PREVALENCE AND ASSOCIATED RISK FACTORS OF TUNGIASIS IN CHIPATA AND VUBWI DISTRICTS OF EASTERN ZAMBIA

BY

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LUSAKA

ABSTRACT

Tungiasis, also commonly known as *jiggers*, is a zoonotic ectoparasitosis caused by the permanent penetration of the epidermis by a gravid female sand flea *Tunga penetrans* in the skin of a host. The skin disease is known to occur throughout Latin America, the Caribbean and sub Saharan Africa especially among resource poor communities. There is no or little epidemiologic data on the skin disease in Zambia.

A cross-section study was conducted in rural communities in Chipata and Vubwi districts of Zambia to determine the prevalence, risk factors and levels of awareness of tungiasis. A total of 384 households were visited from where at least one member was interviewed and examined. A household was considered infested if at least one individual had tungiasis. Collected data included among others social demographic characteristics, household living conditions, localization of the lesions, nature of lesions (avital or vital), number of embedded female *T. penetrans* and tungiasis specific disease related information including knowledge.

The overall prevalence of active tungiasis in the study area was 13.5% (95% CI= 10.1 – 16.9) with Chipata recording 12.6% (95% CI= 8.5 – 16.7) and Vubwi 15.3% (95% CI= 9.3 – 21.3). On assessment of risk factors, individuals in age categories ≤ 15years and those ≥ 60 years showed higher prevalence 68.8% (95% CI= 46.1 – 91.5) and 18.2% (95% CI=2.1 – 34.3), respectively, compared to those in the intermediary age groups (p=0.001). The type of sanitary facilities and marital status as it related to age were found to be significant predictors of tungiasis (p<0.05). Living in a households with a pit latrines reduced the risk of tungiasis by 25.4% (95% CI= 0.071 – 0.913), when compared to those that practiced open defecation (p=0.036). Those that were of marriageable age and single (>20 years old and single) were 19.7 (95% CI= 5.152 – 75.469) more likely to have tungiasis than those that were married and ≥20 years old (p=0.001). All other variables were not significantly associated with *T. penetrans* (p>0.05). The levels of awareness of the disease in the study area were very good, ranging from 80% to 83% on the basis of having experienced tungiasis and 3.3% to 71% on disease specific knowledge.

Tungiasis is prevalent among the studied rural communities in Chipata and Vubwi districts. Although the awareness of the disease was high in the studied communities, people were not able to link tungiasis with risk factors. More research work needs to be done in order to rank the risk factors and to determine the burden tungiasis exerts in economic terms.

DECLARATION

I **Kelvin Mulenga Kampamba** do hereby declare that this dissertation represents my own work and that it has never been submitted before for the award of a degree or any other qualification at this university or indeed any other university.

Signature: ---------------------------- Date: -------------------------

CERTIFICATE OF APPROVAL

This dissertation of **Kelvin Mulenga Kampamba** is approved as fulfilling the requirements for the award of the Degree of Master of Science in One Health Analytical Epidemiology of the University of Zambia.

(Internal Examiner)	Signature	Date
(Internal Examiner)	Signature	Date
(Supervisor)	Signature	Date
(Chairperson)	Signature	Date

DEDICATIONS

This dissertation is a dedication to my late father Phillip Victor Kampamba for his unwavering love during his life on earth, my three children Bwalya, Victor and Kellie, my mother, brothers and sisters and my uncle Simon for the encouragement they gave to me during my studies.

'***Jiggers like mosquitoes will always be there'*, A.S Ward 1914.**

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ABBREVIATIONS AND SYMBOLS

%	Percent
CEO	Camp extension officer
CI	Confidence Interval
CATS	Community Approaches to Total Sanitation
CLTS	Community Led Total Sanitation
COII	Cytochrome Oxidase II
CSO	Central Statistics Office
DNA	Deoxyribonucleic Acid
E	East
EHT	Environmental health technician
ITS2	Internal Transcriber Spacer 2
IQR	Interquartile Range
Mm	millimeter
MspI	Restriction enzyme
n	Sample size
OD	Open Defecation
OR	Odds Ratio
PAF	Population Attributable Fraction
PCR	Polymerase Chain Reaction
PHCC	Primary Health Care Centres
PHO	Provincial Health Office
PVO	Provincial Veterinary Office
RFLP	Restricted Fragment Length Polymorphism
RHC	Rural Health Centre

RsaI	Restriction enzyme
S	South
VA	Veterinary Assistant
WHO	World Health Organization
$\geq$	Greater or equal
$>$	Greater than
$<$	Less than

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CHAPTER ONE

1.0 INTRODUCTION

The parasitic skin disease, tungiasis, is caused by the permanent penetration of the female sand flea *Tunga penetrans* in the epidermis of the host. The disease is known by a variety of names such as *Jiggers*, *Chigoe* and *pique* depending on geographic location (Heukelbach, 2004). In the traditional folklore in the Eastern province of Zambia, it is referred to as *Matekenya.*

The gravid female *T. penetrans* infestation mostly occurs on the extremities of the limbs but may occur anywhere on the body, such as hands, elbows, neck, buttocks and the genital region (Heukelbach, 2006). Severe complications are common in areas with high intensity of infestation and poor hygienic conditions (Heukelbach, 2006). Associated pathologies include bacterial super infection, gangrene, lymphadema, pain, fissures, lameness, deformation, as well as loss of toe nails and digits (Litvoc *et al*., 1991; Ugbomoiko *et al*., 2007). Tungiasis lesions may also serve as portal of entry for tetanus infection (Greco *et al*., 2001; Feldmeier *et al*., 2002).

Tungiasis has many features of a neglected tropical disease and can thus be considered as a paradigm (Heukelbach *et al*., 2004). It is prevalent in resource poor rural areas and is associated with stigma (Heukelbach *et al*., 2004). The situation is worsened by the lack of commercial products targeting the control and treatment of the disease (Heukelbach, 2004).

Tungiasis is not among the World Health Organization (WHO) listed epidermal parasitic skin diseases (EPSD), a category of poverty associated plagues that has been neglected by the scientific community and health care providers (Feldmeier *et* *al*., 2006). *T. penetrans* has not been implicated for direct transmission of infectious diseases and as such is rated as a passive vector in the transfer of bacterial pathogens (Feldmeier and Heukelbach, 2008). However, their readiness to parasitize humans as alternative hosts, gives fleas of domestic animals, *T. penetrans* inclusive, a lot of relevance in public health (Urquhart *et al.,* 1987; Kramer and Mencke, 2001).

Tungiasis, although a self-limiting infestation by nature, is actually a debilitating disease associated with considerable morbidity (Heukelbach, *et al.,* 2005).

Tunga penetrans infestation is an important disease in some rural communities in the Eastern Province of Zambia in that it has been seen to exacerbate the current high poverty levels. In most rural communities in Chipata and Vubwi districts, production technologies are far from being mechanized and the rural livelihoods are solely dependent on human strength and wellbeing e.g. the use of hand - held hoes, ox - drawn ploughs etc. Therefore infestation with *T. penetrans* results in them being unable to be productive and they are secluded from communities because of stigma associated with the disease (Heukelbach *et al*., 2004). Furthermore, livestock, especially pigs, being the most treasured endowment, are usually subjected to indiscriminate mass slaughter in outbreak times, thereby perpetually relegating the rural communities in poverty doldrums.

The attention given to this pro poor ectoparasitosis is very inadequate and this is manifested by the non-availability of scientific publications on prevalence, temporal and spatial distribution of the disease condition, knowledge attitudes and practices of people living in suspected endemic areas in Zambia.

The aim of this study was to determine prevalence, associated risk factors and levels of awareness of tungiasis prevention and control in Chipata and Vubwi districts in the Eastern Province of Zambia.

Specifically, the study aimed to:

1. Determine the prevalence of tungiasis among households in Chipata and Vubwi districts.

2. Investigate risk factors associated with tungiasis among household members in Chipata and Vubwi districts.

3. Determine awareness and knowledge levels on tungiasis in the study areas.

CHAPTER TWO:

2.0 LITERATURE REVIEW

2.1 Background

The first cases of tungiasis were described by Gonzalez Fernandes Oveido Valdes at the turn of the 16th Century when Spanish conquerors of the crew of the Santa Maria were shipwrecked at Haiti and became infested with the flea (Heukelbach *et al*., 2001). In the 17th century, A Portuguese physician working with the Government of Brazil provided the world's first description of *T. penetrans* (Heukelbach *et al*., 2001).

The sand flea is believed to have originally been an arthropod of America from as far back as the 18th Century. In the late 19th Century the parasite spread from South America, the Caribbean Islands to Madagascar and Sub Saharan Africa by travelers, mostly voyagers on ships during the slave trade era. The spread of *T. penetrans* is traced along trade routes during ancient times and has established itself (Kettle, 1984).

Beside the African colonization event, several reports describe further accidental introductions of *T. penetrans* in temperate countries, through infected humans coming back or emigrating from endemic areas (Fein *et al*., 2001; Caputo *et al.*, 2005; Luchette *et al.*, 2007).

In Northern Rhodesia, presently known as Zambia, George Grey (1901), who commanded a commercial expedition in 1899 reported; "When we first found the jigger, saw many of the natives so lame that they could hardly walk in consequence of its attack."

2.2 The taxonomy, morphology and biology of T. penetrans

Tunga penetrans has over the centuries been described by several synonyms as a result of different perspectives in taxonomical changes since 1743 (Pampiglione *et al*., 2009) and these are *Pulex minimus cutem penetrans* (Catesby, 1743); *Pulex minutissimus nigricans* (Barr`ere, 1743); Acarus *fuscus sub-cutem nidulans* (Brown, 1756) ; *Pulex penetrans* (Linneo, 1758); *Pulex reptans* (Illiger, 1805); *Rhynchoprion penetrans* (Oken, 1815); *Dermatophilus penetrans* (Lucas,1839); *Sarcopsylla penetrans* (Westwood, 1840). The current description is based on *Tunga penetrans* (Linneo, 1758; Jarocki, 1838; Pampiglione *et al*., 2009).

Tunga penetrans (Figure 2.1(A), is the smallest of all the known fleas' species (Connor, 1976; Kramer and Mencke, 2001). It belongs to the order Siphonaptera, family Hectopsyllidae and genus *Tunga*. The basic body length of adults is only about 1mm, the gravid female (figure 2.1(B) is bigger and can increase to about 80 times relative to its body size (Nagy *et al*., 2007; Witt *et al*., 2004). The male *Tunga penetrans* has the longest intromittent organ relative to its body size known in the whole animal kingdom (Durden and Traub, 2002).

Tunga penetrans (sand fleas) interestingly and comparably, to other flea species, are poor jumpers and only capable of jumping to only about 20–30 cm but are known for rapid lateral displacement (Service, 1960).

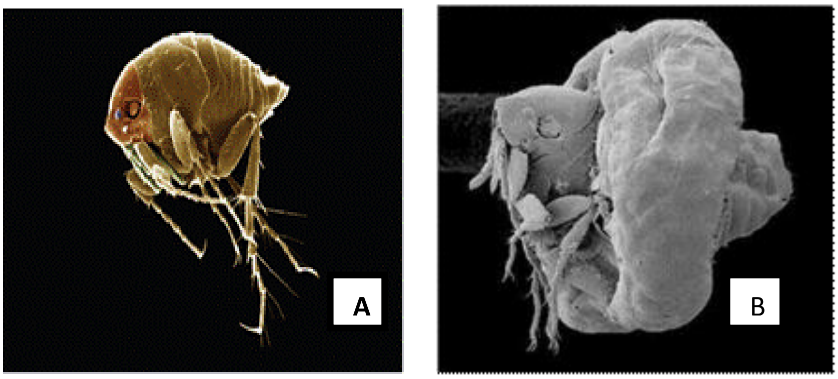

Figure 2.1: Morphology of a free living flea (A) and classic electron micrograph (B) of an in situ image of a gravid *T. penetrans* (Nagy *et al*., 2007)

2. 3 Life cycle of Tunga penetrans

In the natural environment the sand fleas are maintained both on and off the host (Heukelbach *et al*., 2001). Both the adult male and female fleas intermittently feed on their warm blooded hosts. Fleas are holometabolous insects which develop from eggs to larvae (usually consisting of three instar generations), to pupae and finally into the adult stage (Figure 2.2). The mating of fleas may occur at both times when either the female has embedded into the epidermis or during the free living stages (Nagy *et al*., 2007). The next phase of life for the female flea, as the male flea dies after copulation, is *in vivo* ecto-development, and it is commonly referred to as the Fortaleza classification of tungiasis (Nagy *et al*., 2007).

