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Leishmania Parasite Infections and Blood Meal Source Apportionment in Sand Fly Vector Species in Mt. Elgon Habitats (Kenya)

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Abstract
Aim: The aim of the current study was therefore to determine the *leishmania* parasite infections and blood meal sources in Mt. Elgon, Kenya.
Methodology: A total of 400 blood fed female sand flies were randomly selected from the study sites for blood meal source determination. Samples analyzed by ELISA method. Test for blood meal sources was done for bat, hyrax, cat, and human.
Results: The results indicated that females constituted 71.7% of the flies collected, while males constituted 28.3%. There were significant differences in *Leishmania* infection between the vector species ($F = 4.1671$, $df = 3$, $P = 0.022$). The genus was dominated by *Phleobotomus pedifer* (81.54%), *P. longipes* (12.83%), *P. elgonensis* (4.40%) and *Sergentmoyia schwetzi* (1.23%). The temporal trends showed significant differences relative to sampling months ($\chi^2 = 7.1934$, $P = 0.013$) where higher abundance of species occurred in dry months of February to April and November to April. A total of 5,688 sand flies were collected during the twenty four (24) month sampling period from the five study caves (for the period February 2017 to January 2019). There were significant spatial differences in species infection of vectors in the study area ($\chi^2 = 14.9445$, $df = 12$, $P = 0.0012$). Analysis by ELISA on 200 blood fed sand flies showed that 60% specimens fed on humans, 28% of blood fed sandflies fed on bats, 8% fed on hyraxes and 5% fed on cats.
Conclusion: Transmission of *Leishmania* parasites involved three reservoir hosts (bats, hyraxes and cats) and one vector species (sandfly). Therefore disruption of the life cycle of the *Leishmania* parasites should be done targeting these animals.
Keywords: Blood meal analysis; *Leishmania* parasites, Mt. Elgon, ELISA test.
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1. Introduction

Leishmaniasis remains a major vector-borne disease caused by a protozoa of the genus *Leishmania* (Torres-Guerrero *et al.*, 2017; Cunze *et al.*, 2019; Ghatee *et al.*, 2020), which is endemic in areas of the tropics, subtropics, Southern Europe and Central America (Hailu *et al.*, 2016). There is notable expansion of disease globally over the last decades (Wamai *et al.*, 2020) mainly because of climate change (Cotton, 2017). The transmission and epidemiology of leishmaniasis disease is complicated due to the complex life cycle of the parasites and the involvement of vectors, and reservoir animals besides human hosts (Al-Bajalan *et al.*, 2018).

There are more than 20 flagellate parasites of the genus *Leishmania* subgenera *Leishmania* and *Viannia* which are causative agents of Leishmaniasis (Cotton, 2017; Salah *et al.*, 2020). The Eurocentric worldview groups *Leishmania* parasites into Old World species: *L. major*, *L. aethiopica*, *L. infantum*, *L. donovani* and *L. tropica* (Maurício, 2018; Feres, 2019) and New World species such as *L. amazonensis*, *L. chagasi, L. mexicana*, *L. naiffi*, *L. braziliensis*, and *L. guyanensis* (Kevric *et al.*, 2015). In Eastern Africa Region, including Kenya, the more prevalent visceral leishmaniasis (VL) is usually caused by *L. donovani* and *L. infantum* (Kühne *et al.*, 2019; Bhunia and Shit, 2020).

The current only known vector of the leishmaniasis is the small dipteran fly known commonly as a sand fly. Sandflies play a major role in the epidemiology of leishmaniasis (Courtenay *et al.*, 2017). Phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae) are medically important group due to the fact that they are confirmed as natural vectors of *Leishmania* parasites (Trájer and Sebestyén, 2019; Waitz *et al.*, 2019). There are 900 species of sandflies of which about 10% of these species belonging mainly to genus *Phlebotomus* and *Lutzomyia* have been implicated as the vectors of Leishmaniasis (Cotton, 2017; Salah *et al.*, 2020; Sousa-Paula *et al.*, 2021).

