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# Peri-urban Cutaneous Leishmaniasis Transmission Dynamics with Regard to Associated Risk Factors in Mt. Elgon Focus, Kenya

**Mukhwana Wafula Dennis[1*], Makwali A. Judith[1], Ngeiywa Moses[1] and Anjili O. Chris[2]**

*[1]School of Biological Sciences, University of Eldoret, P.O.Box 1125-30100, Eldoret, Kenya.*
*[2]Centre for Biotechnology Research and Development, Kenya Medical Research Institute, P.O.Box 54840-00200, Nairobi, Kenya.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MWD designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MWD, MAJ, NM and AOC reviewed the study design and all drafts of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

The cutaneous leishmaniasis infection status caused by *Leishmania aethiopica* and the vector sand flies distribution in relation to risk factors were investigated in the sites of Bungoma and Trans-Nzoia counties of Mt. Elgon region. These sites are allopatric. The sand flies Collections from the sites were carried out for five nights of each month using Center for Disease Control (CDC) light traps. Mean monthly data of ambient temperature, rainfall, and relative humidity in the two Counties were recorded during the 2015 study period. These data was obtained from Kimilili and Kitale weather stations of Bungoma and Trans-Nzoia study sites respectively. Soil temperatures were recorded monthly in the study sites. Thirty four patients clinically suspected of the disease were tested through microscopic examination for the presence of amastigote forms in their tissue biopsies upon signing the consent form. 657 sand fly specimens were collected. In the two sites, both homesteads

**Corresponding author: E-mail: mukhwanadennis14@gmail.com;*

and caves where sand flies were trapped only *Phlebotomus pedifer* were found. Sand fly abundance 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. Basing on the results of this research, it is recommended that People should not enter the caves since unprotected to avoid infection. Furthermore, the actual reservoir host within the peri-urban study sites needs to be investigated.

*Keywords: Environmental risk parameters; allopatric; Phlebotomus pedifer; climate change; active transmission.*

## 1. INTRODUCTION

*Leishmania aethiopica* (Kinetoplastida: Tyrpanosomatidae) has been the only known foci in Kenyan Mt. Elgon slopes at the border of Kenya and Uganda Since 1970s [1-2]. A detailed investigation of the vectors in the old focus of Mt. Elgon has revealed the existence of a cavernicolous anthropophilic species, *Phlebotomus pedifer* (Diptera: Psychodidae) currently known to be the vector of Leshamaniasis [3]. The breeding sites and biology of *Phlebotomus pedifer* was investigated in the caves of the old focus of cutaneous leishmaniasis in Mt Elgon, Bungoma County, Kenya. *Phlebotomus pedifer* were found to rest mainly in the poorly lighted areas of the caves, on the roof, the floor of caves, under the objects, in the cracks and in the crevices inside the caves. The flies also were abundantly found in cracks and crevices outside the caves where hyraxes were found to rest. Immature stages of *P. pedifer* were recovered from wet areas of the floor of the caves [4].

Various factors have also been associated with the distribution of phlebotomone sandfly vectors. 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 [5]. The Changes in the environmental factors such as rainfall, temperature, humidity, wind patterns, soil factors, forest structure among other factors have resulted in changes in the vector population densities [6]. Furthermore, the increase in human travel has also enabled the spread of infectious agents of human and animal origin such as pets by introducing them into areas from which they had been hitherto absent [7].

Climate change and unpredictable weather patterns have resulted in shifts in distributions of several species such as that of sand fly [8]. These has seen emergence of sand fly species in areas where there were none in the past [9]. Most studies on the emergence of sand fly vectors have been conducted in Europe [10]. However, there is paucity of information on sand fly vector in many countries in Africa including Kenya, with most of the research done over a decade ago [11,12]. There is therefore limited research on the occurrence and distribution of the various species of sand fly in several parts of Kenya despite the evidence of climate change affecting the region. Studies have indicated that the distribution of sand fly 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 [13,14]. These factors may dictate the temporal and spatial distributions of sand fly vectors and thereby also alter the epidemiology and dynamics of disease transmission [14]. The limited studies that have been conducted in Kenya regarding the ecological niches of sand fly vectors have established that several species have overlapping ranges of distribution in areas of recurrent transmission of leishmaniases with some showing extensive distribution.