The female flea burrows into the skin of the host and causes cutaneous lesions (Audy *et al*., 1972; Goff *et al*., 1982; Nagy *et al*., 2007). The female flea attaches to the dermis by anchoring mouth parts and claws violently into the epidermis. In the skin, the female *T. penetrans* burrows a cavity with the head turned toward the upper dermis in order to feed on the host's blood and begins to produce eggs (150–200) over a period of two to three weeks (Leung *et al*., 2007; Rosmanihno *et al*., 2010).

The eggs are discharged into the environment from the gravid female flea. In the dry season, given the right environmental conditions (i.e. loose and dry soils), the larvae emerge. The larvae feed on organic residues and moult twice to reach a length of close to 1mm (Hicks, 1930; Pampiglione *et al*, 2009). Between 10 and 18 days after emerging, the larvae burrow into the soil and pupate (Hicks, 1930). Pupae development varies according to humidity, temperature and season but usually takes three to four weeks. Under laboratory conditions it may take five to 18 days (Nagy *et al*., 2007). The ability of off host stages survival of the *T. penetrans* is still considered to widely vary in different environments (Linardi *et al,* 2010). Adult females are believed to survive without food for a month or more. According to Bonnet (1867) the sex ratio is female biased and it could be as a result of *Wolbachia* infection which naturally depopulates male sand fleas (Luchette *et al,* 2005; Pampiglione *et al.,* 2009).

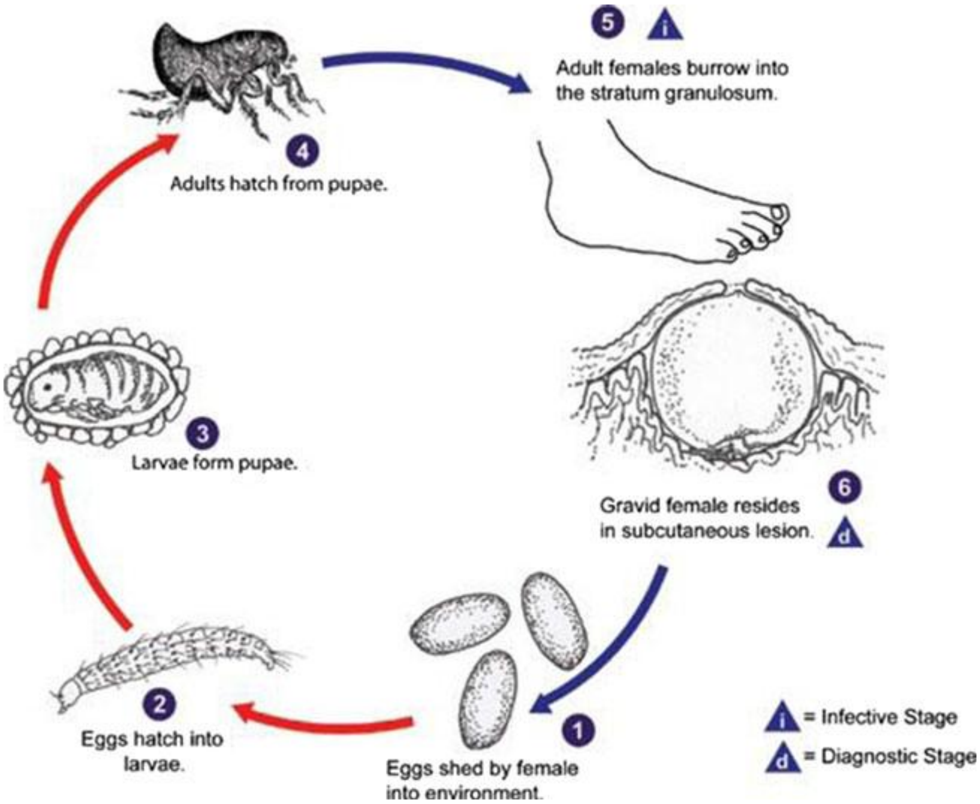

Figure 2.2 Lifecycle of *T. penetrans*. Eggs are discharged into the environment from gravid female flea, with right environmental conditions (i.e. loose and dry soils), the larvae emerge and feed on organic residues and moult twice to reach a length of close to 1mm adult (Karunamoorti, 2013).

2.4 Epidemiology

2.4.1 Transmission and Host range

The term transmission is more of a misnomer when describing tungiasis considering that the sand flea disease is in effect the *T. penetrans* itself. True to its Latin name, the adult female *T. penetrans* burrows into the skin of an unsuspecting host and causes cutaneous lesions (Nagy *et al*., 2007). *Tunga penetrans* parasitizes various mammalian hosts including humans (Hopkins and Rothschild 1953; Linardi and

Guimarães 1993; Heukelbach *et al.* 2004). The host spectrum includes about 94% mammals and 6% birds and normally, 99% of the flea population live (as larvae and pupae) on the ground and are not perceived by humans (Nagy *et al.*, 2007). Thus, host attacks are only done by 1% of the living flea population (Linardi and Guimarães 2000).

2.4.2 Distribution

Worldwide, about 3,000 flea species are known (Lewis, 1972; Nagy *et al.*, 2007). In contrast to most of other flea species, the genus *Tunga* occurs only in the tropics (Heukelbach *et al.,* 2001).

Originally, the ectoparasite occurred only on the American continent (Figure 2.3) The sand flea is one of the few parasites, which has spread from the Western to the Eastern hemisphere.

All the fleas in the genus *Tunga* are hematophagus ectoparasite and can parasitize a single or few closely related hosts and have been known to show a geographically restricted distribution (Li and Chin 1957; Barnes and Radovsky1969; Heukelbach *et al*., 2001). The parasite was introduced accidentally on the African continent during the colonization period and now is considered endemic in paleo and neo areas (Heukelbach *et al.*, 2004).

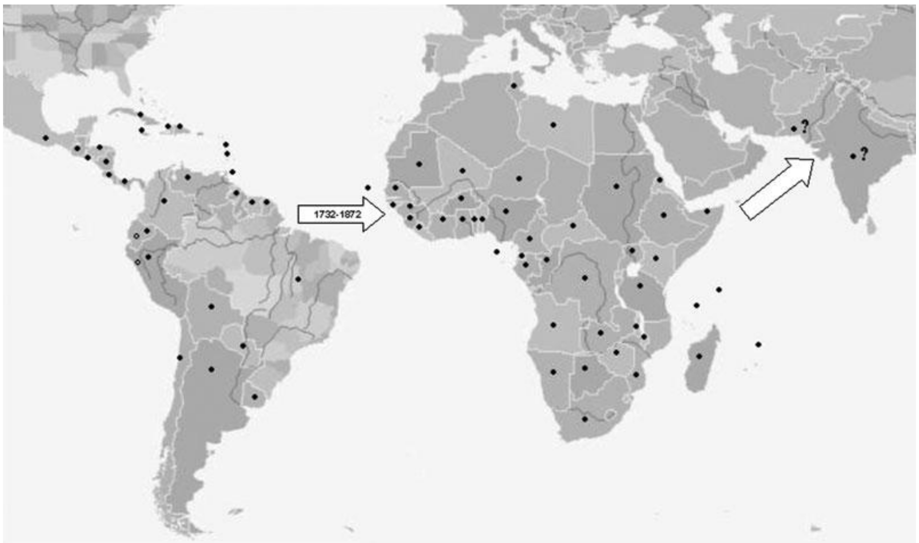

Figure 2.3 Distribution of animal and human tungiasis and arrows indicate transcontinental spread of *T. penetrans.* Distribution of *T. penetrans* (●) and *T. trimamillata* (○) unverified reports (●?). Pampiglione *et al*. (2009).

2.4.3 Prevalence and associated risk factors of tungiasis

According to (Heukelbach *et al.*, 2001) the true prevalence of tungiasis in endemic communities in Latin America and sub Saharan Africa could be as high as 50% (Table 2.1) and within endemic areas, the ectoparasitosis has a patchy distribution (Heukelbach *et al.*, 2001). Recent studies indicate that point prevalence in Trinidad, Cameroun, Brazil and Nigeria are estimated to range from 12% to 54% (Ade Serano and Ejezie 1981; Chadee 1998; Wilcke *et al*., 2000; Muehlen *et al*., 2003; Pilger *et al*., 2008).

Table 2.1: Documented prevalence of tungiasis in Latin America and sub Saharan Africa

Country	Prevalence of Tungiasis (%)	Reference
Brazil	19 – 50%	Heukelbach *et al*., 2001
Cameroun	59%	Collins *et al*., 2010
Haiti	75.4%	Joseph *et al*., 2005
Nigeria	45.2%	Ugbomoiko *et al*., 2009
Tanzania	42.5%	Mazigo *et al*., 2010

Studies conducted by Ugbomoiko *et al.* (2007) and Mazigo *et al*. (2009) in a rural community in the dry season in Nigeria and Tanzania respectively, revealed similar associations between age category and prevalence to tungiasis. In both studies those in the <15 years and >60 years were the most susceptible and for the majority, lesions were found in the feet.

In these two studies an S- shaped prevalence pattern was evident and was attributed to age related behavioural tendencies. The increased prevalence in the elderly according to Mazigo *et al*. (2009) was as result of poor sightedness and this led to failure to remove embedded fleas. Commonly the tendency of having rest in the same places which are considered preferred breeding sites of the flea, such as underneath shady trees predisposed people to *T. penetrans* infestation (Mazigo *et al*., 2012). Almost all lesions were localized in the feet and individuals aged 60 years and above had significantly more lesions (Ugbomoiko *et al*., 2007).

A study on health seeking behaviours by communities in Vincent Pinzoin in Northern Brazil revealed that the desire to seek medical attention was low. Following ectopic examinations of the patients for tungiasis in the community based sample, the prevalence was 36% and for those that visited the Primary Health Care Centres (PHCC) was 33.6% (Heukelbach *et al*., 2003).

Studies on associated risk factors to try and explain the transmission and maintenance of tungiasis in endemic communities have been conducted in Northern Brazil and Nigeria. Notably in Brazil the factors that contribute to the maintenance of *T. penetrans* among animal species vary between urban and rural settings (Verhulst *et al*., 1976). According to Verhulst *et al*. (1976) and Franco da Silva *et al*. (2001), *Rattus rattus* were considered to be the most important host in urban setting, while cattle were considered to be instrumental in rural settings. Additionally, Heukelbach *et al.* (2004) and Pilger *et al*. (2009) revealed that pets i.e. dogs and cats were also important hosts and play a role in the intra and peri domiciliary transmission cycle.

In sub Saharan Africa, the facets that make up the transmission and maintenance of *T. penetrans* are similar. According to Ugbomoiko *et al*. (2009), in endemic rural setting of Lagos in Nigeria, 83% of all tungiasis lesions were found in pigs thus making the porcine species the most important animal reservoir in endemic communities and it was associated with the presence of dry sandy soils (Ugbomoiko *et al*., 2008). Furthermore, the peri-domiciliary transmission of the fleas is enhanced by the notable close contact between communities dwelling places and animal shelters.

