scope especially in vulnerable age groups in endemic areas need to be pursued. In Brazil some work on cytokines has been documented where a mixed Th₁ and Th₂ immune responses have been determined in wistar rats as well as in human peripheral blood (Feldmeier *et al.*, 2004). However immunological reactions of the host to the parasite antigens have not been documented in Kenya. More over the parasite's immunodominant antigens need to be characterized which may help exploring the possibilities of immunization as part of

the control strategies. Thus, medical practitioners are yet to have good access to detailed information about the parasite in endemic regions in Kenya.

1.3 Study justification

Tungiasis is a health issue that has agonized people quietly for a long time with its serious conditions going unreported. It has always been considered as just a social problem that does not warrant medical intervention. For a long time tungiasis has been largely associated with myths and superstition and therefore ignored. More over its high attack rate and social neglect is intricately linked to poverty and inadequate health care behavior. At least in Brazil the medical profession wholly neglects this ectoparasite and physicians do not diagnose tungiasis during consultation unless the condition is mentioned by the patient (Eisele *et al.*, 2003). The pathophysiological and immunological characteristic of this ectoparasite are not well understood. Therefore no effective therapy is currently available (Nagy *et al.*, 2007). The epidemiological data on tungiasis in Kenya is scarce; lack of in depth understanding of the biological behavior of these parasites and characteristic of its antigens may not lead to lasting reliable intervention strategies. Communities have all along depended on traditional methods such as extraction of embedded fleas that can lead to spread of HIV through sharing contaminated sharp objects.

However, in the recent past NGOs like Ahadi Kenya Trust have exposed tungiasis to level that demand immediate intervention, scientific study being an important approach to supplement social approaches. Murang'a County has been suggested to have the highest prevalence, but scientific data to support these claims are scarce. General

observations have shown that tungiasis interfere with normal learning of school children being a major cause of school dropout in Murang'a County. Severe itching, pain, difficulty in walking to school and stigma are some of the factors that make it hard for pupils to concentrate in class, or remain in school (Muehlen *et al.*, 2003). This contributes to school dropout. Pupils were therefore the main study subjects.

In tungiasis, clinical pathology is frequently accompanied by pathological alteration of the epidermis (predominantly hyperplasia, parakeratosis, hyperkeratosis and spongiosis) and dermis (Feldmeier *et al.*, 2004). Deep ulceration and tissue necrosis have led to denudation of bones resulting to amputation of digits, physical disability and stigma (Eisele *et al.*, 2003). The infested are therefore unable to attend to their economic activities such as farming. They are unable to feed well and malnutrition is not uncommon. Urgent solution to tungiasis must be found. This study therefore sought to investigate the epidemiology of tungiasis in Kiharu constituency and characterize immunodominant antigens in *T. penetrans* that could be utilized in formulation of improved disease control and management strategies.

1.4 Research questions

- i. What is the prevalence of tungiasis among pupils and adults in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency?
- ii. What risk factors are associated with tungiasis in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency?
- iii. What immunodominant antigens exist in *T. penetrans* isolates from Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency?

1.5 Hypotheses

- i. There is no difference in mean prevalence of tungiasis in adults in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu Constituency.
- ii. There is no difference in mean prevalence of tungiasis in pupils in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu Constituency.
- iii. There are no risk factors associated with tungiasis in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency.
- iv. There are no immunodominant antigens in *T. penetrans* isolates from Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency.

1.6 Objectives

1.6.1 General objectives

- i. To determine epidemiology of *T. penetrans* in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency.
- ii. To characterize *T. penetrans* antigens from Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency.

1.6.2 Specific objectives

- i. To determine the prevalence of tungiasis among pupils and adults in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency.
- ii. To identify risk factors associated with tungiasis in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency.
- iii. To characterize *T. penetrans* antigens and identify immunodominant antigens from Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu constituency.

1.6.3 Significance of the study

It is anticipated that data on distribution, prevalence, pathological effects and causes of tungiasis in this study population would be made available to government for planning purposes especially in rural community health. The data would shed more light on tungiasis and expose it as an important health problem. This is expected to attract and maintain the attention of both health professionals and researchers, sensitize pupils and parents as well as help in alleviating stigma. More over the data obtained would be utilized for formulation of improved disease control and management policies. Characterization of *T. penetrans* isolates antigens could give in-depth information on immunological characteristics of the parasites which could be utilized in designing of effective preventive strategies against the parasite. This when coupled with campaign for proper hygiene would effectively control tungiasis.

CHAPTER TWO: LITERATURE REVIEW

2.1 Historical background

Tungiasis is caused by jigger fleas the *T. penetrans* (Myers *et al.*, 2014). *Tunga penetrans* is native to the West Indies. The first case of tungiasis was described in 1526 by Gonzalo Fernández de Oviedoy Valdés where he discussed the skin infection and its symptoms on crew members from Columbus's Santa Maria after they were shipwrecked on Haiti (Pampiglione *et al.*, 2009). The sand flea was first discovered in Latin America in 17th century (Heukelbach *et al.*, 2001). It was accidentally introduced in Senegal in the same century due to human transportation across the ocean. The fleas were transported to Angola in 1873 in the sand and ballast on board English sailing vessel of the 'Thomas Mitchel' (Feldmeier *et al.*, 2004). The fleas reached Tanzania during the travels of Henry Morton Stanley (Dr. Livingstone) in 1871, during colonial period. In 1899, the fleas reached Madagascar with Senegalese troops of the French army and later spread to several parts of Africa (Heukelbach *et al.*, 2003). By 1909 severe sequelae of tungiasis were not only observed in native South American population by European travelers but were also reported by military physicians in East Africa (Heukelbach *et al.*, 2003; Feldmeier *et al.*, 2004).

2.2 Worldwide distribution of Tungiasis

As of 2009, tungiasis was present worldwide in 88 countries with varying degrees of incidence (Figure 2.1). This disease is of special public health concern in highly endemic areas like Nigeria, Trinidad, Tobago, and Brazil where its prevalence,

especially in poor communities, has been known to approach 50% (Heukelbach *et al.*, 2005).

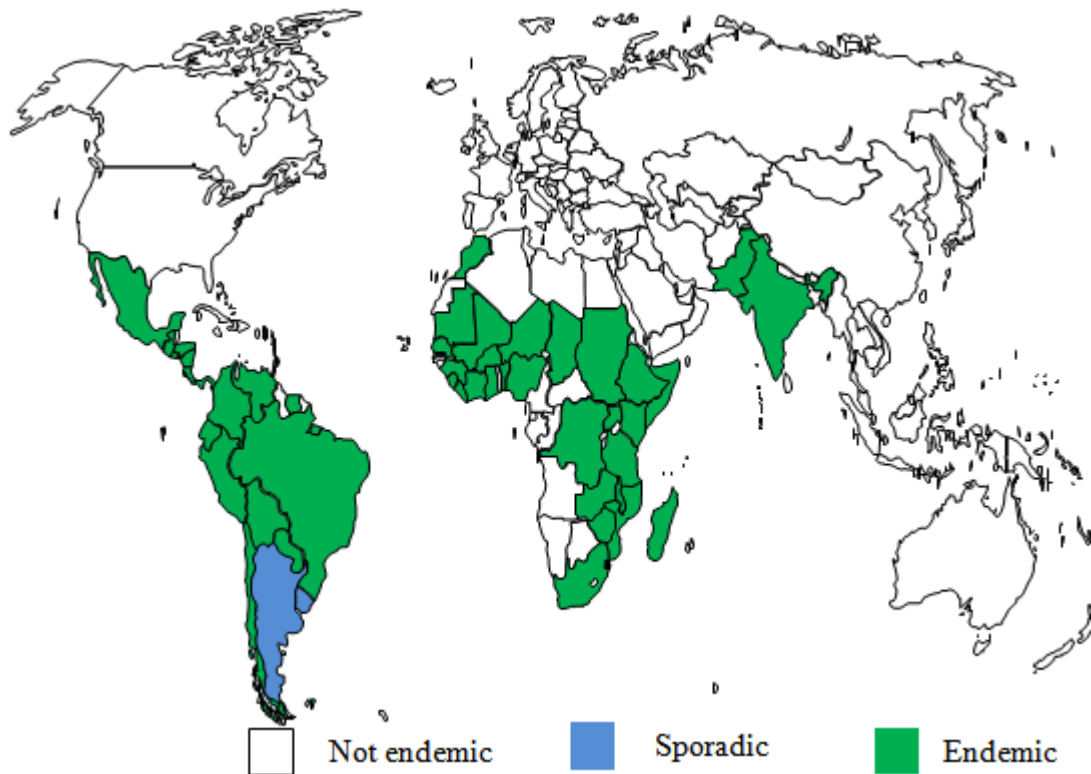


Figure 2.1: A map showing the worldwide distribution of Tungiasis as of 2009 (Feldmeier *et al.*, 2004).

2.3 Classification of *Tunga penetrans*

Myers *et al.* (2014) classified the sand flea into kingdom-Animalia, Phylum-Arthropoda, class-insecta, order-Siphonaptera, family-Hectopsyllidae, Genus-*Tunga*, species-*penetrans*; and *tramamillata* (Pampiglione *et al.*, 2002). *Tunga penetrans* is commonly found in Africa, while *T. tramamillata* is found in Latin America. The genus *Tunga* also includes about 13 subspecies most of which are hematophagous ectoparasites (Pampiglione *et al.*, 2002). The great majority of *T. penetrans* are parasitic of single or

few closely related hosts, especially rodents (Pampiglione *et al.*, 2002). However species of *T. tramamillata* Pampiglione, Trentini, Fioravanti, Onore, and Rivasi, (2002) have a wider range of possible hosts such as wild and domestic mammals and are the unique sand fleas that parasitize humans (Pampiglione *et al.*, 2002).

2.4 General morphology of adults flea

Fleas are small, black to brown-black insects, with complete metamorphosis. They are wingless, having lost their wings in the course of their evolutionary development. Adult fleas are 1 to 4 mm long, depending on the species, and are laterally flattened (Appendix IV, a). They have stout, spiny legs, adapted for leaping, and short, 3-jointed, clubbed antennae that fit into depressions along the sides of the head (Nagy *et al.*, 2007). In a study of 1000 freshly-laid *T. penetrans* eggs, it was found that females are generally smaller than males for all criteria (Figure 2.2). In some cases though, females had a bigger epipharynx and maxillar palpus. Overall, the fleas' head is relatively flattened, which again aids in burrowing through the epidermal and dermal layers (Public Health Image Library, 2009).

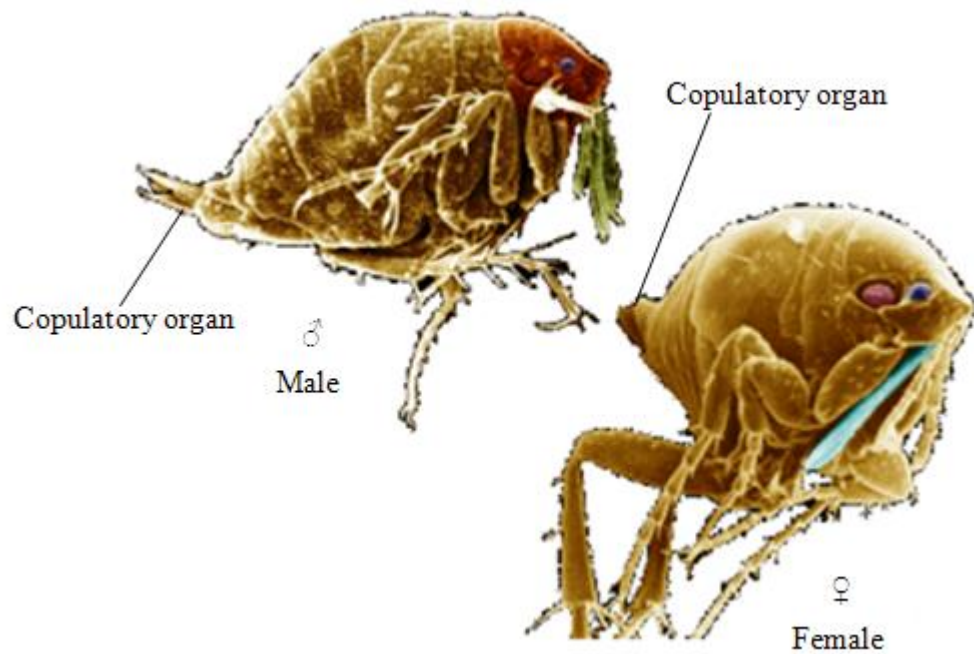


Figure 2.2: Scanning Electron Microscope images of the adult male and female *T. penetrans* fleas. The protruding copulatory organs of the male can be seen, as well as the shortened rear end of the female where, through a groove (not shown), copulation will occur. The male exhibits the ski-jump like head formation (Mehlhorn, 2008).

Due to its burrowing activity, the chigoe flea has a well-developed lacinia and epipharynx that is used to penetrate the skin; the piercing-sucking mouthparts whose principal elements are the grooved labrum and a pair of sharp, sword shaped mandibles (Figure 2.3). The concave inner sides of the mandibles, together with the labrum, form the sucking channel (Nagy *et al.*, 2007).

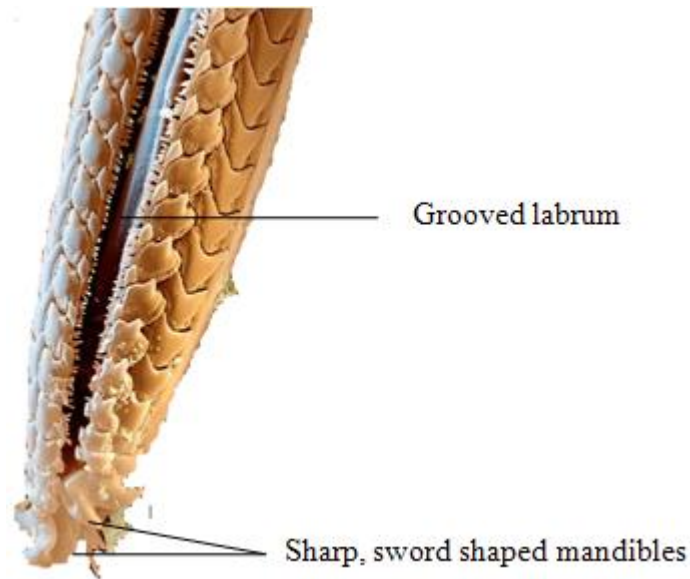


Figure 2.3: Scanning Electron Microscope image of the piercing and sucking mouth part of *T. penetrans* (Mehlhorn, 2008).

In some species, a conspicuous transverse row of spines is located just above the mouthparts, and is called the genal comb or ctenidium. In others, the pronotum bears a transverse row of heavy spines called the pronotal comb (Appendix IV, a). When flea specimens are "cleared" in lactic acid for microscopic examination, the combs show up prominently, and can be used to identify species with the aid of low magnification.

Certain species that infest rodents have the pronotal but not the genal comb. The human, oriental rat, and stick tight fleas lack both combs. It has long been assumed that the combs or ctenidia assist fleas in locomotion through fur, hair, and feathers. However this is apparently not the case; locomotion is facilitated by the strong setae on the legs, projecting almost at right angles. The combs serve to make dislodgement or capture by the host more difficult. They are admirably adapted to resist a backward pull, and so must have a considerable survival value (Nagy *et al.*, 2007).

Investigators have also found that adult *T. penetrans* have different morphologies with respect to the shape of their head. Some have a rounded head; others have head shapes that resemble ski ramps. Others demonstrate head shapes that are very linear with a slight bulge at the nose. These morphologies were seen to be host-specific. This is because only fleas of some head-types were found in specific hosts. This, along with genetic differences among the *T. penetrans* fleas that infect different host animals, one may suggest that there are several species of closely related species have been grouped taxonomically under one binomial nomenclature (Nagy *et al.*, 2007).

Though the chigoe flea resembles most others in morphology, the flea has a hypertrophic region between tergites 2 and 3. As stated in Eisele *et al.* (2003), tergites 2 and 3, as well as the abdominal sternites, stretch considerably and are bent apart. Chitinous clasps that are built for the abdominal enlargement surround these regions and hold onto the hypertrophic zone, giving them the appearance of a three-leafed clover. Surprisingly, the rest of the flea, including the head and the thorax, do not change in shape (Eisele *et al.*, 2003). With the rapid expansion of the flea, the morphology of the flea becomes vastly different. It changes from a small flea, into a bulging mass that measure 5-10mm in diameter in just a few days (Appendix IV, b). This results in a volume that is 2000 to 3000 times its original size, before burrowing into the skin (Eisele *et al.*, 2003). Neosome is the most marked characteristic of the genus *Tunga* and also the most frequently observed from its hosts, causing serious ectoparasitosis and harmful infections. It is an enlarged structure of about 5-13 mm in size. It is formed as a result of the hypertrophy of gravid females and the growth of new

tissue, following the penetration and mating of adult females in to the skin of their hosts (Pampiglione *et al.*, 2002). Of the 13 species of the genus *Tunga*, only one species has no known neosome. After oviposition, the neosome involutes with the death of the parasite being absorbed or sloughed by the host epidermis tissue repair mechanism. This makes the specific identification more difficult (Eisele *et al.*, 2003).

2.5 Life cycle

The parasitic flea is about 1mm in length, generally lives in the soil or sand, and feeds intermittently on warm blooded host such as human beings, pigs, dogs, cats and chicken (Pilger *et al.*, 2008). The young flea is very active and very jumpy on the ground. In order to reproduce, the fertilized female flea burrows head first in to the host's skin until only the last 2 abdominal segments are exposed (Eisele *et al.*, 2003). The orifice allows the chigoe flea to exchange gases while feeding from blood vessels in the cutaneous and sub cutaneous dermal layer. In two weeks time, it releases about 200 eggs through the orifice which falls to the ground or adheres on to the host (Appendix IV, c and d). The flea then dies and is sloughed by the host's skin leaving a hole full bacteria and pus (Eisele *et al.*, 2003). The eggs hatch in the next 3-4 days and mature into adult fleas within 3-4 weeks of the cycle (Figure 2.4). The male flea dies after mating (Nagy *et al.*, 2007).

Eggs are shed by the gravid female into the environment. The smooth, oval or rounded, light-colored egg, about 0.5 mm long, (Appendix IV, c and d) are deposited on, but not firmly attached to the body, the bedding, or the nest of the host animal. Although they are a little sticky, those laid on the host's body may fall or be brushed off. This accounts

for their being found in crevices in the floor, under the edges of carpeting, in sofas, or in cat or dog boxes and kennels, where they usually hatch in about 10 days. Eggs hatch into larvae in about 3-4 days and feed on organic debris in the environment (Nagy *et al.*, 2007).

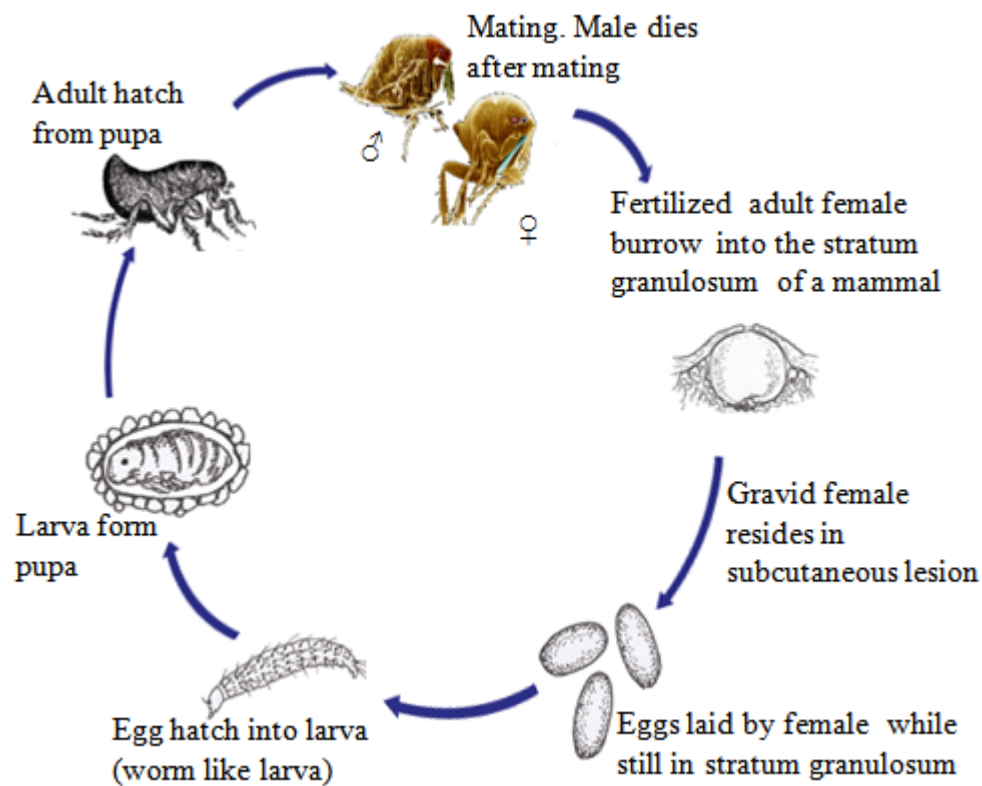


Figure 2.4: The life cycle of *T. penetrans* flea showing a complete metamorphosis cycle. Male flea (♂) which is distinguishable by its conspicuous copulatory organs dies after mating. The fertilized female flea (♀) infests mammalian skin to develop and mature its eggs to perpetuate the cycle.