Despite existence of close relationships among climate and weather conditions, phlebotomine sandfly spread and leishmaniases are well documented. Limited investigations have been done in Western Kenya, despite the emergence of leishmaniasis in such areas. On the basis of the foregoing, this study evaluated the environmental risk factors associated with peri-urban *Leishmania aethiopica* transmission in the Mt. Elgon focus.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out in four sites of Mt. Elgon Region in Kenya (Fig. 1). The study sites were Cheptobot 1 and 2 (0°59'43.32"N and 34°49'8.76"E), Chemai (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) (Fig. 1). Annual mean temperature, rainfall and altitude ranged from 18°C–21°C, 1300 mm-1800 mm and 1500 m to 2000 m above sea level respectively. Cheptobot 1 and Cheptobot 2 are about 500 m apart and separated from Chemai and Kimkug by about 23 to 25 km. The study sites were selected after a survey and interviews with the local population and the Kapsokwony and Kwanza hospital staff in Bungoma and Trans-Nzoia counties respectively.

### 2.2 Collection and Identification of Sand Fly Species

Field collections were done monthly from January to December 2015. Collections of sand flies were carried out in four caves and nearby homesteads on simultaneous days. The cave were natural underground chamber in a hillside sometimes upto 500 m deep. Eight CDC (Centers for Disease Control) light traps were set up at each study site from 6:00 pm to 6:00 am during the study period. There were a total of 16 nights of collection during the entire study period. Sand flies collected from each catch were immediately preserved in ethanol.

### 2.3 Sorting of Sand Flies, Slide Mounting and Identification

Sand flies were preserved in 70% ethanol in the field. Specimens were cleared, dissected and permanently mounted on microscope slides and 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 [15]. Sand flies were identified by external morphology under a dissecting microscope and separated by sex before being counted. They were categorized by examination of the spermathecea (females) and the external genitalia (males) [16].

