# SPATIO -TEMPORAL DISTRIBUTION AND RISK FACTORS ASSOCIATED WITH PHLEBOTOMINE SAND FLIES AND LEISHMANIASIS IN Mt. ELGON REGION, KENYA

**BY**

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# DECLARATION

**Declaration by the candidate**

This thesis is my original work and has not been presented for the award of a degree or any other award in any other university/college. No part of this thesis may be reproduced without prior permission of the author and or the University of Eldoret.

…… ……

Dennis Wafula Mukhwana Date

SC/PGB/013/07

**Approval by supervisors**

This thesis has been submitted for examination with our approval as university supervisors.

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## DEDICATION

This research thesis is dedicated to all those who are dear to me. To the Almighty God without whose grace I would not have been able to undertake this study, to my parents who sacrificed their meagre resources to see me through school and to my wife for always being there for me.

## ABSTRACT

This study determined the spatio–temporal distribution, natural infection and risk factors associated with phlebotomine sand flies and leishmaniasis in Mt. Elgon focus in 2015. Collections of sandflies were carried out for five nights of each month at two sites each in Bungoma and Trans Nzoia. Eight CDC light traps were set up at each study site from 6:00 pm to 6:00 am and the sandflies from each catch immediately preserved in ethanol for laboratory analyses. Seasonal fluctuation in sandfly population was compared using densities obtained from the CDC light trap collections. Sandfly abundance determined as quantitiative counts per site. Differences in abundance were analyzed using Kruskall Wallis Test. Sex ratio of sandflies was calculated as: No. of male/No. of female × 100 and differences in sex ratio values determined using chi-square test. Shannon Weiner index was calculated to determine the diversity of the sandflies among sites. The environmental factors were recorded as mean per replicate and the differences per site analyzed using One Way ANOVA and spatial and temporal differences analyzed using Two Way ANOVA. The relationships between environmental factors and abundance of the sandfly were determined using correlation coefficient tests. A total of 657 sandfly specimens belonging to one species of *Phlebotomus pedifer* were collected from both sites, where higher abundance of the flies occurred in Trans Nzoia than at Bungoma, with higher female to male ratios. Seasonality was an important factor causing differential distrubution of *Phlebotomus* where highest abundance occurred during the dry season. The natural infection rate with *Leishmania* was lower (8.9%) in Bungoma compared to Trans Nzoia where the rate was over 18.5%. Abundance of sandfly was negatively correlated with soil temperature, rainfall and relative humidity. The variation of case age-groups and the fact that all the cases were found in peri-urban areas suggests that there is an active transmission going on with *Phlebotomus pedifer* as the only vector in all the allopatric areas studied. It can also be concluded that, like *Phlebotomus papatasi, Phlebotomus pedifer* can also cause transmission away from rural areas. Based on the results of the current study, it is recommended that there is need molecular identification of sandfly are required to distinguish between subtypes. The environmental characteristics identified as risk factors should inform implementation of targeted vector control strategies. People should be advised not to enter the caves because they can be infected when they are are unprotected.

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## LIST OF ABBREVIATIONS/ACRONYMS

| | |
|---|---|
| AVL | Anthroponotic Visceral Leishmaniasis |
| CBRD | Centre for Biotechnology Research and Development |
| CL | Cutaneous Leishmaniasis |
| DNA | Dioxyribo Nucleic Acid |
| IR | Infection rate |
| KEMRI | Kenya Medical Research Institute |
| MCL | Muco-cutaneous leishmaniasis |
| MIC | Minimum Inhibitory Concentration |
| NFE | Non Feed Effect |
| NK | Natural Killer cells |
| NO | Nitric oxide |
| PBS | Phosphate Buffered Saline |
| PKDL | Post-Kala-zar Dermal Leishmaniasis |
| TNF | Tumor Necrosis Factor |
| VL | Viceral Leismaniasis |
| WHO | World Health Organisaton |
| ZCL | Zoonotic Cutaneous Leishmaniasis |

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# CHAPTER ONE

# INTRODUCTION

## 1.1. Background of the study

Leishmaniasis is one of the most important emerging and resurgent vector-borne protozoal diseases, endemic in 98 countries in Europe, Africa, Asia and America of which 72 are developing countries. Approximately 350 million individuals are at risk of contracting the disease with an annual incidence of 1.5–2 million new cases (de Andrade *et al*., 2014; World Health Organization, 2016; Leelayoova *et al*., 2017). Therefore, World Health Organization (WHO, 2016) has classified Leishmaniasis among the tropical neglected, emerging, and uncontrolled diseases that affect mainly poor regions around the globe. The WHO estimates that worldwide, about 500,000 new cases of leshmaniasis occur every year (World Health Organization, 2016). In East Africa, the annual number of cases is estimated at 50,000 (World Health Organization, 2007) and related deaths at 4000, especially in Sudan, Ethiopia and Kenya (Reithinger *et al*., 2007).

Leishmaniasis infection is caused by the kinetoplastid parasites of the genus *Leishmania*. Approximately 53 *Leishmania* species have been described (without considering the synonyms and including all five subgenera and complexes: *Leishmania, Viannia, Sauroleishmania, L. enriettii* complex, and *Paraleishmania*); of these, 31 species are parasites of mammals and 23 species are pathogenic for humans (Khademvatan *et al*., 2017). *Leishmania* species responsible for a diversity of clinical symptoms belong to two subgenera: *Viannia* and *Leishmania*, which have a wide range of geographical distribution in the tropics, subtropics and the Mediterranean

basins (Pigott *et al*., 2014). In most tropical areas, the genus *Leishmania* is the main causative agent of leishmaniasis. There are more than 20 Leishmanial species, of which *L. aethiopica*, *L. major* and *L. tropica* are common, causing different forms of leishmanianisis (Chappuis *et al*., 2007; Adegboye *et al*., 2017).

Infection by *Leishmania* parasites can result in a variety of clinical manifestations ranging from single self-healing ulcers to life threatening infections (Alexander *et al*., 2000; Desjeux, 2004). The four main clinical syndromes include: cutaneous leishmaniasis; muco-cutaneous leishmaniasis (also known as espundia); visceral leishmaniasis (VL; also known as kala-azar); and post-kala-azar dermal leishmaniasis (PKDL). Cutaneous and muco-cutenous form are not fatal and the patient generally presents with ulcer(s) or nodule(s) in the skin (Karram *et al*., 2012; Alves da Silva *et al*., 2013; Alidadi and Oryan, 2014; Es-Sette *et al*., 2014; Khademvatan *et al*., 2017) while Visceral leishmaniasis (VL) is systemic fatal if left untreated (Lukes *et al*., 2007; Singh *et al*., 2012). The PKDL is highly infectious and characterized by a mascular, or nodular rash and is a complication of VL that is frequently observed after treatment (Basher *et al*., 2015).

In Kenya, the expected annual cases of leshmaniasis average about 600 annually, with a case fatality rate of up to 7% seen in outbreak situations. However, the cases rise to over 1,000 in an epidemic year. Both visceral (VL) and cutaneous (CL) forms of Leishmaniasis have been reported in Kenya (Ngoka and Mutinga, 1978; Schaefer *et al*., 1994; Tonui, 2006). VL cases are seen in: Rift Valley districts of Baringo and Turkana that neighbours South Sudan, Pokot districts that neighbour Nakapiriprit district in North Eastern Uganda and North Eastern districts of Isiolo, Wajir and

Mandera. Previously the disease was present in Central Eastern and Kerio Valley districts and Kajiado area (Ho *et al*., 1982). In the meantime, CL is endemic in the Central Rift Valley districts of Naivasha and Laikipia (Githure *et al*., 1986).

Phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae) are a group of medical importance due to the fact that they are considered natural vectors of *Leishmania* (Euglenozoa: Trypanosomatidae), the etiological agents of leishmaniasis (Ready, 2013; Killick-Kendrick, 1999). The life cycle starts when parasites are picked up by the sand fly during a blood meal where it evolves and multiplies inside the sandfly (Brisola-Marcondes, 2007). With few exceptions, phlebotomine sandflies are the unique haematophagous proven to transmit leishmaniases through the bite of infected female that have previously fed on an infected mammal. Although a total of 900 sand fly species have been described, approximately 70 have been implicated in the epidemiology of leishmaniasis (Sang *et al*., 1993; Young and Duncan, 2012; Grimaldi *et al*., 2012; Akhoundi *et al*., 2016).

In Kenya, there are reports on the occurrence of phlebotomine sandflies dating back to 1921, and later in 1930 and 1932 (Sinton, 1930, 1932). Comprehensive studies so far undertaken indicate presence of only two genus: *Sergentomyia* Franca and Parrot and *Phlebotomus* Rondani and Berte. Until 2011, 48 species of sandflies and subspecies had been reported to occur in Kenya (Anjili *et al*., 2014). The sandflies of the genus *Phlebotomus* are important vectors of the leishmaniases whereas those in *Sergentomyia* are not known to transmit any disease but can be a biting nuisance (Killick-Kendrick *et al*., 1986). The genus *Phlebotomus* in Kenya is represented in five subgenera, namely *Phlebotomus, Larroussius, Synphlebotomus,*

*Paraphlebotomus* and *Anaphlebotomus.* The genus *Sergentomyia* has the largest number of sandflies, and is represented by four subgenera, namely: *Sergentomyia, Sintonius, Grassomyia* and *Parvidens*. There are about 48 species recorded, which are suspected to be the cause of Leishmaniasis. Further evidence of speared of leshmaniasis beyond the traditional aread of the Rift Valley districts of Keiyo, Naivasha, Laikipia, Baringo, Turkana, Pokot and North Eastern districts of Isiolo, Wajir and Mandera (Ho *et al*., 1982). There is more evidence of emergence and spread of leishmaniasis in Western Kenya (Kolaczinski *et al*., 2008; Ngure *et al*., 2009; Ashford, 2010; Elnaiem, 2011). However, research on the occurrence and prevalence of the sandfly vectors are rare in these areas.

A number of factors have also also been associated with the distribution of phlebotomone sandfly vectors. In most instances, the appearance of new and the resurgence of old diseases and pathogens can be associated with ecological and climatic changes that have favoured an increase in vector densities (Mutinga and Odhiambo, 1986; Sang *et al*., 1993; Githeko *et al*., 2000; Kolaczinski *et al*., 2008). Changes in environmental factors such as rainfall, temperature, humaidity, wind patterns, soil factors, forest structure, among others have all resulted in changes in vector population densities (Leelayoova *et al*., 2017). Furthermore, the increase in human travel has enabled the spread of infectious agents of human and animal origin by introducing them into areas from which they had been hitherto absent (Andrade-Narváez *et al*., 2003; Negera *et al*., 2008; Ashford, 2010). Although the close relationship among climate and weather conditions, phlebotomine sandfly seasonality and leishmaniasis is well documented, limited investigations have been done in Western Kenya, despite the emergence of leishmaniasis in the area.