Studies by Heukelbach *et al*. (2001); Muehlen *et al*. (2005) and Ugbomoiko *et al*. (2010), further revealed that other than the mere presence of the flea, *T. penetrans*, poor community hygiene (living in squalor) further enhances the survival of fleas. In a study conducted in an urban slum in Brazil where waste and faecal material littered the entire households, a prevalence rate for tungiasis of 34% among humans was reported (Wilcke *et al*., 2002). Stray dogs, cats and rodents, which are important reservoirs for *T. penetrans* were particularly attracted to such areas. Organic material contaminating the soil have been reported to increase the risk of tungiasis in two different ways: by attracting animals that can act as suitable hosts for *T. penetrans* and by providing a sheltered environment for the development of the free-living stages (Muehlen *et al*., 2005).

Prevalence in human populations in endemic areas was directly related to the extent of parasite burden in animals, thereby qualifying the assertion that presence of heavily infested animal population is a very serious risk factor that leads to human tungiasis (Heukelbach *et al*., 2004). Furthermore studies have revealed that tungiasis usually shows seasonal variation with highest prevalence in the dry season (Heukelbach *et al*., 2004).

A study by Ponnighause *et al*. (1994) in Malawi associated the occurrence of diseases such as leprosy, helminthosis and tungiasis in resource poor communities to a low educational level and particularly illiteracy. It was noted that individuals with no or only basic reading and writing ability had different odds of being affected by tungiasis (i.e. 4.8 and 2.0 respectively). In contrast, if shoes were worn only irregularly, the odds for tungiasis increased only marginally (Ponnighause *et al*., 1994).

Ngunjiri *et al*.(2015) also documented that acquiring education among children can be derailed for various reasons which could range from ill health, need for children to work or care for sick family members, insecurity, natural calamities, inability to afford uniforms to inadequate sanitation and lack of basic amenities.

According to Ngunjiri *et al*.(2015), in the year 2010, 1.01 million primary school children were not attending school in Kenya due to various reasons which may have included disabilities caused by infectious diseases (UNESCO, 2012). This is despite major efforts made to increase literacy levels by offering free compulsory education in Kenya (Ingubu and Wambua, 2011). Acquisition of education has been thought to be a tool in which the socio economic status can be improved thus enhancing the living standards and access to health care of individuals, families and communities (Bogonko, 2006; Ojiambo, 2009).

Another negative impact that might ensue from infestation with *T. penetrans* according to Kamau *et al*. (2010), theoretically, is a risk of HIV transmission when jigger-infested individuals share HIV-contaminated sharp instruments for jigger removal. In Northern Brazil, identified risk factors for *T. penetrans,* were poor housing conditions, having a dog on the compound and use of traditional remedies (Muehlen *et al*., 2005). The same author reported also that being female and use of insecticides use were protective factors against *T. penetrans* infestation. The analysis made on location and type of building materials according to Muehlen *et al*. (2005) show that population attributable fraction (PAF) for housing conditions was highest for houses built on dunes or on swamp land followed by houses made of crude adobe walls, houses built of palm stems and leaves and houses with sand floors in that order.

In endemic areas of Brazil, human population dynamics were quite static i.e. very minimal migration takes place. Therefore prevalence of up to 50% had been reported as being as result of repeated infestation and considerable morbidity was also very evident (Muehlen *et al*., 2005). The study comprehensively reported that conditions related to housing were the most important factors associated with presence of tungiasis. The same study reported that routine household maintenance activities like plastering with clay or cement mortar of floors in endemic areas would reduce the prevalence of tungiasis. In the final analysis Muehlen *et al*. (2005) observed that, physical characteristics of such houses and not primarily the financial security of the owners made individuals susceptible to severe infestation. Thus, in this study, poverty per se did not seem to be a determinant for the presence of disease nor the occurrence of severe infestation.

According to the community led total sanitation newsletter of March 2013, 52% of the population in sub Saharan Africa do not have access to toilets and clean piped water and mostly practice open defecation (OD), CLTS newsletter, (March, 2013).

2.5 Knowledge, Attitudes and Practices

On aspects of specific knowledge on tungiasis, individuals in most rural areas where tungiasis was endemic were able to diagnose the condition clinically with a higher degree of certainty than health workers (Heukelbach *et al*., 2004; Ariza *et al*., 2009). A study conducted in a selected fishing camp and an urban slum in Brazil, all household leaders had good knowledge levels of tungiasis (Winter *et al*., 2009). In both the fishing camp and urban slum, most of the respondents stated that they had experienced tungiasis, at least once in their life time. In that study, most respondents associated tungiasis with sandy soils. Other factors that were perceived to be associated with the disease among the respondents were the presence of infested animals, i.e. rats, dogs, pigs and cats (Winter *et al.*, 2009). As regards the practices, tungiasis was treated by patients themselves or a family member and rarely was medical help sought from health care providers (Feldmeier *et al*., 2006).

Comparing studies conducted in North east Brazil and the sub-Saharan Africa, the levels of knowledge about modes of transmission, pathophysiological presentation, indigenous knowledge and coping up strategies with tungiasis infestation, the levels are very similar. But specific to a study by Kimani *et al*., 2010, the statistics were such that 70.1% of the 271 respondents attributed the presence of tungiasis to poor environmental hygiene, 55.7% poor personal hygiene, 36.2% thought poverty was the main driver of tungiasis and 16.6% believed that tungiasis was transmissible between human beings.

2.6 Clinical manifestation and pathology of Tungiasis

The scientific description of the disease dates back to the 16th Century (Herbig and Geigy, 1949; Eisele *et al*., 2003 Pampiglione *et al*., 2009). In recent times studies have been conducted based on the "Fortaleza classification", a staging system which takes into account the dynamic nature of tungiasis and allows the division of this peculiar ectoparasitosis into five consecutive stages and these are penetration, hypertrophic, white halo, involution and residual stages (Eisele *et al*., 2003). The progression of tungiasis from infestation to eventual resolution of the condition is characterized by unique pathophysiological changes in the host skin (Feldmeier *et al*., 2002; Eisele *et al*, 2003; Pampiglione *et al*., 2009).

A recent elaborate description starts with the penetration stage. In the penetration phase, a penetrating flea may or may not be perceived by the host. Patients in the endemic area frequently feel the pain associated with penetration and described it as being similar to the bite of a midge. Sometimes, early in penetration, an erythema develops (Heukelbach *et al*., 2002; Eisele *et al*., 2003). Some patients reported a particular itching, which was described as ‘‘coceira boa’’ (a pleasant scratching); (Pampiglione *et al*., 2009), This is followed by the hypertrophic phase after complete penetration into the host’s epidermis and only the rear end of the female flea remains visible. The last two abdominal segments of the flea are pushed in and form a crater from which the genital opening and the stigmata protrude like a miniature cone (Eisele *et al*., 2003). The only constant sign during this stage is an erythematic discolouration of up to 30 mm in diameter around the embedded flea (Eisele *et al*., 2003; Feldmeier *et al*., 2004).

The white halo stage ensues and the hypertrophy zone develops into a sphere. The head of the flea, about 72 hours after penetration is completed and the enlargement of the flea’s abdomen becomes macroscopically visible (Eisele *et al*., 2003). Since the stratum corneum is stretched thinly by the continuously increasing size of the hypertrophy zone, the lesion appears as a white halo around the black rear cone.

Consequently after the majority of the eggs are expelled, the hypertrophy zone begins to involute (Eisele *et al*., 2003). This is a continuous process starting about three weeks after penetration. It ends with the complete elimination of the carcass of the dead parasite at the end of the fifth week.

The appearance of signs which indicate loss of viability of the flea is considered as the main clinical characteristic (Eisele *et al*., 2003). The excretion of faeces becomes infrequent and eventually stops completely. The watery secretion is only rarely observed and the pulsation phenomenon no longer occurs (Eisele *et al*.,2003) The size of main characteristic of the white halo stage reduces. The size of the sphere continues to shrink and the surface of the lesion appears more and more wrinkled. The last abdominal segments remain protruding above the skin-level surface, so that the rear cone can be easily seen with the naked eye (Eisele *et al*., 2003).

Once the carcass of the parasite is expelled, a round depression remains in the stratum corneum. As the reorganization of the epidermis continues, the site previously occupied by the lesion is flattened from below (Eisele *et al*., 2003). The reorganization needs one to four weeks. Particularly at sites where the keratin layer is thick, a circular, punched out-like residue of five to 10 mm in diameter remains (Eisele *et al*., 2003). Such residues persist for several months and are a good indicator for the number of sand flea infestations the patient had previously experienced (Eisele *et al*., 2003).

In the residual stage, histopathological sections will show a thickened stratum spinosum, acanthosis and papillomatosis of the epidermis. In the dermis, an intense inflammatory infiltrate will be found in the stratum papillare and reticulare, mainly consisting of lymphocytes. Hypervascularization is also frequent (Eisele *et al*., 2003).

2.7 Diagnosis

2.7.1 Clinical examination

This is usually based on the pathophysiological presentation of the lesions during the progression to the state of tungiasis.

The most effective common methods of diagnosis in the resource poor setting in endemic areas is that patients and the unskilled health workers use macroscopic clinical signs based on the natural history of the disease and a locally validated case definition is a practical alternative (Feldmeier *et al*., 2006).

2.7.2 Microscopy

In the laboratory, flea species have particular morphological characteristics which can easily be identified by means of a light or stereomicroscope (Lane and Crosskey 1993). More robust methods such as the Scanning Electron Microscopy (SEM) is used to give evidence based diagnosis which brings out the morphological features of the *T. penetrans*. However differentiating fleas from the genus *Tunga* morphologically presents some difficulties because of closely related sub species (Pampiglione *et al*., 2004; Vobis *et al*., 2004; Nagy *et al*., 2007).

The current understanding of *T. penetrans* adult morphology is based on the descriptions by Hopkins and Rothschild (1953); Westwood (1860), Blanchard (1890), Karsten (1865), Linardi and Guimarães (2000); Pampiglione *et al*., 2009).

2.7.3 Biopsies and Histopathological techniques

Biopsies and hispathological techniques are not indicated but may be used in atypical tungiasis, such as a lesion with a pseudo-epitheliomatous appearance to demonstrate the presence of the ectoparasite or its chitinous fragments (Feldmeier *et al*., 2006).

2.7.4 Molecular methods

The taxonomic status of *T. penetrans* has been explored and validated using both mitochondrial and nuclear DNA markers from South American and African samples (Luchette *et al*., 2005). The mitochondrial genes, cytochrome oxydase II (COII) and the large ribosomal subunit (16S), as well as the nuclear ribosomal internal transcribed spacer 2 (ITS2) loci have been sequenced and used for diagnosis of *T. penetrans* (Pampiglione *et al*., 2009). In fact, although the morphological identification of gravid females is very easy, the identification of non-gravid females, males or body fragments is difficult and time-consuming, and often requires taxonomic experience (Pampiglione *et al*., 2009).

2.8 Differential diagnosis

In animals, the differential diagnosis of tungiasis must refer to infectious vesicular diseases (mainly in swine and ruminants), mastitis of swine, coronary band disease of the bovine hoof, inter-digital and pododermatitis, mange, Pustular dermatitis, abscesses, foreign body granulomas and other diseases involving the skin in the foot or hoof, perineal and genital areas, udder and tail (Pampiglione *et al*.,2009).

2.9 Treatment and Control

The standard treatment is usually the sterile surgical removal of the engorged flea or remnants of the dead flea (Heukelbach *et al*., 2004). There is no drug that has been proven to be effective against embedded fleas. Oral niridazole was once considered a therapeutic drug, but well-designed studies are lacking and, given the severe adverse effects, this is one drug that is likely to cause more harm than good (Heukelbach , 2005). However, it has some anecdotal evidence of lysing the fleas altogether (Heukelbach, 2005). Oral ivermectin is considered by some in endemic areas to be a panacea against fleas though in real practice, this knowledge is not consistently practiced (Winter *et al*., 2009) but studies using high doses have failed to validate this hypothesis (Gibbs *et al*., 2008; Winter *et al*; 2009).