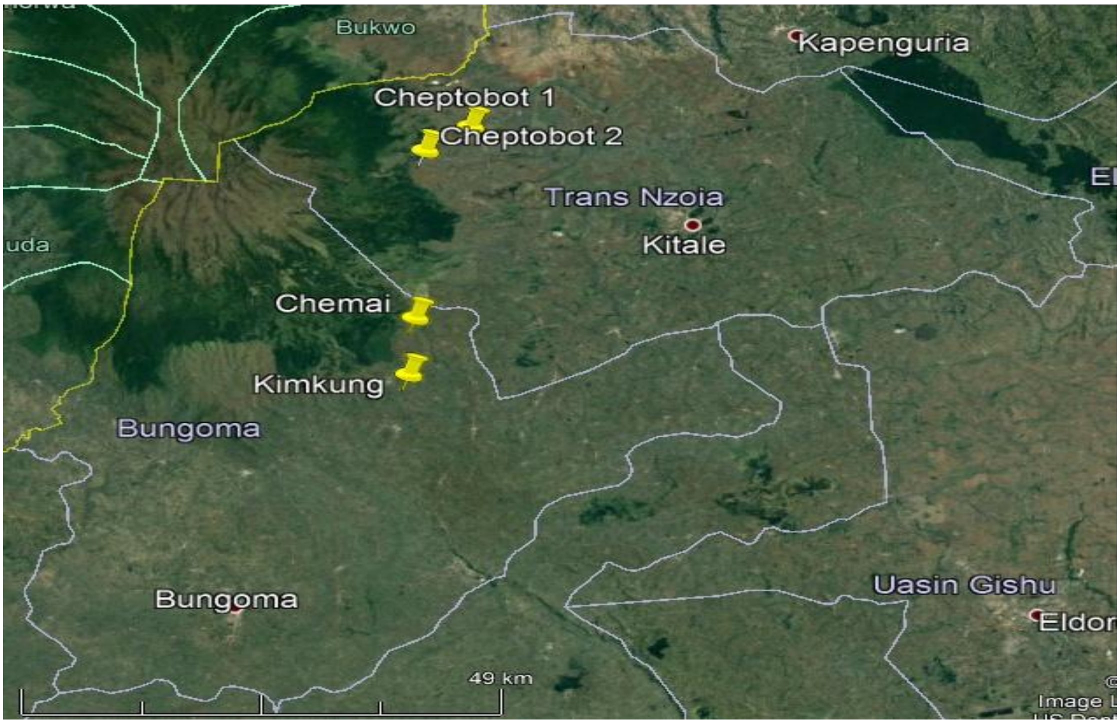


**Fig. 1. Map of Bungoma and Trans-Nzoia County showing the position of the sampling sites (Courtesy of Google maps)**

### 2.4 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 Counties was recorded during the 2015 study period. These data were obtained from the Kimilili and Kitale weather stations for the Bungoma and Trans-Nzoia study sites respectively. These stations are located about 5.5 to 9 km from the sampling sites respectively. Soil temperatures were recorded at 2 inches below the soil surface monthly in the study sites using soil thermometer.

### 2.5 Diagnosis of *L. aethiopica*

A total of 34 patients clinically suspected of leishmaniasis were tested through microscopic examination for the presence of *L. aethiopica* amastigote forms in their tissue biopsies upon signing consent form.

### 2.6 Data Analyses

Collected species of sand fly were counted and the abundance determined quantitatively per site. Data count for all the 4 sites were summarized in the figures and trends determined since the four sampling sites are in the same geographical area with very little differences in weather conditions. The environmental factors were recorded as mean per replicate and the differences per site analyzed using One Way ANOVA. The relationships between environmental factors and abundance of the sand fly were determined using correlation coefficient. Correlation analysis was done between individual data pairs for example count data versus environmental data for each station for 12 months. All statistical analyses were performed with a version of STATISTICA 6.0 [17].

### 2.7 Ethics Statement

The study received approval from the Ministry of health. Capture sites were located in private areas, and all owners gave permission to conduct the study in these sites. The study subject gave an informed consent.

## 3. RESULTS

### 3.1 Distribution of Phlebotomine Sand Fly Vectors in Mt. Elgon Region

A total of 657 sandfly of subgenus *Larroussius* (*Phlebotomus pedifer*) were collected. The distribution of *Ph*. *pedifer* sandfly in terms of abundance of species at the two sites in Trans-Nzoia (Cheptobot cave 1 and Cheptobot cave 2) and in Bungoma (Chemai and Kimkung caves) (Table 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 were 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.

### 3.2 Environmental Risk Factors for the Transmission of *Leishmania* by Phlebotomine Sand Fly

To understand the conditions that determines 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 (Fig. 2). 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 1. Phlebotomine sand flies captured with CDC light traps in the four sampling sites in Mt. Elgon Region during the 2015 study period**