### 1.2 Statement of the problem

Climate change and unpredictable weather patternss have resulted in shifts in distribution of several species including that of sandfly (González *et al*., 2014; Carvalho *et al*., 2015). There has seen emergence of sandfly species in areas where there was none recorded in the past (Ready, 2008). Most of the studies on the emergence of sandfly vectors have been conducted in Europe (Semenza and Menne, 2009; Gálvez *et al*., 2011; Depaquit *et al*., 2010; Ready, 2010; Fischer *et al*., 2011; Christodoulou *et al*., 2012; Arce *et al*., 2013; Poeppl *et al*., 2013; Medlock and Leach, 2015). However not much information is available on sandfly vector in many countries in Africa including Kenya, with most of the research done over decades ago (e.g. Heisch *et al*., 1962; Mutinga and Kamau, 1986; Killick-Kendrick *et al*., 1994; Robert *et al*., 1994; Marlet *et al*., 2003; Ryan *et al*., 2006). There is therefore lack of information on the occurrence and distribution of the variuos species of sandfly in several areas of Kenya despite the evidence of climate change affecting the region. The ability of sandfly to transmit Leishmaniasis is determined by the infectivity of the sandlfy with *Leishmania* species (Desjeux, 2004; Kamhawi, 2006; Quinnell and Courtenay, 2009). However studies on the infection rates of the sandfly in Kenya with leisshmania species are few and isolotaed making the knowledge of the epidemiology of Leishmaniasis in Kenya not to be readily available and thus complicate the control and spread of Lesihmaniasis. Many studies have indicated that the distribution of sandfly species are associated with a number of biotic and abiotic factors, of which those related to rainfall, temperature, altitude, latitude, soil type and physical barriers, as well as the abundance and distribution of vertebrate hosts, are of particular importance (Moo-Llanes *et al*., 2013; Kock, 2015; Pech-May *et al*., 2016). These factors may dictate the temporal and spatial distributions of sandfly vectors, and

thereby also alter the epidemiology and dynamics of disease transmission (Pech-May *et al*., 2016). The few studies that have been conducted in Kenya regarding the ecological niche of sandfly vectors have found that several species have overlapping ranges of distribution in areas of recurrent transmission of leishmaniases with some showing wide distributed. Nevertheless there is consistent lack of studies in Kenya on the risk factors affecting the distribution of sandflies.

### 1.3 Justification of the study

Studies on sandfly species population dynamics are crucial and must refer to seasonality, abundances and infection rates because, in combination, these provide strong evidence on the relative vectorial role of each sandfly species (Pech-May *et al*., 2010). In addition, studies on the ecology of sandfly vectors are equally important because they are helpful for understanding the processes involved in the complex dynamics of transmission of *Leishmania*. In the present study, patterns of abundance, as well as natural infection rates of sandfly species were investigated in two sites in Mt. Elgon Region which had not been studied previously despite reported cases of leishmaniases (e.g., Mebrahtu *et al*., 1993; Sang *et al*., 1993)

The study also aimed to document the species composition of sandfly communities in the four villages and, using that information, to model the pattern of ecological niche distribution of sandfly species in relation to the prevailing environmental and other risk associated factors. Such information is important in developing models for predicting the influence of environmental factors on the distribution of sandfly vectors.

## 1.4 Objectives of the study

### 1.4.1 Main objective

The main objective of this study was to investigate the spatial distribution of phlebotomine sandflies (Diptera: psychodidae) and risk of transmission of leishmaniasis in Mt. Elgon Region, Kenya.

### 1.4.2 Specific objectives

The specific objectives of the study were:

1. To assess the spatio-temporal variation in phlebotomine sandfly vector composition and abundance in Mt. Elgon Region, Kenya.
2. To determine the spatio-temporal variation in natural infection of phlebotomine sandfly with *Leishmania* in Mt. Elgon Region, Kenya
3. To evaluate the risk of ambient and soil temeprature, rainfall and relative humidity for the transmission of Leishmaniasis by phlebotomine sand fly
4. To determine the incidence of *L. aethiopica* infection in the human population in the study areas.

## 1.5 Hypotheses

$H_{01}$: There is no significant spatio-temporal variation in phlebotomine sandfly vector composition and abundance in Mt. Elgon Region, Kenya.

$H_{02}$: There is no significant spatio-temporal variation in infection of phlebotomine sandfly species by *Leishmania* in Mt. Elgon Region, Kenya.

$H_{03}$: There are no significant effects of ambient and soil temeprature, rainfall and relative humidity for the transmission of Leishmaniasis by phlebotomine sand fly in Mt. Elgon Region, Kenya.

$H_{04}$: There are no significant incidences of *L. aethiopica* infection in the human population in Mt. Elgon Region, Kenya.

**1.6 Scope of the study**

In terms of scope in content, the study included the occurrence, distribution and infectivity of phlebotomine sand flies in Bungoma and Trans Nzoia Counties of Kenya and also determined the risk factors in the transmission of *Leishmania* within the study areas.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Phlebotomine sandfly taxonomy and distribution

The name ‘sandfly’ can be misleading, as it wrongly suggests to laypeople that they may be at risk of vector-borne disease while on holiday on the beach. Actually, the English connotation refers to the pale (sandy) colour of this fly. There is further confusion because in certain parts of the world, midges of the genus *Culicoides* (Diptera: Ceratopogonidae) and blackflies (Diptera: Simuliidae) are also referred to by the same name. A distinction must therefore be made for the vectors of the leishmaniases and other diseases of public health concern, which are correctly termed ‘phlebotomine sandflies’ (Killick-Kendrick, 1990; Killick-Kendrick, 1997). To date, over 800 species have been estimated to exist in different regions of the world. They are grouped in the suborder Nematocera of the order Diptera, family Psychodidae, Subfamily Phlebotominae. Phlebotomine sandflies share the family Psychodidae with the non-vector, non-biting moth flies (subfamily Psychodinae), often seen around shower drains. Currently, the classification of phlebotomine sandflies remains controversial, cumbersome and far from being definitive.

Based on the pioneering classification of Theodor (1948), Lewis *et al.* (1977) proposed two genera for Old World species, *Phlebotomus* Rondani and *Sergentomyia* Franca, and three for New World species, *Lutzomyia* Franca, *Brumptomyia* Franc¸a and Parrot, and *Warileya*, Hertig. The genus *Chinius* is a distinct taxon used for some Chinese sandfly species with primitive characters (Leng and Lewis, 1987; Seccombe *et al*., 2017). These three genera (*Phlebotomus*, *Sergentomyia* and *Chinius*) are widely

accepted by modern Old World taxonomists. A few other species have been or are about to be named, but so far these are of unknown medical importance (WHO, 2007). In the genus *Phlebotomus*, 11 subgenera, 96 species and 17 subspecies have been recognized by Lewis and Ward (1987). For the Neotropical sandflies, most entomologists still follow the classification of Lewis *et al*. (1977), later reviewed by Young and Duncan (2012), who recognize the three genera named above, *Lutzomyia*, *Brumptomyia* and *Warileya*, which include 15 subgenera and 11 species groups. More recent revisions have been proposed, but none has been universally accepted. The most recent and comprehensive is that by WHO (2007), who recognized 464 species of Neotropical phlebotomine sandflies grouped into 23 genera, 20 subgenera, three species groups and 28 series.

Phlebotomine sandflies are principally present in the warm zones of Asia, Africa, Australia, southern Europe and the Americas (Killick-Kendrick, 1997). Their distribution extends northwards to just above latitude 50°N in Southwest Canada and just below this latitude in Northern France and Mongolia (Maroli *et al*., 2013). Their southernmost distribution ends at a latitude of 40°S, but they are absent from New Zealand and the Pacific islands while their altitudinal distribution extends from below sea level (Dead Sea) to 3300 m above sea level in Afghanistan (Marcondes, 2007).

The life cycle of phlebotomine sandflies is well dsecribed (Kellick-Kendrick, 1990; Gossage *et al*., 2003; Bates, 2007). Phlebotomine sandflies undergo complete metamorphosis through four developmental stages: egg; larva (four instars); pupa, and adult (Killick-Kendrick *et al*., 1994). The immature stages, unlike those of mosquitoes, do not require standing water to complete their development, although

they need relatively moist and warm habitats (Killick-Kendrick, 1990). The eggs are laid by adult females in a suitable habitat rich in organic content, such as animal excreta and soil, which provides the newly emerged larvae with shelter, nutrition and moisture (Desjeux, 2004). Eggs (0.3–0.5 mm in length) are initially white or light grey in colour but often turn dark brown or black within a few hours of oviposition. Egg hatching is highly temperature-dependent and subsequent larval development is generally slow. Embryonic and larval development periods were recently determined over a 1-year period for nine sandfly species belonging to six genera or subgenera, by the study of 15 laboratory colonies. After the female has taken a bloodmeal and completed oviposition, first-instar larvae emerge in 12–19 days, pupae in 25–59 days, and adults in 35–69 days (Elnaiem, 2011).

Larvae are caterpillar-shaped with head capsules and small leaf-like antennae. They have long caudal setae that can help in their identification as sandfly larvae, although these are not usually employed in taxonomy because they are rarely collected in nature (Gossage *et al*., 2003). The larvae move very little distance from the oviposition site. Pupae are similar to small chrysalises in which the fourth-stage larval exuvia are attached at one end to a solid substrate. Adults are small and seldom exceed 3.5 mm in body length (Ready, 2013). They are covered with dense hairs and hold their wings in a characteristic ‘V’ shape over their backs when at rest. They range in colour from almost white to almost black. The legs are very long and delicate. Both males and females feed on sugary secretions from plants or from honeydew produced by homopterous aphids (Hemiptera: Aphidoidea). Females require at least one bloodmeal in order to complete development of egg batches. Only a few phlebotomine sandfly species are able to produce viable eggs without a

bloodmeal. Unlike mosquitoes, their attack on the host is silent. Adults are mainly active in the evening, at night and in the early morning, although they can bite during the day if disturbed.

The flight speed of phlebotomines is considerably slower than that of mosquitoes and is <1 m/s (Killick-Kendrick *et al*., 1986). They are unable to fly at wind speeds higher than this rate, which is the main factor limiting the range of their dispersal. Their flight range is typically very short (about 300 m) and thus adult activities are usually restricted to the vicinity of larval breeding sites. Evidence from mark–release–recapture studies indicates that forest species disperse at shorter distances than peridomestic ones. For example, *Phlebotomus ariasi* may disperse over 2 km (Killick-Kendrick *et al*., 1994). By contrast, Neotropical forest species seldom appear to disperse over distances of >1 km (WHO, 2016). The males congregate in leks on or near the host and produce sex pheromones. Vibration of the wings by males can be important in encouraging females to mate (Lawyer *et al*., 2017). Resting sites are often near to larval breeding sites and consist of cool, humid and dark micro-habitats (Killick-Kendrick, 1999). The seasonal activity of adult sandflies is affected mainly by temperature and rainfall.

Among the over 800 phlebotomine sandfly species estimated to exist, only 98 species of *Phlebotomus* and *Lutzomyia* genera are currently proven or suspected vectors of human leishmaniases. The role of species belonging to the genus *Sergentomyia* in *Leishmania* spp. Transmission among mammal hosts needs to be elucidated. Most of the information on the phlebotomine sand flies are based on collection and collation by Killick-Kendrick (1999) and the WHO (2007), integrated with information in the

most recent literature and personal evaluations in cases of doubtful reports. Only countries that have reported indisputable endemic human leishmaniases are listed. In the Old World, proven or probable vectors account for a total of 42 species, of which 20 are implicated in the transmission of *Leishmania infantum*, six in the transmission of *Leishmania donovani*, seven in the transmission of *Leishmania major*, seven in the transmission of *Leishmania tropica* and three in the transmission of *Leishmania aethiopica* (Lukes *et al*., 2007). Each species appears to be involved in the transmission of one *Leishmania* agent only, except *Phlebotomus sergenti*, which has been incriminated in the transmission of both *L. tropica* and *L. aethiopica* in parts of Ethiopia (Maroli *et al*., 2013), and *Phlebotomus alexandri*, the role of which in the transmission of both recognized species of the *L. donovani* complex (*L. donovani s.s*. and *L. infantum*) in parts of China, and probably in other countries, is still to be ascertained.