Tunga penetrans has two larval stages before forming pupae. The small, hairy, wormlike larvae, are almost white, with a distinct, brown head, and do not have eyes or legs (Figure 2.4). They have 3 thoracic and 10 abdominal segments, with a single row of bristles around each segment. They move forward by using their backward-projecting

bristles and a pair of hooked, chitinous processes located at the end of the abdomen by which they can obtain a grip on a surface. When disturbed, they may "flip" in circles to escape. The larvae of some species require dried blood for food, but others do not. Those that do not need blood feed on the many kinds of organic debris that are present in the crevices in which the eggs commonly hatch. They also feed on their own cast skins (Nagy *et al.*, 2007). They require high humidity. Larvae then hatch into pupae that are in cocoons (Figure 2.4) and often covered with debris from the environment such as sand and pebbles. The pupae are initially white but they change to brown before the adults emerge. In the pupa, the appendages are not closely pressed to the body, and it has the general shape and characteristics of the adult. Under favorable conditions, the adult emerges in about a week, but under adverse conditions, the pupal period may be prolonged to as much as a year. The adult may remain in the cocoon for a long time until vibrations indicating the presence of a possible host stimulate it to emerge and become active (Heukelbach *et al.*, 2001).

The potentially long pupal stage, besides the fact that adult fleas can live without food for remarkably long periods, accounts for the fact that people may enter a house after it has been unoccupied by humans or pets for months, yet be rapidly and severely attacked by fleas (Eisele *et al.*, 2003). Depending on the species and weather conditions, 2 or 3 weeks, to many months, and rarely as long as 2 years, are required for many species of fleas to complete a life cycle.

The larval to pupal stages take about 3-4 weeks under normal conditions. The adults hatch from pupae and seek out a warm-blooded host (mammals and birds) for blood

meals. Both males and females feed intermittently on their host, but only mated females burrow into the skin epidermis of the host, where they cause a nodular swelling (Feldmeir *et al.*, 2013). Females do not have any specialized burrowing organs, and simply claw into the epidermis after attaching with their mouthparts. After penetrating the stratum corneum, they burrow into the stratum granulosum, with only their posterior ends exposed to the environment. The female fleas continue to feed and their abdomens extend up to about 1 cm. Females shed about 200 eggs over a two-week period, after which they die and are sloughed by the host's skin. Secondary bacterial infections are common with tungiasis (Heukelbach *et al.*, 2001).

2.6 Reproduction and fitness

Females have a depression or groove at their abdominal end whereas the males have their protruding copulatory organs in that same region. These morphological differences reflect the way the male and female copulate. In the first step toward copulation, the female penetrates an organism. It is only there that the male will find her and copulate. Copulation of adults has not been observed in the wild. With the female reproductive organs pointing outward, the male places its reproductive organs to copulate (Nagy *et al.*, 2007). Copulation takes a few seconds to 2 minutes after which, the male takes search for another female. After copulation, the male dies, although sometimes it may take a blood meal before dying. Interestingly, eggs will be expelled whether or not they have been fertilized (Witt *et al.*, 2009).

Tunga penetrans have been observed not to do very well in terms of its Darwinian fitness (Nagy *et al.*, 2007). In a laboratory setting in which different mediums were

provided for larval growth, the rate of survival from egg to adult in the best medium was 1.05%. Only 15% of the eggs were found to develop into larvae, and of those, only 14% formed a cocoon. Moreover, only half of the pupae reached the adult phase (Nagy *et al.*, 2007). Although these results reflect a laboratory setting, the general lack of success for *T. penetrans* K-strategy is surprising given the number of fleas that a single person can attract. The low survival suggests that a concerted public health effort directed at any point in the flea's life cycle is likely to deal a crippling blow to the overall population of the flea in the area.

2.7 Demographic characteristics of tungiasis

Tungiasis is caused by *T. penetrans*, a female flea when it burrows in to the host skin. Jigger transmission directly from one person to another is not possible, but easily occurs through insanitary environment (Kimani *et al.*, 2012). When eggs are laid, the fleas hatch and find the next host. *Tunga penetrans* have been documented to use various mammals as reservoir host. These include humans, pigs, cats, rats, bats, sheep, cattle, donkeys, monkeys, and birds (Pilger *et al.*, 2008). These hosts indirectly propagate the disease by being the origin of the next generation of fleas. Once the female fleas expel 100-200 eggs the cycle of transmission begins again. Several studies in this area have consistently shown tungiasis prevalence of between 16-55 % in typical endemic areas with a peak in children of about 5-14yrs, and to a lesser extent in old age of 55 and above years (Muehlen *et al.*, 2003). In these studies, males have been observed to be slightly more susceptible (Wilcke *et al.*, 2002). In Githunguri and its surrounding villages, a prevalence of 18.8 % was observed (Kimani *et al.*, 2012). Ngomi, (2010)

observed a prevalence of 57% in children of 5-12yrs in Murang'a south whereby boys with a prevalence of 59.2% were more infested compare to girls who had a prevalence of 54.8%.

2.7.1 Seasonal variation of the prevalence of tungiasis

A study conducted in Brazil revealed that the disease has a considerable seasonal variation (Figure 2.5). The prevalence of tungiasis was observed to be highest at the peak of the dry season in the month of September, which was 54.4% while the lowest prevalence of 16.8% was recorded after the first rain of the rainy season in the month of January (Heukelbach *et al.*, 2005). A similar trend has been suggested in Kenya, as reported by a number of respondents during the Ahadi Kenya team visit to Murang'a District.

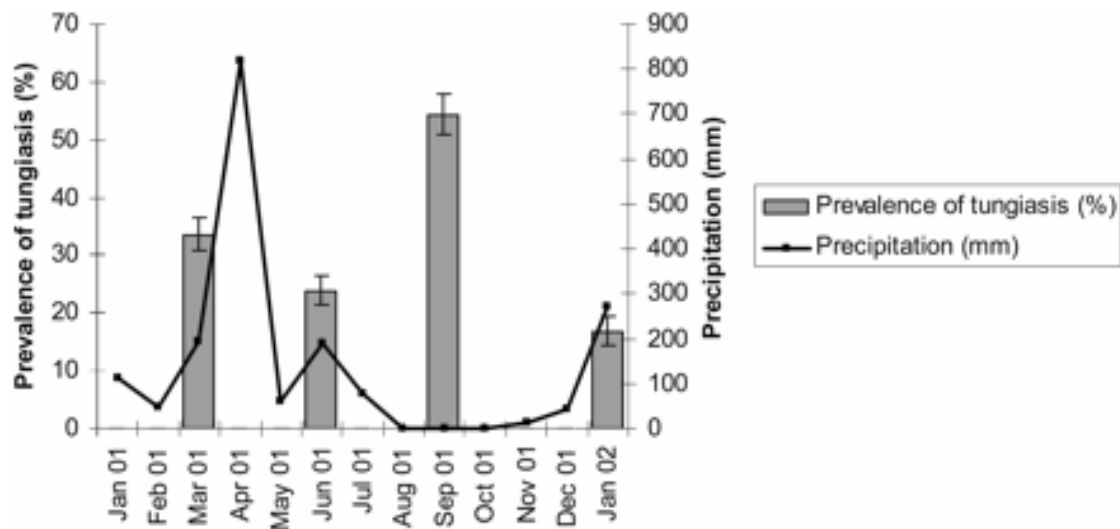


Figure 2.5: Seasonal variation of the prevalence of tungiasis (bar graph) and monthly precipitation from January 2001 to January 2002 (line graph) in Fortaleza, North Eastern Brazil. Y-error bars shows 95% confidence interval (Heukelbach *et al.*, 2005).

From the graph on variation of the infestation with seasons, the prevalence is seen to increase with drier weather and reach its peak when precipitation is zero (Heukelbach *et al.*, 2005). It then decreases with the onset of rains at the end of the year. High humidity in the soil was also observed to impair the development of free-living stages of *T. penetrans*. Furthermore, heavy rains may simply wash away the eggs, larvae, pupae, nymphs, and adult fleas. Therefore, control measures aimed at reducing morbidity should be scheduled to be in place before the attack rate increases, that is, at the beginning of the dry season and focusing on the most vulnerable population groups, namely children and the elderly, for effective control.

2.8 Pathogenesis and clinical manifestation of tungiasis infestation

Once burrowed in the skin of its host, the female flea becomes hypertrophic producing and releasing eggs (Eisele *et al.*, 2003). Acute inflammation with erythema, edema, pain and severe itching results due to tissue damage induced by metabolically high active and continuously enlarging parasite (Heukelbach *et al.*, 2001; Feldmeir *et al.*, 2013; Veraldi *et al.*, 2007). The parasite enlarges by a factor of about 2000 (Feldmeier *et al.*, 2004; Appendix IV, b). It consists of smooth inter-segmental skin and newly formed chitinous clasps. These make up a structural matrix to which the micro organisms can easily adhere. Lesions itches immediately after the flea penetration and the patient usually start to scratch, which in turn promotes the entry of bacteria through the persistent sore in the epidermis (Heukelbach *et al.*, 2002). When the skin's surface is linked by the means of a foreign body to underlying tissue, bacterial infection of the

epidermis may occur. This is presumably due to the formation of a biofilm on the surface of the foreign body (Heukelbach *et al.*, 2002).

In vertebrate's host, the infestation with *T. penetrans* is a self limiting process but the risk of secondary infection complication is obvious (Eisele *et al.*, 2003; Heukelbach *et al.*, 2002; Feldmeier *et al.*, 2004). In many patients, bacterial super infection is as a result of inappropriate manipulation of lesion with non sterile instruments by the patient. Remarkable desquamation of the skin is observed around the late stage lesion. This has its histopathologic correlation to hyperkeratosis and parakeratosis of the stratum corneum (Heukelbach *et al.*, 2002). Literature abounds with observations on severe pathological finding associated with tungiasis. It mentions debilitating sequelae such as phagedenic ulcers, nail loss, tissue necrosis, auto amputation of digits (Appendix IV, f) and the loss of entire limbs (Feldmeier *et al.*, 2004; Heukelbach *et al.*, 2001). Gangrene is another common complication of severe infestation and superinfection (Pilger *et al.*, 2008). Death resulting from secondary infection with *Clostridium tetani* is common in non vaccinated persons (Eisele *et al.*, 2003). In fact infection by *C. tetani* that causes lethal tetanus account for 10% of deaths among infested people in Brazil (Feldmeier *et al.*, 2002). If the patient is not vaccinated tetanus is often a complication due to secondary infections.

Recently *Wolbachia* species of bacteria have been identified in the ovaries of *T. penetrans*. Antigens of these bacteria endosymbiont have been associated with the pathologic immune response in some filarial diseases such as Onchocerciasis. Part of the intense immune response in tungiasis might also be evoked by *Wolbachia* antigens

being released from decaying fleas (Eisele *et al.*, 2003). *Staphylococcus aureus* and *Wolbachia* endobacteria can also be transmitted by the chigoe flea as well as nearly 150 other different pathogens such as bacillus species. For these reasons the chigoe flea should be removed as soon as possible and the hole left sterilized immediately (Eisele *et al.*, 2003).

2.9 Diagnosis and clinical examination of tungiasis

This parasite is an ectoparasite with visible symptoms (Figure 2.6). Identification of the parasite through removal, and a patient's traveling history, should suffice for diagnosis, though the latter is clearly more useful than the former. Localization of the lesion may be a useful diagnostic method for the clinician. The diagnosis of tungiasis is however clinically established (Heukelbach *et al.*, 2001). The untrained physician can diagnose the ectoparasite by taking into account the typical topographic localization (Figure 2.6) and natural history of the disease.

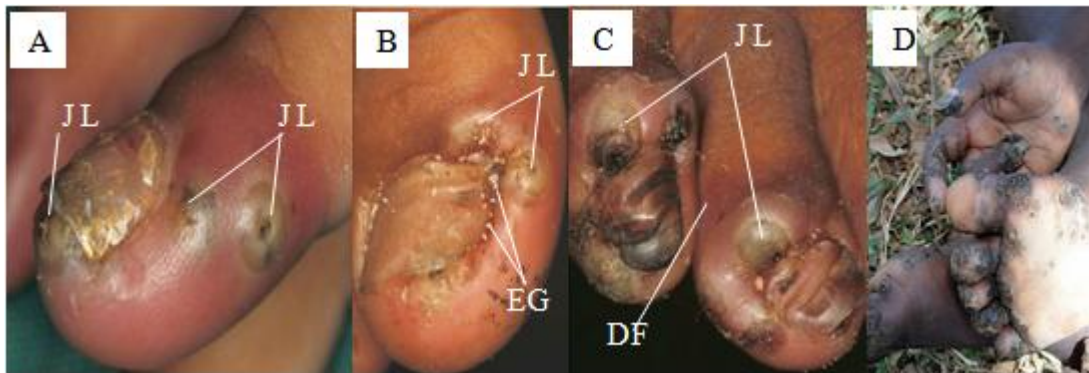


Figure 2.6: *Tunga penetrans* lesions. A, B and C shows localized jigger lesions (JL) on a finger and toes; EG in B are eggs being hatched. DF in C shows deformities on toes and finger nails due to jigger infestation. D shows severe lesions in clusters (Heukelbach *et al.*, 2005).

The patient typically complains about local itching, pain and sensation of a foreign body (Heukelbach *et al.*, 2001). Most lesions occur on the nail rim, periungual area of the toes, the heels, the soles and almost every part of the body such as hands, elbows, neck, buttocks and the genital region (Ahadi, 2008; Heukelbach *et al.*, 2002; Feldmeir *et al.*, 2013; Veraldi *et al.*, 2007). If severe lesions occur simultaneously, they are usually located in clusters (Figure 2.6, D). Severe infestation (defined as the presence of >20 lesions in an individual) with hundred of embedded sand fleas are not rare (Ariza *et al.*, 2010). In single cases, lesions may take the aspect of a growth, and histological sections appear as pseudoepitheliomatous hyperplasia (Figure 2.7 and 2.8; Scott *et al.*, 2013).

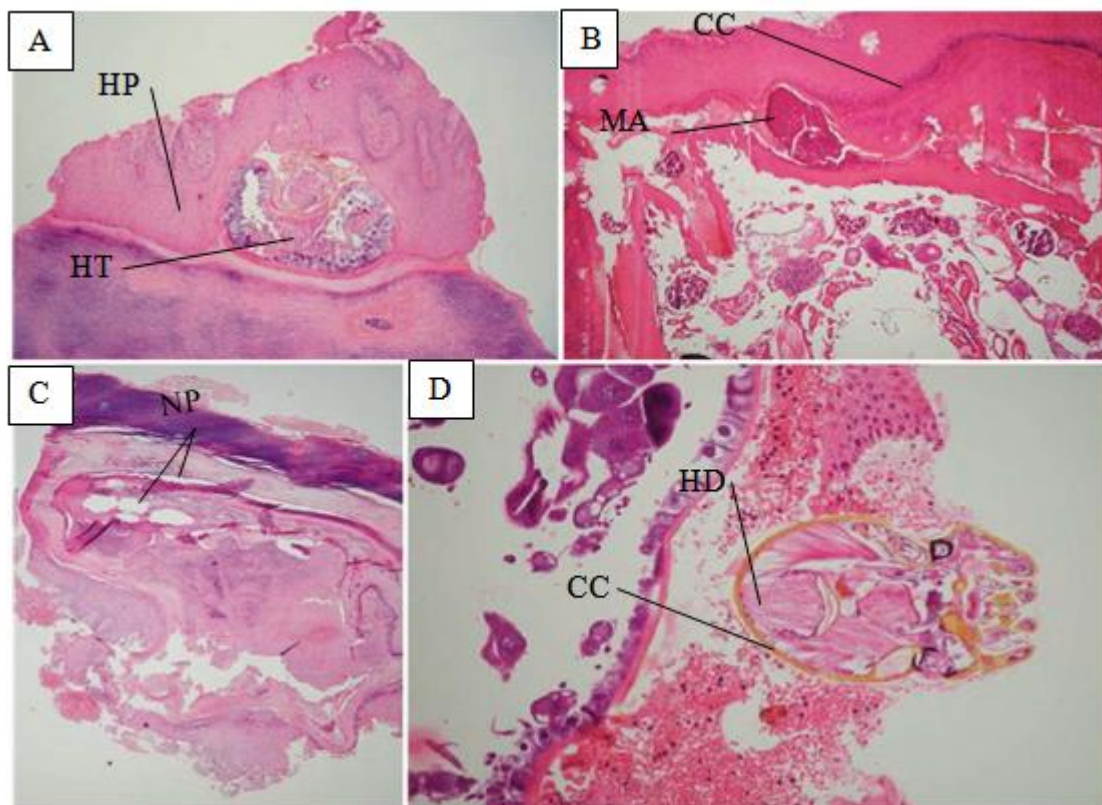


Figure 2.7: Histopathologic findings in tungiasis. A. Tangential cut through a fully developed, gravid flea embedded in the stratum corneum of the epidermis. The flea's head and thorax (HT) are enfolded in the hypertrophic anterior abdominal segments.

The epidermis is hyperplastic and shows papillomatosis, parakeratosis, and hyperkeratosis (HP). B. Tangential cut through the posterior abdominal segments of an embedded sand flea. Next to the chitinous cuticle (CC), a microabscess (MA) has formed. C. Dead parasite; the exoskeleton of the posterior abdominal segment has remained intact; the cuticle has disintegrated at the epidermal–dermal interface. The carcass is infiltrated by neutrophils, and pus has formed (NP). D. The head of the flea (HD) is located at the epidermal–dermal interface, has penetrated the basal membrane, and is surrounded by many erythrocytes, presumably having leaked from a blood vessel. The abdomen of the parasite is separated from host tissue by a thick, chitinous cuticle (CC) (Scott *et al.*, 2013).



Figure 2.8: Histologic examination of engorged *T. penetrans*. EC- reveals an intra-epidermal cavity lined by an eosinophilic cuticle, which represents the body of the flea. In the cavity are round to oval eggs (EG). CC shows hollow ring like components of the tracheal system and the digestive tract. A thick band of striated muscle runs from the head to the terminal orifice (SM). Usually, an inflammatory infiltrate is present in the subjacent dermis (Scott *et al.*, 2013).

Examination of tungiasis is however well understood by studying Fortaleza classification which formally describes the last part of the female flea's life cycle where it burrows into its host's skin, expels eggs, and dies (Figure 2.9; Eisele *et al.*, 2003). This classification is based on clinical Scanning Electron Microscopy and histological

findings to enable the assessment of chemotherapeutical approaches and help evaluate control measures at the community level.

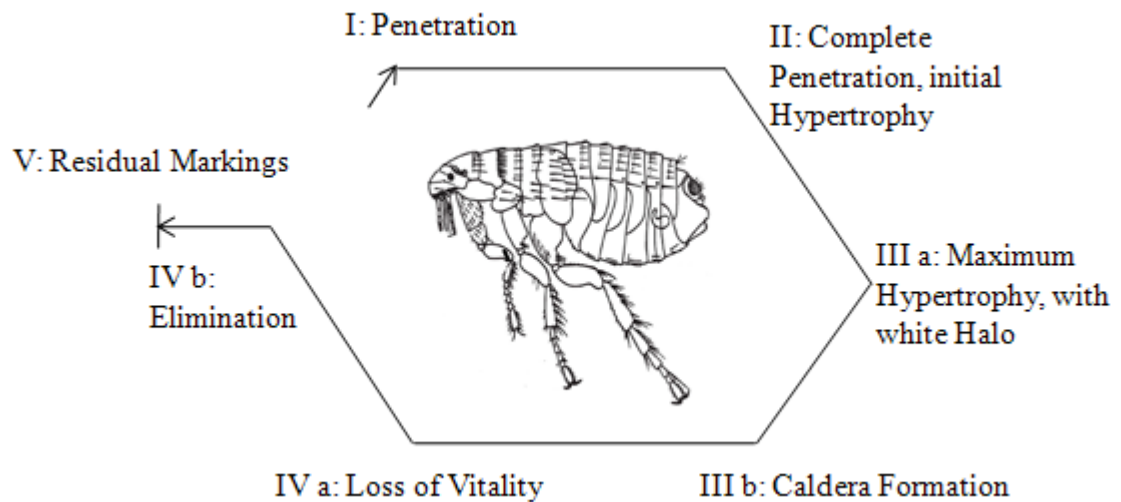


Figure 2.9: Stages in Fortaleza classification

Stage 1 of Fortaleza classification is characterized by the penetration of the skin by the female chigoe flea (Eisele *et al.*, 2003). Running along the body, the female uses its posterior legs to push its body upward by an angle between 45-90 degrees. Beginning with its proboscis, penetration starts (Figure 2.10, A and B), on the epidermis. By stage 2 (day 1-2), penetration is complete and the flea has burrowed most of its body into the skin. Only the anus, the copulatory organs, and four rear air holes in fleas called stigmata remain on the outside of the epidermis (Figure 2.10, C). The hypertrophic zone between tergites 2 and 3 in the abdominal region begins to expand a day or two after penetration (Figure 2.10, D). During this time, the flea begins to feed on the host's blood. Stage 3 is divided into two morphologically and clinically different sub stages, the first of which being 2-3 days after penetration is complete.