A successful transmission requires the presence of pathogen, vector and host species (de Oliveira *et al.*, 2017; Martín-Sánchez *et al.*, 2020). The transmission of *Leishmania* parasites starts when parasites are picked up by the sand fly when feeding on the blood of an infected individual or an animal reservoir (Conceição-Silva and Morgado, 2019). Therefore analyses of blood meals for sand fly vectors must be carried out in habitats where reservoir hosts (co)exist with the flies. During blood-feeding, *Leishmania* parasites are either ingested with the blood meal or

regurgitated from an infected sandfly into the host (Dostálová and Volf, 2012). Since sandflies may feed on a variety of vertebrate hosts which may carry different species of *Leishmania* parasites, identification of the sandfly species and their bloodmeal sources is crucial in incriminating potential vectors, understanding the disease transmission dynamics and identifying the potential ecological reservoirs of the disease (Gitari *et al.*, 2018). Availability of such information is useful data in developing appropriate disease control and response strategies (Ngere *et al.*, 2020). Host blood-meal source analysis in sandflies is useful in determining the reservoir hosts of the *Leishmania* parasite species and whether the flies are anthropophilic or not. Rodents, dogs, goats and sheep (Mutinga *et al.*, 1989) have been found to be naturally infected with *Leishmania* parasites in Kenya. In the Mediterranean region, the dog is considered a major reservoir of visceral leishmaniasis and seemingly canine infections are mainly responsible for human visceral leishmaniasis (Ngere *et al.*, 2020). Man gets in touch with these animals in one way or the other, for example, rodents invading houses, goats sleeping inside human huts and the dog always being near its owner; so transmission can easily take place from these reservoirs to the human hosts so long as the vector is also present. Similarly, potential wild reservoir hosts such as the bats which inhabit caves where sand fly also inhabit have not been studied much and thus there is need to assess their status in the transmission of leishmaniasis.

2. Materials and Methods

2.1 Study Area

The study was conducted in Chemai and Chepkutunyi caves located in Mt Elgon in Bungoma County, Western Kenya. The study sites were Chepkutunyi A cave (N $00^0$49 881/E034^0 42 994/ elevation 1829 M), Chepkutunyi B cave (N $00^0$49 877/E $034^0$43 881/ elevation 1776M), Chepkutunyi C cave (N $00^0$45884/ E$034^0$44 998/ elevation 1845M) Chemai A ($0^0$49 881N/$034^0$43 233'E elevation 1827 M), Chemai B (N0°50 546'E $034^0$43 233/ elevation 1800M). This area was selected based on the increase in suspected CL cases among the people seeking medication at health facilities in Mt. Elgon Sub-County Hospital in Kapsokwonyi and other health facilities in the area. All the study sites had numerous fault scarp ranges, rock crevices and caves which are potential habitats for sandflies, giant rats and rock hyraxes (*Procavia capensis*) (Mukwana *et al;* 2018, Anjili *et al;* 2011). There was also evidence suggesting human activities in and around the study sites that included: observation of children playing, foot prints of animals, social evidence such as used condoms and Hyrax defecation. Consequently all these evidence may suggest a close interaction of humans, wild animals and sand flies found in the caves.

2.2 Sample size determinations

Sample sizes of bats, hyraxes, and cats were obtained using Fisher's Formula (Rosner. 1995):

$$n = \frac{Z^2 P(100 - P)}{d^2}$$

Z = 1.96

d = was taken from any value between 3 and 10

P = Value was based on prevalence rate of a study in Ethiopia (Kassahun *et al.,* 2015). That was 4.9% *Leishmania* kDNA detection in bats

The expected sample size for bats in each of the two caves was therefore

$$n = \frac{1.96^2 * 0.049(100 - 0.951)}{0.05^2} = 199$$

2.3 Bat, hyraxes and sand fly infection status with *Leishmania* parasites

2.3.1 Direct microscopy

Direct microscopy of the crushed spleen tissue smears of bats and hyraxes were fixed with methanol, and then stained with Giemsa as for thin film to view *Leishmania* bodies (amastigotes of *Leishmania*). *Leishmania* bodies are usually found within the macrophages. Some of the L.D bodies can be seen extra-cellularly, released from the macrophage cells ruptured during making of the film as previously described (Cheesbrough, 2005).