| 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** | |

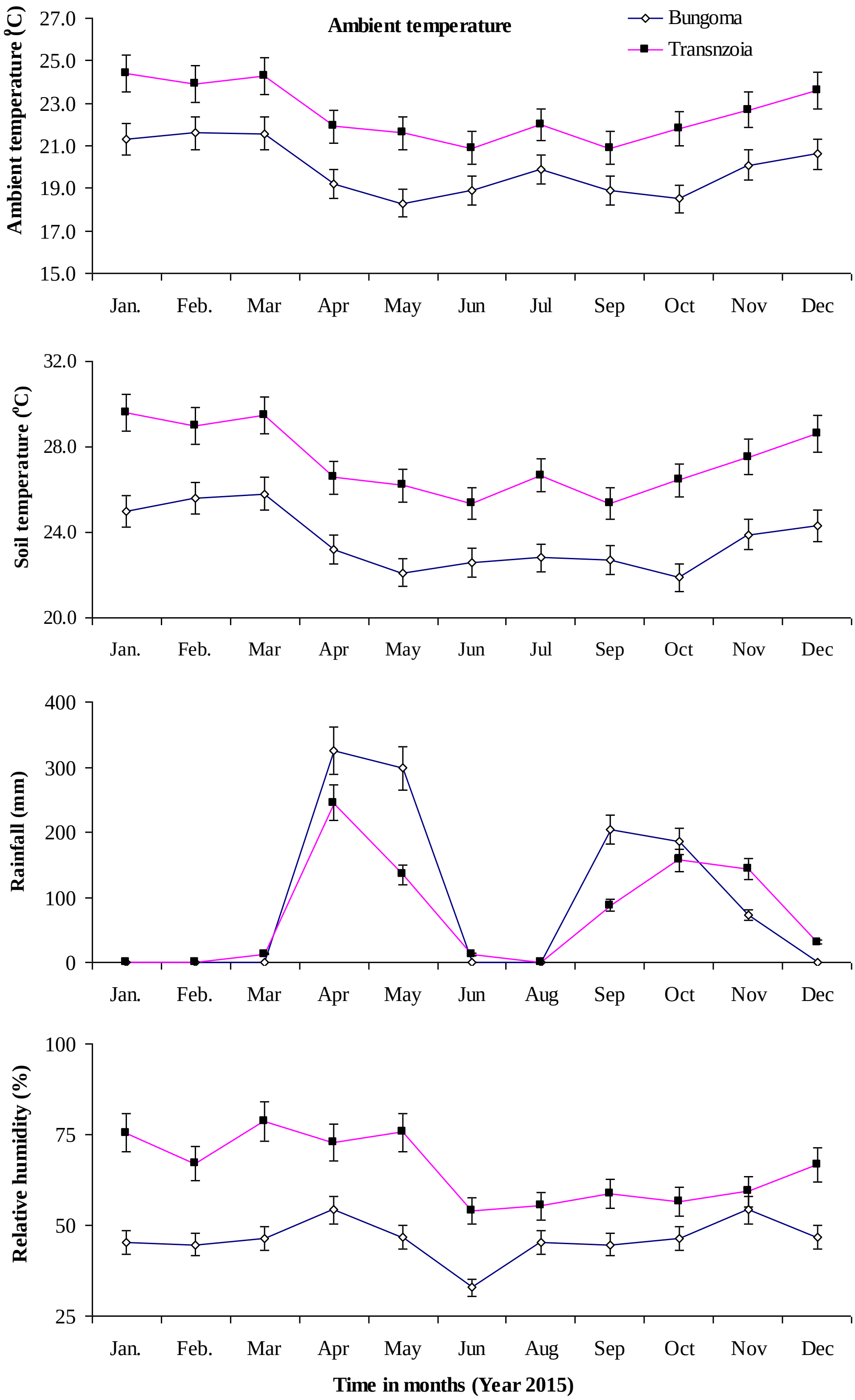


**Fig. 2. Spatial variation in the environmental conditions of the study locations. The correlation between the abundance of sandfly and environmental conditions are shown in Table 3. Abundance of sand fly was negatively correlated with soil temperature, rainfall and relative humidity**

**Table 2. Comparisons of environmental variables recorded at the sampling locations between Bungoma and Trans-Nzoia Counties during the 2015 study period**

| Environmental variable | Counties | | ANOVA | |
|---|---|---|---|---|
| | 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 |

**Table 3. 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 | 0.0148 | -0.0792 | -0.5097 | -0.4314 | 1 |

The seasonal variations in the environmental parameters are in Fig. 2. 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.

### 3.3 Incidences of Leishmaniasis Infections

*Leishmania aethiopica* is the causative organism of leishmaniasis in this focus. Clinical picture of the most identified cases in this study were consistent with *L. aethiopica* infection. This study established four cases of suspected *L. aethiopica* infection. The effect of *L. aethiopica* was observed in one case in Trans-Nzoia County with the upper face and body indicating nodules of the victims (Plate 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 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. This cave has never been studied for the presence of animal reservoirs in Kapsokwony.