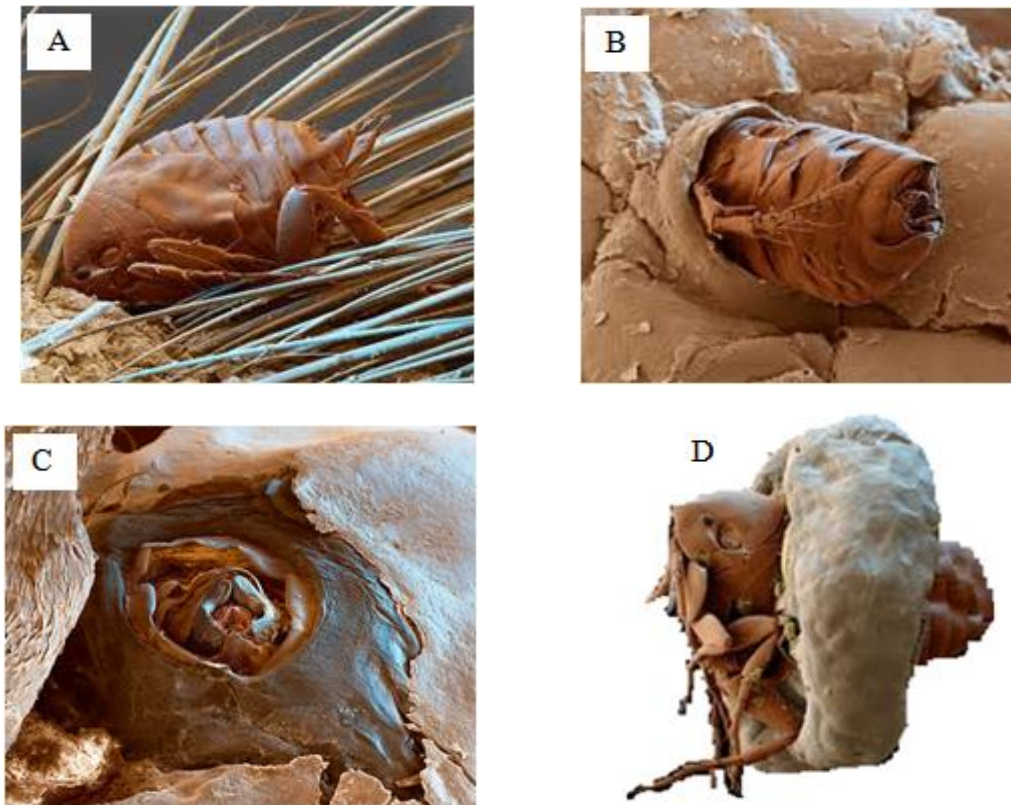


Figure 2.10: Penetration of *T. penetrans* into the host skin. A. The flea burrowing into the host skin. B. The flea head completely burrowed into the host skin. C. The whole flea fully burrowed into the host skin. D. Tergites 2 and 3 in the abdominal region expand (Heukelbach *et al.*, 2005).

In stage 3a, maximum hypertrophy is achieved and the flea's midsection swells to the size of a pea. Due to the expanding flea, the outer layer of the skin is stretched thin, resulting in the appearance of a white halo around the black dot (rear end of the flea) at the center of the lesion. In sub stage 3b, the chitin exoskeleton of tergites 2 and 3 increase in thickness and gives the structure the look of a mini caldera. Egg release is common in sub stage 3b, as fecal coils. The eggs tend to stick to the skin, possibly through adhesive proteins (Figure 2.6, B; Appendix IV, C).

At about the 3rd week after penetration, stage 4 begins, which is also divided into two sub stages. In sub stage 4a, the flea loses its signs of vitality and appears near death. As a result, the lesion shrinks in size, turns brown, and appears wrinkled. The death of the flea marks the beginning of sub stage 4b (around day 25 post-penetration) as the body begins to eliminate the parasite through skin repair mechanisms such as shedding and subsequent skin repair. At this phase, the lesion is seen as brown or black. By the 5th stage of tungiasis, the carcass of the *T. penetrans* flea is expelled but there are residues of the infection that remain. In all the cases, tungiasis by itself only causes morbidity, though secondary infection may lead to mortality. This life cycle section presents the Fortaleza stages from the flea's developmental perspective, specific to symptoms of human infection. The dates for stages and symptoms may be different for different animals due to anatomical variations (Nagy *et al.*, 2007).

The patient with a single flea may complain as early as stage 2 when a boring pain and the curious sensation of 'pleasant' itching occur resulting in inflammation. This inflammatory reaction is the initial immunological response to the infestation. Heavily infested patients may not notice a stage 2 infection due to the other fleas' causing irritation as well. Feces of fleas may be seen, but this is more common in the 3rd stage. Around the third day after penetration, erythema and skin tenderness are felt, accompanied by pruritus (severe itching) and a black furuncular nodule surrounded by a white halo of stretched skin caused by the expansion of the flea. Fecal coils may protrude from the center of the nodule where the flea's anus is facing upward. They should be washed off quickly as the feces may remain in the skin unless removed.

During this 3a sub stage, pain can be severe, especially at night or, if the nodule is on the foot, while walking. Eggs will also begin to be released and a watery secretion can be observed.

The radical metamorphosis during the 3rd to 6th day after penetration, or neosomy, precedes the formation of a small caldera-like rim rampart as a result of the increased thickness of the flea's chitin exoskeleton. During the caldera formation, the nodule shrinks a bit and it looks as if it is beginning to dry out; this takes 2 weeks and comprises sub stage 3b. At the 3rd week after penetration and sub stage 4a, the eggs' release will have stopped and the lesion will become smaller and more wrinkled. As the flea is near death, fecal and water secretion will stop altogether. Pain, tenderness, and skin inflammation will still be present. Around the 25th day after penetration, the lesion looks like a black crust, the flea's carcass is removed by host repair mechanisms and the skin begins to heal.

With the flea gone, inflammation may still persist for a while. Although patients would not complain of itching in the 5th stage of tungiasis as the flea would be dead and no longer in the body, this stage is characterized by the reorganization of the skin (1-4 weeks). A circular residue of 5-10mm in diameter around the site of penetration is observed. An intraepithelial abscess, which developed due to the presence of the flea, will drain and later heal. Although these residues would persist for a few months, tungiasis is no longer present. In severe cases, ulcers are common, as well as complete tissue and nail deformation. A patient may be unable to walk due severe pain if too many of the lesions are present in the feet (Figure 2.6, D). Suppuration, auto-amputation

of digits and chronic lymph-edema may also be seen (Appendix IV, F; Heukelbach *et al.*, 2003; Ahadi, 2008).

2.10 Immunological responses against tungiasis

Tungiasis causes a mixed Th₁ and Th₂ immune responses, characterized by significantly increased concentration of the pro-inflammatory cytokines IFN- γ and TNF- α with a slightly increased concentration of IL-4 (Feldmeier *et al.*, 2004). However this has not been utilized in chemotherapeutical procedures. *Tunga penetrans* releases proteolytic enzymes during penetration and growth, causes acute inflammation responses on the skin with erythema, edema, pain and itching due to tissue damage (Eisele *et al.*, 2003). The immune responses of the host might contribute to the intense inflammation observed soon after penetration. However a lot on immunology of the parasite is yet to be discovered. The concept of effective therapy based on immunology is not well researched. In fact, currently no effective therapy is available (Feldmeier *et al.*, 2004). More over antibody responses during infestation have not been studied. Immunological reactions of the human host when infested by jiggers in Kenya have not been documented. Consequently, data on *T. penetrans* antigen-antibody reactions is scarce. However, at molecular level genetic diversity of *Tunga* species has been investigated in Brazil. Jigger mitochondrial DNA isolates from cats, dogs, and humans were found to have identical sequence presuming infestation by the same species of *Tunga*. However, when these isolates were compared to those of fleas from pigs and rats differences in sequence of up to 49% was found so that the existence of one or more new species of *Tunga* may be presumed (Vobis *et al.*, 2005).

2.11 Treatment and control of tungiasis

Immediate mechanical removal of the embedded fleas and disinfection of the hole left is so far the most efficient way of getting rid of jiggers (Feldmeier *et al.*, 2003). The disease is self-limiting, at least when exposure to the parasite is limited. So management is mostly confined to treatment. Due to the secondary infection that can cause serious medical issues, care should be taken to avoid tearing the flea during the extraction procedures. Otherwise severe inflammation would result. The same would occur if part of the flea is left behind (Eisele *et al.*, 2003). Sterile equipment should always be used, as contaminated instruments could act as mechanical vectors for pathogens to enter the body or enhance spread of HIV.

Prevention consists of regular wearing of closed well fitting shoes instead of walking barefooted or with wide sandals. More over socks that are left lying on the floor should be avoided. General hygiene has been found to be effective (Eisele *et al.*, 2003). Ointment with Lysol has also been found to protect the feet (Heukelbach *et al.*, 2003). It has also been established that 5% oral thiobenzadole in case of super infection is effective against the parasite but controlled studies are unavailable (Heukelbach *et al.*, 2004). A randomized controlled trial realized more than 20 years ago showed a good efficacy of oral niridazole, an anti schistosomal compound but with severe side effects; thus it has been withdrawn from the market ever since (Feldmeier *et al.*, 2004). Additionally there are some reports by health providers about the efficacy of oral ivermectin for the treatment of tungiasis (Heukelbach *et al.*, 2003). However a recently conducted controlled trial with oral ivermectin at a relatively high dose ($2 \times$

300µg/body weight) did not show any efficacy as compared to placebo (Heukelbach *et al.*, 2002).

In Kenya dettol has been tried with some success (Ahadi, 2008). Occlusive petrolatum suffocates the organism. Twenty-percent salicylated petroleum jelly (Vaseline) applied 12-24 hours in profound infestations causes the death of the fleas due to suffocation and facilitates their manual removal (Clyti *et al.*, 2003). However, these treatments do not remove the flea from the skin, and they do not result in quick relief from painful lesions (Cabrera and Daza, 2009). The gum of *Saint Domingo Apricot* fruit (common name - Mammee apple), has also been used to kill the chigoe flea (Heukelbach *et al.*, 2006). One very successful repellent is called Zanzarin, a derivative of coconut oil, jojoba oil, and *Aloe Vera*. In a recent study involving two cohorts, twice daily application of this plant based repellent reduced the infestation rate by 92% on average for the first one and by 90% for the other (Schwalfenberg *et al.*, 2004). The non-toxic nature of Zanzarin combined with its “remarkable regression of the clinical pathology” makes it a tenable public health tool against tungiasis (Schwalfenberg *et al.*, 2004). In an endemic community, the use of an effective repellent would be a better approach to reduce tungiasis other than treatment after infestation (Eisele *et al.*, 2003).

Regular use of insecticide indoors especially in sand and earthen floors is also effective. Use of pesticide, like DDT, led to elimination of the *T. penetrans* in Mexico in 1950 (Feldmeier *et al.*, 2003), however this control/prevention strategy should be very carefully utilized, if at all, because of the possible side effects it can have on the greater biosphere (Wilcke, 2002). In the 1950's, there was a worldwide effort to eradicate

malaria. As part of that effort, Mexico launched the National Campaign for the Eradication of Malaria. By spraying DDT in homes, the *Anopheles* genus of mosquitoes known to carry the deadly *Plasmodium falciparum* and its other malarial cousin pathogens was mostly eliminated. As a consequence of this national campaign, other arthropods were either eliminated or significantly reduced in number, including the reduviid bug responsible for Chagas's disease (American Trypanosomiasis) and *T. penetrans* (Feldmeier *et al.*, 2003). Controlled in-home spraying of DDT is effective as it gives the home immunity against arthropods. However it can lead to contamination of water supplies and doing as much ecological damage as was once the case when DDT was first introduced (Allison, 2009).

While other species gradually gained resistance to DDT and other insecticides that were used, *T. penetrans* did not (Eisele *et al.*, 2003); as a result, incidences of tungiasis in Mexico are very low when compared to the rest of Latin America, especially Brazil, where rates in poor areas had been known to be as high as or higher than 50% (Heukelbach *et al.*, 2005). In fact, there was a 40-year period where there were no tungiasis cases in Mexico. It was not until August 1989 that three Mexican patients presented with the disease. Though there were other cases of tungiasis reported thereafter, all were probably reintroduced from Africa (Eisele *et al.*, 2003).

Prevalence rates of tungiasis were further reduced with the coincidental rise of shoes wear (as opposed to sandals). Though the use of socks and shoes provide some measure of protection against jigger infestation, it does not offer a long lasting solution as neither eradication nor elimination of the parasite will occur. Nevertheless, social efforts to

improve hygiene, welfare, and standard of living do provide additional protection against the chigoe flea as tungiasis is mostly a disease of the poor. Through increased efforts aimed at socio-economic improvements, coupled with the use of repellants, insecticides, and knowledge of proper extraction techniques, tungiasis has the potential to be overcome and defeated, finally ending the life of a disease that has plagued the poor all too often (Eisele *et al.*, 2003).

Murang'a County is believed to have a high prevalence of jiggers (Ahadi, 2008). However data to prove this theory is scarce. Recently a prevalence of 57% in children of 5-12 yrs old was observed on Murang'a South (Ngomi, 2010). A prevalence of 18.8% was also observed in Rukoroi, Githunguri and its surrounding areas (Kimani *et al.*, 2012). The soil factors in Murang'a County have been found to be important risk factors. Soil moisture, organic matter content, soil pH, soil texture and soil colour influences the prevalence of tungiasis by up to 33% and the *T. penetrans* population by up to 39.7 % (Ngunjiri *et al.*, 2011). However the epidemiology of this parasite is still poorly understood in Kiharu constituency and in the County in general. The body immune responses against jigger infestation have not been assessed. In addition *T. penetrans* antigens and corresponding antibody responses has not been characterized. This is important because if immunodominant antigens were identified perhaps it would form the basis for immunoprophylaxis studies aimed at controlling the infestation among the afflicted communities. Therefore this study sought to fill these study gaps. It has been documented that children of 5-14yrs old are the most vulnerable and to a lesser

extent those in age bracket of 55 yrs and above (Muehlen *et al.*, 2003). For this reason this study has put emphasis in school going children.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study site

This study was carried out in Kiharu constituency. Four administrative locations were targeted namely Gaturi, Kimathi, Mugoiri and Kahuhia (Figure 3.1). Rainfall in parts of Kimathi, Gaturi and Mugoiri is poorly distributed and receives as less as 900mm per year (Murang'a District Environmental Action Plan, 2006-2011). Day temperatures are about 26-29°C during dry seasons and an average of about 22°C during cold weather. The area normally experience famine as soon as the rain fails for at least a season (Kiharu Constituency Strategic Development Plan, 2010-2030). Clustered pattern of settlement is evident in the relatively flat areas of lower Murang'a County. The study area has a population density of 181,076 people according to Kenya Population and Housing Census (2010). The inhabitants of this study area do not have a proper regular source of income. This is mainly because coffee, the only cash crop that existed was in 1990s neglected and left to dry due to poor returns. The inhabitants are mainly peasant farmers growing food crops like maize, beans and bananas.

3.2 Study population and study design

This study was carried out in 2012 when the primary schools were in session during dry weather seasons. This is because the study population mainly comprised of primary school children (≥ 4 rs) and rainy seasons made some places inaccessible due to poor road network. The relatives of the recruited pupils were also recruited. Before the onset of the study, information meetings were held with teachers, parents, pupils and community members. Kiharu constituency comprise of nine locations including the

township. Purposive sampling was used to select four of the nine locations to be recruited in the study guided by records from Murang'a County hospital, local health centers and information from magazines by Ahadi Kenya. These are Gaturi, Kimathi, Kahuhia and Mugoiri (Figure 3.1).

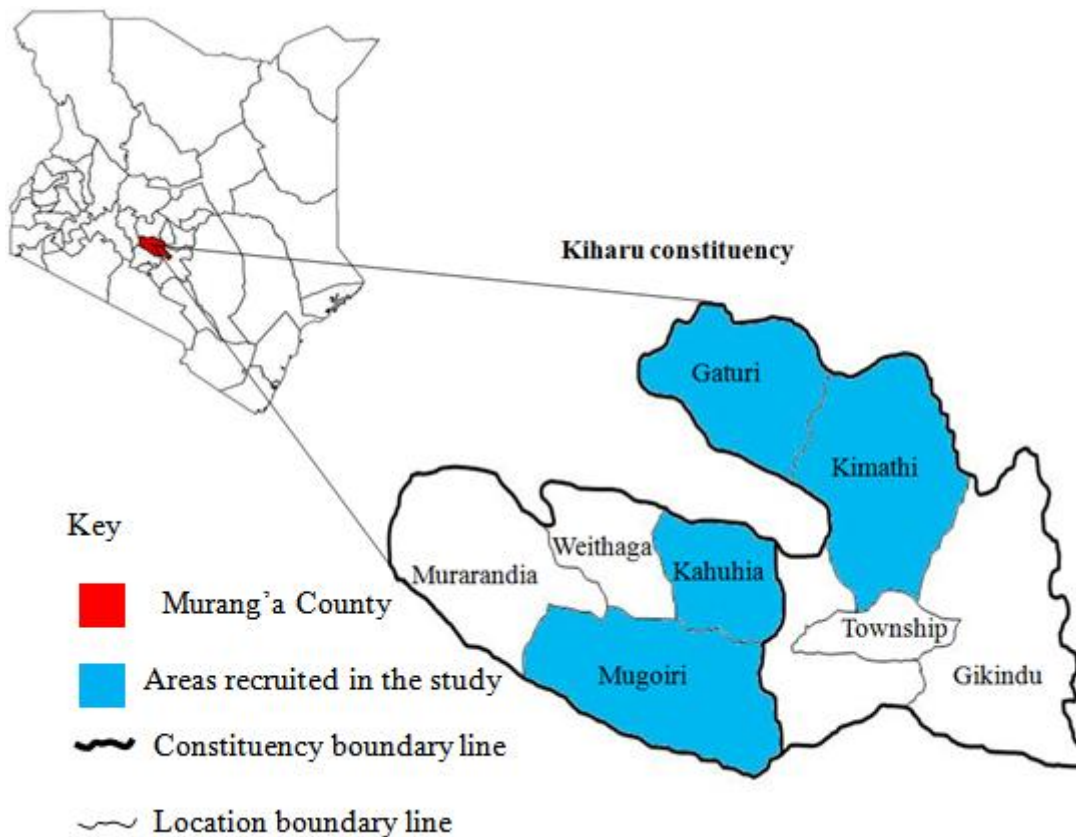


Figure 3.1: A Map of the study area; Kiharu constituency (Source: National Environmental Management Authority, (2006)

Random sampling was used to select three primary schools in each of the location recruited in the study. In each school, three classes (pre-unit/nursery school up to standard eight) were randomly sampled. Interviews guides, focused group discussions and observations were used to collect data on prevalence. Questionnaires were applied

to both infested and non infested pupils in the sampled classes to collect demographic characteristics of the study population. Public Health Officers, Clinical Officers and Community Health Workers were engaged in extraction of jigger specimens, collection of blood samples and treatment of tungiasis. Jigger infested pupils were then followed into their homes whereby their family members were also recruited, interviews and questionnaires were administered.

Participants were thoroughly examined for the presence of embedded jiggers. Clinical examination was performed by carefully inspecting the legs, feet, hands and arms. To guarantee privacy, other regions of the body were not examined. This approach was taken because in endemic communities more than 99% of tungiasis lesions occur on these areas (Heukelbach *et al.*, 2002). For clinical examination the following was considered diagnostic for tungiasis: an itching red-brownish spot with a diameter of 1-3 mm, a circular lesion presenting as a white patch with a central black dot, black crust surrounded by necrotic tissue, as well as partially or totally removed fleas leaving a characteristic sore in the skin. Localization and number of lesions were recorded. As defined by Ariza *et al.* (2010) the presence of more than 20 lesions on an individual was regarded a clinical disease.

3.3 Inclusion criteria

Pupils sampled in the identified primary schools, both infested and non-infested by jiggers; their family members and guardians at home.

3.4 Exclusion criteria

Pupils below 4yrs and those in classes that were not sampled; their family members and guardians.

3.5 Sample size determination

The sample size was arrived at using the following formula (Suresh and Chandrashekara, 2012);

$$N = \frac{Z_{\alpha/2}^2 \times P(1-P) \times D}{E^2}$$

N is the sample size.

$Z_{\alpha/2}$ is normal deviate for two-tailed alternative hypothesis at a level of significance = 1.96 at 95% confidence interval.

P is the estimated prevalence rates of people with jiggers in Kiharu Constituency = 57% (a proportion of 0.57), the prevalence rate of tungiasis in Murang'a South, Ngomi (2010).

D is the design effect = 2 (For designs other than simple random sampling).

E is the level of precision (or margin of error) which is 10% of P.

$$\text{Therefore; } N = \frac{(1.96)^2 \times 0.57 \times (1-0.57) \times 2}{(10/100 \times 0.57)^2} = 579$$

3.6 Sample collection for immunological studies

Samples for laboratory analysis were gravid female fleas that were mechanically removed from infested individuals. The specimens were put in 100µl of PBS and kept in low temperature of about 4°C. Venipuncture was used to collect 5 milliliters of blood

samples from patient's antecubital area of the arm and drained into EDTA tubes. This was later centrifuged at 1000 rpm for 10 minutes; serum collected and kept in a -20°C deep freezer.

Blood sample from immunized rats was drawn from the tail vein each time just before the next immunization. The rats were later sacrificed and more blood samples that was collected in veils for serum preparation. The serum was kept in a -20°C deep freezer until used.

3.7 Data collection tools

3.7.1 Questionnaires

Questionnaires (Appendix I) were applied alongside interviews, focused group discussions and observations to gather both demographic and epidemiological data on tungiasis. These included questionnaires for adults and for school going children.

3.8 Laboratory procedures

3.8.1 Protein extraction (freeze and thaw)

Protein extraction involved mechanical crushing of 30 gravid female jiggers in 2ml of PBS in clean sterilized 5ml bijou bottles using a clean sterilized glass rod to avoid contamination. This was followed by freezing the proteins in a deep freezer at -20°C for 1 hour, and thawing it for 1 hour at 25°C to extract soluble proteins. This process was repeated five times after which centrifugation of the products were done at 1000 rpm for 10 minutes at room temperature (25°C). The debris was discarded and the supernatant retained as the extracted protein sample.