Other drugs such as topical ivermectin and metrifonate have been somewhat successful, but not enough to be significant (Heukelbach; 2005; Nagy *et al*., 2007). For super infections, trimethoprim, sulfamethoxazole, metronidazole amoxicillin (with/without clavulanate) have been used successfully, though these treat only secondary infections (Joseph *et al*., 2009)

Successful topical treatments also include cryotherapy and electrodessication of the lesion. If formaldehyde, chloroform or DDT is used topically, care should be taken when dealing with the resulting morbidity. The embedded *T. penetrans* can also be suffocated using occlusive petrolatum, while Vaseline® will kill the organism as well, most likely due to suffocation as the stigmata would be covered (Gibbs *et al*., 2008).

Prophylaxis and control of human tungiasis is based on protecting the feet with closed shoes, use of repellent liquids or ointments, control of rodent populations, disinfection and hygiene of soil in houses and stables, and the prohibition of free movement of pigs, goats, sheep or cattle in the vicinity of houses (Feldmeier *et al*., 2006). Many authors feel that daily inspection of bare feet in order to detect initial infestations is useful (Jolly, 1926; Wilcocks and Manson-Bahr, 1972; Pampiglione *et al*., 2009).

In domestic animals at risk, inspection of the lower limbs and parts of the body which come into contact with the ground is recommended. This should be followed by extraction of the sand fleas and the disinfection of resulting ulcers. Use of locally manufactured liquid repellents represents the essential prophylactic regime (Feldmeier *et al*., 2006). The following disinfectants in order of descending frequency have been known to be effective are mercurochrome, ethanol, organic iodine and hydrogen peroxide (Feldmeier *et al*., 2006).

Possible interventions to control *T. penetrans* can be targeted towards treatment of infested areas (off host treatment), such as infested soil or spraying resting places for animals with insecticides. Treatment of domestic animals with anti-flea compounds (on host treatment) is another avenue. Impregnating clothes might be a cost- effective option in areas were long garments are worn. Wearing foot wear prevents tungiasis to a certain extent but does not guarantee complete protection (Heukelbach *et al*., 2001).

In Kenya, National Policy Guidelines on prevention and control of Jigger infestation has been developed based on the fact that 4% of the population in Kenya is a victim of tungiasis (NPGPCJI, 2014). At least in Kenya the control of tungiasis is addressed at policy level and has a multi-sectoral approach. The National policy guidelines are anchored on the public health mantra “Prevention is better than Cure” implying that tungiasis does not have any effective cure and disease burden is most of the time reduced by effective vector control.

CHAPTER THREE:

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in Chipata and Vubwi districts (Figure 3.1) located on latitudes and longitudes $13^0$40"S and 32^0 40"E and 14^0 03"S and 32^0 23"E, respectively in the Eastern province of Zambia. The study area is located in agro ecological zone II that receives average annual rainfall of between 800 – 1000mm and has characteristic open savannah grasslands mixed with moderate Miombo woodlands (Seigel, 2008). Both districts share international borders with Malawi (Figure: 3.1). Vubwi further south shares a short stretch of border with Mozambique.

The total human population in the Eastern province is estimated at 1,707,731. Chipata has a population of about 452,428 with 90,809 households, while Vubwi has a population of 104,255 with 20,199 households. Generally, as for the whole country, 39% of the people are in urban areas where as 61% are in rural areas (CSO, 2010).

The rainy season in this province starts in November and ends in April. Annual rainfall from 1995 to 2001 in Petauke and Katete ranged from 740.3mm to 1067.2mm (mean 887.6mm). Mean annual maximum temperatures in the same period ranged from 28.1ºC to 29.4ºC (mean 28.6ºC) in the two districts, while the mean annual minimum temperatures ranged from 16.7ºC to 17.5ºC (mean 14.2ºC). The vegetation in Eastern province includes "Miombo" woodland dominated by *Brachystegia* and *Julbernardia* species, the "Munga" woodland, where the principal tree species are *Acasia*, *Combretum* and *Terminalia* and the "Mopane" woodland with *Colophospermum mopane* being the dominant tree species (Berkvens *et al.*, 1998).

Eastern province is dominated by the indigenous breed of pigs called *Nsenga* (Figure 3.4). Theses pigs are normally kept in small shelters or Kraals called *makola* (*kola* = singular) during the rainy season and are left free to scavenge during the dry season. The common type of pig house is seen in Figure 3.5. Eastern province also kept other types of livestock such as cattle, goats and chickens.

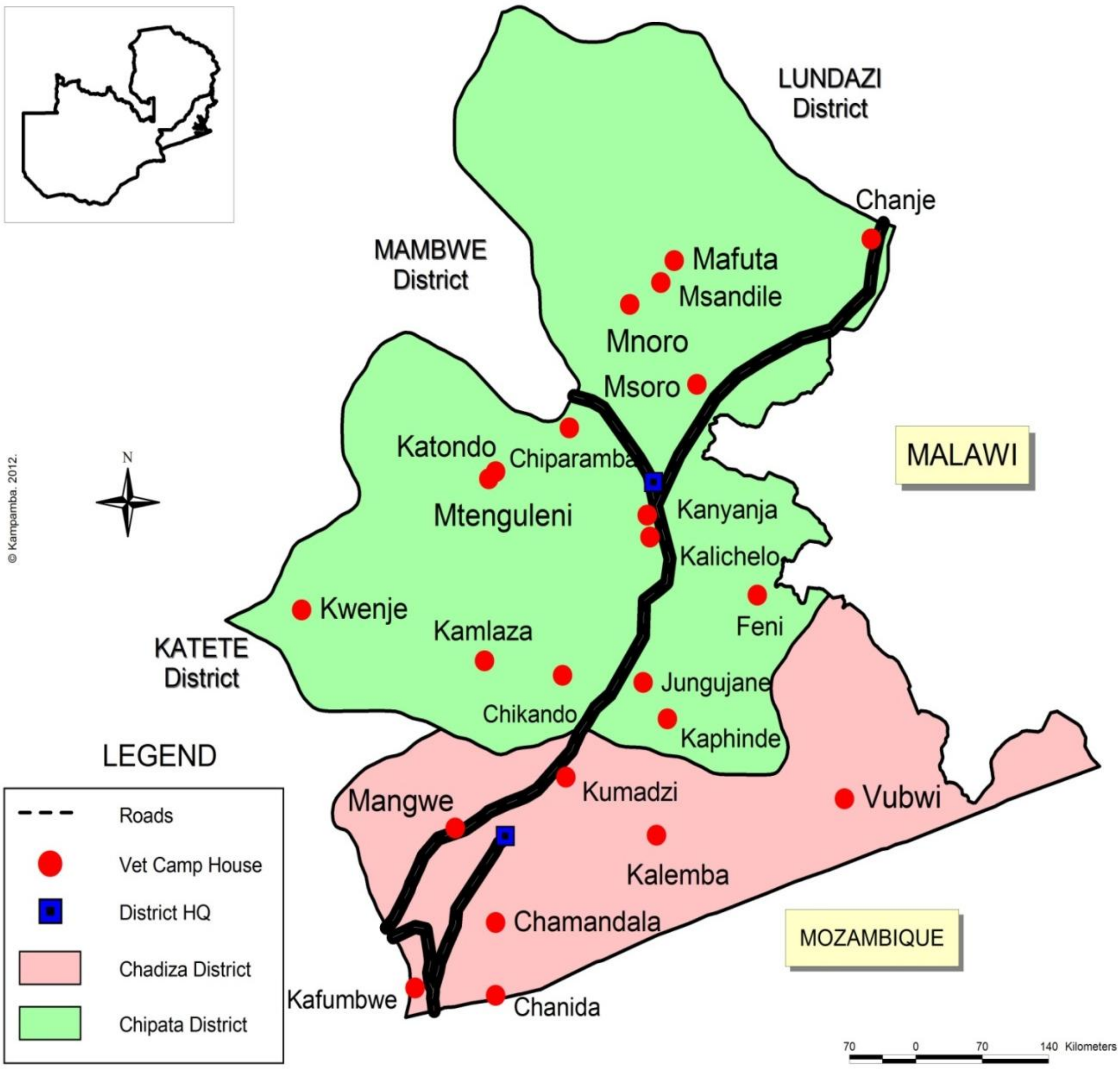

Figure 3.1 Map of study area; Chipata and Vubwi districts located on latitudes and longitudes $13^0$40"S and 32^0 40" E and 14^0 03"S and 32^0 23"E, respectively in the Eastern province of Zambia.

The livelihoods of the rural communities in the two districts are, by and large, dependent on small to medium scale agricultural activities. The activities are mainly crop and livestock production with isolated communities engaged in fish farming. Eastern province has the third largest livestock population in Zambia and is considered as the grain basket of country. In the eastern province, land available for agricultural activities is relatively scarce compared to the other provinces in Zambia (Areghore, 1987).

Figure 3.2 The indigenous black dwarf (*Nsenga*) pigs found in the rural areas of Eastern province at the homestead mixing with people without shoes.

Figure 3.3 A typical pig house (*kola*) found in the study area which easily let pigs escape and contaminate the people's resting places with Fleas.

3.2 Study design and sample size determination

A cross-sectional study was conducted. Villages were treated as primary units while the occupants of individual households were secondary units. The tertiary units were the households Table 3.1. The unit of study was the household. The household was considered infested with *T. penetrans* if at least one house hold member was diagnosed to be positive for tungiasis

Table 3.1 Sampling at each level and the sampling method used

Levels	**Units**	**Sampling Method**
Primary Unit	Villages	Random
Secondary Unit	House members	Random
Tertiary Units	Households	Random

The sample size was calculated using the formula by Martin *et al*. (1987) as follows:

$$n = \left(\frac{Z_{\alpha/2}}{E} \right)^2 p\, q$$

The value of Z_α is the (1- $\alpha/2$) critical value for the 95% confidence level in the standard normal distribution ($Z_{\alpha/2}$ =1.96) p=50% was the prevalence, q was (1-p) = 0.5.The prevalence (p) of 50% was used to get the maximum possible sample size since the prevalence of tungiasis in Zambia is not known. , and E was the margin of error which was estimated at 5%. Therefore, **n** = $1.96^2 \times 0.5 \times 0.5/0.05^2 = 384$. This gave a total sample size of 384.

3.3 Sampling

The study was conducted between November 2012 and April, 2013. A total of three constituencies were purposively selected for the study in the two districts. These were Chipangali and Luangeni in Chipata and Vubwi in Vubwi district. The constituencies were selected based on anecdotal data on the spatial occurrence of the ectoparasitosis. A total of 384 households were randomly selected in the two districts using the table of random numbers based on the number of villages.

3.4 Clinical Examination

As tungiasis may occur at any topographic site (Heukelbach *et al*., 2002), the whole body surface of the patient was examined for the presence of vital, egg-producing, involuting or dead fleas. Lesions were classified according to the Fortaleza Classification, a recently elaborated staging system (Eisele *et al*., 2003). The following findings were considered diagnostic for tungiasis: flea in statu penetrandi, stage I, a dark and itching spot in the epidermis with a diameter of one to two millimeters with or without local pain; stage II (early lesion), lesions with as a white halo with a diameter of three to 10 mm with a central black dot; stage III (mature flea), a brownish-black circular crust with or without necrosis of the surrounding epidermis; stage IV (dead parasite), circular residue punched out in the keratin layer of the sole of the foot or irregular thickening of the nail rim; and stage V, lesions altered through manipulation by the patient (such as partially or totally removed fleas, which leaves a characteristic crater-like sore in the skin) and suppurative lesions, mainly caused by using non sterile perforating instruments such as needles and thorns. During the examination, location and number of lesions were noted. The presence of less than five lesions were considered as mild, six to 30 as moderate and of more than 30 as heavy infestation (Muehlen *et al.,* 2003).

The following signs and symptoms were observed: erythema, oedema, tenderness, itching, pain, shining skin, desquamation, hyperkeratosis, fissures, pustules, suppuration, ulcers, deformation of the toes (defined as deviation of the normal axis of the toe caused by intense swelling), deformation of nails, loss of nails, and difficulty in walking or gripping.