In stained preparations, the cytoplasm surrounded by a limiting membrane appears pale –blue. The nucleus is relatively large and stains red. The kinetoplast is situated at right angle to nucleus. The kinetoplast is slender, rod-shaped and is stained deep red. Axoneme arises from the kinetoplast and extends to the margin of the body. The unstained vacuole space is seen alongside the Axoneme (Mohaghegh *et al.*, 2013).

2.3.2 Analysis of blood meals sources in sandflies

Four hundred blood fed female sand flies were randomly selected from the study sites for blood meal source determination. Samples analyzed by ELISA method. Test for blood meal sources was done for bat, hyrax, cat, and human.

2.4 Data analyses

Details of all the collected sandflies including the trapping sites and compared for consistency using SPSS (version 23.0). Descriptive statistics were used to determine the distribution using frequency and percentage for each sandfly species per site. Species abundance was determined as the quantitative counts per trap per trapping night per site. Differences in the species prevalence were analysed using the Chi-square (χ^2) test. Differences in abundance of parasites in host tissues and blood, sandfly and reservoir hosts species per site and monthly distributions were analyzed using One-Way ANOVA. Significance was determined at $P \leq 0.05$.

3. Results

A total of 5,688 sand flies were collected during the two years sampling period from the five study caves (Table 1). There were significant spatial differences in species infection of vectors in the study area ($\chi^2 = 14.9445$, df = 12, P = 0.0012). Most of these collected species were *P. pedifer* (81%) followed by *P. elgonensis* (12%) and least was *S. schwetzi* (1.2%).

Table 1. Distribution of collected sandfly species and sex in five caves of Mt. Elgon area

Locality	*P. pedifer*	*P. elgonensis*	*P. longipies*	*S. schwetzi*	Total
Chemai A	694	103	60	18	875
Chemai B	250	15	5	3	273
Chepkutunyi A A	647	295	28	15	985
Chepkutunyi B	1075	122	47	14	1258
Chepkutunyi C CC C	1972	195	110	20	2297
Total	4638	730	250	70	5688

There was a significant sex differences (Table 2). Females constituted 71.7% (4079/5,688) of the flies collected, whereas males constituted 28.3% (1,609/5,688). After identification, 3006 (83.29%) female sandflies were identified to be from the genus *Phleobotomus pedifer,* 387(10.72%), *P. elgonensis,* 173(4.8%) were *P. longipes* and 43(1.2%) were *S. schwetzi* that was the least recorded species.

Table 2. Sex ratios of sandflies

Locality	*P. pedifer*	*P. elgonensis*	*P. longipies*	*S. schwetzi*
Chemai A	0.31	0.41	0.50	0.38
Chemai B	0.30	0.80	1.20	0.40
Chepkutunyi A	4.35	2.69	1.15	2.00
Chepkutunyi B	0.14	0.17	0.34	0.40
Chepkutunyi C	0.70	0.70	0.38	0.67
Total	0.54	0.89	0.45	0.63

All sites were positive for sand flies. A total of 5688 (100%) sand flies specimens were collected, Chepkutunyi C had the highest number of sand flies; 2297 (40.4%), followed by Chepkutunyi B, 1258 (22.1%), then Chepkutunyi A 985(17.3%). Chemai B yielded the smallest number of collected sand flies, 273 (4.8%). Three species were identified in the surveyed region belonging to the *Phlebotomus* genus. In terms of distribution based on sex, Chepkutunyi C had the highest number of females (1367/5688 = 24.03%) followed by Chepkutunyi B, (19.23%), Chemai A (11.53%), Chemai B (4.80%) and Chepkutunyi A (3.85%) had the lowest number of female flies. *P. pedifer* was the most prevalent species totaling a number of 4638 specimens (81.54%, followed by *P. longipies* with 12.83%, *P. elgonensis* (4.40%) *and Sergentmoyia schwetzi* (1.23%). In terms of abundance, *P. pedifer* were 3006 (52.85%), *P. elgonensis* 387(6.8%), *P. longipies* (3.04%) and *S. schwetzi* (0.75%).

The monthly distribution of sand flies is shown in Fig. 1. The temporal trends were similar for both male and female flies where most of the vectors were significantly differently distributed during the sampling months ($\chi^2 = 7.1934$, $P = 0.013$). Higher abundance of the species occurred between July and September while the least abundance occurred between November and April which coincided with long rains period and drought seasons respectively. In terms of seasonal distribution, it was revealed that the November to April which were dry months seasons recorded the highest abundance of female sand flies, 2450/4403 (55.64%) in the dry season as compared to 1953/4403(44.36%) in May to September , the wet season.