3.8.2 Immunization of rats

Immunization of rats involved mixing 2ml of the extracted antigens with 1ml of complete Freud's adjuvant (ratio of 2:1). This was used to immunize 5 rats each with 0.5ml of the mixture in the thigh muscle (intra-muscular injection). Four immunizations were done in intervals of three weeks. Before each immunization, blood from the tail vein was withdrawn, serum extracted and later tested for antibodies using double immunodiffusion method. Three weeks after the fourth immunization the rats were sacrificed and serum prepared for immunodiffusion.

3.8.3 Ouchterlony double immunodiffusion and Immunoelctrophoresis

Ouchterlony double immunodiffusion, an immunological technique used in the detection, identification and quantification of antibodies and antigens was used. Briefly, on agar gel prepared on a glass slide, six wells in a row parallel to another were made (Figure 3.2). Extracted jigger proteins (12.5 μ l) was added into each of the four wells against equal volume of rat serum in the corresponding wells, the first well having a positive control and the last one a negative control. The slides were then incubated at 25⁰C for 48hrs for Ag-Ab complexes to form. The actual protocol used is as described by Bailey and Graham (1996).

Immunoelctrophoresis (IEP) procedure was used to determine the level of antibodies in blood, whereby electrophoresis and immunodiffusion techniques are combined. To run this test, a gel was prepared and a well punched on to the left end. The antigen mixture (jigger protein extracts) was placed in the well. Then electrophoresis and immunodiffusion were performed, as described by Csako (2012).

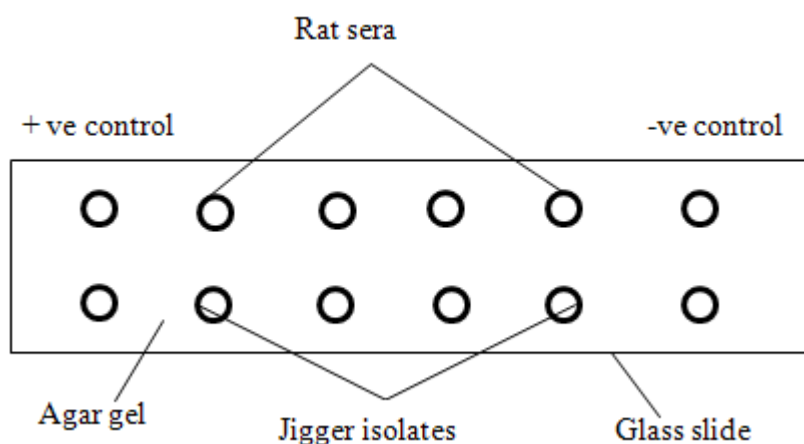


Figure 3.2: Agar gel slide showing how rat sera and jiggers isolates were put in the wells.

3.8.4 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)

In this study SDS-PAGE technique was used whereby protein molecules with similar charge to weight ratio were separated according to their size and shape by applying an electric current to them. The current forced the molecules through pores in a thin layer of gel so that smaller and lighter protein molecules traveled further than large heavy ones (Kurien and Scofield, 2009).

In brief, a BIO-RAD mini gel apparatus was used for this technique whereby two gels; the separating gel and the stack were prepared. When separating gel polymerized, stack gel was applied and combs fixed to draw wells where the protein samples in serial dilutions of 1:2, 1:4, 1:5, and 1:10 were loaded. Pharmacia standard sample markers (+ ve control) were prepared and utilized in accordance to manufactures instructions. The gel apparatus terminals were then subjected to 100V and 100amp from a power pack for 45 minutes for complete migration of protein bands. This procedure was conducted in

accordance to the standard protocol described by Kurien and Scofield, (2009) and as per instructions given by manufacturer manual.

3.8.5 Western Blot

Briefly, Western blot was used to whereby protein bands on gel electrophoresis were electrophoretically transferred onto a nitrocellulose paper where they were probed with antibodies that are specific to the protein being targeted in the serum in serial dilutions of 1:25, 1:50, 1:100, 1:200, and 1:400. This was then incubated with secondary antibody conjugate (Anti human Horseradish peroxidase) and then treated with a substrate for chemiluminescence. The actual procedure was conducted according to the standard protocol in Western blot as described by Alegria *et al.* (2009).

3.9 Ethical clearance

School going children were recruited in this study. Permits (Appendix V) to visit their schools were obtained from local District Education Officers in recently created Districts, Murang'a East and Kahuro. This study also involved handling jigger infested individuals and extraction of jiggers for laboratory analysis. Therefore research ethical clearance was obtained from Murang'a general hospital ethical committee and Institute of Primate Research (IPR).

Before the study, the objectives and the study protocol were explained during meetings with the teachers and community leaders who approved the study. In addition consent was also obtained after explaining the objectives from all study participants, or in case

of minors, from their parents or guardians. In such cases the objectives were translated into the local language.

3.10 Data analysis and presentation

Data was presented in graphs and tables. Chi square was used to compare data on prevalence rates between various groups in the study locations. One way ANOVA was used to analyze data comparing mean infestation within different locations in the study area and across age group. Correlation was used to determine the relationship between level of income of the parents and some risk factors. Multivariate logistic regression analysis was used to compare independent risk factors associated with tungiasis to determine their relationship (odds ratio) to prevalence of jigger infestation. Regression analysis was used to estimate the molecular weights of the unknown immunodominant antigens in jigger crude antigen preparations.

CHAPTER FOUR: RESULTS

4.1 Demographic characteristics of the study population

4.1.1 Level of education of adults in the study area

The sample size for this study was 579 individuals. However responses from 551 individuals were finally analyzed. Responses from 28 individuals were found inappropriate and information from them could not be properly analyzed. Five hundred and eight were school children (127 from each location) and 43 were adults in 40 households.

Education background of the study subjects was assessed. It was observed that in Gaturi, 4.5% of the adults recruited in the study never went to school, 31.8% went up to lower primary, 40.9% stopped schooling at upper primary level while 22.7% went up to secondary level. In contrast 7.7% of the adults sampled in Mugoiri district never went to school, 23.1% did not go beyond lower primary while 53.8% stopped schooling at upper primary. Only 15.4% managed to reach the secondary level. In Kahuhia 20% of adults never went to school, 20% others reached lower primary, 40% did not go beyond upper primary while 20% managed to reach the secondary level. In Kimathi some uniformity in the levels of education reached by adults was observed. All the adults sampled went to school while 33% would graduate from lower primary to secondary level in each case (Table 4.1). When compared using ANOVA the mean differences in level of education reached among the four study locations was however not significantly different ($F = 0.101$, $df = 3$, $p > 0.05$).

Table 4.1: Level of education reached by adults in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency.

Location	Adults level of education			
	Never went to school %	Lower primary %	Upper primary %	Secondary %
Gaturi	4.5	31.8	40.9	22.7
Mugoiri	7.7	23.1	53.8	15.4
Kahuhia	20.0	20.0	40.0	20.0
Kimathi	0.0	33.3	33.3	33.3

The level of education of eldest child above 18yrs in the four study locations was assessed. It was found that in Gaturi location 4.5% of the first children never went to school, 22.7 % reached the lower primary, 50% went up to upper primary and 22.7% managed to get to secondary level. In Mugoiri location it was found that 7.7% of the eldest children never went to school, 15.4% reached the lower primary level, 61.5% went up to upper primary and 15.4% managed to get to secondary level. In Kahuhia location all the first children of the adults studied went to school. However, 40% of their first borns did not go beyond lower primary, 40% of those who went to upper primary failed to go to secondary school, while only 20% managed to finish secondary education. In Kimathi location, 33% of the eldest children did not go school. However, all of those who went to primary school managed to go to upper primary but only 66.7% who manage to finish the upper primary. None managed to reach secondary level (Table 4.2). When compared using ANOVA, the mean differences in highest education reached by the eldest children among the four study locations was not significantly different ($F = 0.604$, $df = 3$, $p > 0.05$).

Table 4.2: Comparison of level of education reached by eldest child in each family in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency.

Location	Highest level of education of eldest child			
	Never went to school %	Lower primary %	Upper primary %	Secondary %
Gaturi	4.5	22.7	50.0	22.7
Mugoiri	7.7	15.4	61.5	15.4
Kahuhia	0.0	40.0	40.0	20.0
Kimathi	33.3	0.0	66.7	0.0

4.1.2 Marital status of parents in the study population

It was observed that most of the children in the four study locations have been raised in nuclear family setups. In Gaturi location only 18.4% of the adult recruited are not married, 9.1% are single parents, 59.1% are married, 4.5% divorced and 9.1% widowed. In Mugoiri location 7.7% adults recruited in the study are not married, 7.7% others are single parenthood, 76.9% married, 7.7% divorced and none windowed. In Kahuhia location 20% of the adults recruited are not married, none have single parenthood, 60% are married, none divorced but widowed were 20%. In Kimathi it was observed that all the adults recruited in the study are married (Table 4.3). However the mean differences in marital status within the four study locations was not significantly different ($F=0.384$, $df = 4$, $p > 0.05$). Majority of the parents in the four study locations (41.9%) had an average of 4-6 children, 32.6% of parents had an average of 1-3 children and only 7% had an average of 7-9 children.

Table 4.3: Marital status of parents in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency.

Marital status of parents in the four study locations					
Location	Not married	Single	Married	Divorced	Widowed
	%	%	%	%	%
Gaturi	18.2	9.1	59.1	4.5	9.1
Mugoiri	7.7	7.7	76.9	7.7	0.0
Kahuhia	20.0	0.0	60.0	0.0	20.0
Kimathi	0.0	0.0	100.0	0.0	0.0

4.1.3 Livelihoods of parents in the study area

Result shows that most of the parents in the four study areas are peasant farmers (41.9%) growing food crops like maize, beans and bananas as a major source of food and income. Wages as a source of income for the families accounts for 37.2% of the families while 9.3% depend on income from small businesses. Making bricks as a source of income for the family accounts for 7% while mining stones in quarries accounts for 4.7% (Figure 4.1).

These economic activities however were observed to bring low income to the families. This is because on average, majority of the parents (48.8%) earned between Ksh. 1000-3000 per month while 34.9% would earn less than Ksh. 1000. Those who earned above Ksh. 3000 were only 16.3% (Figure 4.2).

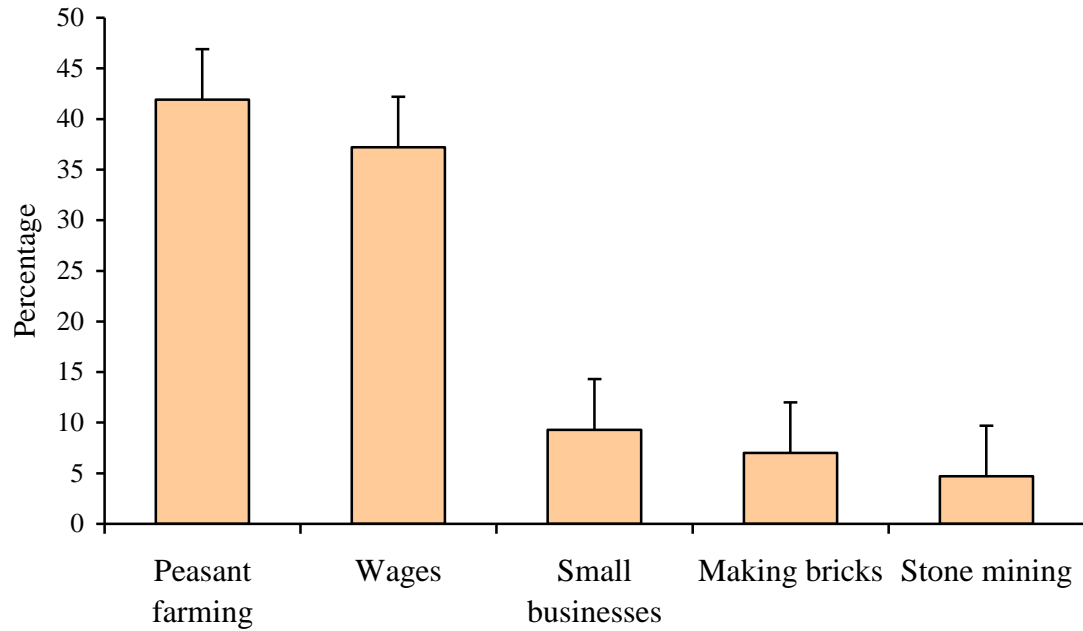


Figure 4.1: Main sources of family income collectively compared of in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y- Error bars shows 95% confidence interval.

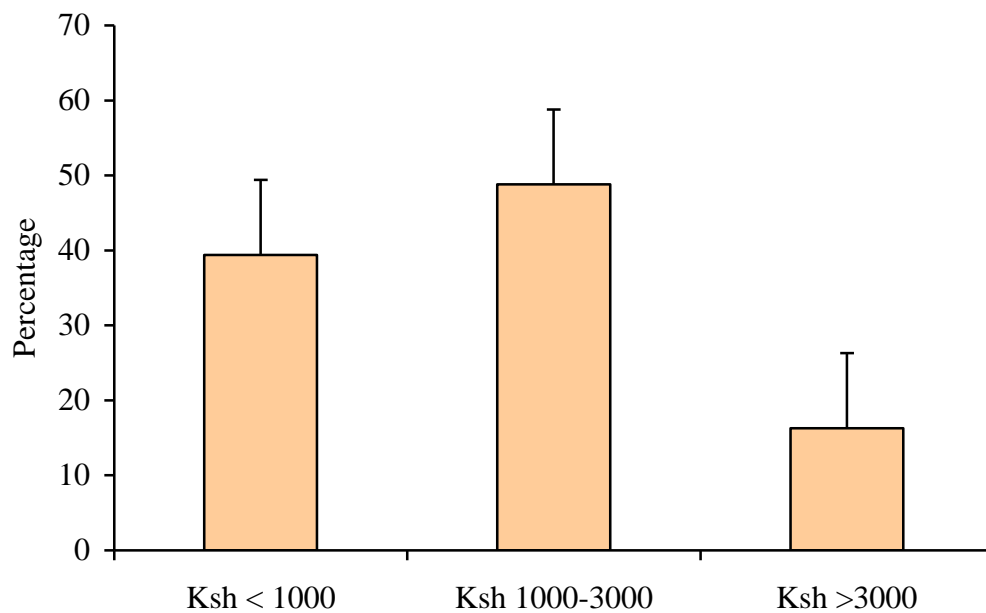


Figure 4.2: Estimated average family income per month collectively compared in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y- Error bars shows 95% confidence interval.

4.2 Diagnosis, clinical examination and treatment of tungiasis in the study population

Diagnosis and clinical examination of tungiasis in the study population was done mainly through observations taking into account the typical topographic localization of the embedded fleas. Patients typically complained of itching, pain and sensation of foreign bodies. Most lesions were observed on the foot; nail rim, periungual area of the toes and the sole. Knees, fingers and elbows were also infested in some patients (Figure 4.3).



Figure 4.3: Jigger infestation on various parts of the body of the patients. A. Severe tungiasis; spongiosis and tissue necrosis are observed on the foot. B. Jigger infestation in the periungual region of the toes. C. Hyperkeratosis and parakeratosis of a jigger infested foot. D. Jigger infested elbow. E. Jigger infested fingers. F. Jigger infested knees. G. An extracted jigger from a patient –Magnification; x400. (Pictures taken using a digital camera; a light microscope used to magnify the jigger specimen)

Severe tungiasis in adults (>20 lesions) was observed in 11.6% of the infested individuals compared to 2.3% (<20 lesions) who suffered from less severe tungiasis. This was significant ($df=2$, $p<0.05$). In pupils, 17.7% were severely infested compared to 2.3% who suffered less severe tungiasis (Figure 4.4). This was significant ($df=2$, $p<0.05$). Severe tungiasis was observed to be high in pupils as compared to adults.

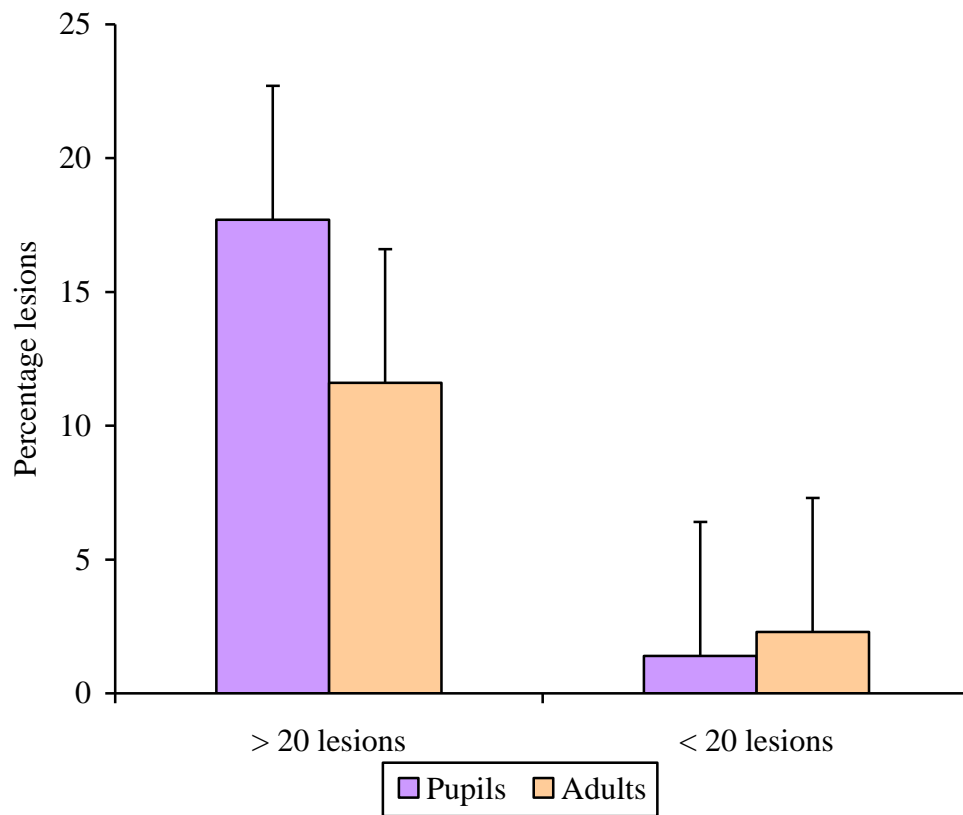


Figure 4.4: Severe tungiasis in adults jointly compared to pupils in Gatari, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y- Error bars shows 95% confidence interval.

The lesions were observed in various stages of life cycle described by Eisele *et al* (2003). Symptoms of pathological secondary infections such as inflammation, tissue necrosis, nail loss, parakeratosis, hyperkeratosis and spongiosis were observed in

severely infested individuals. These were the main causes of high morbidity especially in pupils (Figure 4.4 and 4.5). In some cases of severe tungiasis and secondary infections, injection against *C. tetani* that causes tetanus was necessary (Appendix IV, k).



Figure 4.5: Acute inflammation on some infested parts of the body. A and B. Inflammation on infested feet and toes; nail loss is also observed on some of the infested toes. C. Inflammation on infested fingers. (Pictures taken using a digital camera).

Results show that majority of the jigger infested (93.7%) treat jiggers at home through mechanical removal. It was also observed that (78.4%) do not use any form of disinfection. Others, 8.2% combine extraction with use of herbs such as sap from Sodom apple fruit (*Calotropis procera*), *Aloe vera* sap and tobacco (*Nicotiana tabacum*) powder. Those who exclusively use herbs alone were 7.2% while 6.2% do not attempt to treat tungiasis.

4.3 Risk factors associated with tungiasis

4.3.1 Type of housing

The type of housing material in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency was observed as a major risk factor in transmission of tungiasis.

Most of the houses in the study area were semi permanent with earthen floors (67.4%), whereby 37% of the houses were made of iron sheets. Wooden walled houses were 35.5% while mud/mud brick walled houses were 27.8%. The floors of these houses were all earthen (Figure 4.6).

A high percentage (78.4%) of those who were infested by jiggers lived in houses whose walls were made of mud or mud bricks while 17.5% lived in iron sheets walled houses and 4.1% in wooden walled houses (Figure 4.6). A multivariate analysis of this data revealed the type of housing material in the study locations increased the chances of jigger infestation by 13.78 times (OR=13.78; 95% CI=3.127-60.69). This means that some housing materials especially mud walls with earthen floor put the occupants at a high risk of jigger infestation because the fleas could have various places to hide and breed.