Figure 3.4 Ectopic clinical examinations of tungiasis lesions in patients during a visit of the study area

Clinical pathologic findings were classified as follows: acute inflammation or painful lesion surrounded by erythema, oedema, and tenderness; chronic inflammation, oedema, tenderness, shining skin with or without desquamation, or deformation of digits; superinfection, presence of pustules, suppuration, or ulcers; and physical disability, difficulty in walking, or gripping (if lesions were located on the hands), based on patients' statements that pain restricted their movements.

The basis of diagnosis was made on the natural history of the disease i.e. presence of a red-brown itching spot (evidence of an embedded flea) with a diameter of one to two millimeters; presence of a yellow-white watch glass-like patch with a diameter of three to 10 mm with a central dark spot; or a brown-black crust with or without surrounding necrosis (Eisele *et al*., 2003).

Where the participant had the embedded parasite and was willing, the parasite was removed by clinicians from respective rural health centres, themselves or with the aid of community members that had proven experience in using indigenous methods**.**

In instances where more than one individual from a household offered to be examined, they were all subjected to it. For minors permission was sought from the parents or guardians (Heukelbach *et al*., 2001). The clinical examination was made by inspecting carefully the legs, hands and arms for *T. penetrans* infestation excluding the genitals areas to guarantee privacy. The approach is considered acceptable, as in endemic communities more than 99% of tungiasis occurs in those regions except in children who are less than five years old (Ade Serano *et al*, 1981; Chadee, 1998; Pampiglione *et al*., 2009).

3.4.1 Parasite collection and preservation

The removed gravid *T. penetrans* was the preserved in 70% alcohol and examined using a stereo microscope. Morphologically, embedded gravid *T. penetrans* were relatively easy to identify than free living adult fleas (Lane and Crosskey 1993) and it was of major interest in this study.

Figure 3.5 Punched – in tungiasis lesion following removal gravid *T. penetrans*

3.5 Investigation of risk factors

A questionnaire (Appendix 1) was developed and used to collect information on risk factors and other related information from pig farmers. The questionnaire was orally administered by veterinary assistants in charge of the respective areas using the native language. A number of households in each village were randomly selected depending on the willingness of the farmers to participate in the study. This structured questionnaire was used as a survey tool to collect qualitative and quantitative data. The information collected included socio-demographic characteristics (i.e. household size, age and sex of respondent or victim, marital status),. Other information that was captured included level of education attained, religious affiliation and occupation of victims and their knowledge of tungiasis.

Figure 3.6 Administering of a structured questionnaire to a male member of the house hold in the study area

Furthermore, questions on the type of house (material used in construction), type of floor, access to water and sanitation facilities, the types of livestock species that were kept was also collected and living conditions were directly observed and noted.

The head of the household or where not available a senior member of the family was interviewed. At each household, at least one member of the family was examined for the presence of the disease.

The households were only visited twice only if the occupants were unavailable. In an event that all visits proved futile and no interview conducted, a nearby household was co-opted in the survey.

3.6 Ethical Consideration and Clearance

Participants consented and assented to participate. Confidentiality and anonymity was guaranteed throughout the study period using codes. Data were stored in a computer under password protection. Prior to data collection, each participant was required to fill the written consent form showing their acceptance to participate in the study. Those found to be infested with tungiasis were advised on the best way to prevent and control of parasitic infestations. Ethical clearance was sought from the review board of Ethics and Research Converge (ERES CONVERGE) IRB No. 00005948, FWA No. 00011697, approval Ref. No. 2012 –Oct- 005.

3.7 Data Analysis

Data were entered into MS excel database (Microsoft) and checked for entry errors and analysis was done using SPSS v21.0, (IBM, USA).

Descriptive statistics were displayed either in graphs or tables. The Chi squared test or Fisher's exact test were used to determine association between categorical variables. Step-wise binary logistic regression (Hosmer and Lemeshow, 2000), was used to determine predictors of tungiasis. All variables with p-values less than 0.250 were included in the model. The logic link function reported the coefficient, p-value, odds ratio (OR) and the 95% confidence interval (CI) of the OR. The criteria used in determining whether the constructed model adequately fitted the data were a non-significant Hosmer and Lemeshow test $p > 0.05$ and a significant Omnibus Test for Model Coefficients $p < 0.05$. All statistical tests were considered significant at $p \leq 0.05$.

CHAPTER FOUR:

4.0 RESULTS

4.1 Overall Prevalence of tungiasis in the study area

The total number of households that were sampled from the two districts was 384. Of these 247 were from Chipata (Table 4.1) and 137 from Vubwi (Table 4.1). The overall prevalence (active or partially manipulated lesions) of tungiasis in the two districts was 13.5% (95% CI= 10.1 – 16.1). Of these persons that had active or partially manipulated lesions of tungiasis, 8.1% (95% CI=5.4 – 10.8), were from Chipata and while 5.5% (95% CI=3.2 – 7.8), were from Vubwi. There was no significant difference in the prevalence of active (intact or partially manipulated lesion) tungiasis between the districts (p=0.270, Table 4.1)

When new and old lesions were combined, the prevalence in the study area was 84.9% (95% CI = 81.3 – 88.5). The overall prevalence of old and new cases combined was 86.3% (95% CI=81.9 – 90.5) in Chipata, while in Vubwi it was 82.5% (95% CI=81.3 – 88.5) (Table 4.1). There was no significant difference in prevalence between the two districts (p=0.201) Table 4.1.

Table 4.1 Overall prevalence of tungiasis in the study area with 95% in brackets

District	N	Old and new cases	p-value	Intact (active) or partially manipulated cases	p-value
Chipata	247	86.3% (81.9 – 90.5)	0.201	12.6% (8.5 – 16.7)	0.270
Vubwi	137	82.5% (81.3 – 88.5)		15.3% (9.3 – 21.3)	

n = sample size

4.1.1: Intensity of infestation around the body

During the clinical examination of participants in the study, the highest of cumulative number tungiasis lesion were located on the feet (total = 1188, max=30; average =4.3), followed by legs (total =172, max=12 , average 3.07); in the hands, (total 55, max=20, average = 2.9) and the least was located in the arms (total=16, max=4 average = 2.6) (Table 4.2).

Lesions tended to occur in clusters, which were arbitrarily defined as a group of five or more lesions that occurred in close proximity (e.g., on the periungual region of the toe, the heel, or the fingertip). Overall from the infestation ratings, in the study area were that household members were mildly infested i.e. <5 tungiasis lesion.

Table 4.2 Body location and number of tungiasis lesion on legs and arms with a highest number (30) on the feet and the lowest (4) found on the arms.

Location	n	Maximum	Sum	Mean
Hands	19	20	55	2.9
Legs	56	12	172	3.07
Arms	6	4	16	2.6
Feet	279	30	1188	4.3

4.2 Investigation of Risk Factors

4.2.1 Prevalence of tungiasis according to age Category

Out of the individuals examined from each household during the study, 4.2% (95% CI=2.5 – 7.0) were 15 years old and below, 29.9% (95% CI=27.1 – 34.5), were between 16 and 30 years old, 37.5% (95%CI=29.6 – 45.4) were between 31 – 45 years, 22.4% (95% CI=17.9 – 26.9) were between 46 – 60 years and 6.0% (95% CI=3.9 – 9.3) were above 60 years old. The prevalence based on active or partially manipulated tungiasis lesions was; among those who were 15 years and below, 68.8% (95% CI =46.1 – 91.5), for those between 16 – 30 years of age the it was 13.1% (95% CI= 6.9 – 19.1), for those between 31 - 45 years of age, 9.0% (95% CI= 4.3 – 13.7) , for those between 46 – 60 years, 10.5% (CI= 4.0 – 17.0) and for those above 60 years, it was 17.4% (95% CI=1.9 – 32.9). There was a significant difference in prevalence among the age groups (p=0.001, Table: 4.3 and Figure 4.1).

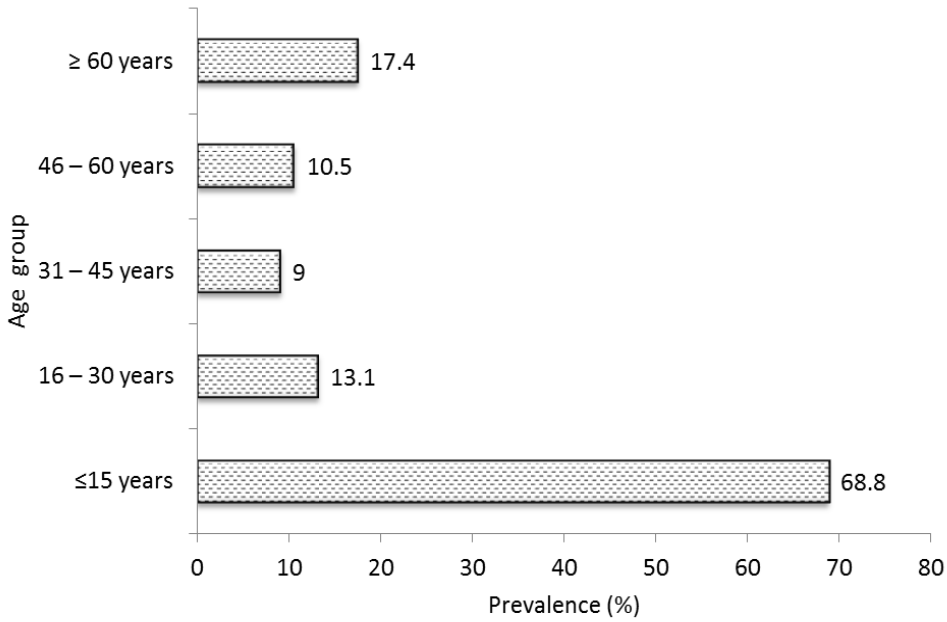

Figure 4.1 The prevalence of tungiasis lesions according to age groups with the young people being most affected and those between 31 to 45 years being least affected.

Table 4.3 A combined prevalence of tungiasis from Chipata and Vubwi Districts according to age groups

Variable	Categories	n	Prevalence (%)	95% CI	p- value
Age category	≤15 years	16	68.8	46.1 – 91.5	
	16 – 30 years	115	13.1	4.3 – 13.7	
	31 – 45 years	144	9.0	6.9 – 19.1	0.001
	46 – 60 years	86	10.5	4.0 – 17.0	
	≥ 60 years	23	17.4	1.9 – 32.9	

4.2.2 Prevalence of tungiasis according to gender , marriageable age and marital status

The prevalence of tungiasis among males 15.2%, (95% CI = 4.9 – 15.5) was higher than that of females 10.2%, (95% CI = 10.8– 19.6) and there was no statistical difference (p=0.119, Table: 4.4).

Out of the 384 households that participated in the prevalence survey, 318 participants fell in the marriageable age group (i.e. ≥ 20 years old) and were married. Of the 318, 9.7% (95% CI= 7.4 – 12.0) were positive for tungiasis. The second category involved those that were from the unmarriageable category (i.e. < 20 years old) and these were 22. The prevalence for tungiasis in this category was 72.7% (95% CI= 60.8 – 84.6). The other group that was subjected to analysis was those of marriageable age group and single. These statistics were as follows; total number was 44, representing 11.5% (95% CI= 2.1 – 20.9) and the prevalence was 11.4% (95% CI= 2.0 – 20.8).

There was a significant difference in prevalence when the age and marital status were analyzed concurrently. p = 0.001.

Table 4.4: Prevalence of tungiasis lesions according to gender and marital status

Variable	Category	n	Prevalence (%)	95% CI	p-value
Sex	Female	127	10.2	4.9 – 15.5	
	Male	257	15.2	10.8 – 19.6	0.119
Marital status	Married	317	9.5	6.3 – 12.7	
	Single	67	32.8	21.6 – 44.0	0.001

4.2.3 Prevalence of tungiasis according to level of educational

Level of education was found to be significantly associated with prevalence of tungiasis (Table: 4.5). The prevalence was higher among those that had little or no form of education 24% (95% CI= 14.3 – 33.7) compared to those that had attained primary school education, 9.9% (95% CI= 5.9 -13.9) and those that had attained secondary school education or higher 13.4% (95% CI= 6.6 – 20.2), (p=0.013).