## 4. DISCUSSION

This study was carried out at four sites within Mt. Elgon Region, in the outskirts of two major urban centres of Kitale and Kimilili Towns as well as within the vicinity of Kapsokwony Town thus the areas are regarded as rural-urban transition areas or peri-urban and currently have massive settlements of human populations. It was determined that abundance of sand fly was negatively correlated with soil temperature, rainfall and relative humidity. Although rainfall frequency and quantity were not measured and compared between the villages in the present study, qualitative observations indicated that more days of low intensity of rain occurred in the region, which encourages the occurrence of the vector. It is possible that the area is usually more humid because its vegetation has been better conserved. One of the determining factors in the development of the immature sand fly is humidity and some species have been observed to present neotropical quiescence in the egg stage or in the pupal stage during periods of drought and heavy rain [18], affecting the productivity of adults. Thus, damp environments favour the hatching of larvae, yet excessive rain reduces the number of larvae.

The distance from known Mt. Elgon Endebess foci where *L. aethiopica* was first reported [1], and later [19], where the two sand fly species *Phlebotomus pedifer* and *Phlebotomus elgonensis* were reported are far from the new Trans-Nzoia sites of Cheptobot and the Kimkung' sites of Kapsokwony Bungoma, range from 15-43 km. 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 [20], Since *Phlebotomus pedifer* were caught in the peri-urban areas where *Leishmania 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.

**Plate 1. The Kwanza case of *L. aethiopica* infection showing nodules on the face and upper body (arrow)**

**Plate 2. Kimkung *L. Aethiopica* infection with fresh developing nodules (arrow)**

The spread of Leishmaniases is always determined by the presence of the vector and a human or animal reservoir host [18], 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 *Leishmania tropica*is anthropotonic and the vector, *Phlebotomus papatasi* is found both in urban and rural areas.

*Phlebotomus elgonensis* whose females are inseparable form *P. pedifer* reported to be non-vector on higher altitudes of Mt. Elgon [21], and its absence in the peri-urban sites, this is a confirmation that the only vector in the study area is *P. pedifer.* The finding of *Leishmania aethiopica* cases aged 7-10 years who have never left the homestead in Kimkung' which is 43 kilometers away from the previously known endemic Endebess area suggests that active transmission is still going on. 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. A part from this, peri-domestic transmission due to either presence of vectors or them flying to household areas from caves may result in sand fly bites on uncovered back and chest of people within the houses. Inhabitants coming in to close contact with cave areas or adjacent peri-domestic areas may also be subjected to sand fly bites on uncovered body parts. 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 43 km in order to establish a transmission. The maximum life span of sand flies has been reported to be 1.54 gonotrophic cycles for *Phlebotomus ariasi* Tonnoir [22], 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. 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 beginning of anthroponotic cycle since information since information given by the locals states that they come from different parts of the country. Within Kimkung homestead are caves where children play even though only one *P. pedifer* was found, this could be the vector in the homestead. 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 human or animal reservoir host within the peri-urban study sites needs to be investigated.

## 5. CONCLUSION

The occurrence of moderate to high abundance of a single species in sand fly suggest increased spread of these species beyond their areas of endemicity. The occurrence of *Phlebotomus pedifer* in the peri-urban areas where *Leishmania aethiopica* cases were. It can be concluded that transmission is actively going on in these sites. This represents a newly identified focus of cutaneous leishmaniasis due to *L. aethiopica.*

## 6. RECOMMENDATIONS

The environmental characteristics identified as risk factors of cutaneous leishmaniasis in this study could help implementation of targeted vector control strategies.

Further investigations on identification of the actual reservoir host in the within the peri-urban study sites needs to be done.

## CONSENT

Thirty four patients clinically suspected of the disease were tested through microscopic examination for the presence of amastigote forms in their tissue biopsies upon signing the consent form. The study subject gave an informed consent.

## ETHICAL APPROVAL

The study received approval from the Ministry of health. Capture sites were located in private areas, and all owners gave permission to conduct the study in these sites.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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