By contrast with *Phlebotomus* spp., some *Lutzomyia* species are probably able to transmit more than one *Leishmania* species; for example, *Lu. migonei* has been found to be infected with four different parasite species (see below). The incrimination of a species as a vector is based on a series of widely accepted criteria (Killick-Kendrick, 1990; WHO, 2007): (a) the vector must feed on humans; (b) in zoonotic entities of leishmaniasis, the vector must also bite the reservoir host(s); (c) the vector must be infected in nature with the same *Leishmania* species as occurs in humans, and this must be ascertained by comparison of isolates using isoenzymes or DNA; (d) the vector must support the complete development of the parasite after the infecting bloodmeal has been digested, and (e) the vector must be able to transmit the parasite by bite to a susceptible host while taking a bloodmeal. With regard to the ‘degree’ of

incrimination (species can be ‘proven’, ‘strongly suspected’ or ‘suspected’ vectors), it must be admitted that even these reference standard criteria may be subject to interpretation or may be extremely difficult to meet. An example refers to the fourth criterion concerning the ability of a species to support parasite development: the description of permissive vectors by Myskova *et al.* (2007) indicates that no rule should be taken into account separately and isolated from an epidemiological context; these authors detected massive promastigote infections after blood digestion in 80% of Neotropical *Lutzomyia longipalpis* artificially fed on blood containing Old World wild-type *L. major*. Furthermore, meeting the fifth criterion by (laboratory) demonstration of *Leishmania* spp. transmissibility to susceptible hosts is notoriously difficult because sandflies must first be infected ‘naturally’ through the ingestion of the appropriate stage and number of parasites (the best option being by biting on infected reservoir hosts), and those that survive in laboratory conditions after blood digestion must be induced to feed again on a näıve susceptible host, a procedure that is quite difficult to accomplish (Oliveira *et al*., 2005).

Given these limitations, the present analysis of the literature takes into account the following minimal requirements for robust vectorial incrimination: (a) epidemiological evidence indicated by the overlapping of the geographical distributions of the vector and the human disease; (b) evidence that the vector feeds on humans, and (c) evidence that the vector supports natural gut infections with promastigotes of the same *Leishmania* species as occurs in humans. Evidence for species incrimination is further reinforced in endemic settings from which the usual proven vectors are apparently absent, or in which species meeting the criteria listed here are the only human-biting phlebotomine sandflies (Gonzalez and Ferro, 2014).

In the Old World, updated evidence suggests *Phlebotomus argentipes* as a possible vector of *L. donovani* cutaneous leishmaniasis (CL) in Sri Lanka (Maroli *et al.*, 2013) and confirms the vectorial role of *Phlebotomus orientalis* for *L. donovani* in Kenya (Mutinga *et al*., 1989), *Phlebotomus salehi* for *L. major* and members of the *Phlebotomus major* complex for *L. infantum* in Iran (Azizi *et al*., 2012a,b). Furthermore, *P. sergenti* has been confirmed as the vector of CL caused by *L. tropica s.l.* in Algeria (Khezzani and Bouchemal, 2017) and Tunisia (Bousslimi *et al.*, 2012). With regard to the identity of the leishmanial agent *Leishmania killicki*, to which the latter two records actually refer, this species belongs to the largely polymorphic *L. tropica* taxon. The evidence provided by Leng and Lewis (1987) indicate that *Phlebotomus chinensis* and *Phlebotomus sichuanensis* are indeed two separate species, both of which are involved in *L. infantum* transmission, although in different VL endemic areas of China.

In the New World, recent evidence incriminates *Lutzomyia forattinii* and *Lu. migonei* as new potential vectors of VL. In the state of Mato Grosso, Brazil, Pita-Pereira *et al.* (2008) found *Lu. forattinii* together with the isomorphic *Lutzomyia cruzi*, which was macroscopically distinguishable by its external coloration; both species were naturally infected by parasites identified as *L. infantum* by molecular methods. *Lutzomyia migonei*, recently suspected to be the VL vector in La Banda, Argentina (Salomón *et al*., 2010), has also been indicated as a possible vector in Brazil (Pernabuco state) because *L. infantum* DNA has been detected in wild-caught specimens. This finding suggests that this species may be responsible for the transmission of the disease in areas from which the usual VL vector, *Lu. longipalpis*, is absent. As far as the vectors

of CL, the following information is added: (a) *Lutzomyia nuneztovari anglesi* is a vector of *L. amazonensis* in Bolivia, as confirmed by anthropophily, biochemical identification of wild isolates and successful experimental infection (Martinez *et al*., 1999); (b) *Lutzomyia ayacuchensis* was recently found in Peru naturally infected by promastigotes typed as *L. guyanensis* (Kato *et al*., 2015); (c) *Lutzomyia fischeri* is included as a proven vector because of repeated observations in Brazil of natural promastigote infections identified as *L. braziliensis*, associated with anthropophily and a spatial distribution related to human CL (Pita-Pereira *et al*., 2008); (d) in Venezuela, *Lu. migonei* has been reported as a putative vector of *L. guyanensis* and *L. mexicana* (Feliciangeli *et al*., 1999), and past reports have incriminated *Lutzomyia gomezi* as a proven vector of *L. braziliensis* and *Lutzomyia ovallesi* as responsible for the transmission of not only *L. braziliensis*, but also *L. mexicana* (Maroli *et al*., 2013); (e) in the Yucatan Peninsula of Mexico, *Lutzomyia cruciata*, *Lutzomyia panamensis*, *Lutzomyia shannoni* and *Lutzomyia ylephiletor* are considered to represent possible vectors of *L. mexicana* because recent investigations using molecular techniques have detected natural infections (Pech-May *et al*., 2010).

It has been estimated that there are currently 988 valid phlebotomine species and subspecies from all continents except Antarctica, including 29 fossils, with 512 extant and 17 fossil taxa found in the Americas. The genus *Phlebotomus rondani* and Berté, 1840 has been split and supplemented during the 20$^{th}$ Century. Most specialists now accept at least six genera: *Phlebotomus* and *Sergentomyia* in the eastern hemisphere; *Brumptomyia*, *Lutzomyia* in the Americas. However, doubts about the monophyly of the most speciose genera - *Phlebotomus*, *Sergentomyia* and *Lutzomyia* - have been re-inforced by phylogenetic studies based on morphology and morphometry

(Feliciangeli, 2004) as well as by more limited molecular datasets (Maroli *et al*., 2013), making it increasingly difficult to support the practical classification and its modifications (Myskova *et al.*, 2007), which place most mammalophilic species and all vectors of human leishmaniasis in the genera *Phlebotomus* (eastern hemisphere) and *Lutzomyia* (Americas).

The phylogenetic analyses based on a non-numerical cladistic approach, identified two tribes: Phlebotomini and Hertigiini. The latter contained two sub-tribes, one from each hemisphere, but only 28 extant species classified in 5 genera and no vectors of human leishmaniasis. In contrast, Phlebotomini was far more species, containing 931 extant species classified in 30 genera in six sub-tribes: Phlebotomina (eastern hemisphere), Australophlebotomina (Australia and Asia), Sergentomyiina (both hemispheres), and the exclusively *American Brumptomyiina*, *Lutzomyiina* and Psychodopygina. Some of the generic proposals were supported by numerical phylogenetic analyses of faunas from China and the Oriental region (Pita-Pereira *et al*., 2011), and most of the American genera are accepted by many specialists in Latin America and some others.

**2.2 Natural infection of phlebotomine sandfly species with *Leishmania***

*Leishmania* parasites occur in two forms; the rounded forms referred to as amastigotes found in vertebrate hosts tissues, and the promastigote found in the vector, the sand fly. They are the causative organisms of leishmaniases and belong to the genus *Leishmania,* family Trypanosomatidae. They are transmitted by a host of sand fly species. At least thirty species have been identified that can transmit human leishmaniasis which affects more than 2 million people world wide each year (Oryan

*et al.*, 2007). Leishmaniases occurs as dermal (cutaneous, muco-cutaneous or diffuse cutaneous) and visceral infections. Old world dermal leishmaniasis (oriental sore) is caused by *L. tropica* transmitted by various sand fly species, including *Phlebotomus papatasi, P. caucasicus* and *P. longipes* in Asia and Middle East. American dermal leishmaniasis (also known as "espundia" in Mexico) is caused by *L. braziliensis* and *L. mexicana,* and is spread to humans through bites of *Lutzomyia* species (Kock, 2015).

Visceral leishmaniasis, also known as "kala-azar", is caused by *L. donovani* in Kenya, Asia and Sudan. *Phlebotomus* species are known to be vectors in Kenya and Iran (Lewis, 1987*). Phlebotomus martini* has been incriminated as a vector in Kenya (Tinui *et al.*, 2006; Ngure *et al.*, 2009). The sand fly *Phlebotomus duboscqi* is widely spread from North Africa to east Africa and transmits *Leishmania major* that causes cutaneous leishmaniasis in the vast Savanna and desert areas of Africa (Bates, 2007). *Phlebotomus pedifer* transmits *Leishmania aethiopica,* the causative agent for diffuse cutaneous leishmaniasis in Kenya and Ethiopia.

Leishmaniasis is primarily a zoonotic disease in which wild and domestic animals such as the fox, jackal, rodents, hyraxes and wolves serve as reservoir hosts. Other animals in the surrounding areas can become infected and these are referred to as secondary or incidental hosts. Among the potential animal hosts, domestic dogs by far play the most important role in harboring and transmitting the disease to humans due to the close association between humans and dogs as pets (WHO, 2016). In anthroponotic VL, due to *Leishmania donovani,* such as in India and Sudan, man is the principal reservoir host. Ashford (2010) recognized hosts as reservoir when they

are abundant or gregarious, long lived or survive at least during non transmission season of the parasite, remain infected for long time without acute disease and present the parasite in their skin or circulation for sandfly bite.

*Procavia capensis* and *Heterohyrax brucei* in the family Procavidae are the main animal reservoir hosts of CL. *Procavidae* is the only extant family in the order *Hyracoid* which is considered to have evolved in Africa before the Oligocene or 40 million years ago (Walker, 1975). *Procavia*, *Heterohyrax* and *Dendrohyax* are the three genera in the family Procavidae. Hyraxes live in a wide variety of habitats and are found in altitude ranging from sea level to the height of 4650 m in Kenya (Walker, 1975). A narrow temperature range (from 3 to 10°C) within hyrax holes as opposed to the large temperature range (41.8°C to 5°C) of external environment in which they are known to live, augmented their poor thermoregulatory system (Marlet, 2003). Rock hyraxes select suitable habitats which provide protection from predators including inhabiting the crevices in rock outcrops, cliffs and boulder rockformations, mountains and escarpments. It has however been shown that *H. brucei* live entirely in large trees in the absence of outcropping rocks (Marcondes, 2007). More reearch is needed to clearly elucidate the distribution of CL and a delineate the boundaries of the major foci, the essential characteristics of each major focus, including etiological agents, known or suspected reservoir hosts and known or suspected phlebotomine vectors.