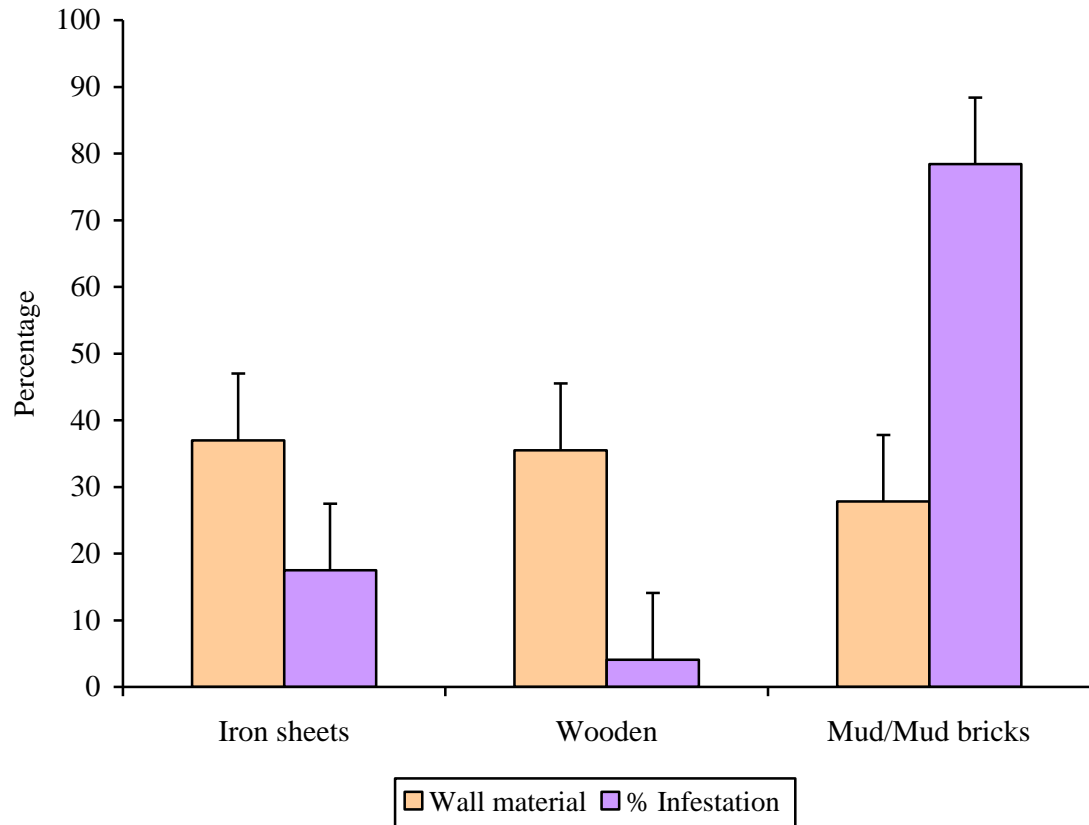


Figure 4.6: Percentage type of materials used to make the wall of earthen houses compared to average percentage jigger infestations in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y- Error bars shows 95% confidence interval.

4.3.2 Domestic animals

Tungiasis depend on the risk factors conducive for the breeding of *T. penetrans* fleas. In this study no domestic animal was observed to have embedded fleas on their skin. Nevertheless dogs, cats, chicken and goats were observed to play a role in tungiasis by harboring *T. penetrans* fleas that take blood meals from them. It was observed that 83.3% of the jigger infested shared living quarters with some domestic animals compared to 16.7% who were not infested. When analyzed in a multivariate analysis (OR=0.11; 95% CI=0.003-0.046) it was observed that individuals sharing living quarters with some domestic animals are 0.11 times more likely to get infested by

jiggers which was significant ($p < 0.05$). Therefore people who share living quarters with some domestic animals such as dogs, cats, chicken and goats are exposed to risks of jigger infestation.

More over it was observed that 53% of the children in the study area had knowledge that domestic animals harbor the fleas that causes tungiasis. However 31.1% of the children in both districts indicated that such fleas are transmitted from soil while 15.9% pointed other people who are already infested as the spreading tungiasis (Table 4.4). Based on this observation, it was clear that majority of the pupils know that domestic animals were important risk factor for *T. penetrans*.

Table 4.4: The average percentage knowledge of the respondents on transmission of fleas that causes tungiasis in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency.

Knowledge of the respondents on transmission of fleas		
Domestic animals %	Soil %	Infested people %
53	31	16

4.3.3 Foot wares

Study results revealed that majority of the school children (84.8 %) walk to school without closed shoes and only 15.2% walked to school with closed shoes regularly. Among the infested children, 93.8% would attend school without closed shoes while 6.2% of the infested children would attend school with closed shoes (Table 4.5). When analyzed in a multivariate analysis (OR=10.45; 95% CI=1.49-73.23) it was observed that individuals without shoes are 10.45 times more likely to get infested by jiggers

which was significant ($p < 0.05$). Therefore that lack of regular use of closed foot ware predisposes a child to jigger infestation.

Table 4.5: The average percentage of jiggers infested pupils with and without closed shoe in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency.

Jigger infestation	
Infested without shoes %	Infested with shoes%
93.8	6.2

4.3.4 Status of classroom floors

Dusty conditions at school were observed to play a major role in spread of tungiasis. Although most of classrooms in primary schools recruited in the study were cemented and clean (78.5%), 5.1% were not, while 16.3% were cemented but dusty. It was found that 86.7% of the jigger infested children in both districts were learning in classes that were cemented and dusty. Some classes that were not cemented accounted for 53.8% of the jigger infested children while clean cemented classes accounted for only 2.8% of the jigger infested children (Figure 4.7). When analyzed in a multivariate analysis ($OR=14.657$; 95% $CI=2.262-94.95$) it was observed that pupils learning in classes with cemented dusty floors and floors that are not cemented are 14.657 times more likely to get infested by jiggers which was significant ($p < 0.05$). This is summarized in table 4.6.

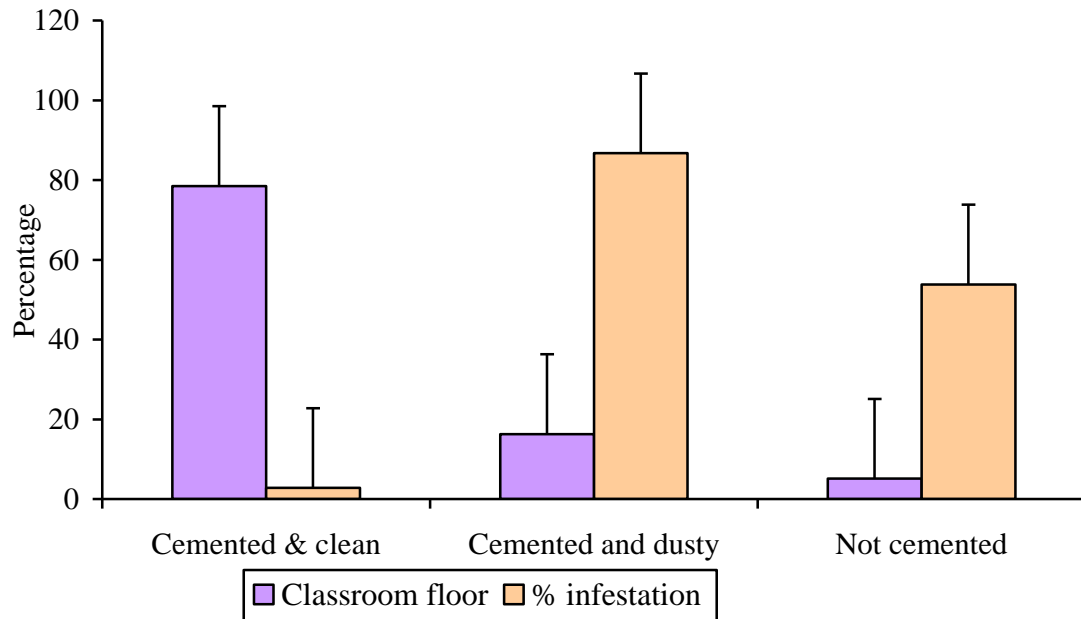


Figure 4.7: Percentage type of classroom floors compared to percentage jigger infestations in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y- Error bars shows 95% confidence interval.

Table 4.6: Summary of the relationship between tungiasis risk factors odds ratio (OR) in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency.

Multivariate logistic regression analysis of factors associated with tungiasis.							
Risk factor	B	Std. Error	Wald	df	Sig.	Exp(B) (OR)	95% C.I for (OR)
Conditions of classroom floor	2.685	.953	7.931	1	.005	14.656	(2.262-94.951)
Housing materials	2.623	.757	12.019	1	.001	13.775	(3.127-60.685)
Sharing living quarters with domestic animals	-4.537	.739	37.681	1	.000	0.011	(.003 -0.046)
Use of closed foot ware	2.346	.993	5.578	1	.008	10.448	(1.491-73.228)

4.3.5 Relationship between level of income and some risk factors

The level of income of the parents was observed to be associated with some risk factors indicating that poverty is a factor in jigger infestation. This is because the parents average low levels of income positively correlated ($r=0.4$, $p < 0.05$) to average low level of education of their eldest children (18yrs and above) whereby only an average of 25% of their eldest children in the four locations went up to secondary level. On average, 39.5% did not go beyond upper primary level, 23.3% only went up to lower primary level and 11.6% never went to school. Moreover, the parents' low levels of income positively correlated to sharing living quarters with some domestic animals such as chicken, goats, cats and dogs ($r=0.403$, $p < 0.05$). This means that the parents' financial weaknesses could be a stabling block to separately housing of these domestic animals. The parents' low level of income was also observed to positively correlate to the type of hosing materials used to build their housing facility ($r=0.438$, $p < 0.05$). This demonstrates that jigger infestation is associated with general poverty of the communities affected.

4.4 Prevalence rates

The prevalence of jigger infestation in adults and children in each of the four study locations was studied, analyzed and compared. Result shows that the prevalence of tungiasis in adults (>18years) in Kimathi, Kahuhia, Mugoiri and Gaturi, was 33.3%, 20% 15.4%, and 9.1% respectively (Figure 4.8). The mean prevalence rate of jigger infestation in adults in the four study locations was 14%. When compared the mean differences in jigger infestation in adults within the four study locations was not

significant ($F=0.485$, $df=3$, $p>0.05$). However it was observed that adults in Kimathi location had the highest burden of tungiasis than the rest of the locations with Gaturi having the least.

Result also shows that the prevalence of jigger infestation in children in Mugoiri was 23.3%, 20.7% in Kahuhia, 17.6% in Kimathi and 11% in Gaturi (Figure 4.8). The mean prevalence rate of jigger infestation in children in the four study locations was 19.1%. When compared the mean differences in jigger infestation in children between Mugoiri and Gaturi was significant ($F = 3.197$, $df = 3$, $p < 0.05$). However there were no significance differences in the means prevalence within the other locations ($p > 0.05$). It was noted that children in Mugoiri had the highest prevalence of jigger infestation, compared to the rest of the locations and Gaturi the least.

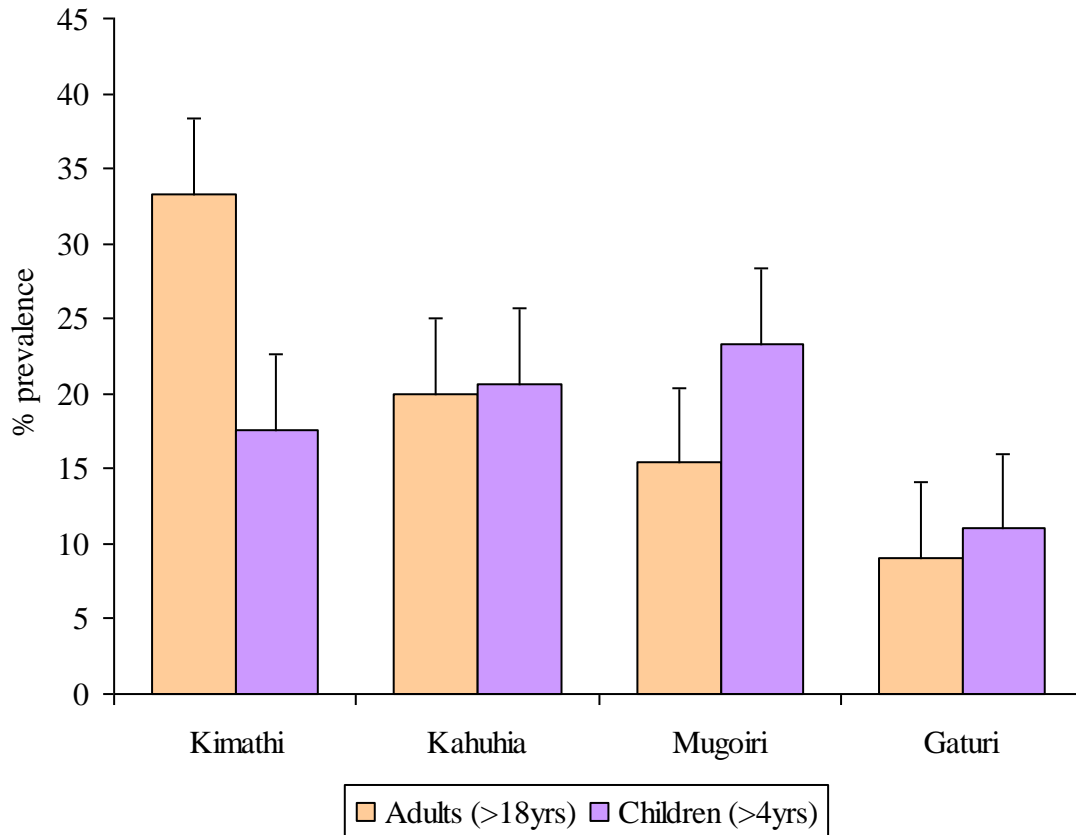


Figure 4.8: Percentage jigger infestation in adults (above 18yrs) compared to children above 4 years in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y- Error bars shows 95% confidence interval.

The prevalence of jiggers between boys and girls in the four study locations was jointly analyzed and compared. Boys with a mean prevalence of 20.1% were more infested compared to girls whose mean prevalence was 18.1% (Figure 4.9). However this difference in infestation was not significant ($df = 1, p > 0.05$).

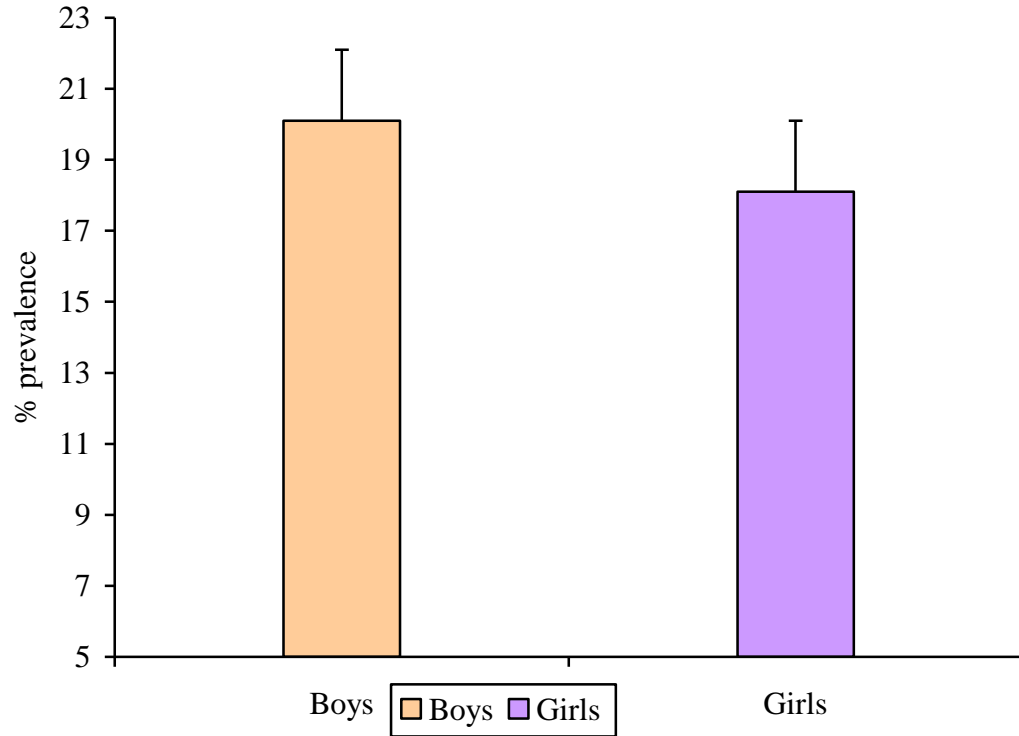


Figure 4.9: The average prevalence of jigger infestation in boys compared to girls in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y-Error bars shows 95% confidence interval.

A unique trend in prevalence of jiggers in certain age brackets in children in the four study locations was observed. Children of 4-6yrs had a prevalence of 12.5%, while those of 7-9yrs had a prevalence of 18.3%. The peak was in children of 10-12yrs whose prevalence was 21.5%. This prevalence then decreased such that children between 13-15yrs had a prevalence of 16.5% and those of 16-18 yrs had a prevalence of 8.3% (Figure 4.10). One way Analysis of Variance did not show any significant differences in mean infestation within the age brackets in children ($F = 0.711$, $df = 4$, $p > 0.05$). However it is evidently clear that younger children were more vulnerable compared to older ones to jigger infestation.

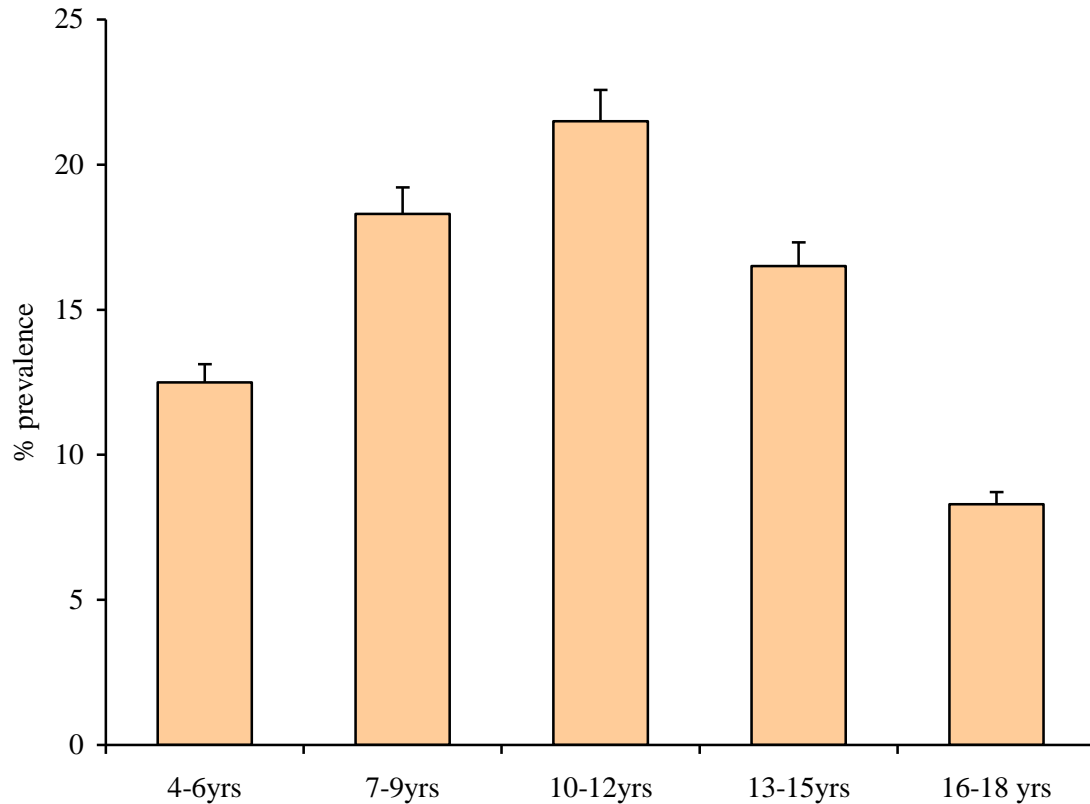


Figure 4.10: Trend in the average jigger infestation in different age brackets in Gatari, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y- Error bars shows 95% confidence interval.

The trend in prevalence of jigger infestation in adults was observed to increase towards the old age. This is because 18-55yrs old had a prevalence of 13.6% while those with 56yrs and above had a prevalence of 14.3% (Figure 4.11). This difference in prevalence was not significant ($df=1$, $p>0.05$).

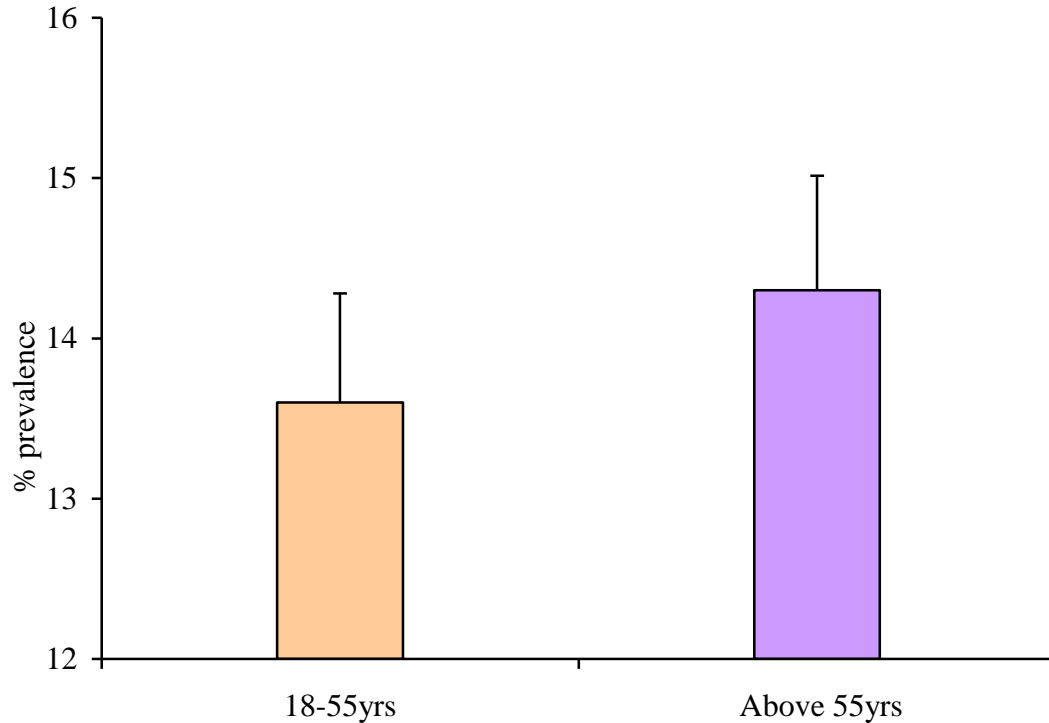


Figure 4.11: Trend in the average jigger infestation in different age brackets in adults in Gaturi, Mugoiri, Kahuhia and Kimathi locations in Kiharu constituency. Y- Error bars shows at 95% confidence interval.