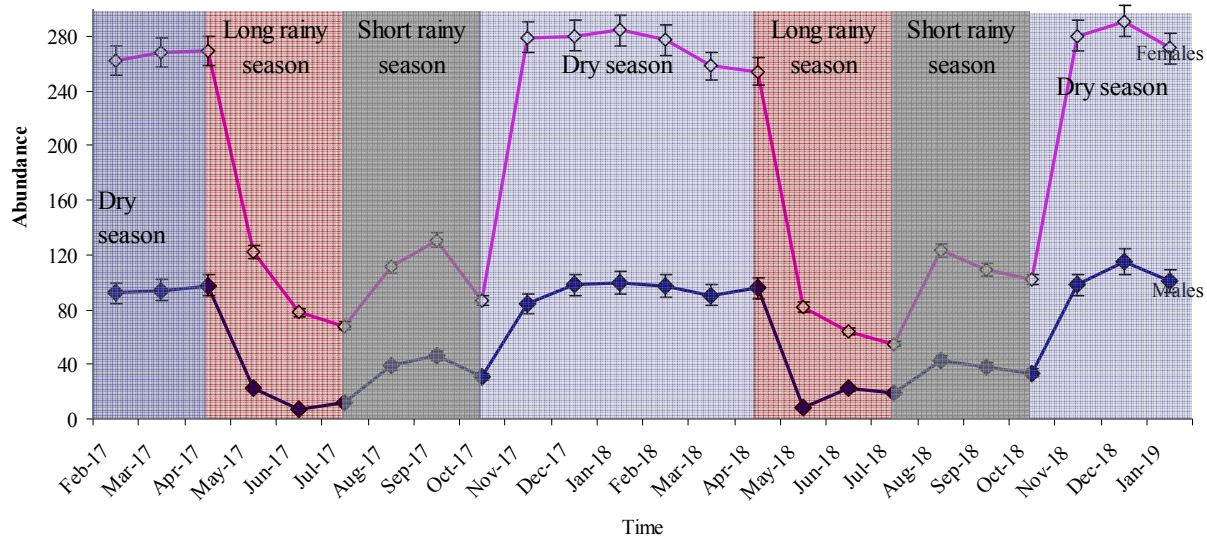

Fig 1. Monthly distributions of sand flies in Mt. Elgon caves

There were more males during rainy season 879/1483 (59.27%) compared to dry season 606/1483 (40.86%) (F = 10.4531, *P* = 0.003).

Table 3: Seasonal distribution of sand flies by sex

Season	Male	Female	Total
Dry season	606	2450	3056
Rainy Season	879	1953	2832
Total	1485	4403	5888

The prevalence of *Leishmania* infection of the vector during the survey is shown in Fig. 2. There were significant differences in *Leishmania* infection between the vector species (ANOVA; F = 4.1671, df = 3, *P* = 0.022).