### 2.3 Risk factors associated with the transmission of *Leishmania*

Leishmaniasis is caused by species of parasites of the genus Leishmania and transmitted by vectors in the family Psychodidae, Phlebotomus or Lutzomyia genera (Reveiz *et al*., 2013). Human leishmaniasis can be divided into four main forms

namely cutaneous, mucosal, muco-cutaneous and visceral leishmaniasis (Oryan *et al*., 2007; Daneshbod *et al*., 2011; Shirian *et al*., 2012). Cutaneous leishmaniasis (CL) makes up approximately three quarters of these new cases (Reveiz *et al*., 2013). CL is caused by *Leishmania major*, *L. tropica*, *L. infantum* and *L. aethiopica* in the old world and by *L. Mexicana*, *L. braziliensis*, and *L. guyanensis* in the new world (Motazedian *et al*., 2006; Oryan *et al*., 2008; Hatam *et al*., 2013; Alidadi and Oryan 2013; Shrian *et al*., 2014). Cutaneous leishmaniasis exists in two epidemiological forms namely zoonotic (or wet form, in rural areas, by *L. major*), and arthroponotic CL (or dry form, in urban areas) by *L. tropica*.

Rodents such as gerbils and humans are the main reservoir hosts with *Phlebotomus* as the main vector in zoonotic CL (Motazedian *et al*., 2006; Mehrabani *et al*., 2007; Mehrabani *et al*., 2011). Visceral leishmaniasis (VL), also known as kala azar, is caused by *L. tropica* transmitted by *P. argentipes* in an arthroponotic cycle (Shirian *et al*., 2013; Mengesha *et al*., 2014; Picado *et al*., 2014). Nowadays, especially CL and VL forms have undoubtedly a wider geographical distribution than before. The increase in leishmaniasis incidence is mainly attributed to several risk factors that will be mentioned here. Generally, factors including environmental conditions, human behavior, socioeconomic status, immunogenic profile, and genetic factors pose a major risk to human populations (Votýpka *et al*., 2012).

Important environmental risk factors including living in houses with cracked mud or thatched plastered house walls, damp earthen floors, sleeping on floor or outside, and vegetation near house can facilitate sand fly survival and enhance vector abundance via providing diurnal resting places, breeding sites, and humidity (Reithinger *et al*.,

2010). Sand flies can hide in cracks and fissures in the un-plastered house walls, ceiling or floor. Additionally, living close to a previous case of leishmaniasis strongly increases infection risk (Reithinger *et al*., 2010). Lack of phlebotomine sandflyicide spraying in the houses is associated with increased risk (Coura-Vital *et al*., 2013). Sleeping outside especially during summer months without bed nets can place people at risk of sand fly exposure. So, the use of bed nets impregnated with phlebotomine sandflyicide is often very important for people in protecting against leishmaniasis transmission (Votýpka *et al*., 2012). Migration from rural to urban areas due to low quality of life and social facilities or socioeconomic conditions and improper climate or even migration into villages can increase cases of leishmaniasis (Reithinger *et al*., 2010; Coura-Vital *et al*., 2013).

High prevalence of zoonotic VL observed in urban areas may be attributed to high population density, increased migration, environmental changes, inadequate living condition, and the presence of vectors and reservoirs in the domestic environment (Coura-Vital *et al*., 2013). Factors such as low educational level, lack of land, and socioeconomic concerns all reflect the increased risk related to poverty. Poverty in many ways increases the risk of leishmaniasis, for example it can increase sand fly access into poorly built houses, and human exposure to infected flies (Ghatee *et al*., 2013). Moreover, poor housing and sanitary conditions such as lack of waste management and open sewerage can increase breeding of sand flies, and their access to people (Coura-Vital *et al*., 2013; Dawit *et al*., 2013).

Other environmental factors including elevation, forest coverage, proximity to woodland, new agricultural projects, irrigation, the storage of waste products close to

the city and increase in sand flies population all are associated with risk of CL or VL (Mehrabani *et al*., 2011; Asgari *et al*., 2007; Valderrama-Ardila *et al*., 2010). On the one hand, global warming, changes in temperature, rainfall and humidity, via influencing survival and population size of vectors and reservoir hosts and altering their distribution, exert strong effects on their ecology. On the other hand, situations such as drought, famine and flood may result in extensive displacement and migration of people to endemic areas (Dawit *et al*., 2013). In addition, natural disasters like earthquakes can exert dramatic effects on creation a breeding place for sand flies, the abundance and propagation of the vectors, and transmission of the parasite (Sharifi *et al*., 2011). Presence of dogs and rodents are regarded as the most important risk factors for CL reflecting their role in the transmission cycle of Leishmania (Belo *et al*., 2013). Susceptibility of dogs, the main reservoirs for *L. infantum* in human beings, to canine VL is associated with their fur length, the presence of manure or dry leaves in the backyard as a food source for sand fly larvae and housing conditions reflecting socioeconomic status (Coura-Vital *et al*., 2013; Belo *et al*., 2013). Dogs with short hair have a higher likelihood of being seropositive than those with long hair. Purebred dogs are more likely to be infected compared with mixed-breed dogs (Coura-Vital *et al*., 2013). Co-existence of dogs with other animals such as pigs, horses, cows, chickens or other domestic fowls has been shown to be associated with high prevalence of canine VL (Belo *et al*., 2013).

Presence of these animals, mostly cattle can increase the prevalence of leishmaniasis cases via increasing the density of sand flies around houses, as their dung provides a rich environment for the sand flies, drawing the vectors into closer association with humans and increasing the risk of their being bitten (Bern *et al*., 2010; Belo *et al*.,

2013). Immunosuppression is one of the major factors responsible for reactivation of a silent *Leishmania* infection or for increased susceptibility to the primary infection (Alves da Silva *et al*., 2013; Mengesha *et al*., 2014). Immunosuppressed peoples and graft and renal transplant recipients who live with dogs and cats and in VL endemic areas are at high risk for VL. Intestinal parasitic infections causing malnutrition and HIV are other risk factors sensitizing people to leishmaniasis (Mengesha *et al*., 2014). HIV/VL co-infections exist in countries in which leishmaniasis is endemic and the increase in cases of leishmaniasis is widely associated with the spread of HIV (Alves da Silva *et al*., 2013). In addition to HIV itself, severe malnutrition due to HIV is involved in the increased prevalence of VL (Mengesha *et al*., 2014). Malnutrition, low dietary protein, energy, iron, vitamin A, and zinc levels increase the risk of VL and mucocutaneous leishmaniasis. This effect has been shown to be related to functional failure of the lymph node barrier and increased early visceralization of the Leishmania (Dawit *et al*., 2013). Genetic factors are also involved in incidence of leishmaniasis, so that specific genes coding associated with the immune response to *Leishmania* in animal models and human beings have been discovered (Blackwell *et al*., 2009). Men seem to be at higher risk of VL compared with women probably due to the role of sex hormones in modulating the response to Leishmania (Mengesha *et al*., 2014; Votýpka *et al*., 2012).

In addition, intense agriculture has been associated with increased incidence of sandfly bites particularly in rural areas (Mangesha *et al*., 2014). It should be mentioned that resistance of *Leishmania* species in some regions to antimonial drugs can be a novel risk factor for the increased incidence of the disease (Negera *et al*., 2008). In order to develop strategies to improve the management and control of the

disease and design the surveillance programs for the early detection and reduction of lethality, it is necessary to understand the risk factors associated with leishmaniasis. Therefore, there is hope that the identification of risk factors for leishmaniasis could greatly help in designing preventive strategies.

Different species of sandflies show different distribution and seasonal variation, but in general, they survive at temperatures above 16°C, up to 44°C, and are mostly found between the months of May to November, showing maximum activity on warm, clear nights with low wind speed. Certain species, like *P. bergeroti*, *P. papatasi*, and *P. arabicus*, prefer indoor habitats, while others, like *P. alexandri*, are found in outside environment (Doha and Samy, 2010; Maroli *et al*., 2013). Sandflies are generally no more than 3.5 mm in length and covered with dense hair, holding their wings in a characteristic V-shaped position. Both male and female adults survive on sugary secretions from plants; females, though, require blood meal for development of egg batches. They are generally active during the night and early morning and characteristically hop across the skin to find a blood meal. The eggs are deposited in batches in warm and moist places close to the larval food sources (Maroli *et al*., 2009; Ready, 2013). There are four different modes of transmission of leishmaniasis. In the Middle East Zoonotic Cutaneous Leishmaniasis (ZCL), caused by *L. major*, is transmitted through *P. papatasi*, with rodent species of Psammomys obesus, Meriones libycus, *Nesokia indica*, and *Rhombomys opimus* serving as nonhuman reservoirs. Zoonotic visceral leishmaniasis (ZVL) is caused by *L. infantum*, spread through *P. galilaeus*, *P. syriacus*, *P. tobbi*, *P. halepensis*, and the dog species of *Canis familiaris* acts as nonhuman reservoirs.Anthroponotic cutaneous leishmaniasis (ACL), caused by *L. tropica* and spread through *P. sergenti*, circulates exclusively in humans.

Anthroponotic visceral leishmaniasis (AVL) caused by *L. donovani* spreads through *P. alexandri* without any non-human reservoir (Colacicco-Mayhugh *et al*., 2011; Jacobson, 2011).

Due to the absence of a vaccine and emergence of drug resistance, leishmaniasis continues to be a burden on society. Additionally, with human population and migration increasing, there is a chance that this infectious disease could spread to other areas, introducing the pathogen to newer environments and leading to mutations and emergence of more virulent strains. Under these circumstances, there is an increasing pressure for the development of novel vaccines, therapeutic targets, and biomarkers of infection (Kedzierski, 2010; Ben Salah *et al*., 2013). There is also an urgent need to report and document cases of leishmaniasis from endemic and non-endemic regions that can give government and health agencies an idea of the prevalence, disease-causing species, vector species, nonhuman reservoirs, and inform the efforts to control the infection (Singh *et al*., 2010).

# CHAPTER THREEE

# MATERIALS AND METHODS

## 3.1 Study area

This study was carried out in four sites in Mt. Elgon Region in Kenya (Figure 3.1). Mount Elgon region covers areas adjacent the extinct volcano in the border of Kenya and Uganda, in the north of Kisumu and western part of Kitale. The mountain's highest point, named "Wagagai", is located entirely within Uganda.It has been estimated by geologists that Mount Elgon is at least 24 million years old, making it the oldest extinct volcano in East Africa. It covers about 80 km in diameter, rises 3,070 metres above the surrounding plains. Mt. Elgon consists of five major peaks: Wagagai (4,321 m), in Uganda, Sudek (4,302 m) on the Kenya/Uganda border, Koitobos (4,222 m), a flat-topped basalt column in Kenya, Mubiyi (4,211 m) in Uganda and Masaba (4,161 m) in Uganda. There are notable feature such as: The caldera which is the larges in the world, the warm springs by the Suam River, Endebess Bluff (2,563 m) as well as Ngwarisha, Makingeny, Chepnyalil, and Kitum caves: Kitum Cave is over 60 m wide and penetrates 200 m. The Mt Elgon traverse Bungoma and Trans Nzoia Counties in Kenya.