4.5 Characterization of *T. penetrans* antigens

Result shows that immunized rats reacted to *T. penetrans* antigens after 3rd and 4th immunization whereby only one precipitate band was formed in double immunodiffusion assay (Figure 4.12). This was comparable to results of immunoelectrophoresis, whereby only one precipitate arc was formed (Figure 4.13).

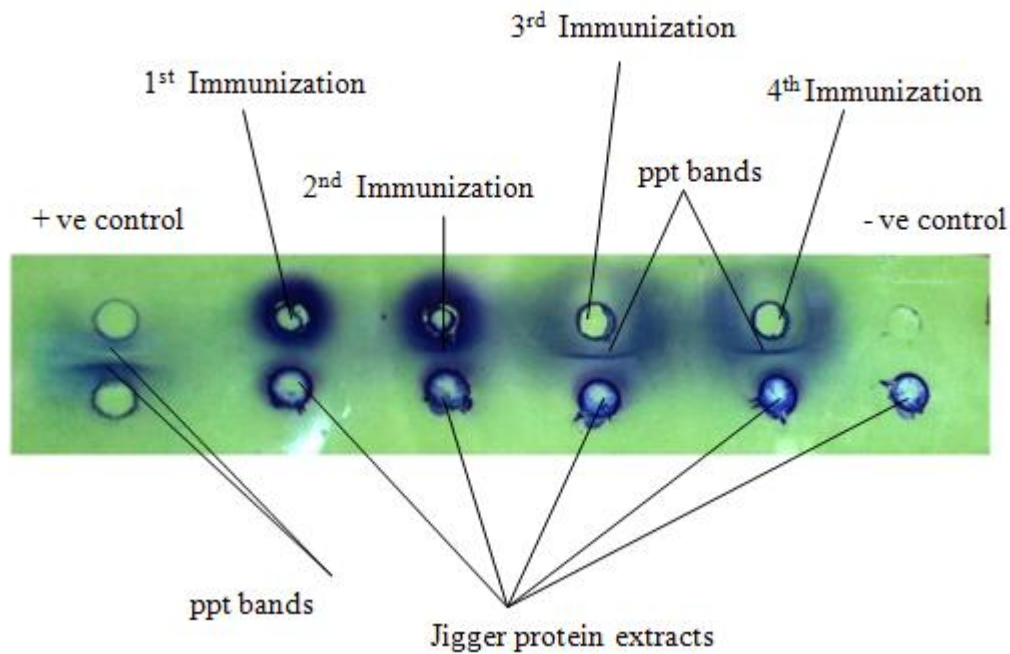


Figure 4.12: Analysis of *T. penetrans* antigens using sera from immunized rats in Ouchterlony double immunodiffusion assay. Positive controls include sheep serum as antigen and anti sheep as antibodies. Negative controls used were jigger protein extracts as antigens and buffer (PBS) in place of serum. Test samples were jigger protein extracts antigen. Anti jigger serum was from immunized rats.

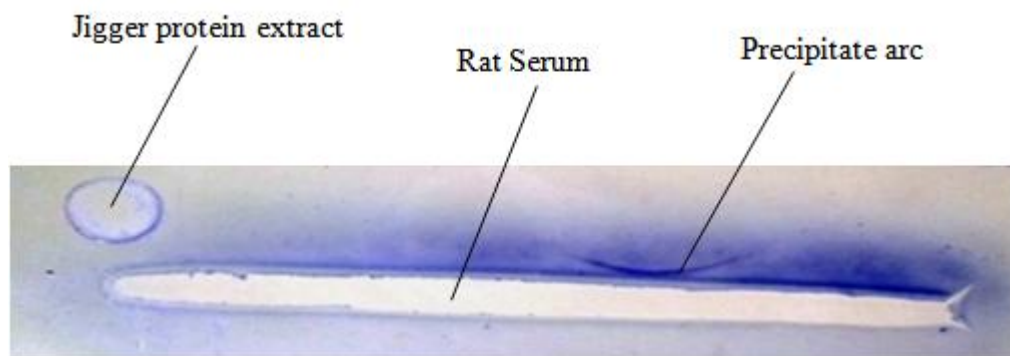


Figure 4.13: Analysis of *T. penetrans* antigens using sera from immunized rats in immunoelectrophoresis assay. The antigens comprised of jigger protein extracts antigen that was electrophoresed in the gel before addition of serum. The antibodies (anti jigger) was serum from the rats in their 4th immunization stage.

Tunga penetrans antigens were further analyzed in Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE, Figure 4.14). Result shows that separated jigger antigenic protein are all of medium to low molecular weights.

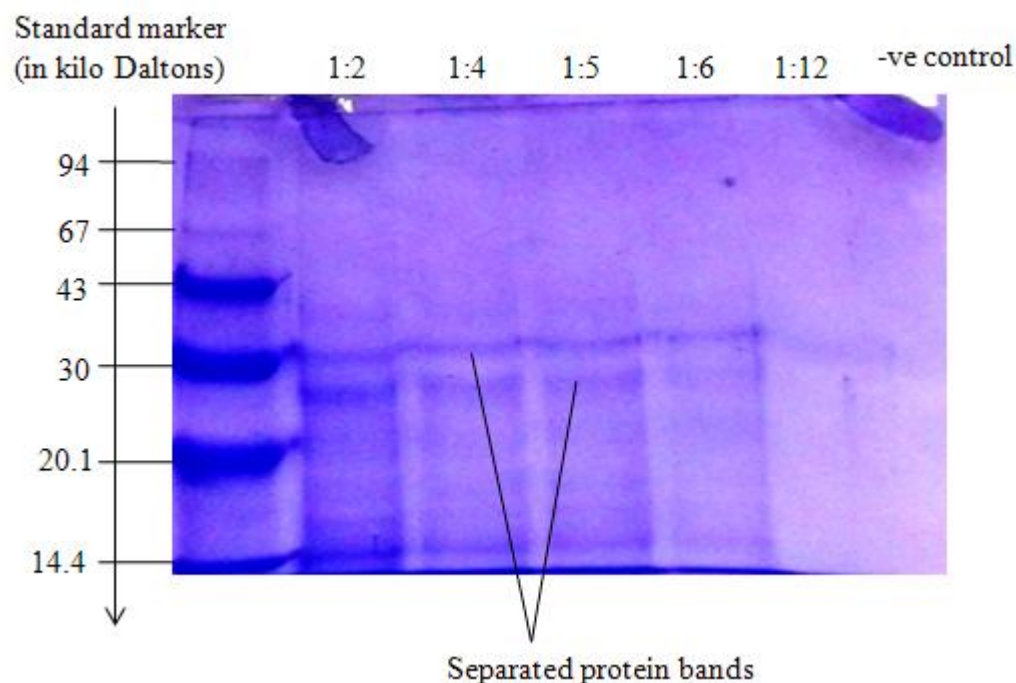


Figure 4.14: Analysis of *T. penetrans* antigens in Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE). The positive control was a low molecular weight marker from Pharmacia Ltd. Negative control was Phosphate Buffered Saline (PBS). Sample proteins were jigger extracts prepared in dilutions of 1:2, 1:4, 1:5, 1:6 and 1:12 respectively.

Separated *T. penetrans* antigens were further characterized in Western blot (Figure 4.15). Strips numbered 1-5 in I and strips numbered 7-11 in II are replicas comparing results of Western blot whereby *T. penetrans* antigens reacted with pooled human sera from infested victims. Result shows the most immunodominant antigens labeled A, B, and C in strip 5 (Figure 4.15, I) as compared to strip 11 (Figure 4.15, II).

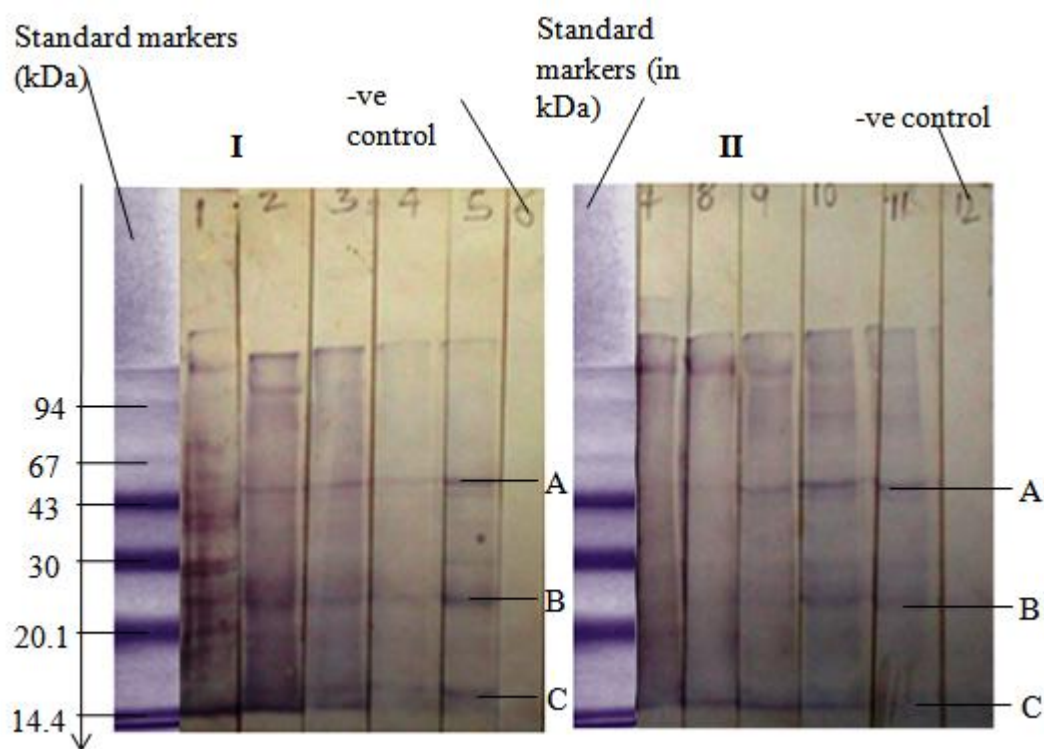


Figure 4.15: Analysis of *T. penetrans* antigens in Western blot using pooled sera from jigger infested patients. The positive control is a standard protein marker from Pharmacia Ltd. Negative control in I (strip 6), is a buffer (PBS) control. Negative control in II, (strip 12) is a conjugate control (Anti human secondary Antibody bound to Horseradish peroxidase enzyme). Strips No. 1-5 and 7-11 are replicas (except negative controls) of protein bands that reacted with pooled sera from patients infested with jiggers at various dilutions. Serum dilutions of 1:25, 1:50, 1:100, 1:200, and 1:400 corresponds to strips number 1-5 and number 7-11 respectively. Immunodominant antigens are labeled A, B, and C.

4.6 Molecular weight determination of unknown immunodominant protein molecules

Using simple regression analysis (Table 4.7) molecular weights of unknown immunodominant antigens labeled A, B, and C (Figure 4.15, I and II) were estimated as 51.795, 23.395 and 15.38 kDa respectively (Table 4.8). Therefore human immune system does react mainly against three immunodominant antigens in *T. penetrans*.

Table 4.7: Molecular weights of Pharmacia standard protein markers with Log₁₀ and R_f values. R_f values were calculated using the formula; R_f = (migration distance of the protein) / (migration distance of the dye front).

Molecular weight(Daltons)	Log₁₀	Electrophoretic mobility (R_f)
14,400	4.15836	0.96
20,100	4.30319	0.73
30,000	4.47712	0.52
43,000	4.63346	0.29
67,000	4.82607	0.23
94,000	4.97312	0.08

Table 4.8: Immunodominant antigenic proteins, their R_f values and Molecular Weights in kilo Daltons.

Immunodominant proteins	antigenic	R_f values	Molecular Weights (kDa)
Antigen A		0.31	51.795
Antigen B		0.67	23.395
Antigen C		0.86	15.380

$$y = -1.043x + 5.227$$

CHAPTER FIVE: DISCUSSIONS

5.1 Demographic characteristics of the study population

This study suggest that tungiasis is a disease of economically disadvantage communities characterized by low literacy levels in Kiharu constituency. Majority of the parents (46.5%) whose children are infested with jiggers never went beyond upper primary school level in their education. Similarly a higher percentage of their eldest children (53.5%) did not go beyond upper primary school. Therefore it is clear that low literacy level and ignorance could be an important factor associated to jigger infestation. Majority of parents in families infested with jiggers (41.9%) have about five children in the family whom they have difficulties providing basic needs such as food, proper housing and clothing. This when accompanied by poor monthly income of less than K.sh 3000 demonstrates a characteristic general poverty associated with jigger infestation. This data is supported by earlier observations in Brazil where it was established that tungiasis is mainly a problem of populations living in poverty (Heukelbach *et al.*, 2001). In fact, in resource poor communities in the endemic area, severe infestations occur, and high morbidity is common (Feldmeier *et al.*, 2003).

An earlier study by Ahadi (2008) further supports this observation that jigger infestation in Murang'a contributed to the appalling poverty levels in the area. The report revealed that jigger infestation had made the residents to languish in the quagmire of vicious cycle of poverty. People afflicted with jiggers are less economically active, which further raises poverty levels (Heukelbach *et al.*, 2001). Since jiggers are usually a result of already existing poverty, their infestation perpetuates the vicious cycle of poverty;

the poor are unable to rise out of poverty or actually often sink deeper into it. This frustrates Kenya's attempts to achieve the Millennium Development Goal, which aims at reducing levels of poverty in the country by a half by the year 2015 (Ahadi, 2008).

This study observed that jigger infestation affects the education of children. This is because they experience difficulties in walking to school, write properly, or participate in normal learning activities at the same level with other children who are not infested. These when coupled with stigma emanating from intimidating ridicule from their peers, prompt repeated absenteeism from school. This results into dropping out of school. Hence majority fail to go beyond upper primary.

5.2 Diagnosis, clinical examination and treatment of tungiasis in the study population

This study observes that severe tungiasis (>20 lesions) in the study area mainly affect pupils as compared to adults. This is because pupils have a higher prevalence (17.7%) of severe tungiasis compared to adults (11.6%). It was further observed that where parents were infested, children from the same family suffered from severe tungiasis with higher morbidity. This shows some level of negligence of the children by their parents. This observation is consistent to that of Eisele *et al.* (2003) who observed that where parent suffer from less severe tungiasis, children from the same family are hard hit. Such families were observed to helplessly accept tungiasis as a problem to live with without much effort to deal with it. More over such families have limited social interactions with the neighborhood due to stigma. In several cases of severe tungiasis, hundreds of embedded fleas were observed in various parts of the body, but mostly on

the feet and fingers. In such cases tungiasis was accompanied by secondary pathological conditions such as inflammation, tissue necrosis, nail loss, hyperkeratosis and parakeratosis hence high morbidity. For such individuals tetanus vaccine is important.

This study observes that immediate mechanical removal of embedded fleas and disinfection of the wound could be the best method of treating tungiasis. This would destroy the flea before it could lay eggs, break the life cycle and also prevent secondary infections. However there is danger of sharing contaminated sharp objects that can spread HIV, hepatitis, Ebola or other blood related diseases. More over mechanical removal of jiggers, as practiced in the villages, is a painful and traumatic experience, especially for children who would scream in pain during extraction. In fact some would rather opt to remain with the embedded jiggers. In addition, if mechanical removal is not done well it can cause injury to the infested parts seriously impeding movement and performance of many of life's chores. If disinfection is not done, secondary infection would occur. In view of this observation, exploring other ways of treating tungiasis without actual mechanical removal, such as washing with disinfectant, topographic application of anti-parasitic agent, use of anti-inflammatory creams and use of repellants should be preferred. Nevertheless, in this study it was observed that majority of jigger infested people (93.8%) preferred remove jiggers, using sharp objects such as safety pins. Unfortunately disinfection of the wound left was hardly done. This paves way for serious secondary infections. This behavior could simply be attributed to ignorance as suggested by Ahadi (2008). A more striking observation was that a good percentage of those infested (about 6.2%) do not attempt to treat tungiasis in any way

unless someone intervenes. This portrays high levels of desperation and hopelessness as a result of stigma. This would make a person dependent on others who may themselves be unable to help due to their own affliction with the affected family.

This study has established that majority of jigger infested (78.4%) prefer self treatment of tungiasis at home (which in most cases is inappropriate) other than seeking medical intervention. This could be attributed to stigmatization that cultivates fear of being ridiculed by their neighbors and even health workers (Ahadi, 2008). Further more physical deformities such as ulcerations and auto-amputation of the digits make the victims feel ashamed of being in social places and generally reduce their self esteem and gets isolated further perpetuating stigma and poverty. This has contributed to persistent jigger infestation and re-infestation making the life cycle of the parasite to persist while continued spread of the flea is enhanced (Eisele *et al.*, 2003).

5.3 Risk factors associated with tungiasis

In this study presence of infested domestic animals in the home such as cats, dogs, chicken and goats was observed to be an important risk factor to jigger infestation. This observation is confirmed by a similar study done in traditional fishing community North East Brazil. The study revealed that occupants of households with infested pets and domestic animals had tungiasis that correlated to infestation in the animals (Pilger *et al.*, 2008). More over Ugbomoiko *et al.* (2007) observed that presence of pigs in the compound in Erikit, a rural community in Lagos State of Nigeria was an important risk factor for tungiasis.

The fleas take blood meal from warm blooded organism and sometimes cause tungiasis in them (Eisele *et al.*, 2003). Such animals are seen as reservoirs for tungiasis. However, strictly defining reservoirs as organisms that directly or indirectly transmits a pathogen while being virtually immune to its effects, then tungiasis would have no reservoirs. Reservoir host - a host that serves as a source of infection and potential re-infection of humans and as a means of sustaining a parasite when it is not infecting humans could be the best term. The host themselves (especially human beings) are also vectors since the gravid female expel eggs spreading tungiasis to other mammals though indirectly (Pampiglione *et al.*, 2009). This means that the fleas have a relatively large number of hosts and victims (Eisele *et al.*, 2003). This is epidemiologically important since tungiasis often cause secondary infections. Therefore to combat tungiasis in Gaturi, Kimathi, Kahuhia and Mugoiri locations in Kiharu constituency, separate housing of domestic animals such as goats, chicken, dogs and cats are important. Regularly dusting these animals with insecticides such as sevin dust is crucial to reduce the fleas' burden.

Poor living condition was found to be another major risk factor in transmission of tungiasis. This study observes that most of the houses (79.1%) in the four study locations were semi permanent, made of mud walls and earthen floors. A high percentage of those who were infested by jiggers (78.4%) lived in such houses. The houses are usually dusty and have cracks on the walls and floors providing good breeding environment for *T. penetrans* fleas. More over maintaining high levels of cleanliness in such houses is challenging. This could increase the flea population hence

high attack rate. This observation is supported by a similarly observation in a recent study done in Murang'a South (Ngomi, 2010). The study demonstrates that the highest chance of infestation exist in individuals living in houses with earthen floors. The findings are also consistent to a similar survey conducted in Nigeria (Ugbomoiko *et al.*, 2007; Pilger *et al.*, 2008) which revealed that such housing conditions are a major risk factor.

Jigger flea has been observed to mostly live 2-5cm below the sand an observation which helps explains its overall distribution (Nagy *et al.*, 2007). The temperature on the surface is generally too hot for the larvae to develop and the deeper sand does not have enough oxygen. This preferred ecological niche offers a way to decrease transmission among humans if they invest in concrete floors as opposed to the sand or just earthen as is normally the case. Indeed, Nagy *et al.* (2007) reported that in shacks with concreted floors being cleaned every day with water, *T. penetrans* larvae were hardly found. However low economically disadvantaged victims, unless supported are not able to improve their housing from the current state. For this reason to combat tungiasis in Gaturi, Kimathi, Kahuhia and Mugoiri locations in Kiharu constituency, it is important to engage the affected in income generating activities through social groping such as women and youth groups. This would raise their living standards and afford better housing facilities.

In this study dusty classrooms as well as those which are not cemented were found to significantly influence jigger infestation in school going children. A high percentage of jigger infested children in the study locations (86.7%) were observed to learn in classes that were cemented, dusty or earthen. These results are comparable to a report on the

jigger situation in rural schools in Busia and Teso Districts by Ahadi (2008) whereby classrooms which are often dusty were identified as points of infestation which was aggravated by earthen floor. This study is also supported by earlier observations by Ruttoh *et al.* (2012) who reported that in Kenya, classrooms which are normally dusty because of either mud walls and earthen floors increase the chances of jigger infestation in pupils. Dusty cemented floor or earthen floors offer conducive environment for *T. penetrans* fleas. Therefore to reduce tungiasis in such classrooms, daily cleaning of cemented floors with water is important. Sprinkling water on floors that are not cemented, accompanied by sweeping on daily basis is important. Cementing such floors however would be a better idea. However, water availability in such rural primary school is a challenge; rivers may be a bit far and buying tanks for rain water harvesting could be another challenge. This makes daily cleaning of classes difficult. Funding of such projects should be enhanced by CDF.

Majority of the school children (84.8%) were observed to walk to schools barefooted. In fact a high percentage of those infested in the study locations (93.8%) walk to school without closed shoes. The results of this study is supported by earlier observations by Ugboimoiko *et al.* (2007), who identified lack of regular use of closed foot ware as an important risk factor for jigger infestation. Muehlen *et al.* (2005) made similar observation when in his studies on tungiasis concluded that certain risk factors such as age, type of housing, level of education, lack of shoes and a low socio-economic profile predispose individuals to jigger infestation. Lack of proper regularly washed closed foot

ware is attributed to high poverty levels whereby parent crucial choice is provision of food which is scarce hence forego buying of shoe which would be taken as a 'luxury'.