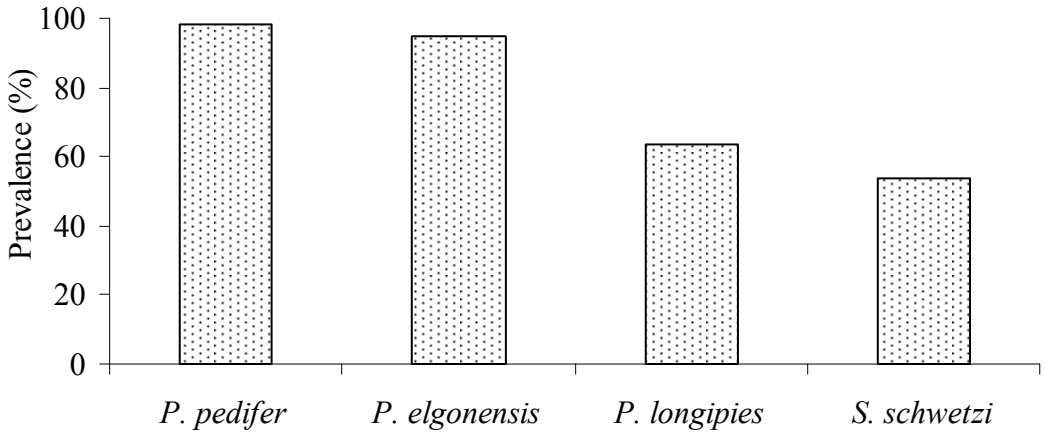

Fig. 2: Prevalence of *Leishmania* infection in vector species

Blood source analysis from a sample of 200 females was done. The results are as shown in Table 4. Serologic analysis by ELISA on 200 blood fed sand flies showed that the flies fed on human, bat, hyrax and cat blood. Percentage distribution showed that 120 (60%) specimens fed on human, 55 (28%) of sandflies fed on bat, 16(8%) fed on hyrax and 9 (5%) fed on cat. Chepkutunyi C had the highest number of blood fed sandflies at 80 (40%) and showed high preference for human blood recording the highest 50(63%) frequency, bat at 20 (25%) hyrax at 7 (9%) and finally cat at 3 (4%). The blood-meal analysis results revealed that the sandflies in Mt. Elgon mostly fed on bats as the wild main reservoir host. However, the sandflies caught and analyzed revealed anthropophillic tendencies as far as the blood meal analysis was concerned with 60 % of the flies having fed on human while 28 % fed on bats, 8% fed on hyraxes while 5% fed on cats. The flies that were engorged and whose blood-meals were assessed belonged to only one species namely *P. pedifer*.

Table 4. Host blood meals sources in Mount Elgon, Kenya determined by ELISA method

Capture site	No of blood fed (%)	No of human blood meal (%)	No of Bat blood meal (%)	No of hyrax blood meal (%)	No of cat blood meal (%)
Chepkutunyi A	20(10)	10(50)	5(25)	3(15)	2(10)
Chepkutunyi B	50(25)	25(50)	15(30)	6(12)	4(8)
Chepkutunyi C	80(40)	50(63)	20(25)	7(9)	3(4)
Chemai A	20(10)	15(75)	5(25)	0	0
Chemai B	30(15)	20(67)	10(33)	0	0
Total	200(100)	120(55)	55(30)	18(8)	9(5)

4. Discussion

An entomological survey was carried out in five different caves in Mount Egon (Chemai B, Chemai A, Chepkutunyi B, Chepkutunyi A and Chepkutunyi C) in Mount Elgon sub County, Bungoma County, in Western Kenya. One trapping method (CDC light traps) was used from February 2017 to January 2018. All monitored sites (Chemai B, Chemai A, Chepkutunyi B, Chepkutunyi A and Chepkutunyi C) were positive for sand flies. A total of 5,688(100%) sand flies were collected during the five (5) month sampling period from the five study caves. Of the 5688 flies collected, Chepkutunyi C yielded the highest number of sand flies; 2297 (40.4%), followed by, Chepkutunyi B, 1258 (22.1%), then Chepkutunyi A 985(17.3%). Chemai B yielded the smallest number of collected sand flies, 273 (4.8%) This may be explained by the narrow entrance nature of Chepkutunyi C, which made it retain more moisture and warmth. Lack of disturbance by human and livestock in Chepkutunyi C due to its small entrance could have allowed uninterfered developments of the fly's immature stages on the floor of the cave. The lager population of bats in the same cave is another factor, bat's excreta provides organic nutrients to developing sandfly larvae. Only two genera of sand fly were identified and presented in each surveyed region, belonging to the *Sergentomyia schwetzi* and *Phlebotomus pedifer*, however *Phlebotomus* genus is the only one that was found with *Leishmania* parasites and thus can be considered as the vector of leishmaniasis in the study area.

In this study of sandflies in five caves found on the slopes of Mount Elgon, Kenya, the *Phlebotomus (Larroussius)* species: *P. pedifer, P. elogonensis P.longipies* and *S. schwetzi* were found. The females of the genus *Phlebotomus* encountered in the study were morphologically indistinguishable using morphological techniques. On the basis of the morphological identification of the males, however, *P. pedifer* occurred mainly at the lower altitudes (1750-1900 m) while *P. elgonensis* predominated at higher altitudes. *P.longipies* and *S. schwetzi* were found in all the habitats studied. The presence of promastigotes in the guts of female sandflies are often restricted to lower altitudes (below 1900 m), the area of maximum distribution of *P. pedifer* and of cases of cutaneous leishmaniasis (Mutinga and Ngoka, 1983; Mutinga and Odhiambo, 1986). This supports the suggestion that *P. pedifer* is the vector of cutaneous leishmaniasis in Mount Elgon.