The areas and its surrounding contain the red laterite soils. The mountain act as catchment for the several rivers such as the Suam River, River Nzoia and Lwakhakha River. River Swam flows to Turkwel downstream and drains into Lake Turkana, while Nzoia and Lwakhakha Rivers flow to Lake Victoria. The town of Kitale is in the foothills of the mountain. The area around the mountain is protected by two Mount Elgon National Parks, one on each side of the international border.

The study sites were Cheptobot 1and 2 (0°59'43.32"N and 34°49'8.76"E), Chemai ($0^0$50'35.43"N and $34^0$43'14.32"E) and Kimkung' (0°49'53.01"N and $34^0$42'59.44"E) are also shown. Annual mean temperature range from $18^oC$–$21^oC$. Annual average rainfall: 1300 mm-1800 mm. Altitude range: 1500 m to 2000 m above sea level.

### 3.2 Collection and identification of sandfly species

Field collections were conducted monthly in the year 2015 from January to December. Collections of sandflies were carried out in four caves and nearby homesteads. Eight CDC (Centers for Disease Control) light traps were set up at each study location from 6:00 pm to 6:00 am during the study period (Dinesh *et al*., 2008). Upon collection, sandflies from each catch were immediately preserved in ethanol. According to Dinesh *et al* (2008), most of the sandfly are nocturnal and occur in caves thus most of the sampling occurred in caves.

### 3.3 Sorting of sand flies, slide mounting and identification

Sandflies were preserved in 70% ethanol in the field. Specimens were cleaned, dissected and permanently mounted on microscope slides following the procedure outlined by Ibáñez-Bernal (2005).

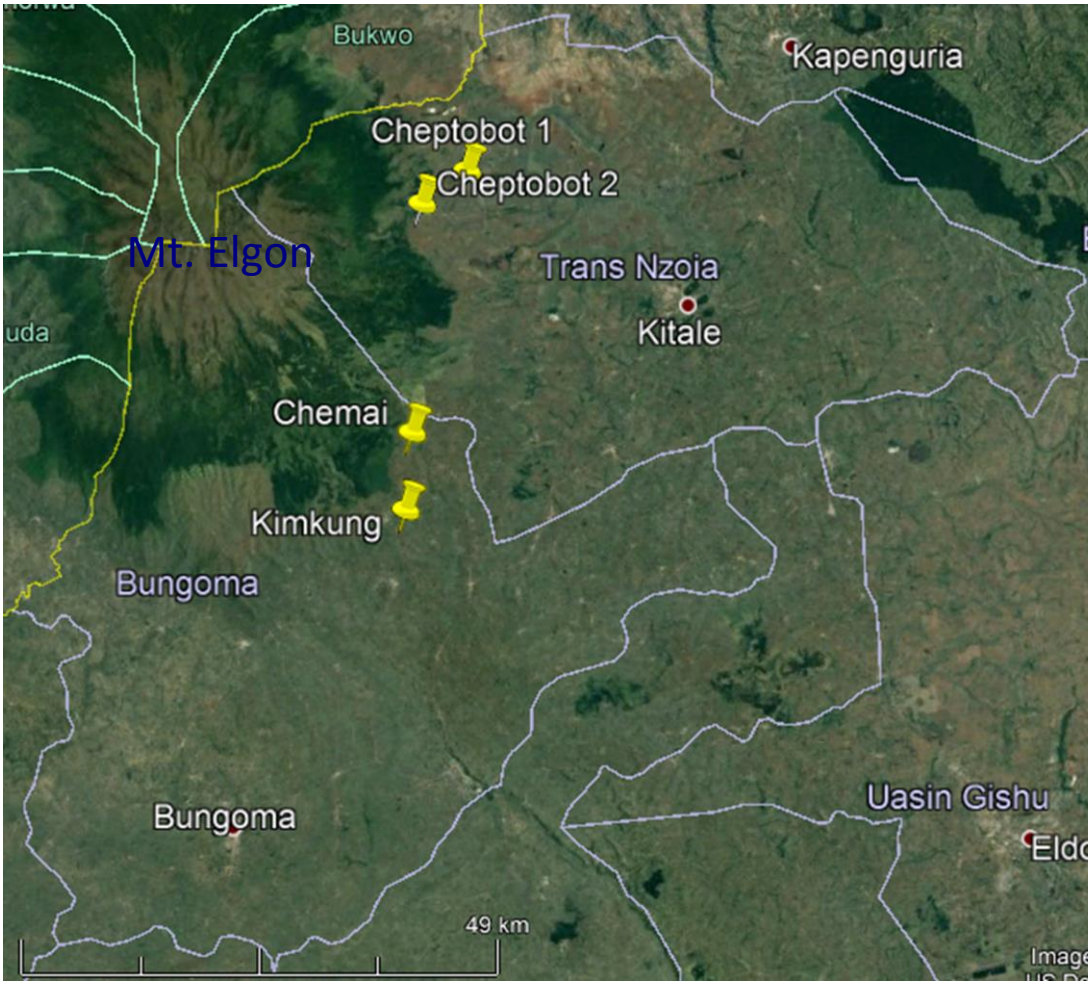


**Figure 3.1**: Map of Bungoma and Trans Nzoia County showing the position of the sampling sites (Courtesy of Google Earth)

Specimens were examined using a Nikon Eclipse 50i compound microscope equipped with phase contrast. Drawings were made with the aid of a Nikon Y-IDT drawing tube and digitally processed using Corel Photo Paint X3 (Version 13). All specimens were deposited in the laboratory at the Kenya Medical Research Institute (KEMRI) in Nairobi. Abbreviations for genera and subgenera followed the proposal of Marcondes (2007). Sandflies were identified by external morphology under a dissection microscope and separated by sex before being counted. They were categorized by

examination of the spermathecea (females) and the external genitalia (males) (Abonnenc and Minter, 1965).

### 3.4 Natural infection of sandflies with *Leishmania*

All the sandfly species were dissected and their gut examined for *Leishmania* promastigotes using a compound microscope. The total number of sandflies infected with *Leishmania* species was then used to determine the infection rates of sandfly with *Leishmania*.

### 3.5 Environmental risk parameters

To understand the conditions that determine the temporal distribution of sand flies, mean monthly data of ambient temperature, rainfall, and relative humidity in Bungoma and Trans Nzoia Conties was recorded during the 2015 study period. These data was obtained from the nearest weather stations to the study sites. Soil temperature was recorded monthly in the study sites.

### 3.6 Diagnosis of *L. aethiopica*

To demonstrate the incidence of *L.aethiopica* in the study area, thirty four patients clinically suspected of of the disease were tested through microscopic examination for the presence of amastigote forms in their tissue biopsies.

### 3.7 Data analyses

All the collected species of sandfly were counted and the abundance determined as the quantitiative counts per site. Differences in abundance were analyzed using Kruskall Wallis Test. Sex ratio of all the species was calculated as: No. of male/No. of

female × 100 and differences in sex ratios determined using ($\chi^2$) chi- square test. Shannon Weiner index was calculated to determine the diversity of the sandflies among sites. The spatial variation in abundance of the sand fly as well as natural infection was determined using chi square test. The environmental factors were recorded as mean per replicate and the differences per site analyzed using One Way ANOVA and spatial and temporal differences analyzed using Two Way ANOVA. The relationships between environmental factors and abundance of the sandfly were determined using correlation coefficient. In case where data was found not to follow normal distribution (heteroscedastic), log (x+1) transformation was used to normalize all the biological data before applying any statistical procedure (Carroll, 2017). All statistical analyses were performed with a version of STATISTICA 6.0 (StaSoft, 2001).

# CHAPTER FOUR

# RESULTS

## 4.1 Distribution of phlebotomine sandfly vectors in Mt. Elgon Region

A total of 657 sandfly specimens belonging to one subgenus (*Larroussius*) and one species of *Phlebotomus pedifer* were collected. The distribution of *Ph. pedifer* sandfly in terms of abundance of species at 2 sites in Trans Nzoia (Cheptobot cave 1 and Cheptobot cave 2) and in Bungoma (Chemai and Kimkung caves) is provided in Table 4.1. There were significant differences in the abundance of *Ph pedifer* among the four sampling sites (Kruskall-Wallis Test; H = 13.564, df = 1, P = 0.0002). The abundance of *Ph. pedifer* sandfly was observed to be highest in Cheptobot Cave 2 followed by Cheptobot Cave 1 both in Trans Nzoia followed by Kimkung cave and was lowest in Chemai cave both located in Bungoma County.

**Table 4.1**: Phlebotomine sandflies captured with CDC light traps in the four sampling sites in Mt. Elgon Region

| Counties | Sampling sites | No. of sandfly captured | Percentage |
|---|---|---|---|
| Bungoma | Chemai | 72 | 40.0 |
| | Kimkung | 108 | 60.0 |
| | **Sub-total** | **180** | **100** |
| Trans Nzoia | Cheptobot cave 1 | 288 | 60.4 |
| | Cheptobot cave 2 | 189 | 39.6 |
| | **Sub-total** | **477** | **100** |
| | **Grand total** | **657** | |

The study also determined the distribution of the sandflycatches by sex ratio (Table 4.2). The sex-ratio indicated higher number of female than males were collected at both sites ($\chi^2$ = 21.6532, df = 1, P = 0.0000). The overall male/female ratio was 1:2.75 in Bungoma and 1:2.6 in Trans Nzoia. These traps also collected large numbers of non-target organisms, including non-biting flies and Lepidoptera.

**Table 4.2**: Phlebotomine sandflies captured by CDC light traps by sex in the four sampling sites in Bungoma and Trans Nzoia

| County | Sampling sites | Male | Female |
|---|---|---|---|
| Bungoma | Chemai | 10 | 55 |
| | Kimkung | 38 | 77 |
| | **Sub –totals** | **48** | **132** |
| Trans Nzoia | Cheptobot cave 1 | 57 | 161 |
| | Cheptobot cave 2 | 76 | 183 |
| | **Sub –totals** | **133** | **344** |
| | **Grand totals** | **181** | **476** |
| Chi-square | $\chi^2$ | *21.6532* | |
| | *Df* | *1* | |
| | *P value* | *0.0000* | |

The Shannon-Weinner diversity index (H') on the species sampled in Bungoma and Trans Nzoia is shown in Figure 4.1. Highest diversity index was recorded for the females in both study areas with Trans Nzoia recording higher diversity index than Bungoma.