Table 4.5: Prevalence of tungiasis according to educational level

Variable	n	Prevalence %	95% CI	p-value
No form of education	75	24	14.3 – 33.7	
Primary school	212	9.9	5.9 – 13.9	0.013
Secondary School of higher	97	13.4	6.6 – 20.2	

The direct influence of tungiasis by respondents' education levels, age groups and gender is summarized in figure 4.2 below;

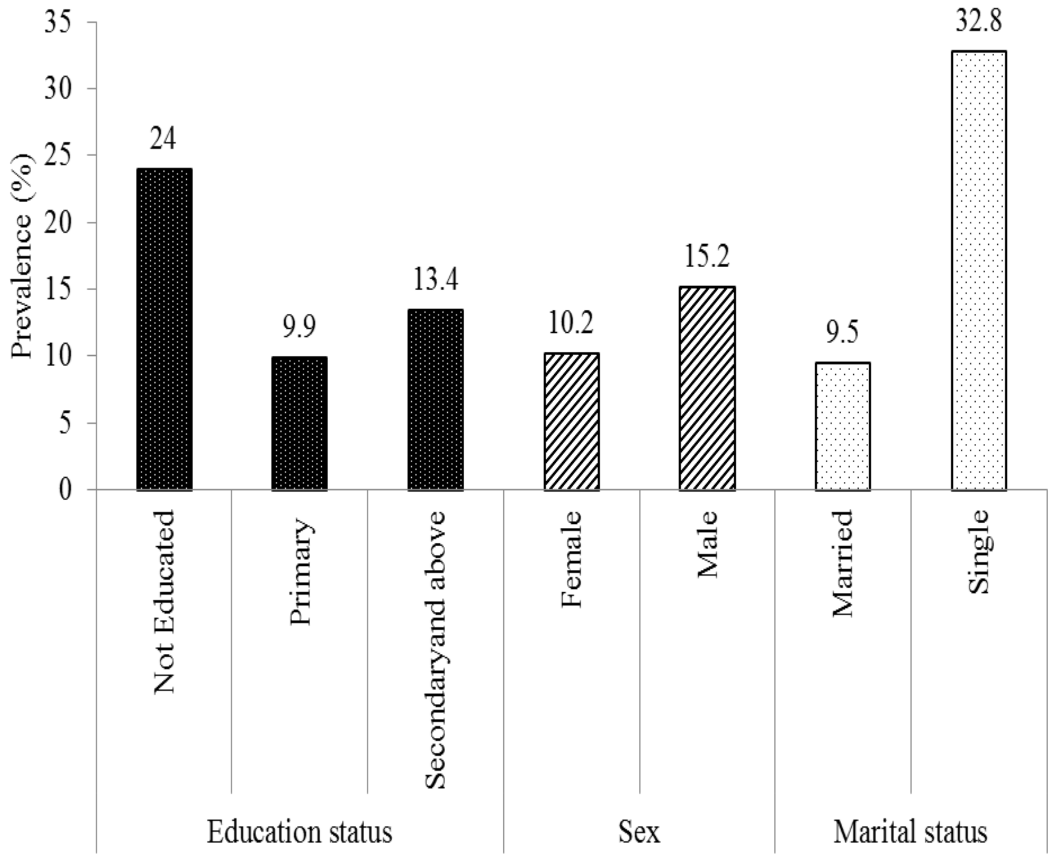

Figure 4.2 A summary of prevalence of tungiasis lesions as it relates to Education, Sex (gender) and marital status of the members of the households examined.

4.2.4 Prevalence of tungiasis according to sanitary facilities

Among households that had indicated practicing open defecation, the prevalence was 4.4% (95% CI= -0.5 – 9.3), whilst among those that reported to use pit latrines, it was 16.3% (95% CI = 11.5 – 19.5). There was a significant difference in prevalence between households that practiced open defecation and those that used pit latrines (p=0.008, Table 4.6). Garbage disposal practice was significantly associated with the prevalence of *T. penetrans* (p= 0.012). The prevalence was higher among those households that reported burning out refuse around homesteads 30.3%, (95% CI = 14.6 – 46.0); than among those households with no specific dump sites 7.2% (95%

CI = 3.3 – 14.5), and those that reported to use refuse pits 13.2% (95% CI =9.0 – 17.4), (Table 4.6).

Table 4. 6: Prevalence of tungiasis according to type of toilet and garbage disposal.

Variable	categories	n	Prevalence %	Confidence interval (95%)	p-Value
Type of toilet used	Open defecation	68	4.4	-0.5 – 9.3	
	Pit latrine	316	15.5	11.5 – 19.5	0.008
Garbage disposal practice	Burning of refuse	33	30.3	14.6 – 46.0	
	No specific dump-site	101	8.9	3.3 – 14.5	
	Use of refuse pits	250	13.2	9.0 – 17.4	0.012

4.2.5 Prevalence of tungiasis according to socio-cultural behavior

The prevalence of tungiasis among those who reported not using foot wear regularly was 10.5% (95% CI = 2.5 – 18.0), while among those who reported to using closed foot wear regularly it was 14.1% (95%CI = 10.3 – 17.9) (Table 4.7). There was no significant difference in prevalence between the two categories (p=0.315). Among those that had a tendency of taking a rest inside the houses after a day's work, the prevalence was 23.1% (95% CI= 9.9 – 36.3), among those that rested outside on corridors (veranda) the prevalence was 15.2% (95%CI=7.9 – 22.5) and among those that rested under tree shades around homesteads the prevalence was 11.5% (95%CI=7.6 – 15.4) (Table 4.7). There was no significant difference in prevalence among the three resting places of the households (p=0.120).

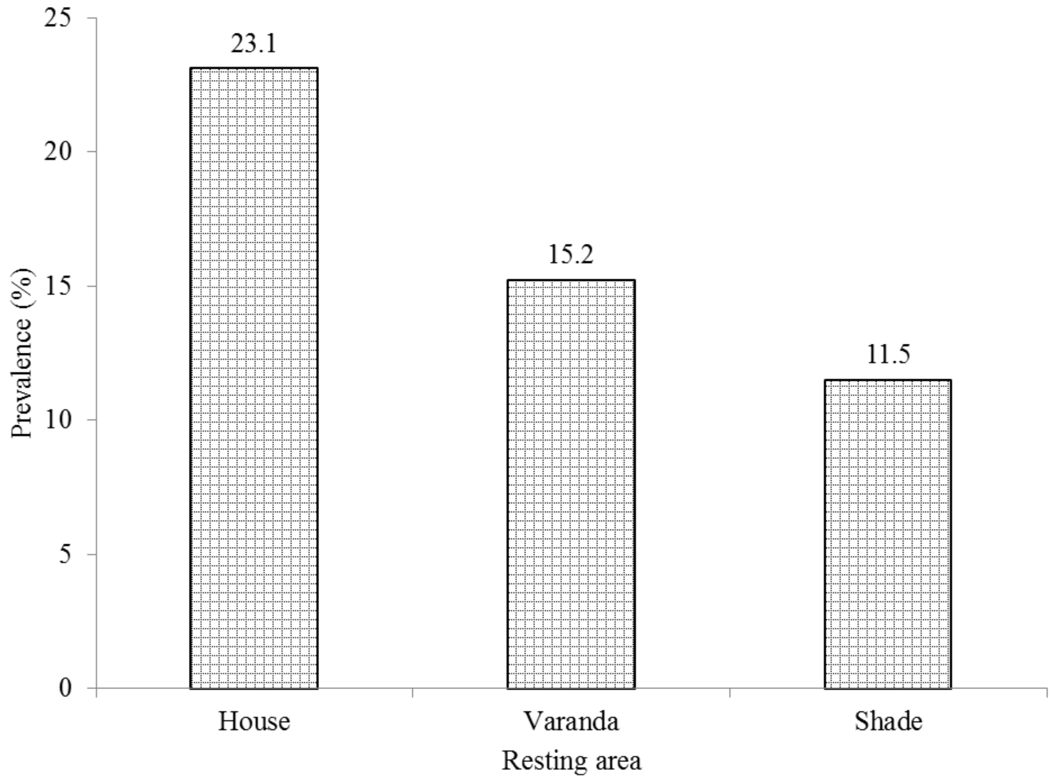

Figure 4.3: Prevalence of tungiasis according to areas of rest during the day at household level

The prevalence of tungiasis among those that slept on beds had was 13.2% (95% CI=10.0 – 16.2), while it was 13.7 % (95% CI=9.3 – 18.1) among those that slept on reed mat or beddings directly on the floor (Table 4.7). There was no significant difference in prevalence between those that slept on a bed and those that slept on a mat or bedding directly on the floor at night (p=0.510).

Table 4.7: Prevalence of tungiasis according to Behavioural tendencies

Variable	Category	n	Prevalence (%)	Confidence interval (95%)	p-Value
Use of Closed footwear	No	57	10.5	2.5 – 18.0	
	Yes	327	14.1	10.3 – 17.9	0.315
Sleep on bed at night	No	233	13.7	9.3– 18.1	
	Yes	131	13.2	10.0 – 16.4	0.510

4.2.6 Prevalence of tungiasis according housing building materials

The prevalence of tungiasis among those whose dwelling places were constructed using non burnt brick walls was 13.8% (95% CI= 6.2 – 21.4), among those having houses made of burnt bricks it was 12.9% (95% CI= 7.9 – 17.9), among those with houses made of cement bricks it was 30.8% (95% CI = 5.7 – 55.9) and among those with houses made of mud and tree poles it was 12.4% (95%CI = 6.5 – 18.3) (Table 4.8). There was no significant difference in prevalence based on the type of housing material used (p=0.332). The prevalence among those whose houses had a floor of clay in nature was 14.5% (95% CI= 10.3 – 18.7), those houses that had concrete floors it was 10.1% (95% CI = 3.0 – 17.2). Houses that had floor predominantly characterized by loose sand particles it was 13.0% (95% CI = 3.3 – 22.7) (Table 4.8). There was no significant difference in prevalence of tungiasis among the different house floor types. p=0.730.

Table 4.8: Prevalence according to type of housing construction material

Variable	Category	n	Prevalence (%)	95%	p-value
Type of housing	Non burnt brick wall	80	13.8	6.2 – 21.4	0.332
	Burnt brick wall	170	12.9	7.9 – 17.9	
	Cement brick wall	13	30.8	5.7 – 55.9	
	Mud/Tree poles	121	12.4	6.5 – 18.3	
Type of floor	Clay	269	14.5	10.3 – 18.7	0.730
	Concrete	69	10.1	3.0 – 17.2	
	Sand	46	13.0	3.3 – 22.7	

4.2.7 Prevalence according to access to water

In the study area 284 of the households had access to water from the boreholes and the prevalence was 14.4% (95% CI = 10.3 – 18.5), 76 got water from open wells and the prevalence was 11.8% (95% CI = 4.5 – 19.1) and, 24 got water from either rivers or streams, the prevalence was 8.3% (95% CI = -2.7 – 19.3) (Table 4.9). There was no significant difference in prevalence among households depending on their sources of water (p=0.720).

Table 4.9: Prevalence according to water source

Variable	Category	n	Prevalence	95%	p-Value
Water source	Borehole	284	14.4	10.3–18.5	
	Open well	76	11.8	4.5 – 19.1	0.720
	River/stream	24	8.3	-2.7 – 19.3	

4.2.8 Prevalence of tungiasis according to presence of domestic animals

The keeping of animals around homesteads is a traditional practice in the rural areas of the study sites and this practice was not significantly associated with the prevalence of tungiasis in the study area (p=0.587). Of those that indicated that they had no domestic animals of their own around their households, the prevalence was 12.9% (95% CI= 1.1 – 24.7), while among those that kept livestock the prevalence was 13.6 (95% CI=10.0 – 17.2) (Table 4.10).