Regarding monthly distribution of sand flies, it was found to be the same for all detected species, there were fewer sand fly population with the onset of wet season in May , since, sand fly larvae undergo diapauses, permitting them to survive the cold environment and emerge as adults in the following warm weather (Cissé *et al.*, 2020) with a population increase by November to April, then drops between May to September then increases to peak at January. However in a similar study in Agool in Egypt, sand fly numbers peaked at September, October and December in addition to June. Such pattern of seasonal abundance in Abyar Almashy, Almaliliah and Mondasa was observed by El-badry *et al*. (2008) in El-Nekheil and by other studies in Asir (Ibrahim *et al*., 2005), Hail (Bakr, 1995) in Saudi Arabia and Egypt (Hanafi *et al*., 2007).

There were seasonal variations in male/female (sex) ratio of different species of the same area and within the same species of different areas, which implicate more study on the geographical and ecological systems and fecundity of Mt. Elgon sand flies. This is likely to shed light on the mating season habits of sand flies in Mt. Elgon caves region and may be used to design novel control strategies. The proportions of gravid flies may be a valid indicator of the physiological age and of epidemiologic importance at this focus. The *Leishmania* infection rate by culture of sand flies intestinal contents ranged from 0.3 to 1.5% in November, and April which shed light on infectivity of sand flies and plan for control strategies of cutaneous *Leishmania*sis (Bi *et al.*, 2018).

The blood-meal analysis revealed that phlebotomine sandflies in Mt. Elgon fed on bat, hyraxes and cat. There are other animals in Mt. Elgon such as rats, snakes and monkeys. It would appear that the species tested did have preferred hosts for feeding, that is, Human and bats. Blood analyses from 55 out of 200 *Phlebotomus pedifer* adults trapped in the caves in Mt Elgon, Kenya revealed that this sandfly species fed on bats and 120 out of 200 flies fed on human. However these numbers were only recorded in Chepkutunyi A, Chepkutunyi B and Chepkutunyi C. This could be attributed to the fact that the three caves are close to human habitation sites. Similarly 9 out of 200 *P. pedifer* tested positive for blood meal analyses for Cat. This was recorded in Chepkutunyi A, Chepkutunyi B and Chepkutunyi C which may be due to proximity of the three caves to human habitation where cat reside. Host

blood-meal source analysis in sandflies is useful in determining the reservoir hosts of the *Leishmania* parasite species and whether the flies are anthropophilic or not. Rodents, dogs, goats and sheep (Mutinga *et al.*, 1989) have been found to be naturally infected with *Leishmania* parasites in Kenya. In the Mediterranean region, the dog is considered a major reservoir of visceral leishmaniasis and seemingly canine infections are mainly responsible for human visceral leishmaniasis (Ngere *et al.*, 2020). Man gets in touch with these animals in one way or the other, for example, rodents invading houses, goats sleeping inside human huts and the dog always being near its owner; so transmission can easily take place from these reservoirs to the human hosts so long as the vector is also present. Similarly, potential wild reservoir hosts such as the bats which inhabit caves where sand fly also inhabit have not been studied much and thus there is need to assess their status in the transmission of leishmaniasis. The most predominant sandfly species in the Mt. Elgon caves were those of the *Phelobotmus* group. This is in agreement with Mutinga' findings (1975 a,b) that both *P. pedifer* and *P. elgonesnsis* are common and widespread in Mt. Egon, Kenya. *Sergentomyia schwetzi* was previously reported (Heisch *et al.* 1956) to have a high incidence in Kitui and was caught throughout the year. Similar results were reported by Mutinga *et al.* (1982) in West Pokot for the *Phelobotomus* members.