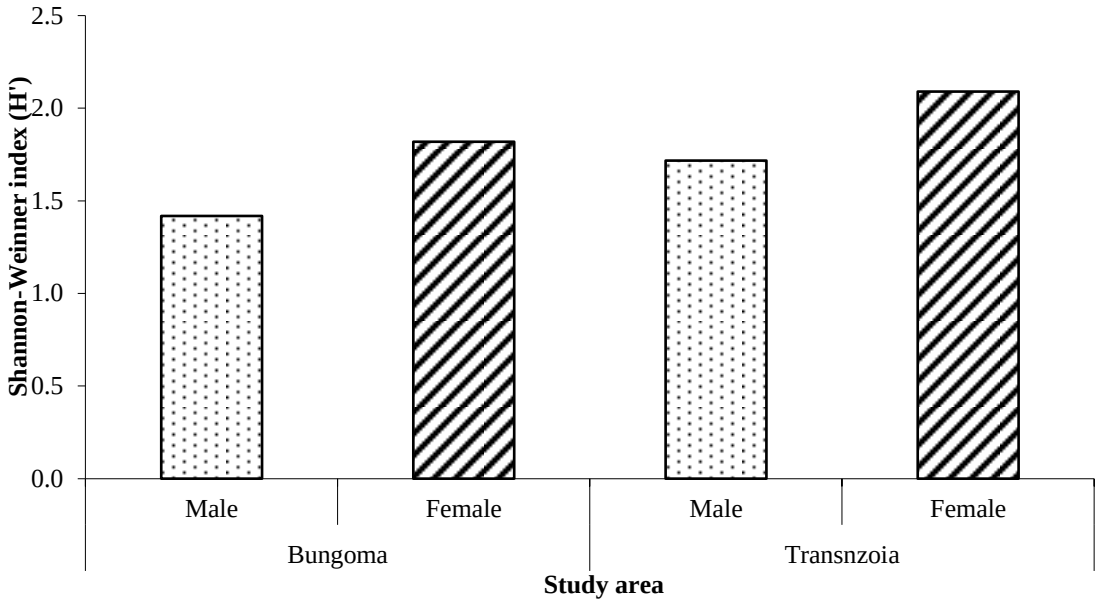


**Figure 4.1**: Shannon-Weinner diversity index (H') on differences in sex ratios of sandfly in Bungoma and Trans Nzoia sites.

The overall spatio-temporal variation of *Ph. pedifer* during the study period is shown in Figure 4.2. There was a singnificant spatio-temperal variation in *Ph. pedifer* catches in Trans Nzoia ($\chi^2$ = 11.6543, df = 11, P = 0.0031) and Bungoma ($\chi^2$ = 23.7122, df = 11, P = 0.0000) Counties. The *Ph. pedifer* sand flies total population was strongly marked with changes during the dry and the rainy seasons: Highest peak abundance of *Ph. pedifer* in both Trans Nzoia and Bungoma oocured during the dry seasons starting January to April and lowest peak occurred during the June to August dry season. The long rainy season resulted in the lowest recorded abundance of *Ph. pedifer* followed by the short rainy periods. Thus the temporal fluctuations of population density of *Ph. pedifer* was due to the seasonality

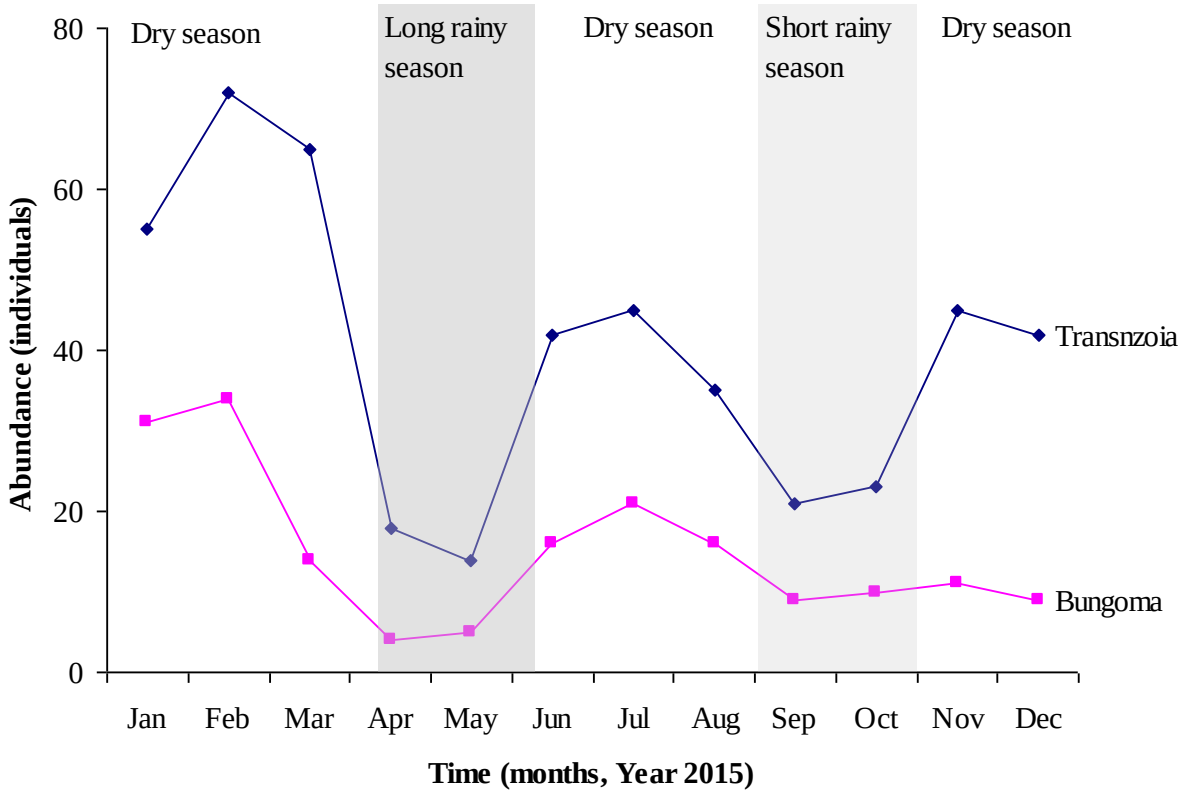


**Figure 4.2**: Spatio-temporal variation of *Ph. pedifer* sandfly in Bungoma and Trans Nzoia during the year 2014

### 4.2 Distribution in natural infection of phlebotomine sandfly by *Leishmania* promastigotes

Results showing natural infection of *Ph. pedifer* sandflies with *Leishmania* spp among the dissected species from Bungoma and Trans Nzoia are shown in Table 4.3. There were significant differences reported in the natural infection rates between Bungoma and Trans Nzoia ($\chi^2$ = 17.5432, df = 9, P = 0.0001). In Bungoma, natural infection rates of 8.9% while in Trans Nzoia infections rates over 18.5%. All the infected sandfly were females, with males showing no sign of natural infections.

**Table 4.3:** Results of dissection for natural infection with *Leishmania* species in phlebotomine sandflies by sex in the four sites of Bungoma and Trans Nzoia Counties. Percentage infection are in parenthesis

| | | Number dissected | Natural infection | |
|---|---|---|---|---|
| County | Sampling sites | (Male:Female) | Male (%) | Female (%) |
| Bungoma | Chemai | 25 (12:13) | 0 (0.0) | 2 (15.0) |
| | Kimkung | 20 (9:11) | 0 (0.0) | 2 (18.1) |
| | **Sub-totals** | **45 (21:24)** | **0 (0.0)** | **4 (16.7)** |
| Trans Nzoia | Cheptobot cave 1 | 68 (32:36) | 0 (0.0) | 9 (25) |
| | Cheptobot cave 2 | 56 (29:27) | 0 (0.0) | 8 (29.6) |
| | **Sub-totals** | **124 (61:63)** | **0 (0.0)** | **17 (26.9)** |
| | **Grand- totals** | **169 (82:87)** | **0 (0:0)** | **21 (42:16)** |

Monthly monitoring of natural infections of sand fly with *Leishmania* spp during the 1-year-study period is shown in Figure 4.3. Peak natural infections of *Ph pedifer* with *Leishmania* spp were observed during the dry seasons between January and April and also during the June to September in both Bungoma and Tranzoia. Long rainy season recorded the lowest natural infections rates in both Bungoma and Trans Nzoia.

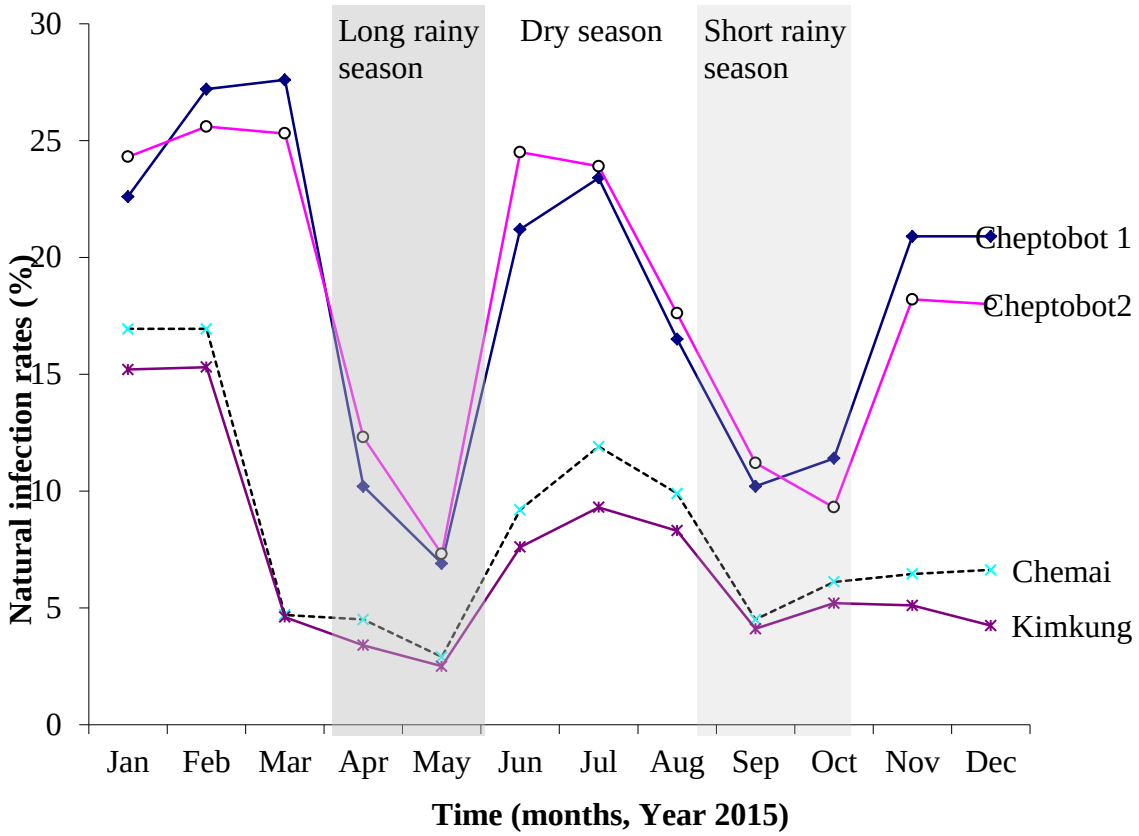


**Figure 4.3**: Spatio-temporal variation of *Ph. pedifer* sandfly natural infection with *L. aethiopica* in Bungoma and Trans Nzoia during the year 2015

### 4.3 Environmental risk factors for the transmission of *Leishmania* by phlebotomine sand fly

To understand the conditions that determines this temporal distribution of sand flies, data of ambient temperature, soil temperature, rainfall, and relative humidity in Bungoma and Trans Nzoia, during 2015 survey was recorded (Figure 4.4). Ambient air as well as soil temperatures were significantly ($P < 0.05$) higher in Trans Nzoia than Bungoma. Relative humidity was consistently higher in Trans Nzoia than Bungoma and showed significant changes during the year.