Generally shoes prevent infestation and re-infestation of feet by fleas from the ground. Closely fitting shoes would suffocate the embedded fleas better compared to loosely fitting shoes. However shoes only protect the feet and not the other parts of the body such as knees or fingers. Furthermore though shoes are protective to the feet, it should be noted that they do not actually eradicate the fleas; maintenance of the shoe general hygiene and that of socks to remove and destroys adhered eggs or larvae is quite important. Nevertheless, provision of cheap shoes such as rubber shoes by County government and NGOs such as Ahadi Kenya Trust would prevent spread of tungiasis to a great extent in Gaturi, Kimathi, Kahuhia and Mugoiri locations in Kiharu constituency.

The low economic status of the parents was observed to be associated with some risk factors suggesting that poverty is an important factor in jigger infestation. This is because the parents low level of income positively correlated to low level of education of their eldest children (18yrs and above) whereby majority 39.5% on average, did not go beyond upper primary level and a significant number 11.6% on average, never went to school at all. This could be an indication that parent were unable to educate their children due to low level of income which may have further worsened the jigger situation indirectly. Furthermore, the parents' low level of income positively correlated to sharing of living quarters with some domestic animals. This could mean that the parents' financial weaknesses were a major reason for failure to separate housing of

some of the domestic animals especially chicken. Moreover, the parents' income was also observed to positively correlate to the type of housing materials used to build their housing facility suggesting that jigger infestation is associated with general poverty of the communities affected.

From these observations there is a strong conviction that improved living standards of the affected communities would reduce tungiasis in Gaturi, Kimathi, Kahuhia and Mugoiri locations in Kiharu constituency by a big margin. All the risk factors observed seem to be centered around low economic profiles of the affected such that they are unable to afford better education, better housing, shoes or separate housing for domestic animals. Intervention by engaging these affected communities in income generating economic activities would help address the economic factors and tungiasis could be no more.

5.4 Prevalence rates

Kiharu Constituency Strategic Development Plan (2010-2030) acknowledges that tungiasis contribute to school dropout (Ndegwa, 2010). However it fails to give its prevalence rates among the other diseases most prevalent in the constituency. A list of most prevalent diseases in the constituency identified in the Plan are; malaria 42.4%, typhoid 14.1%, flue and cold 12.1%, Diabetes 8.7%, pneumonia 6.5%, STDs & AIDS 5.4%, tuberculosis 4.2%, hypertension 2.0%, stroke 2.0%, Arthritis 1.7%, Worm infections 0.6%, and meningitis 0.3%. Tungiasis prevalence rate is missing altogether, yet it ought to be one of the prioritised diseases due to its high prevalence of 19.1% in children and 14% in adults on average. This observation shows that tungiasis is

neglected in Kiharu constituency. Heukelbach *et al.* (2003) supports this finding when he pointed out that tungiasis is indeed neglected being considered as just an entomological nuisance, making it fail to catch the attention of researchers and health professionals.

This project has demonstrated that tungiasis prevalence rate increases with increase in age bracket, from 4-6yrs, 7-9yr, then reaches peak at 10-12yrs and starts decreasing such that children 16-18 yrs had the least prevalence. Although the difference in prevalence rates within the age brackets is not significant, there is evidence that the most prone age bracket in children is 10-12yrs. It also shows that, although not statistically significant, the prevalence in adults increases toward the old age so that those with 55yrs and above have a higher prevalence compared to adults of between 18-55yrs. Furthermore on average, the prevalence of tungiasis of male in the four study locations was observed to be slightly higher compared to that of females, showing a clear preponderance of infestation in the male sex. These results corresponds to the findings of Community-based studies that have consistently shown tungiasis prevalence of between 16% and 55% in typical endemic areas with a peak of age-specific disease occurrence in children of 5 to 14 years and the elderly, and a preponderance of infestation in the male sex (Wilcke *et al.*, 2002; Muehlen *et al.*, 2003). This trend in jigger prevalence is also consistent with Ugbomoiko *et al.* (2007) who observed that a relationship between jiggers and age brackets exist whereby a high prevalence exist between 5-14yrs, decrease in young adults and increase again in elderly.

Boys are slightly more infested when compared to girls in the study area. This is probably because boys of jigger vulnerable age of 3-15 years spend most of their free time with pets like dogs that harbor jigger fleas. More over boys of this age experience challenges in maintaining cleanliness compared to girls (Ahadi, 2008). By the age of about 17yrs the young adults are able to maintain significant levels of hygiene and the prevalence of tungiasis fall. However, in elderly (above 55yrs) the prevalence rises perhaps because they suffer neglect as the youth goes to town to look for jobs. More over they are aged, unable to earn a living and maintaining some level of hygiene becomes a challenge.

According to Kiharu Constituency Strategic Development Plan (2010-2030), Gaturi and Kimathi locations have higher day temperatures compared to Mugori and Kahuhia. Jigger infestation is associated with high temperatures and low precipitation (Heukelbach *et al.*, 2005; Ruttoh *et al.*, 2012). Therefore, one would expect Gaturi and Kimathi to have higher prevalence of jiggers. However, Mugoiri and Kahuhia locations on average have slightly higher prevalence of jigger infestation. This could be attributed to the fact earlier activities by Ahadi (2008) focused their interest in tungiasis in dry areas which were observed to have high jigger infestation. The effect of this was that early intervention by Ministry of health focused in the areas of Gaturi and Kimathi with little attention to cooler areas of Mugoiri and Kahuhia.

5.5 Characterization of *T. penetrans* antigens

Rats are appropriate animal model in tungiasis studies (Feldmeier *et al.*, 2008). In the current study immunized rats were found to react to *T. penetrans* antigens whereby one

precipitate band was formed in both immunodiffusion and immunoelectrophoresis assays. This was an important pre-analysis of jigger antibodies prior to analysis of human sera since human immune system could similarly react to the same antigens. It was observed that, though one can induce immune responses in animals like rats, the response was limited the same type of protein molecules since only one precipitate band was formed. In addition people too do not react to a variety of jiggers antigens since only three major antigens were observed. This could be attributed to the fact that jiggers being ectoparasites are not largely exposed to systemic circulation.

The rate of proteins migration on SDS-PAGE has an inversely proportionality to log their Molecular Weight (Bio-Rad, 2004). Therefore in this study Pharmacia standard protein markers were used to estimate the molecular weight of the unknown proteins that were found to be in the range of 51.795 kDa to 15.38 kDa medium to low molecular weight. So far presences of anti-jigger against these immunodominant antigens in human blood system have not been shown to confer any protection against infestation or re-infestation. Jigger, being a semi-ectoparasite could be protecting itself through mutations, antigenic camouflage or even immune suppression. However research based evidence to this observation is of paramount importance.

Jigger penetration in to the skin of its host is normally accompanied by immediate acute inflammation on the site of the skin penetration which could be as a result immunological responses against identified immunodominant antigens in jiggers. This acute inflammation is more pronounced when compared to other skin diseases caused parasites (Eisele *et al.*, 2003). In fact this inflammation has been found to the cause of

secondary pathological conditions when human are infested. These are remarkable desquamation of the skin, uneven thickening of the skin and debilitating sequelae such as phagedenic ulcers. Others include tissue necrosis, nail loss and complete loss of fingers or toes (Feldmeier *et al.*, 2003). This edema is characterized by high levels of agranulocytes such as lymphocytes and granulocytes such as neutrophils and eosinophils (Feldmeier *et al.*, 2004). If not disinfected, the lesions often become infected by *Clostridium tetani*. This can result to death if the victims are not vaccinated (Eisele *et al.*, 2003). Domestic animals and pets in endemic areas such as cats, dogs, and pigs also suffer from tungiasis (Vobis *et al.*, 2005). However, when compared to human victims the inflammation is less pronounced. Rats other than mice, for instance *Rattus rattus* experience local inflammation as a result of jigger infestation in a similar manner to human victims (Vobis *et al.*, 2005).

Based on results of this study the null hypothesis there is no difference in mean prevalence of tungiasis in adults within Gatari, Kimathi, Kahuhia and Mugoiri in Kiharu Constituency was accepted. The analysis of the mean differences using one way ANOVA did not show any significant differences ($F=0.485$, $df=3$, $p>0.05$). The null hypothesis that there is no difference in mean prevalence of tungiasis in children within Gatari, Kimathi, Kahuhia and Mugoiri in Kiharu Constituency was rejected. This is because there was significant mean prevalence difference ($F = 3.197$, $df = 3$, $p < 0.05$) between Mugoiri and Gatari. Mugoiri, having a proportion of 0.123 higher than that of Gatari (Sig. = $0.012 < 0.05$) in a post hoc test. However there were no significance differences in the mean prevalence of the other locations ($p > 0.05$).

The null hypothesis that there are no risk factors associated with tungiasis in Gaturi, Kimathi, Kahuhia and Mugoiri in Kiharu Constituency was rejected. This is because in a multivariate logistic regression analysis some independent factors were found to be associated with tungiasis. These are lack of regular use of closed foot ware (OR=10.45; 95% CI=1.49-73.23), living in earthen mud walled houses (OR=13.78; 95% CI=3.127-60.69) sharing living quarters with domestic animals (OR=0.11; 95% CI=0.003-.046), and learning in classrooms with dusty floors (OR=14.657; 95% CI=2.262-94.95). These factors were significant ($p<0.05$).

The null hypothesis that *T. penetrans* has no immunodominant antigen was rejected since three immunodominant antigens of molecular weight 51.795 kDa, 23.395 kDa and 15.38 kDa were identified.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- i). The prevalence of tungiasis in adults (≥ 18 years) in Kimathi, Kahuhia, Mugoiri and Gaturi, was 33.3%, 20%, 15.4%, and 9.1% respectively. That of pupils ($\geq 4 < 18$ years) in Mugoiri was 23.3%, 20.7% in Kahuhia, 17.6% in Kimathi and 11% in Gaturi.
- ii). Observed risk factors associated with tungiasis in Kimathi, Kahuhia, Mugoiri and Gaturi include poor housing with earthen floors, sharing of living quarters with some domestic animals, such as cats, dogs, chicken and goats, lack of regular use of closed foot ware as well as learning in dusty classrooms.
- iii). *Tunga penetrans* in Kimathi, Kahuhia, Mugoiri and Gaturi have three immunodominant antigens of molecular weight 51.795 kDa, 23.395 kDa and 15.38 kDa.

6.2 Recommendations

- i). This study recommends development and sustenance of public health awareness campaigns and control strategies against jiggers to reduce stigma, create awareness on risk factors and improve hygiene to combat tungiasis.
- ii). This study discourages people from sharing living quarters with domestic animals to reduce chances of infestation. Regular dusting of infested domestic animals and floors of earthen houses with chemicals such as sevin dust is highly recommended to reduce the flea burden in such animals.

- iii). Strategies to diversify economic activities and empowering of organized social groupings such as women and youth groups are highly recommended to help address economic factors associated with tungiasis.
- iv). County government should consider provision of shoes to infested pupils in Kimathi, Kahuhia, Mugoiri and Gaturi help curb tungiasis.
- v). This study recommends further characterization of jigger immune-dominant antigens since such research work could be fertile bases for future development of immune prophylaxis.

6. 3 Suggestions for further research

- i). During the study it was observed that some families members were jigger infested while others were not when they in fact share the same social facilities in the family. This study therefore suggests further research on this phenomenon to investigate any significant association between jigger infestation and individual's blood group or genotype.

6. 4 Limitations of the study

A major challenge to this study was limited information in literature especially on the immunology of this parasite. More over jigger infestation to some people is a bad omen and the victims end up being traumatize and isolated both physically and psychologically. Recruiting such people in the study was a challenge. In some cases such people would hide whenever research team visited their homes.

Roads network into the villages was another major issue especially during rainy seasons. The roads were earthen and poorly maintained hence impassable when wet. Therefore collection of specimens would be suspended until the weather improves. Furthermore collection of specimens was interrupted whenever the school closed for vacation.

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APPENDICES

APPENDIX I

Questionnaires

(a) Questionnaire (for adults)

The following questions are for research purposes only. The information given will be treated with utmost confidentiality. You are not required to write your name or your personal identification details. So feel comfortable to give the correct information as much as possible. Use a tick (✓) to mark the appropriate box or write briefly on blank spaces left. Thank you.

1. What is your gender? Male ☐ Female ☐

2. How can you grade your housing facility?

a) Permanent ☐ b) Semi permanent ☐

If semi permanent what building materials are used?

a) Wood and earthen floor ☐ b) Mud/ Mud Bricks with earthen floor ☐

c) Iron sheet and earthen floor ☐

d) If any other

specify.....

3. What is your highest level of Education?

a) Never went to school ☐ b) Lower primary ☐ c) Upper primary ☐

d) Secondary ☐

4. If you never went to school what reasons might have made you not to go to school?

.....

5. What reasons might have made you to stop schooling if applicable?

.....

6. What is your marital status?

a) Not married ☐ b) Single parent ☐ c) Married ☐
 d) Divorced ☐

7. If a parent, how many children do you have?

a) 1-3 ☐ b) 4-6 ☐ c) 7-9 ☐
 d) 10-12 ☐

8. What is the highest level of education of your eldest child?

a) Never went to school ☐ b) Lower primary ☐
 c) Upper primary ☐ d) Secondary ☐

9. What is the main source of income for your family?

.....

10. What is your average income per month?

a) < 1000 ☐ b) 1000- 3000 ☐ c) 3000-5000 ☐

11. What food crops do you grow?

a) Maize ☐ Beans ☐
 b) Any other ☐ Specify

12. From where do you think jiggers come from?

.....

13. Who are mostly infested by jiggers?

a) The children ☐ What age bracket? a) 2-5yrs ☐

b) 6-9yrs ☐ c) 10-13yrs ☐ d) 14-17yr ☐

b) The handicapped ☐

c) The old parents What age bracket? a) 18- 55yrs ☐

b) above 56yrs ☐

14. Which parts of the body do you think are normally infested by jiggers?

.....

15. Are there some domestic animals in your homestead you know, that harbor the

jiggers or fleas that causes tungiasis? a) ☐ Yes b) ☐

If yes, name them in order of prevalence.

.....

16. Are you infested now? Yes ☐ No ☐

17. Have you ever gone to hospital because you have been infested by jiggers?

a) Yes ☐ b) No ☐

If yes how were you treated?

.....

If no why?

.....

18. Do you treat jigger infestation at home?

a) Yes ☐ b) No ☐

If yes how do you treat them?

I) Removing using sharp object?

- a) Yes ☐ b) No ☐

If yes, what sharp object do you use?

- a) Needle ☐ b) Safety pin ☐ c) A thorn ☐

II) Is the sharp instrument shared at home or does each has his or her own?

- a) Shared ☐
b) Each has his/her own ☐

III) Is the sharp instrument sterilized?

- a) Yes ☐ b) No ☐
b) If yes, how it is sterilized

.....

IV) If a sharp object is not used, how else do you treat jiggers?

.....

.....

19. After extracting the jiggers, is the open wound treated?

- a) Yes ☐ b) No ☐

If yes, how is the wound treated?

- c) Using disinfectants. Name the disinfectants used.....
d) Using traditional medicine. Name the medicine used

(b) Questionnaire for school children (class 4-8)

The following questions are for research purpose only. The information supplied will be confidential. Do not write your name. Use a tick (✓) in the appropriate box or write briefly in the blank spaces. Remember to give correct information as much as possible.

School.....

Class.....

- 1) What is your gender? Male ☐ Female ☐
- 2) What is your age? a) 4-6 ☐ b) 7-9 ☐ c) 10-12 ☐
 d) 13-15 ☐ e) 16-18 ☐
- 3) What building material is used to make the wall and floor of your house?
 - a) Wood and earthen floor ☐ b) Mud/ Mud Bricks and earthen floor ☐
 - c) Iron sheet and earthen floor ☐
 - d) If any other specify.....
- 4) Where do you think jiggers come from?
 - a) From soil ☐ b) From other people? ☐
 - c) From domestic animals ☐
 - If from domestic animals, name them.....
- 5) Which parts of the body do you think are normally infested by jiggers?

- 6) Are you infested now? a) Yes ☐ b) No ☐
- 7) Do you go to school with closed shoes? Yes ☐ b) No ☐
- 8) Have you ever been sent home because you are infested with jiggers?
 - a) Yes ☐ b) No ☐

9) If yes, where were you treated?

- a) At home ☐ b) At hospital ☐

10) How do you treat jiggers at home?

- a) Do not treat ☐ b) Treat using herbs ☐ c) Removing them ☐

If herbs are used, name them

11) If you remove them, what do you use to remove them?

- a) Needle ☐ b) Safety pin ☐ c) Thorn ☐

12) With whom do you share your sharp objects to remove jiggers?

- a) Do not share ☐ b) Share with friends ☐

If you do not share, why?

13) a) When you remove jiggers from your feet and hands, what do you do to the

wounds left? a) Nothing ☐ b) Apply disinfectants ☐ c) Apply herbs ☐

b) Name the disinfectant used if applicable.....

c) Name the herbs used if applicable.

14) How can you describe the floor of your class

a) Cemented and clean ☐

b) Cemented and dusty ☐

c) Earthen and dusty ☐

d) If any other, specify.....

APPENDIX II

Laboratory procedures

a) Protein extraction (freeze and thaw)

Protein extraction involved mechanical breakdown of 10 gravid female jiggers in 2ml of PBS in a clean sterilized 5ml bijou bottle using a clean sterilized glass rod to avoid contamination. This was followed by freezing the protein in a deep freezer at -20°C for 1 hour, and thawing it for 1 hour at 25°C to extract soluble proteins. This process was repeated five times after which centrifugation of the products were done at 1000 rpm for 10 minutes at room temperature (25°C).

b) Immunization of rats

Immunization of rats involved mixing 2ml of the extracted antigens with 1 ml of complete adjuvant (ratio of 2:1). This was used to immunize 5 rats each with 0.5ml of the mixture. Four immunizations were done in intervals of three weeks. Before each immunization, blood from the vein in tail region was withdrawn, serum extracted and later tested for antibodies using double diffusion method. Three weeks after the fourth immunization the rats were sacrificed and serum prepared for immunodiffusion.

c) Ouchterlony double diffusion and Immunoelectrophoresis

Two clean sterilized glass slides were overlaid with adhesive agar and allowed to dry at room temperature to enable the agar to stick on the glass. An even layer of boiled and cooled sterilized agar, without air bubbles was put on to two glass slides and allowed to dry at room temperature for 1 hour. Then using a well puncher, six wells in a row parallel to another at a distance of 1cm apart were made on each slide. Using a micro

pipette 12.5µl of extracted jiggers proteins were carefully placed in the five wells on one side of the slide. The first well was filled with 12.5 µl of sheep serum against 12.5 µl of anti-sheep serum- the positive control. The next four wells were filled with same amount of rat serum, each well corresponding to the order of immunization intervals. PBS was put in the last well to act as a negative control. This procedure was replicated on a second slide. The same procedure was repeated on two new slides replacing rat serum with human serum in dilution of 1:1, 1:2, 1:4, 1:8, 1:16, and 1:32 PBS to serum. The gel boxes were over layered with absorbent filter papers to retain moisture in the box. The glass slides were then put in gel boxes which had barbital solution to prevent bacterial and fungal growth. This was then incubated at 25⁰C for 48hrs for Ag-Ab complexes to form.

The slides for immunoelectrophoresis were prepared in a similar manner, except that the jigger proteins extract was first were put in a well and stained with bromothymol blue (BPB) stain to track their movement on the gel. The electrophoretic tank was flooded barbital buffer with a pH of 8.6. The slides were then put into the tank and connected to the buffer using moist filter paper wicks. This was electrophoresed for 90 minutes to separate proteins according to their negative electric charge. After the protein migration, a trough was made 0.5cm away from the edge of each slide and serum was added. This was filled with human serum and the gels incubated for precipitate bands to form.

Staining procedures involved incubating the gels in saline water for 48hrs to fix the bands after which the gels were incubated at 37⁰C for another 48hrs to dry it. Then the gels were put in a tray containing commasie blue (CBB) stain, put in a shaker at 110

rpm at 37°C for 30 minutes. Excess stain was removed using running water followed by destaining for 30 minutes in a destain solution to remove the stain from the gel except from the bands. This step was repeated 3 times to fully destain. Finally the gels were incubated in distilled water overnight and later incubated for 6hrs at 37°C to dry.

d) Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)

SDS-PAGE is a technique in which protein molecules with similar charge to weight ratio are separated according to their size and shape, by applying an electric current to them. The current forces the molecules through pores in a thin layer of gel. Smaller and light protein molecules travel further than large heavy ones.

A Bio Rad mini gel apparatus was used for this technique. It comprise of a tank where electrode buffer was put, a stand where the clamps were assembled and also used to level the clamps. The clamps were used to fix glass plates (shorter and larger plates; the larger one lies to the clamp) in such a way that there was no leakage of the gel. Two spacers (each 0.5mm thick) were put between the shorter and large plates to define the size of the space between them and therefore the thickness of the gel. A comb was used to make wells on each gel where the samples were put.