Table 4.10: Prevalence of tungiasis according to presence of domestic animals around homesteads

Variable	Category	n	Prevalence	95%CI	p-Value
Presence of animals around homesteads	No	31	12.9	1.1 – 24.7	0.587
	Yes	353	13.6	10.0 – 17.2	

4.2. 9 Gender of household head

The prevalence of tungiasis among individuals from female headed households 20.5% (95% CI=7.8 – 33.2) was slightly higher than that of male headed households 12.8% (95% CI= 9.3 – 16.3) although there was no significant difference between the household heads (p=0.138).

4.3 *Assessment of predictors of tungiasis in Chipata and Vubwi districts*

A binary logistic regression analysis was carried out using the forward Likelihood ratio method to assess predictors of being positive for tungiasis in the study area (Table 4.12). The variables included in the analysis were only those with p-values less than 0.25 in the univariate analysis. Hosmer and Lemeshow test was not significant (p= 0.623) and an Omnibus Test for Model Coefficients was significant (p=0.001), indicating that the model fitted the data. However, the confidence intervals were wide, indicating the uncertainty of the estimates. From the analysis, variables that were found to be significant predictors were type of toilet, garbage disposal method, marriageable age combined with marital status. It was found that those that used pit latrines were 25.4% (95% CI = 0.071 to 0.913) times more likely to be positive for tungiasis than those that practiced open defecation (p = 0.036). As for the combined marital status and age, those that were single and of marriageable age (i.e. ≥20 years old) were 19.7, (95% CI = 0.330 to 1.820) times more likely to be positive for tungiasis than those that were married and ≥20 years old (p=0.001) (Table 4.11). All the other variables were not significant predictors of being infected with *T. penetrans* (p>0.05).

Table 4.11: Maximum likelihood estimates of predictors of tungiasis in Chipata and Vubwi districts

Variable	Category	Adjusted (OR)	95% CI OR		P-value
			Lower	Upper	
System of toilet	Open defecation	Ref			
	Pit latrine	25.4	0.071	0.913	0.036
Marital Status and age	≥ 20 year old and married	Ref			
	< 20 years old (Not marriageable)	0.8	0.291	2.199	0.669
	≥ 20 years old and single	19.7	5.152	75.469	0.001

Ref: Reference category

4.4 Awareness Levels

The levels of awareness on tungiasis were assessed on the premise of indigenous knowledge, affirmation of occurrence, having had an infestation in one's lifetime and what the community's reason was for the persistent recurrence of the ectoparasitosis and above all, any other ancillary information that could be explained scientifically.

Overall 315, 82% (95% CI=78.2 – 85.8) indicated that they had experienced tungiasis in their life time in both districts combined. In Chipata, 205; 83% (95% CI=78.3 – 87.7) of the households indicated that they had experienced tungiasis at least once in their life time and had considerable knowledge about the ectoparasitosis whilst in Vubwi (80.3%) they also gave similar responses (Figure 4.1).There was no significant difference in the nominal rates of the households that have experienced tungiasis between the two districts. p=0.299.

On disease specific aspects of tungiasis, 61%(95% CI=56.1 – 65.9) thought that transmission of tungiasis resulted from contact with pigs infested with *T. penetrans*, 14.8% (95% CI=11.2 – 18.4), thought it resulted from the lack of using footwear, 18.3% (95% CI=14.4 – 22.2) indicated that transmission was fostered by poor personal and community hygiene, 19% (95% CI=15.1 – 22.9) gave a combination of poor personal hygiene and presence *T. penetrans* infested pigs and 14.8% (95% CI=11.2 – 18.4) did not know what caused tungiasis.

On presentation of tungiasis, 55.7% (95% CI=50.7 – 60.7) indicated that tungiasis was characterized by a characteristic itch7.8% (95% CI=5.1- 10.5) also affirmed the presence of a characteristic itch and eventual punched in characteristic sore indicating that the parasite had been removed, 33.3% (95% CI=28.6 – 38.0) were able to tell all the stages of the pathophysiology of tungiasis when the host epidermis had a been infested by way of morphological appearance of the lesion and 3.6% (95% CI=1.7 – 5.5) were not able to definitively indicate whether the lesion was because of *T. penetrans*.

CHAPTER FIVE:

5.0 DISCUSSION

The results of this study indicate that tungiasis is very prevalent among households in the areas in Vubwi and Chipata districts of Eastern province. The overall (old and new cases) prevalence of tungiasis was considerably higher when compared to cases that were active or partially manipulated at the time of study. This could be as a result of repetitive infestation that occurs at peak outbreak times (July to Mid-November). However, the values obtained from the study do fall within findings of other studies (Ugbomoiko *et al*., 2007; Collins *et al*., 2009), conducted in the sub-Saharan Africa where it has been reported that the prevalence ranges between 12% and 54%.

There was no significant difference in the extent of tungiasis between Chipata and Vubwi and the logical explanation could be based on social demographic characteristics which are common between the districts and these are; the contiguous spatial distribution, households sharing almost the same cultural and socio economic status. The latter statistics could be an indication that the prevalence may be much higher than what we are reporting in this study as this study started two weeks after the commencement of the rain season. Heukelbach *et al*., (2001) postulated that the prevalence for tungiasis is largely dependent on seasonal conditions (seasonal variability) and free living adult stages of *T. penetrans* is largely influenced by environmental conditions. Normally, 99% of the flea population live (as larvae and pupae) on the ground and are not perceived by humans (Nagy *et al*., 2007). Thus, host attacks are only done by 1% of the living flea population (Linardi and

Guimarães 2000). The adult fleas are known to thrive better in dry and warm conditions. The presence of domestic animals around households was not significantly associated with the disease. This is contrary to the perception that traditionally reared indigenous pigs were responsible for the maintenance and occurrence of tungiasis in the communities (Ugbomoiko *et al*., 2007). However in this study, this association could not have been detected as it did not look at the mere presence of pigs alone but included other species, thus diluting the effect of this risk factor.

The other important risk factors that were found to be associated with tungiasis among the rural resource poor communities in Chipata were the age, type of sanitary facilities such as garbage disposal facilities, type of toilets used and marriageable age as it related to marital status.

To begin with, marital status as a risk factor could be confounded in age relatedness to prevalence and as such the reasons for its inclusion could be explained along the same lines as age categories. The findings of this study show that the prevalence of tungiasis was higher in young and older people. The pattern of prevalence in this study presented a U – shaped pattern, where it was high in young, declined in middle aged and increased again in the elderly. Children had very much higher chance of being infested mainly because they were less selective in their association with community dwelling places as compared to the much older categories. Similarly, those in senile category were very much at risk because of inactivity in as far as day to day household chores were concerned and had the tendency of being in favourable resting places for longer periods, thus increasing chances of exposure to *T.*

penetrans. This pattern blends well with the assertion that tungiasis is a behavior related disease (Ugbomoiko *et al*., 2007; Mazigo *et al*., 2009).

Type of housing was not significantly associated with tungiasis in this study. This may qualify school of thoughts that the causation web of tungiasis is multifaceted (Heukelbach *et al*., 2001).

In this study nominal rates show that prevalence was interestingly higher among the relatively affluent households i.e. inhabiting in dwelling places with cement and concrete floors. It suffices to mention that houses made of locally available materials like mud and tree poles or bamboo stems more often under regular structural maintenance. The maintenance in this respect is the routine mending of cracks on the floors and walls. Furthermore, the regular use of water as on floors to control against dust might be protective. In more permanent structures this is not very common there is a possibility of the lifecycle of *T. penetrans* can be completed within the confines of a room in house (Linardi *et al*, 2007). This gives a possibility that despite living in relatively affluent home chances were personal and household hygiene is compromised tungiasis might find a niche (Muehlen *et al*., 2005; Pilger *et al*., 2009). Housing structures on analysis did not yield any association with tungiasis. Affluence in rural setups again could be said to predispose households to tungiasis.

The source of water was not significantly associated. The essence of accounting for water source as potential risk factor was based on the parameter of community and personal hygiene and also the close interaction between animals and humans in their natural behaviour to utilize water. The assumption was that water points could create an environment for cross species transmission.

As for the use of pit latrine and bath rooms / structures, the logic in this study can attributed to the fact the living conditions in these areas are characterized by close usage of utility structures such as bath rooms and toilets. Animals would in most cases seek shelter in housing for pit latrines and bath rooms more especially during summer.

As for those that practiced open defecation the nominal prevalence rates of tungiasis were lower than those that used pit latrines. On this state of affairs, it can only be mentioned that chances of the infestation per individual practicing open defecation could be much lower because rarely do they use the same spot and commonly the presence of pigs in these communities could reduce the negative impact of open defection because of their coprophagic tendencies.

Level of education was significantly associated with tungiasis. It can all be deduced that those that had at least some form of education were more likely to make more informed decisions by seeking specific knowledge on the control and treatment of tungiasis. This finding is similar to that reported by Ponnighause *et al*., (1994) in Malawi that the level of education had a bearing on the risk of having tungiasis.

Putting on closed foot wear was found not to be associated with tungiasis in Vubwi and Chipata districts. This is contrary to most assertions that tungiasis can be simply controlled by the regular use of closed foot wear. Other studies have found that those that wore shoes regular were actually more at risk of contracting tungiasis, as the skin becomes relatively tender compared to those that didn't use shoes making it an easy barrier for the female *T. penetrans* to penetrate (Ponnighause *et al*., 1994).

Specific disease knowledge on tungiasis in this study was high. Indigenous knowledge could be attributed to the wide knowledge that individuals had in the study area on the control of the ectoparasitosis which has been passed on through generations. This was in agreement with findings from Ariza *et al*. (2003); Heukelbach *et al*. (2004); Feldmeier *et al*. (2006) were most cases in impoverished communities in Brazil treated cases of tungiasis on their own i.e. using non sterile needles, thorn from trees and also applied coconut oil and above all the high accuracy in their diagnosis of the disease. This study also brings out the fact that tungiasis in most cases is handled at household level. In this study other than the use of commercially produced products to control the parasites like cypermethrine used to control parasites in their cotton fields, an indigenously thought out approach is the use of soya bean ash water bath on pigs. A mixture of water with ash collected from soya bean Stover is used as parasiticide or repellant. This is done by spreading this mixture in pig house and as animal baths (usually for pigs the hooves are dipped in the same). Hitherto, Klimpel *et al.,* (2007), advocates for the incorporation of the veterinary aspect to the control of tungiasis. Kerosene was also mentioned as repellant. However, considering that resource base for most households was weak, regular and extensive use of the same could not be tenable.

The important risk factors associated with tungiasis are more socio-cultural in nature and these importantly were the type of toilet used in rural areas and garbage disposal practices. More interestingly in Kenya policy level efforts have been developed which involve a multi-sectoral approach to improve the quality of life among the resource poor in tungiasis endemic areas by developing a standard operating procedures (SOP) called the National Policy and Guidelines for the Prevention and Control of Jigger Infestation.

The study was conducted during the 2012/2013 farming season and as a consequence of this situation it was difficult to find people in certain homes as most of them were working in the fields. Furthermore, for minors, just the mere intention to examination them was vehemently rejected. However, it is unlikely that these shortcomings would have biased our findings.

CHAPTER SIX:

6.0 Conclusions and Recommendations

This study shows that tungiasis is highly prevalent among households in the study area and probably also in other areas apart from Eastern province where the skin condition has been reported.

On the perceived risk factors, by and large, the study revealed that more precise assessment on linkages between various factors needs streamlining in order to fully understand the causation web.

The levels of indigenous knowledge on tungiasis was noted to good and that provides and entry point for intervention by public health service providers should be to intensify on community approaches to total sanitations (CATS) and strengthen Community led total sanitation programs (CLTS).