**Table 4.4**: Comparisons of ambient and soil temeprature, rainfall and relative humidity recorded at the sampling locations between Bungoma and Trans Nzoia Counties during the study period

| | Counties | | ANOVA | |
|---|---|---|---|---|
| Environmental variable | Bungoma | Trans Nzoia | F | P |
| Ambient temperature (°C) | 20.0 ± 1.9 | 22.6 ± 2.1 | 13.4232 | 0.0031 |
| Soil Temperature (°C) | 23.8 ± 2.2 | 28.6 ± 2.5 | 17.9834 | 0.0000 |
| Rainfall (mm) | 1085 ± 119 | 823.5 ± 90.6 | 21.20932 | 0.0000 |
| Relative humidity (%) | 47.1 ± 7.1 | 64.2 ± 0.8 | 9.2312 | 0.0043 |

The seasonal variations in the environmental parameters are in Figure 4.4. Highest ambient and soil temperatures occurred in Trans Nzoia compared to Bungoma throughout the study period with dry season recording markedly higher temperatures than rainy season.

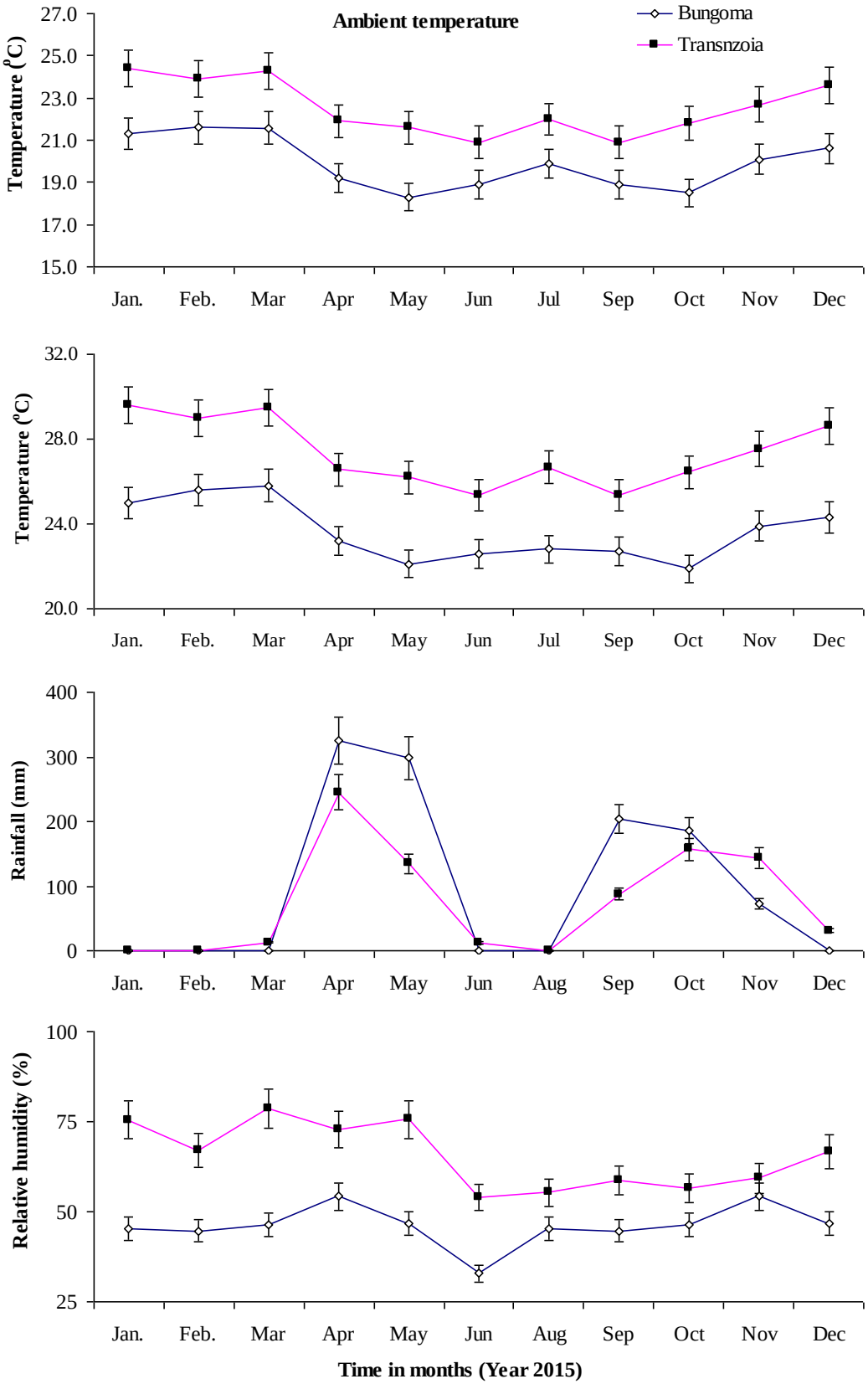


**Figure 4.4:** Spatial variation in the environmental conditions of the study locations (Values are means ± Standard Deviation)

The correlation between the abundance of sandfly and enevironmental condictions are shown in Table 4.5. Abundance of sandlfy was positively correlated with soil temperature, ambient temperature and relative humidity, but negatively with rainfall.

**Table 4.5:** Correlation analysis of environmental variables and sandfly collected during the study

| *Environmental variables* | *Amb. Temp* | *Soil temp* | *Rainfall* | *Rel_humidity* | *Sand fly* |
|---|---|---|---|---|---|
| Amb. Temp | 1 | | | | |
| Soil temp | 0.9903 | 1 | | | |
| Rainfall | -0.4793 | -0.4002 | 1 | | |
| Rel_humidity | 0.7556 | 0.7936 | 0.0355 | 1 | |
| Sand fly | 0.4812 | 0.6792 | -0.8097 | 0.6414 | 1 |

### 4.4 Incidences of leishmaniasis infections

The incidecens of *L. aethipica* infection and Leishmaniasis was observed in Kwanza constituency in Trans Nzoia County with the upper face and body indicating nodules of the victims (Plate 4.1). In Bungoma County, three cases were found two advancing cases were found in Kimkung, a homestead where a young child aged 7 years was developing new nodules (Plate 4.2). Even though the homestead is semi-urban, it also has a cave where the parents reported that children sometimes go inside to play. Within the same area young boy also had nodules developing on the face. In this site which is close to the urbanized Kapsokwony town, there was only one named Chemai that was studied.

**Plate 4.1**: The Kwanza observed case of *L. aethiopica* showing nodules on the face and upper body (Source: Author, 2015)

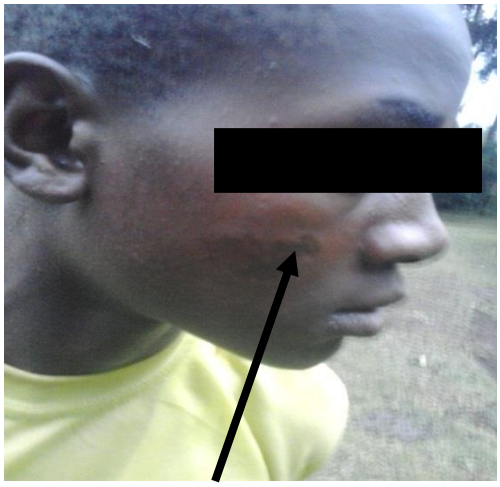

**Plate 4.2**: Observed case of *L. aethiopica* at Kimkung with fresh developing nodules (Source: Author, 2015)

# CHAPTER FIVE

# DISCUSSION

## 5.1 Distribution in phlebotomine sandfly vector in Mt. Elgon Region, Kenya

This study determined the taxonomic composition and the spatio–temporal distribution of sand flies at the micro-geographical level in Mt. Elgon regions within part of Bungoma and Trans Nzoia Counties of Western Kenya. The study established that the sandfly specimens in the region belong to one subhenus (*Larroussius*) and one species of *Ph. pedifer*. In a previous study in Kenya, it was established that *Phlebotomus* genus was represented by only one species, *Ph. (Phlebotomus) duboscqi* Neveu-Lemaire (Anjili *et al*., 2011), which is the only known vector for human cutaneous leishmaniasis caused by *L. major*. However, given the limition of diagnostics during the study where only microscopic examination was used, we cannot rule put occurrence of other species of phlebotomine sandflies. Caution is expressed in this study because of the method of analyis of the sandlfly species which may have not accurately identified other sandfly species. For instance, Young and Duncan (2012) and Depaquit *et al*. (2000), established that when molecular methods of identification of sandlfy was used compared to microscopic methods there were more than 15 species that could be identified compared to only 2 species.

The occurrence of these flies in the region especially in Bungoma in areas closer to Mt. Elgon contradicts earlier studies (e.g. Killick-Kendrick *et al*., 1997) who stated that there was no occurrence of such species. Also the type of traps used most used may have yielded small or no catches (Alexander, (2000), so Bungoma and Trans Nzoia region could include a greater density than that observed in our study.

Nevertheless, during the study, Trans Nzoia had higher phlebotomine sandfly than Bungoma, which suggest that sandfly in Bungoma are new incidences since there have been previous reports of occurrence of Leishmaniasis in TansNzoia (Sang *et al*., 1993). Previous studies have established that distribution and incidence of sandfly are both influenced by human behavior and environmental variables affecting the vector and the reservoir populations (Anderson *et al*., 2013).

During the study, there was higher number of females than males at both sites. The overall male/female ratio was 1:2.75 in Bungoma and 1:2.6 in Trans Nzoia. This is in agreement with other studies done elsewhere (Killick-Kendrick, 1999; De Luca *et al*., 2003; Andrade *et al*., 2007). In another study, Feliciangeli (2004) found that males were almost twice abundant as females, while using CDC light traps, where it was found that female sand flies account for 79% of the catch. The current results could be explained by the type of traps used rather than the disproportion in the natural sex-ratio of sand flies captured in Bungoma and Trans Nzoia Region. The dispersion capacity is reduced for males than for females (Yuval *et al*., 2009) due to the fact that females need blood meals for egg production every few days (Killick-Kendrick, 1997). Since sandflies are poor in flight, the high number of captured females provides evidence of the presence of sand flies breeding sites around human habitations.

There was a significant spatio-temperal variation in *Ph. pedifer* abundance in Trans Nzoia, and Bungoma, where *Ph. pedifer* sand flies population was strongly marked with changes in the rainy seasons: Highest peak abundance of *Ph. pedifer* in both Trans Nzoia and Bungoma ocured during the dry seasons starting January to April

with a lower peak in the June to August during the dry dry season. Thus the temporal fluctuation of population density of *Ph. pedifer* showed marked seasonality. This bimodal profile was nearly the same as those observed in other previous studies in different parts of the world; in Syria for *P. sergenti* and *P. papatasi* (Maroli *et al*., 2009) and in Morocco for *P. papatasi*, *S. minuta* and *S. fallax* (Killick-Kendrick, 1999). Sandfly breed mainly during dry season and this could explain the high population during the dry season. When temperatures were very high and with low rainfall, few species were registered during the survey days or the month prior to sampling, vector activity and thus vector captures were limited (Gálvez *et al*., 2010).

Shannon information index takes into account the number of species and their frequency, so this index expresses more correctly the diversity of each sector. It was observed clearly that the diversity in Trans Nzoia was higher than that of Bungoma. The general theories stipulated that diversity usually is reduced gradually form population origin to newly colonized areas. It appears that Trans Nzoia contains the reservoir of the population source. The differences in the species abundance between the two areas of study point out that the environment disturbance action brought about by man in these regions has caused great alterations regarding these sand fly species distribution rates. It also contributes to visualize the importance of maintaining these forest areas for the balance of the entomological populations and their interaction with the neighboring community.

An important factor in the distribution of sand flies could be the altitude and accompanying differences in bioclimate and vegetation. In the present study sand fly distribution varied notably between sampling sites according to altitude. *P. ariasi*

showed a wide altitudinal range in Andorra, being captured between 800 m and 2200 m a.s.l. (highest point at 2141 m asl), whilst *P. perniciosus* was not located above 1000 m a.s.l. Sand flies around the world are found from below sea level to 3300 m a.s.l. (Killick-Kendrick, 1999), but in temperate regions they do not usually occupy sites over 1000–1500 m a.s.l. The maximum altitudinal distribution of *P. ariasi* and *P. perniciosus* varies among western European countries. Spain is where they have shown the greatest altitudinal level whilst in France and Italy these sand flies are not present at high altitudes in the northern regions or they are substituted by other species.

A smaller number of sand flies were collected than in other studies conducted in Minas Gerais and other areas of Brazil (Moreno *et al*., 2005; Lainson and Rangel, 2005; Young and Duran, 2014). This may be explained by variability in the climatic characteristics between the study sites. Kapsokwony in Mt. Elgon sub-county where Chemai and Kimkung' sites have higher levels of rainfall, colder nights, located near the forest and higher altitude than Kaptobot sites in Trans-Nzoia County.

The findings presented in this study make up the first information regarding the sand fly fauna found at these altitudes, and contribute to the knowledge on insect distribution, as well as to the better understanding of the leishmaniasis vector epidemiology in the lower and upper Mt. Elgon Regions. It also contributes to information on the importance of maintaining these forest areas and the caves for the balance of the entomological populations and their interaction with the neighboring community.

### 5.2 Distribution of natural infection by *Leishmania* in *Phlebotomine* sandfly species in Mt. Elgon Region, Kenya

During the study, a total of 169 sandfly were dissected, of which the natural infection by *Ph. pedifer* was found to be in the range of 16 to 26%, described as moderate levels of natural infection in other studies (Dawit *et al*., 2013). This study concurs with previous studies that established that all proven vectors of *Leishmania* are closely related members of subgenus *Phlebotomus* (Killick-Kendrick, 1997). The infection of the sandfly is controlled by polymorphic, specific structures on the parasite lipophosphoglycan (LPG) as shown by binding of purified LPG to midguts *in-vitro* and by LPG inhibition of the binding of promastigotes *in-vitro* and by failure of LPG-deficient mutants to persist in the sandfly (Oryan *et al*., 2007). Strong vector competence of *Ph. pedifer* is attributed to selection for the unique, highly substituted LPG in *Leishmania* spp, which can bind to specific midgut receptors of these sandflies (Killick-Kendrick, 1999).

In this study, *Leishmania* spp naturally infected phlebotomine sandfly, though at low rates compared to other studies including the the natural infection in *Lutzomyia cruzi* and *Lutzomyia forattinii* by *Leishmania infantum chagasi* as well as natural infection *Lutzomyia (Pintomyia) fischeri* by *Leishmania* (Pita-Pereira *et al*., 2008). The species *Ph. pedifer* is anthropophilic and bite man and the presence of as high as 27% infection by *Leishmania* spp makes the area especially Trans Nzoia high risk for leishmanianis. Mechanisms underlying this susceptibility of the sandfly species are currently under investigation: O-glycosylated epitopes on midgut epithelium seem to be involved in this phenomenon (Volf, unpublished data). This work demonstrates the

feasibility of *Ph. pedifer* being a CL vector, but this species of has not yet been proved to transmit *Leishmania* spp. in nature.

Natural infection of *Ph. pedifer* sandflies with *Leishmania* spp was different betweeen the sampling sites. Bungoma had lower natural infection rates of 8.9% than Trans Nzoia with infections rates of 18.5%. The high natural infection may be associated with differences in the environmental parameters within the area. High natural infection has been established to be higher in areas of high temperature and humidity (Marlet *et al*., 2003; Kolaczinski *et al*., 2008; Maroli *et al*., 2009).

Peak natural infections of *Ph pedifer* with *Leishmania* spp were observed during the dry seasons between January and April and also during the June to September in both Bungoma and Trans Nzoia. During the long rainy season, there were low natural infections rates in both Bungoma and Trans Nzoia., probarbly because the high temperature during dry season favour breeding of the species. All the infected sandfly were females, with males showing no sign of natural infections. These findings show that female *Ph. pedifer* sandflies was highly susceptible to *Leishmania* spp, supporting multiplication and development of parasite species as in their natural vectors.

**5.3 Risk factor of soil and ambient temperature, rainfall and relative humidity on the transmission of *Leishmania* by phlebotomine sand fly**

During the study, the abundance of sandlfy was positively correlated with soil temperature and ambient temperature, suggesting that the population of the sandflies

increased at higher temperature, which has been attributed to optimal condition for breeding.

Higher rainfall however, negatively influenced the population of sandflies which concurs with a a study carried out in Venezuela on the abundance *Lutzomyia spinicrassa* relative to precipitation (Perruolo *et al*., 2006). Although rainfall frequency and quantity were not measured and compared between the villages in the present study, qualitative observations indicated that during periods of higher rainfall, there was less observed incidences of sandfly species in the region, suggesting that reainfall discouraged multiplication of the vector species (Ostfeld *et al*., 2004). Rebollar-Téllez *et al*. (1996) found that abundances of *L. cruciata* were almost null during the rainy months in the village of La Libertad, Campeche. Sánchez-García *et al*. (2010) showed that the biting rates of *B. o. olmeca*, *L. cruciata* and *Psa. shannoni* decreased during the rainy season in the state of Quintana Roo, Mexico. At both study sites, the four most abundant sandfly species were those considered important in the transmission of leishmaniases to humans (*B. o. olmeca*, *L. cruciata*, *Psy. panamensis* and *Psa. shannoni*).

It was further observed that humid conditions encouraged increased population of the sand flies. One of the determining factors in the development of the immature sandfly is humidity and some species have been observed to present tropical quiescence in the egg stage or in the pupal stage during periods of drought and relatively dry season (Killick-Kendrick, 1999). Thus, damp environments favour the hatching of larvae, yet excessive rain reduces the number of larvae (Marcondes, 2007).

The distance from known Mt. Elgon Endebess foci where *L. aethiopica* was first reported (Mutinga, 1975) and later (Dawit *et al*., 2013) where the two sand fly species *Ph. pedifer* and *Ph. elgonensis* were reported are far from the new Trans Nzoia sites of Cheptobot and the Kimkung' sites of Kapsokwony Bungoma, range from 15- 43 kilometres. It is unlikely that the cases that were found within the study area could have acquired the infections elsewhere apart from within the study sites. This is because through Mark - release - capture studies using fluorescent powders on *Lutzomyia shannoni* and *Lu. gomezi* showed that passively, sand flies can fly up to 960 m in 36 hours after release (Alexander and Young, 1992).

Since *Ph. pedifer* were caught in the peri-urban areas where *L. aethiopica* cases were, it can be concluded that transmission is actively going on in these sites. Sand flies even though were caught in small numbers during and after the rainy season, in the same sites; the sand flies breed and rest in these sites where disease transmission takes place. It is yet to be known when transmission takes place.

The spread of Leishmaniases is always determined by the presence of the vector and a reservoir host (Killick-Kendrick, 1997). In the absence of a known reservoir host, apart from what has been reported in Chemai, it is possible that the disease could be evolving from zoonotic to anthropotonic. A similar trend was reported in the Middle East where *L. tropica*is anthropotonic and the vector, *Ph. papatasi* is found both in urban and rural areas Confirmation of the actual ecology of the disease especially in the study areas can only be done through more studies which should involve trapping of suspected animal reservoirs, parasite isolation and surveillance of new cases in the new study sites.

The cave is in the compound and children are known to enter and play in the cave where they can be bitten by infected sand flies apart from the two children where lesions are found only on the exposed face and hand, the adult cases had lesions even on the back and abdomen which indicates that transmission could be occurring indoors on and uncovered body. It is also possible that the infected individuals do not use any protective measures as can be seen by multiple nodules for case one. It would be difficult for a delicate sand fly to cover a distance of between 15 to 43 km to establish a transmission. It would take a sand fly 45 days to reach the nearest peri-urban study site (Kwanza-15 km), which would reflect days to reach kimkung' (43 km). The maximum life span of sand flies has been reported to be 1.54 gonotrophic cycles for *Ph. ariasi* Tonnoir (Dye *et al*., 1987), which cannot be covered in travel energy, breeding, passive flight and hoping.

The low numbers of the sand flies caught suggests that if the sand fly was never originally in the case sites, then it could be having a long flight range. Nodules on back of human cases could be a sign of indoor transmission and failure to use protective mosquito bed nets hence exposing the back to sand fly bites. In Cheptobot cave, people are known to go, sleep inside and light fires as the pray/worship. This could be a source of infection and the begining of anthroponotic cycle since information since information given by the locals states that they come from different parts of the country. Considering that Chemai cave 1 yielded more sand flies, this cave may be the main breeding site of the vectors, which can acquire the disease and transmit within a large area where resting indoors is comfortable for them. Apart from outdoor transmission, indoor transmission is highly suspected since the case found at Kwanza had *L. aethiopica* nodules not only on the face but also on the shoulders and

back which are areas that are not easily accessible to the sand fly. The actual reservoir host within the peri-urban study sites needs to be investigated.

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

During the study, there was a total of 657 sandfly belong to one subgenus (*Larroussius*) and one species of *Ph. pedifer*, where higher abundance of phlebotomine sand flies occurred in Bungoma than Trans Nzoia, with higer females to male ratios. In terms of diversity, highest diversity index was recorded for the females in both study areas with Trans Nzoia recording higher diversity index than Bungoma. Also the highest peak abundance of *Ph. pedifer* in both Trans Nzoia and Bungoma oocured during the dry seasons starting January to April and lower peak in the June to August rainy season. The long rainy season resulted in the lowest recorded abundance of *Ph. pedifer* followed by the short rainy periods.

The highest catch *Ph. pedifer* sandflies was from Trans Nzoia (n = 124) compared to Bungoma (n = 45). There were significant differences reported in the natural infection rates in the sand flies between Bungoma and Trans Nzoia, natural infection rates of 8.9% were observed in Bungoma while in Trans Nzoia infections rates were over 18.5%.

Ambient and soil temperatures were significantly higher in Trans Nzoia than Bungoma. Relative humidity was consistently higher in Trans Nzoia than Bungoma and showed significant changes during the year. The effect of biotope, although it interferes with the number of recovered sticky traps, was a significant factor that

influences spatial distribution of sand flies. One case of *L. aethiopica* infection was observed in Trans Nzoia County and three cases in Bungoma County.

### 6.2 Recommendations

Based on the results of the current study, the following are recommended

1. The dry season should be targeted for intervention since more sandflies were captured during this season.
2. Caves near homesteads should be targeted for intervention.
3. The environmental characteristics identified as risk factors of cutaneous leishmaniasis in this study could help implementation of targeted vector control strategies.
4. People should be advised not to enter the caves because they can get infected when they are unprotected.

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