The first step in this procedure was the preparation of two gels; the separating gel and the stack. Preparation of the separating gel involved addition of 3.4 ml distilled water in a conical flask. This was followed by 4ml of 12% base acryl amide to form a matrix in the gel by forming cross linking co-monomer molecules. Then 2.5 ml of 8.8% Tris HCl

was added to stabilize the pH at 8.8. This was followed by addition 100µl of SDS detergent whose molecules binds to the proteins giving them a similar negative charge to weight ratio such that bigger molecule gets bigger charge. This was then followed by addition of 100 µl ammonium per sulphate (APS) that catalyses the reaction. Lastly after setting up the apparatus, 4 µl of TEMED was added to initiate polymerization. The mixture (separating gel) was the immediately applied into the space between the glass plates using a 10ml pipette up to the level where the combs would reach. Bubbling was avoided as it would interfere with smooth protein separation. Polymerization of the gel took about 10 minute. Parallel to preparation of separation gel was preparation of the stack gel with the same reagents but in different proportions. It was made by putting 3.3ml of distilled water in a conical flask. This was followed by 830µl of 12% base acryl amide. Then 630 µl of 2.5% Tris HCl of pH 6.8 was added followed by 50µl of SDS. Added to the mixture was 50µl of APS and finally 20µl of TEMED to initiate polymerization. Polymerization of the separating gel took 10 minute after which stack gel was applied using a 10ml pipette and combs fixed immediately to draw the wells where the protein samples were put. This was put aside for 10 more minutes, time when samples were prepared.

Sample preparation involved spinning jigger protein extract for 1 minute at 100rpm. Debris was discarded. The supernatant (sample proteins) were diluted; 1:2, 1:4, 1:5, 1:10 serial dilutions, of sample buffer to the protein sample and a buffer negative control. A Pharmacia low molecular weight standard sample marker (+ ve control) was prepared in the ratio of 1:1 protein to sample buffer. Sample buffer comprised of BPB

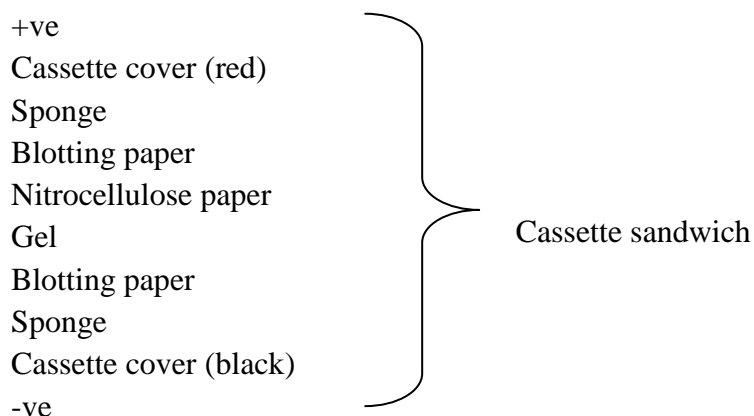
dye that assist in following the migration of the proteins. It also contains metacarpal ethanol that break down all disulphide and hydrogen bonds in between the protein molecules to straight free up the polypeptides. In addition it also comprise of SDS detergent that gives the protein molecules negative charges depending on their sizes, whereby bigger sized molecule get a bigger negative charge and smaller ones a small negative charge. The sample buffer also contain glycerol, high molecular weight molecules that give weight to protein molecule and assist in pushing them down to the bottom of the well.

Finally the protein samples were boiled for 5 minutes to further help in denaturization of disulphide bond between the protein molecules to reduce them to single molecules. Boiling also inactivates endogenous proteases are very active in SDS sample buffer that can cause severe degradation After 10 minutes the combs were removed from the glass plates. The plates were released from the stand, fixed on to the terminals and then immersed into the tank containing electrode buffer made of SDS and Tri HCl that conducts current. Twenty five microlitre of each sample dilution was loaded in the well in that order starting with the standard to the two gels on each side of the plate. The terminals were then subjected to 100V and 100amp from a power pack for 45 minutes for complete migration of protein bands.

e) Western Blot

After 45 minutes the gels were carefully removed from the glass plates under transfer buffer in a tray to avoid breakage. The transfer buffer comprise of methanol for protein resolution, glycerol which has high molecular weight to help push proteins on to the

nitrocellulose paper and Tris HCl to give a pH of 8. The nitrocellulose paper used was of 0.45µm pores diameter. Sponges and blotting papers were soaked in the transfer buffer to make the pore open and ensure that no bubbles are trapped. These were then aligned in a sandwich called cassette in the order shown below.



The cassettes were clamped tightly and fixed on the plate for the protein transfer. Black side of the cassette was placed on to the black side of the terminal and the red side to the red terminal. This ensured that the protein bands were transferred on to the nitrocellulose paper and not lost to the sponge. Then ice was added in to the tray in the assemble unit and then the whole unit was buried in more ice to prevent overheating. This was then connected to electrical terminals from a power pack and set at 200v and 200amp for 90 minutes.

After 90 minutes the power was disconnected and the nitrocellulose papers carefully removed under buffer. The papers were then immersed in Ponceau S stain for 5 minutes in a tray covered with aluminium foil to prevent decomposition of the stain by light. This was done to visualize the transfer of protein bands on to the papers. This was followed by washing the papers 3 times with PBS/Tween washing buffer made by

mixing 0.05% PBS in a litre of distilled water and 500 μ l of Tween detergent. The papers were then labeled No. 1 up to 12 and cut in to strips. The standard marker strips were wrapped in aluminium foil to keep light away and kept in fridge to make them remain moist. The rest of the paper strips were further washed 3 times each for 30 minutes in a shaker at 60 rpm to remove as much stain as possible. This was because the stain could interfere with the results hence giving misleading results.

Serum preparation involved pooling of the human serum from two severely infested individuals, by mixing 125 μ l from individual A and same volume from individual B. Then serum dilutions of 1:25, 1:50, 1:100, 1:200, and 1:400 of 0.1% BSA/Tween to serum were made. The BSA in this instance serves as a protein carrier from the serum to the paper strips since it has high molecular weight. The strips were put in a tray containing 3% PBS/BSA/Tween; a solution made by mixing 1.5g of BSA in 50ml PBS/Tween, and then put on a shaker for 30 min at 60 rpm. This was done to ensure proper blocking of non specific sites. The strips were then washed 3 times, each wash taking 30 minutes on a shaker at 60 rpm using 0.05% PBS/Tween to remove excess BSA. The strips were then put in stoppered tubes each with 4ml of diluted serum whereby strips No. 1 & 7 were put in 1:25 serum dilution, strips No. 2 & 8 put in 1:50, strips No.3 & 9 in 1:100, strips No. 4 & 10 in 1:200 and 5 & 11 in 1:400 serum dilution respectively. Strips 6 & 12 were put in 0.05%PBS/Tween buffer. The strips were incubated for 24hrs in a shaker set at 5⁰C at 40 rpm. At the expiry of 24hrs the strips in the stoppered tubes were removed from the incubator, and subjected to another shaking at 25⁰C for 90 minutes. This was followed by addition of 4ml of 0.05% PBS/Tween

buffer in each tube, tilting it several times and discarding off the buffer to remove excess serum. The strips were then removed from the tubes using a forceps, flooded with the washing buffer in a tray and shaken for 5 minutes. This was repeated four times to remove any excess serum. After the fourth wash strip No. 12 was put together with the other strips and a conjugate (anti human Horse Radish conjugated antibody) added. This was incubated for 90 minutes on a shaker at room temperature. The conjugate was prepared in dilution of 1:2000 by mixing 10 μ l of 20ml of 0.1% PBS/BSA/Tween. Strip No. 6 was incubated separately in 0.1% PBS/BSA/Tween for the same time. This was followed by washing the strips using the washing buffer to remove excess conjugate. Strip No. 6 also subjected to the same washing procedure. In a tray with all the strips, a substrate, 4 Chloro-1- Naphthal made by mixing 500 μ l of the substrate to 50ml of 7.6 pH Tris HCl was added. This was then followed by addition of hydrogen peroxide catalyst made by mixing 10ml of Tris HCl and 10 μ l hydrogen peroxide. This was then put in the dark for five minutes for reaction to complete. The presence of light could degrade the substrate/conjugate complex hence interfering with the results. After five minutes the strips were removed and kept in the dark to dry after which results were viewed. Strip No. 12 was a conjugate control and strip No.6 a buffer (negative) control.

APPENDIX III

Statistical analysis

- i). Cross tabulation on pupils infested by jiggers in Kimathi, Gaturi, Mugoiri and Kahuhia locations in Kiharu constituency.

Pupils infested by jiggers in Kimathi, Gaturi, Mugoiri and Kahuhia locations in Kiharu constituency			
	Infested	Not infested	Total
Kahuhia	20.7%	79.3%	100.0%
Mugoiri	23.3%	76.7%	100.0%
Kimathi	17.6%	82.4%	100.0%
Gaturi	11.0%	89.0%	100.0%
Total	19.1%	80.9%	100.0%

- ii). One way ANOVA on pupils infested by jiggers in Kimathi, Gaturi, Mugoiri and Kahuhia locations in Kiharu constituency.

Descriptives								
Location	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimu m	Maximu m
					Lower Bound	Upper Bound		
Kahuhia	29	.21	.412	.077	.05	.36	0	1
Mugoiri	283	.23	.424	.025	.18	.28	0	1
Kimathi	51	.18	.385	.054	.07	.28	0	1
Gaturi	145	.11	.314	.026	.06	.16	0	1
Total	508	.19	.393	.017	.16	.23	0	1

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.466	3	.489	3.197	.023
Within Groups	77.013	504	.153		
Total	78.478	507			

iii). **One way ANOVA Post Hoc Tests on pupils infested by jiggers in Kimathi, Gatari, Mugoiri and Kahuhia locations in Kiharu constituency.**

Multiple Comparisons						
Dependent Variable: Infested by jiggers Tukey HSD						
(I) Cluster	(J) Cluster	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kahuhia	Mugoiri	-.026	.076	.986	-.22	.17
	Kimathi	.030	.091	.987	-.20	.26
	Gatari	.097	.080	.618	-.11	.30
Mugoiri	Kahuhia	.026	.076	.986	-.17	.22
	Kimathi	.057	.059	.775	-.10	.21
	Gatari	.123*	.040	.012	.02	.23
Kimathi	Kahuhia	-.030	.091	.987	-.26	.20
	Mugoiri	-.057	.059	.775	-.21	.10
	Gatari	.066	.064	.727	-.10	.23
Gatari	Kahuhia	-.097	.080	.618	-.30	.11
	Mugoiri	-.123*	.040	.012	-.23	-.02
	Kimathi	-.066	.064	.727	-.23	.10
*. The mean difference is significant at the 0.05 level.						

Infested by jiggers		
Tukey HSD		
Cluster	N	Subset for alpha = 0.05
		1
Gatari	145	.11
Kimathi	51	.18
Kahuhia	29	.21
Mugoiri	283	.23
Sig.		.299
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 61.996.		

- iv). **Cross tabulation on average jigger infestation in boys and girls in Kimathi, Gaturi, Mugoiri and Kahuhia locations in Kiharu constituency.**

Jigger infestation in boys and girls in Kimathi, Gaturi, Mugoiri and Kahuhia locations in Kiharu constituency			
Gender	Infested	Not infested	Total
Boys	20.1%	79.9%	100.0%
Girls	18.1%	81.9%	100.0%
Total	19.1%	80.9%	100.0%

- v). **Chi-Square Test on jigger infestation in boys and girls in Kimathi and Gaturi, Mugoiri and Kahuhia locations in Kiharu constituency.**

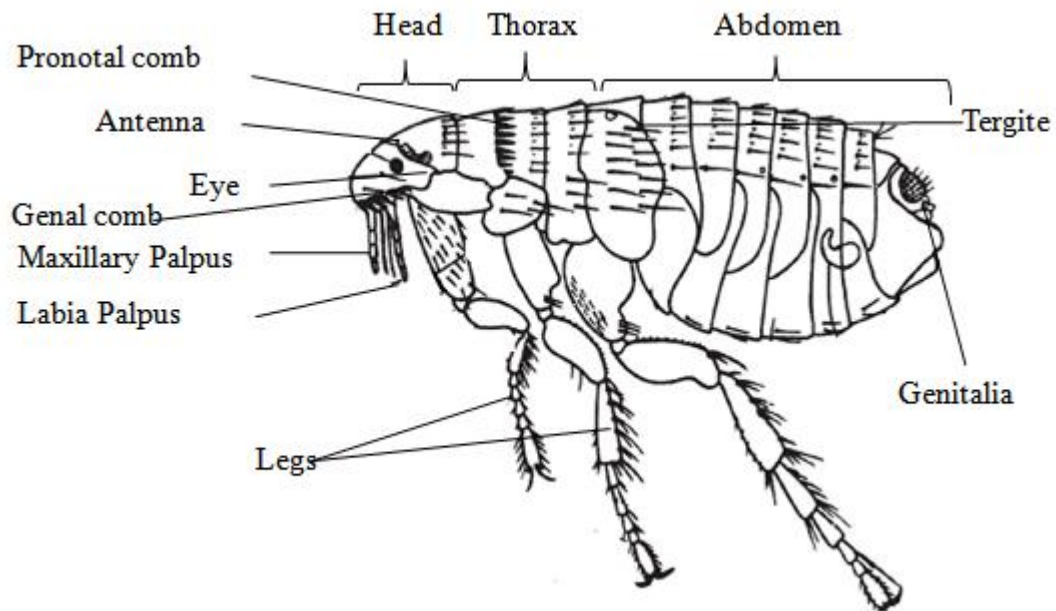
Chi-Square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.330 ^a	1	.565

- vi). **Cross tabulation on age brackets infested by jiggers in pupils in Kimathi, Gaturi, Mugoiri and Kahuhia locations in Kiharu constituency**

Age brackets infested by jiggers in pupils in Kimathi, Gaturi, Mugoiri and Kahuhia locations in Kiharu constituency.						
	4-6years	7-9years	10-12years	13-15years	16-18years	Total
Infested	12.5%	18.3%	21.5%	16.5%	8.3%	19.1%
Not infested	87.5%	81.7%	78.5%	83.5%	91.7%	80.9%
Total	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

APPENDIX IV

Photographs



(a). Labeled diagram of *T. penetrans* (Feldmeier *et al.*, 2005).



(b). Engorged female (Feldmeier *et al.*, 2005).



(c). *T. penetrans* eggs being laid
(Heukelbach *et al.*, 2003)



(d). *T. penetrans* egg x 1000
(Feldmeier *et al.*, 2005)

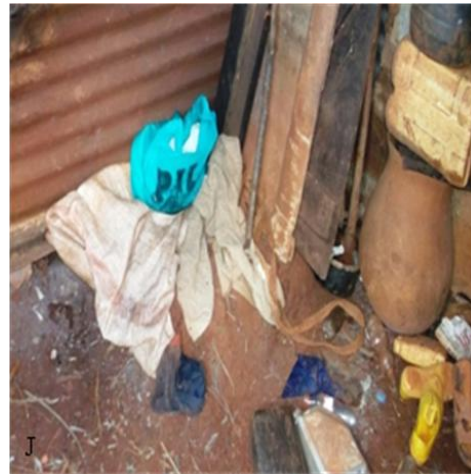


(e) Infested back (Ahadi, 2008).



(f). Amputated toes and fingers
due to jigger infestation (Ahadi,
2008).





(G, H, I, J). Poor housing and living conditions witnessed in Kimathi, Kahuhia, Mugoiri and Gaturi that promotes jigger infestation by offering good breeding places for *Tunga penetrans*. (Pictures taken using a digital camera)



(k). Administration of tetanus jabs for prevention of infection by *Clostridium tetani* through septic wounds left open after jigger extractions in Kahuhia



(l). Nail loss (Ahadi 2008).



(m). Tissue necrosis (Ahadi 2008).

APPENDIX V

Clearance letters



**KENYATTA UNIVERSITY
GRADUATE SCHOOL**

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
 Website: www.ku.ac.ke

P.O. Box 43844, 00100
 NAIROBI, KENYA
 Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School **DATE:** 5th May, 2012
TO: Mwangi Jamleck Ndung'u **REF:** I56/CE/10952/06
 C/o Department of Zoological Sciences
REF: **APPROVAL OF RESEARCH PROPOSAL**

This is to inform you that the Graduate School Board at its meeting of 30th April, 2012 approved your research proposal for M.Sc degree entitled, "Epidemiology and Characterization of Tunga Penetrans Isolates in Murang'a East District".

DAVID NJOROGE
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Zoological Sciences

Supervisors:

1. Dr. Michael M. Gicheru
 Department of Zoological Sciences
 Kenyatta University.
2. Dr. Hasting Ozwara Suba
 Department of Tropical and Infectious Diseases
 Institute of Primate Research
 National Museums of Kenya
 C/o Department of Zoological Sciences
 Kenyatta University.



**KENYATTA UNIVERSITY
GRADUATE SCHOOL**

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dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

**P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530**

Our Ref: I56/CE/10952/2006

Date: 5th May, 2012

The Permanent Secretary,
Ministry of Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

**RE: RESEARCH AUTHORIZATION FOR MWANGI JAMLECK NDUNG'U REG.NO
I56/CE/10952/2006**

I write to introduce Mwangi Jamleck Ndung'u who is a Postgraduate Student of this University. He is registered for a M.Sc degree programme in the Department of Zoological Sciences in the School of Pure and Applied Sciences.

Mr. Ndung'u intends to conduct research for a Proposal entitled, "**Epidemiology and Characterization of Tunga Penetrans Isolates in Murang'a East District**".

Any assistance given will be highly appreciated.

Yours faithfully,

**MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL**

MINISTRY OF PUBLIC HEALTH AND SANITATION

Telephone: (060) 30244,
 Fax: (060) 30244,
 When replying please quote,

Ref:



MEDICAL OFFICER OF HEALTH,
 MURANG'A NORTH DISTRICT,
 P. O. BOX 69,
 MURANG'A

DATE: 21st FEBRUARY, 2011

DR. GICHERU
 KENYATTA UNIVERSITY
 DEPARTMENT OF ZOOLOGY

**RE: PERMISSION TO CONDUCT RESEARCH IN LARGER MURANGA
 NORTH DISTRICT (KAHURO, KIHARU, KANGEMA AND MATHIOYA)**

Reference is made to your letter dated 24th January, 2011. This is to inform you that the undersigned office has no objection to your team conducting research within the stipulated area.

Please limit yourselves to study objectives, "Tungiasis as a risk factor for HIV and Aids in Economic Disadvantaged communities in Murang'a North county and characterization of the most immunodominant antigen in Tunga penetrans potential for vaccine development". The department of health is eager to work with your team in this important study. Team members authorized are: Dr.M Gicheru, Dr. H. Ozwesa, Dr. M.Maree and Mr.J Mwangi.

Thank you.

DR.E.M.MAREE

DISTRICT MEDICAL OFFICER OF HEALTH
 MURANG'A EAST, MURANG'A WEST, MATHIOYA AND KAHURO.

DISTRICT MEDICAL OFFICER
 OF HEALTH
 P.O. Box 69 - 10200,
 MURANG'A

REPUBLIC OF KENYA
MINISTRY OF EDUCATION



Telegrams:
Telephone: 0722-352708
When replying please quote

DISTRICT EDUCATION OFFICE
KAHURO DISTRICT
P.O BOX 27-10201
KAHURO.

7th February, 2011.

Dr. Michael M. Gicheru

Principal investigator/chairman
Department of zoological sciences
Kenyatta University

REF: RESEARCH

This is to authorize the bearer of this letter to visit primary schools in Kahuro District for the purpose of carrying out research on Tungiasis.

Any assistance given to him will be greatly appreciated.

MARK S. ALULU

DISTRICT EDUCATION OFFICER

KAHURO DISTRICT

DISTRICT EDUCATION OFFICER

KAHURO DISTRICT

P.O. Box 27 KAHURO

Date:.....Sign:.....

MINISTRY OF EDUCATION /

Telegrams: "SCHOOLING", Murang'a
 Telephone: Murang'a 30231 AND 30236
 When replying please quote



REPUBLIC OF KENYA

DISTRICT EDUCATION OFFICE
 MURANG'A EAST
 P.O. BOX 118
 MURANG'A

REF: MGA/GEN/26/VOL.III/170

4th February, 2011

The Headteachers
 Primary Schools
MURANG'A EAST

RE: PERMISSION TO CONDUCT A RESEARCH IN PRIMARY SCHOOLS

The education office has no objection for Dr. Gicheru, Dr. Muriuki and Mr. Mwangi to carry out research entitled "*Tungiasis as a risk factor for HIV and AIDS in economically disadvantaged communities in Murang'a East and characterization of the most immunodominant antigen in Tunga penetrans potential for vaccine development*" in our primary schools.

District Education Officer

E.W.NJUGUNA

FOR: DISTRICT EDUCATION OFFICER
MURANG'A EAST



INSTITUTE OF PRIMATE RESEARCH
NATIONAL MUSEUMS OF KENYA
WORLD HEALTH ORGANIZATION COLLABORATING CENTRE



10th December 2009

THE EXECUTIVE SECRETARY
 NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

P.O. BOX 30623-00100

UTALII HOUSE

8TH FLOOR ROOM 822

NAIROBI

TEL: 020-310571; FAX: 020-2213215

E-MAIL: research@ncst.go.ke

WEBSITE: WWW.ncst.go.ke

RE: SUBMISSION OF A RESEARCH PROPOSAL ENTITLED: "TUNGIASIS AS A RISK FACTOR FOR HIV AND AIDS IN ECONOMICALLY DISADVANTAGED COMMUNITIES IN MURANG'A NORTH AND CHARACTERIZATION OF THE MOST IMMUNODOMINANT ANTIGEN IN *TUNGA PENETRANS* POTENTIAL FOR VACCINE DEVELOPMENT"

This to confirm that I am happy to collaborate with Kenyatta University and Muranga District hospital in the above project. Our laboratory facility will be involved in identification and characterization jigger isolates

Thank you

Hastings Ozwara Suba, PhD,
 Head, Department of Tropical and Infectious Diseases,
 Institute of Primate Research,
 P.O. Box 24481, Karen 00502,
 Nairobi.
 Tel: +254 20 882571/4
 Fax: +254 20 882544

APPENDIX VI**Publication**