Similarly the parasites can even be maintained within the lizard host and more research performed on the *Leishmania* parasite as has been done by Forawi (1986) when *L. major* was isolated from a lizard in West Pokot. The current research has shown parasites of *Leishmania* in bats. However, lizard was not investigated in the current study. The current results shows that phlebotomine sandflies in Mt. Elgon harbor *Leishmania* parasites, and the presence of amastigotes in bats suggests that they could the reservoir hosts for the parasites. The Mt Elgon region has several caves with a lot of movements of people, tourists and farming activities which could easily transport and introduce new parasites and vectors of the disease. Some of the fly species caught, for example, *S. schwetzi* and *P. pedifer* has been indicated to be an alternate zoonotic vector in Baringo District (Mutinga *et al.* 1986a), may be the vector that causes the disease in Mt .Elgon as shown by cutaneous lesions among the local residents. Experimental evidence on the vectorial capability of most of the various species of phlebotomine sandflies in Kenya is an important and urgent aspect of the epidemiology of leishmaniases (Kaddu, 1986). This would enhance the knowledge and facilitate the forecasting of potential disease (leishmaniasis) outbreak in any new area, where potential vectors are found. People would then not have to be taken "unawares" as it happened in the mid fifties (Mutinga and Kamau, 1986) when the study of sandflies "received a sudden impetus after a serious epidemic of kala-azar in Kitui District" (Minter, 1964a).

Sand fly vectors show host preferences in feeding and represent an important aspect of vector borne disease dynamics. In experiment involving domestic animals (cow, donkey, sheep, goat) and humans in north Ethiopia, higher numbers of *P. orientalis* females were attracted and engorged on donkey and cow than on other hosts (Gebresilassie *et al.,* 2015). These animals present favored blood meal sources for *P. orientalis* as demonstrated in direct blood meal analysis by enzyme linked immunosorbent assay (ELISA) and PCR (Gebresilassie *et al.,* 2015). In northwest Ethiopia, the results of blood meal analysis (Gebre-Michael *et al.,* 2010) showed 91.6% of engorged *P. orientalis* positive for bovine blood and only about 2% for human blood and 2.6% for mixed feeding (cattle and human). Another blood meal analysis from the same locality (Lemma *et al.,* 2014a) showed that only 8% of engorged *P. orientalis* females fed on cattle. However, 28% blood meals were identified as human and 36% as of mixed origin (bovine and human). Interestingly, in eastern Sudan, the dog-baited trap significantly attracted the highest number of *P. orientalis* females, followed by the Egyptian mongoose (*Herpestes ichneumon*) baited trap and less frequent by the common genet (*Genetta genetta*) and nile rat (*Arvicanthis niloticus*) baited trap (Hassan *et al.,* 2009).

It was concluded from these studies that phlebotomine sandflies in Mt. Elgon harbor *Leishmania* parasites, and the presence of amastigotes in bats means that they could be the reservoir hosts for the parasites. In this study, it was also demonstrated that *P. pedifer* is the most abundant sandfly species distributed across the assessed caves in Mt. Elgon. Transmission of *Lieshmania* parasites involved three reservoir hosts (bats, hyraxes and cats) and one vector species (sandfly). Therefore disruption of the life cycle of the *Leishmania* parasites should be done targeting these animals.

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LIST OF ABBREVIATIONS

ATL Adult T-cell leukemia-lymphoma
CL Cutaneous Leishmaniasis
COI Cytochrome c oxidase subunit 1
DAT Direct Agglutination Test
DAT Direct Agglutination Test
DNA Dioxyribonucleic Acid
DNP Dinder National Park
ELISA Enzyme-linked Immunosorbent Assay
ICIPE International Centre of Insect physiology and Ecology
IFAT Immuno Fluorescence
LDU *Leishmania* donovani Unit
LPG Glycoconjugate Lipophospho Glycan
ML Muco-cutenous Leishmaniasis
NNN Novy- Nicolle –McNeal
NTD Neglected Tropical Diseases
PCR Polymerase Chain Reaction
RFLP Restriction Fragment Length Polymorphism
SSA Sub Saharan Africa
VL Visceral Leishmaniasis
WHO World Health Organization

AUTHORS CONTRIBUTIONS

OKO carried out specimen collection, laboratory investigations, design of the study and statistical analysis
MMN conceived of the study, and participated in its design and co-ordination and guided in drafting the manuscript
JM conceived of the study, and participated in its design and co-ordination and guided in drafting the manuscript.

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