Tungiasis on its own might not attract the attention it deserves but it cannot go without mentioning that it has a negative effect on livestock production more especially traditionally reared pigs which provide the diversified source of income in these resource poor communities.

According to Pampiglione *et al*. (2009) there existed another *Tunga* species *T. trimillata* species that causes tungiasis but only known to occur in Brazil.

Future studies on prevalence and intensity of infestation should target the period from the month of July to late October when conditions are favorable for flea survival in the Eastern province. The effect of seasonal variability on true prevalence can be incorporated in the epidemiological data for tungiasis. Intensity of infestation (parasite load) and morbidity in the rural villages needs to be investigated.

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APPENDICES

Questionnaire on Prevalence, knowledge, attitudes, practices and risk factors for tungiasis

District:……………………………… Chief:……………………………………….. Village……………………………………

Name of administrator:…………………………………Job Title:……………………………

1.0 Social demographic factors

1.1 Name of respondent…………………………

1.2 Age: [ ]

1.3 Sex / Gender [tick or Circle]*:* 1. (M) 2. (F)

1.4 Marital status [tick or circle] : Married / Single / Widow /None

1.5 HH type: [tick] MHH, FHH, OHH 1.5.1 Number of Persons in household [ ]

1.6 Level of Education: (i) University / College (ii) Secondary (iii) Primary (iv) N/A

1.7 Religion: (a) Christian (b) Moslem (c) if others specify……

1.8 Occupation: (i) Student (ii) Employed / Worker (iii) Unemployed (iv) Retiree (v) Peasant (vi) (vii)Others (Specify) ………………………..

Respondents will be briefed on the purpose of the study, and urged to be as free as possible to answer the question to the best of their knowledge and experience. Information given will be treated with highest possible confidentiality and identity will not be made public without their due consent

2.0 Knowledge, attitudes and practices

2.1 Do you have a skin condition called tungiasis in your area? (1) Yes (2) No [ ]

2.1.1 If Ans. Is No? Have you ever heard about it? (1) Yes (2) No [ ]

2.2 Where did you hear about Tungiasis? [ ]

(i) Home (ii) School (iii) I have experienced the condition (iv) Radio (v) Health service provides (vi) From community members

2.3 If answer is YES to 2.1. What aspects make you say it is tungiasis? (Allow respondent to express themselves).

(i) Visible embedded flea (ii) Characteristic sore (iii) Characteristic itch (iv) I can't explain

2. 4 How is tungiasis transmitted from one person to another?

(1). Poor personal hygiene (2) Lack of footwear (3) Contraction from animals (4) I don't know [ ]

2.5 How do you rate or classify tungiasis?

(1) Nuisance (2) Skin Disease (3) Normal skin condition (4) Not a condition to worry about [ ]

2.6 Do you know of any skin diseases that affects humans and animals? (1). Yes (2). No [ ]

2.7 Are there any skin conditions that similarly occur in humans and animals? (1).Yes (2). No

2.8. Are you in a position to make clear distinctions over skin diseases? (1) Yes (2) No [ ]

If ans. Is yes then how?

(1) Evidence of embedded fleas (2) Nature of lesion (3) Characteristic sensation []

2.9. Who are mostly affected by this disease?

(i) Infants (ii) Children (iii) Youths (iv) Adults (v) Aged [ ]

2.9 How often do you see this disease?

(i) Daily (ii) Weekly (iii) Monthly (iv) Yearly [ ]

2.10 What do you think is the major reason why the disease occurs?

(i). Presence of infected animals (ii). Poor sanitation (iii). It is just there in the area (iv). I don't know [ ]

2.11 At what time of the year is Tungiasis (Matekenya) prevalent?

(i) Rainy season (ii) Hot dry season (iii) Cool dry season (iv) Throughout the year [ ]

2.12 What remedies or interventions do local people apply to mitigate against the effects of the disease?

(i). Treat infested animal (ii) Use local herbs/medicines (iii) Seek medical assistance (iv) None

2.13. What is the perception of the affected ?

Affected: (1) Secluded and stigmatized (2) Normal situation (3) [ ]

Unaffected: (1) Skin disease of the poor (2) Disease that affects anyone [ ]

2.14 What effects does this condition have on the victims?

(I) Lameness and deformation (ii) Discomfort (iii) Stigmatisation (IV) No major worrisome effect.

2.15 Have you ever had any assistance in addressing the issue of tungiasis (matekenya) (1) Yes (2) No) . if answer is yes. [ ]

Who assists or assisted?……… …………….. ………………………………………

3.1 If ans is No. What do you think should be done to augment the local efforts if at all any? ………………… …………………….. …………………………… …… ………………………………………………………………………………………

2.17. Have you ever heard someone who has died as result of tungiasis? (1) Yes (2) No [ ]

2.17.1 Have you ever known someone who has died as a result of tungiasis (1) Yes (2) No [ ]

2.18. To the best of your knowledge how many? Which village and RHC centre attended to the deceased. [ ]
………………………………………………………………………………………
……………………………………………………………………………

2.19 How often do the health and veterinary staff discuss the condition?

(i). Never (ii). Rarely (iii). Often (iv). Very often [ ]

Who do you think are more proactive regarding the control of tungiasis?

(i) Veterinary (ii) Health (iii) Community based organizations (NGO) (iv) I can't tell

2. 20 How best do you think this problem can be handled?………………… ………
………………………………………………………………………………………

3.0 Risk factors for Tungiasis

(Make direct observation of Household State and fill in 3.1 & 3.2)

3.1 Type of house construction [ ]

(1) Wall made up of cement bricks

(2) Wall made with tree poles and mud/soil

(3) Wall made with burnt bricks

(4) Wall made with non burnt bricks

3.2 Type of floor

(1) Concrete floor (2) Sand floor (3) Clay floor [ ]

3.3 Water and Sanitation

.1 Do you have any toilets? (1) Yes (2) No [ ]

.2 Which type of toilets do you use? (1) Pit latrine (2) Water borne toilets (3) Open Defecation [ ]

.3 Where do you get water for domestic use?

(1) Open well. (2) Rivers / streams (3) Borehole (4) Dams [ ]

.4 How do you dispose of garbage?

(1) Rubbish pit within the homesteads (2) Burning outside perimeter of villages (3) No specific area [ ]

2.4 Types of animals kept

2.4.1 Do you have animals within homestead perimeter? (1) Yes (2) No

2.4.2 If *ans* is YES. Which animals do you have? Number

1: Pigs (1) Yes (2) No	[ ]	☐
2: Dogs (1) Yes (2) No	[ ]	☐
3: Cats (1) Yes (2) No	[ ]	☐
4: Goats (1) Yes (2) No	[ ]	☐
5: Sheep (1) Yes (2) No	[ ]	☐
6: Cattle (1) Yes (2) No	[ ]	☐
7: Chickens (1) Yes (2) No	[ ]	☐

3.0 Personal behaviors associated with tungiasis transmission

3.1 Do you use closed foot wear (Shoes)? (1) Yes (2) No [ ]

3.2 If yes, How often do you wear them? (1) Regularly (2) Occasionally [ ]

3.3 Do you think wearing shoes help in preventing Tungiasis? (1) Yes (2) No [ ]

3.4 If yes to 3.3 Why do you think it is like that?...

3.5 Where do you rest during the day?..

3.6 Do you sleep on a bed at night? (1) Yes (2) No [ ]

3.7 If answer is No. Where do you sleep?..

3.8 Do you use bathing soap? (1) Yes (2) No [ ]

4.0 Prevalence of Tungiasis

4.1 Have you ever experienced Tungiasis (1) Yes (2) No [ ]

4.2 Do you have it right now anywhere in the community? (1) Yes (2) No [ ]

4.3 Do you have it yourself (1) Yes (2) No [ ]

4.3.1 If *ans* is No. Does any of your family members have it? (1) Yes (2) No [ ]

4.4 If yes, which part of your body (self reported case)………………………..

5.0 Clinical Examination

5.1 For clinical examination consider the following findings diagnostic for tungiasis

1. Itching red brownish spot with diameter of 1-3mm
2. A circular lesion presenting as a white patch with 1-4mm with a central black dot
3. Black crust surrounded by necrotic tissue
4. Partially or totally removed flea lesion/ characteristic sore in the skin

5.2 Record the number of lesions observed in part of the body examined

1. Hands(Fingers and between fingers) [ ]
2. Legs (from knee joint to ankle joint [ ]
3. Feet (plantar / toes / between toes) [ ]
4. Arms (wrist and elbow joint) [ ]

5.3 Classification of Tungiasis Infestation (Examination Sheets to provided)

Classification	Number of Lesion	Tick
Mild infestation	< 5	
Moderate infestation	6 – 30	
Heavy infestation	>30	

Other lesions observed

1. Number of vital lesions for tungiasis (Vital lesion= active lesions)

    1.1 Hands (fingers and between fingers) [ ]

    1.2 Legs (from knee joint to ankle joint) [ ]

    1.3 Feet (Plantar / between toes / toes) [ ]

    1.4 Arms (wrist and elbow joint) [ ]

2. Number of avital lesions for tungiasis (Avital lesion= non active lesions)

    1.1 Hands (fingers and between fingers) [ ]

    1.2 Legs (from knee joint to ankle joint) [ ]

    1.3 Arms (wrist and elbow joint) [ ]

    1.4 Feet (Plantar/between toes / toes) [ ]

3. Other associated pathology as well (signs and symptoms) such as bacterial super infection, pain and deformations (record if observed)…………………………………………………………………….

What else would you want us to know about tungiasis from your own experience? ....……………………………………………………………

Thank you for your time you will hear from us soon

Tungiasis lesion record and clinical examination sheet for other household members

Name:…………………………………………………

Village:……………………………………..House Hold ID………………………

Age: [ ] Gender: M/ F [ ] Position in HH: (a). Head (b). Child (c).Visitor (d). Dependent

Record the number of lesions observed in part of the body examined

1. Hands (fingers and between fingers) [ ]
2. Legs (from knee joint to ankle) [ ]
3. Feet (plantar, toes and between toes) [ ]
4. Arms (wrist and elbow joint) [ ]

Classification	Number of lesion	Tick
Mild Infestation	Less than 5 (< 5)	
Moderate	Between 6 and 30 (6 – 30)	
Heavy infestation	Greater than 30 (> 30)	

2.0 Number of vital lesions (Vital lesions = Active) – *{indicate figure in between brackets provided}*

1. Hands (fingers and between fingers) [ ]
2. Legs (from knee joint to ankle) [ ]
3. Feet (plantar, toes and between toes [ ]
4. Arms (wrist and elbow) [ ]

3.0 Number of avital lesion (Avital lesions = Non active) – {indicate figure in between brackets provided}

1. Hands (fingers and between fingers) [ ]
2. Legs (from knee joint to ankle) [ ]
3. Feet (plantar / toes / between toes) [ ]
4. Arms (wrist and elbow) [ ]

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19th November, 2012

Ref. No. 2012-Oct-005

The Principal Investigator
Dr. Kelvin M. Kampamba
Ministry of Agriculture and Livestock
Department of Veterinary and Livestock Development
P.O. Box 510016,
CHIPATA

Dear Dr. Kampamba,

RE: Determination of the Prevalence, Associated Risk Factors and Levels of Awareness of Tungiasis Prevention and Control in Chadiza and Chipata Districts of Eastern Zambia.

Reference is made to your corrections dated 6th November, 2012. Noting that you have addressed all concerns raised the IRB members resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	Normal	Approval No. 2012-Oct-005
Approval and Expiry Date	Approval Date: 19th November, 2012	Expiry Date: 18th November, 2013
Protocol Version and Date	Nil	18th November, 2013
Information Sheet, Consent Forms and Dates	• English and • Chichewa.	18th November, 2013
Consent form ID and Date	Version-Nil	18th November, 2013
Recruitment Materials	Nil	18th November, 2013
Other Study Documents	Questionnaires.	18th November, 2013
Number of participants approved for study	• 386 Households	18th November, 2013

Where Research Ethics and Science Converge

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON