

## Species Composition of Sand-fly and Occurrence of Cutaneous Leishmaniasis in Owerri North Local Government Area, Imo State

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### ABSTRACT

Cutaneous leishmaniasis (CL) is a neglected tropical disease of public health value vectored by sand-fly in tropical and vegetative regions of the world. This study was carried out between March and September, 2019 to determine species composition of Sand fly and occurrence of cutaneous leishmaniasis in Owerri North Local Government Area of Imo State. Light and paper oil trap were used to trap 204 Sandflies from 3 villages and identified with standard entomological technique based on morphological characteristics. Assessment of the skin for cutaneous leishmaniasis manifestation was carried out on available respondents above 15 years. Blood samples were collected from 42 head of households and emptied into EDTA containers. Laboratory analysis of blood samples was carried out using standard method of staining and microscopy. Result was represented in percentages and subjected to chi-square analysis. The result revealed two sand-fly genera; *Phlebotomus* (135) and *Lutzomyia* (69) with abundant rates of 66.17% and 33.82% respectively. The different species were present in the villages sampled and female flies accounted for 45.59% of the collection. There was no typical CL skin manifestation observed among villagers, however, laboratory analysis of the household respondents showed 3(7.14) persons had amastigote form of leishmaniasis in their blood. The study reveals occurrence of cutaneous leishmaniasis and its possible vectors in the study area. There is need for public health awareness, health education and application of effective control measures for sand-fly in this area.

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### Introduction

Phlebotomine sand flies (Dipteral: Psychodidae) are small insects of medical and veterinary relevance. Sand-fly is about 3 mm in length, characterized as “hopping” flight. They possess dark and large eyes, long antennae, with mouthparts that are oriented downward, dagger shaped, and the legs are delicate and long. Sand flies are nocturnal and sensitive to dehydration. They shelter in caves, termite hill, rocks, animal burrows, tree holes and human rooms or accommodation. In addition to being a tremendous nuisance due to their bites, the females of most species are hematophagous and are responsible for transmitting several etiological agents to vertebrate hosts. Thirty species of sandflies are the vectors for number of species of *Leishmania* (Ross)-Protozoan which cause Leishmaniasis [1,2]. They are known to be vectors of other human pathogens, such as *Bartonella* spp. (Carrion’s disease), and a number of viral agents causing sandfly fever, summer meningitis, vesicular stomatitis and Chandipura virus encephalitis [3].

Leishmaniasis is found in every continent except for Antarctica and Australia. The manifestation of this disease determines the type of disease caused. Visceral leishmaniasis is known to affect internal organs, causing swollen spleen and liver. Mucocutaneous form can also be observed when it affects the mucous membrane presenting nose bleeding signs. Cutaneous leishmaniasis (CL), also known as oriental or tropical sore, manifests as skin lesions in humans and may occasionally extend to the mucous membrane. The lesion starts as a tiny, reddish and often itchy papule that gradually enlarges, burst to an ulcer, the edges of the ulcer become

raised and firm with the surrounding skin a dusky red colour [4]. Generally, cutaneous lesions resolves spontaneously after sometime leaving a disfiguring, mottled, depressed scar, with altered pigment, and which persists throughout one’s existence [5].

Cutaneous leishmaniasis (CL) occurs in various parts of the world, mainly in tropical and subtropical regions. In the African continent, CL due to *L. major*, *L. tropica* and *L. aethiopica* is unevenly distributed from the northern to the southern areas of the continent and it is the common form of leishmaniasis in Nigeria [6]. The leishmaniasis is far more prevalent and of greater medical importance for public health than previously recognized. The epidemiology of leishmaniasis is extremely complex partially because, firstly its taxonomy is still in a state of flux and secondly it is difficult to get accurate figures for the number of leishmaniasis cases in the old world as a whole because in many endemic areas; the patients have no access to health services and many cases thereby remain unreported. A common estimate of the worldwide annual incidence is 600,000 newly reported clinical cases, an overall prevalence of 12 million cases and an estimated population at risk of about 350 million in 88 countries [6].

Leishmaniasis affects mostly people living in the most impoverished parts of developing countries and places further economic stress on already strained meager financial resources [7]. Although CL is associated with low morbidity and mortality, it causes very severe disfiguring scars in infected individuals, which most often leads to stigmatization in both men and women. Leishmaniasis also negatively impacts on the psychological and social status of women. The disfiguring scars lead to social stigmatization, exclusion from community activities, lose of work

hour and may precipitate psychological problems. Young women who have disfiguring scars as a result of CL infection suffer social stigmatization and are usually unable to marry.

It has been on record that this disease is associated with only three Sandfly genera (*Phlebotomine*, *Lutzomyia* and *Sergentomyia*) and these are area specific in their disease distribution in the Old World and New World [8,9]. The disease risk factors which have been on the increase include: poverty, illiteracy, malnutrition, rapid urbanization, deforestation, irrigation, construction of dams [10]. There are documented researches on composition of Phlebotomine sandfly and different forms of leishmaniasis in parts of Africa. However, these information are inadequate in profiling the status of the disease and its vector. In Nigeria, inconsistent attention has been paid to sandfly species and leishmaniasis.

The few reports available on sandfly are from the Northern part of the country which include [11,12]. Which identified among the many genera of *Psychodidae*, only species of *Phlebotomus* and *Lutzomyia* as vectors of human leishmaniasis. Equally, the reports on cutaneous leishmaniasis are limited to Northern Nigeria [13-16]. The forested south eastern parts of Nigeria with preponderance of risk factors for leishmaniasis have only a report by Ikpeama and Obijuru in 2013 study on bionomics of sandfly composition in Mbaise Imo State. Unfortunately, the study did not reveal the species composition which factor in the transmission of specific types of human leishmaniasis. Secondly, the behavior patterns of adults of different species of sand fly vary significantly, hence requiring tailor-made control solutions based upon a profound knowledge of their species-specific biology.

According to the leishmaniasis remains among the top emergent infectious diseases in the world, despite control and treatment measures [10]. The incidence the disease is increasing primarily in the developing countries because of human encroachment into the habitats of animal reservoirs and corresponding exposure to infected vectors, partly as a result of poor housing. In fact, it is considered as second only to malaria as major cause of parasitic burden. There is no available report on leishmaniasis in Imo State. This is unfortunate considering the observed high biting rate of sandfly and the occurrence of several skin neglected tropical diseases.

Besides, there have been documented reports on increase in the severity of malaria infection in malaria and sandfly co-endemic environment. The increase may support the assertion of that malaria outcome can be altered according to the *Leishmania* species based on observed changes in the immune response of patients [17]. The design of better and efficient control strategies for cutaneous leishmaniasis is highly dependent on the availability of information of species composition of sand flies and identification of natural *Leishmania* infection in endemic setting. This study is intended to achieve the aim.

### Study Area

Owerri North LGA of Imo State is situated between longitude 5.4567° N and latitude 7.1144°E (Google search). The area has its headquarters at Orié Uratta and with an area of 198 square km. The population of this area as at 2006 was 175,395 [18]. It is a semi-urban government area that encircles Owerri municipal, capital of the state like a peninsular. It is also presently composed of 12 wards and has water tributaries located in villages close to its boarder lines. Development with incursion into forest by individuals for establishment of homes is on a high increase. The villagers depend on borehole for domestic use. Children and adults

occasionally visit the stream at hot afternoon for a bath to cool their body and for fun. While some farmers after farming activity wash work tool in the stream before returning home.

The practice of domestic farming is carried out by few households in the communities such practice includes; poultry, goat hording. Home keeping of dogs for security is practiced in the communities and very few household identified living close to the thick vegetative area resides in an old rickety house made with bricks and having cracks on their building walls. Hospitals and health centers are situated in almost all the communities of Owerri North but individuals do not often visit them except on critical health situation.

### Ethical Consideration

Clearance was obtained from the Head of Department, Animal and Environmental Biology, Imo State University, Owerri. Consent was gotten from the heads of the three study Communities (Nduhu, Ndokwu and Umunahu) and Administrative head Owerri North L.G.A. The reason for the study was carefully explained to them and other respondents.

### Data Collection

Data for this study was collected between March and September, 2019. Sandfly collection was carried out through trapping with light trap and sticky Paper trap from the three villages in the study area. The paper traps consist of white standard A4 paper size coated with castor oil held upright inside an open black nylon under a touch light rays placed in animal housing, near wall cracks and under vegetation in three villages of the community with sandflies between 5:00 pm to 7:30pm each day and were transported to Zoology laboratory, Imo State University for morphological identification.

Physical body examination was carried out during the house to house visitation for manifestation of Cutaneous Leishmaniasis in the area. Signs like; Pimple-like boil, wound with a crusted surface, an ulcer with a ring edge and some reddish colour in the wound, and a patch on the skin differing in colour with the skin were screened. Forty two heads of households equally drawn from the three villages were purposely selected for blood sample collection. Blood was obtained through the skin using a syringe and was transferred into an EDTA bottle and transported to the Parasitology Laboratory, Zoology department, Imo State University Owerri for haematological analysis.

### Labouratory Identification

Sandflies were sorted from other insects trapped using a hand lens and broom stick, and were transferred into a tube containing about 70% alcohol in 10% potassium hydroxide solution (KOH) for 2 hours. They are then mounted between slides and covered with cover slip in a drop on normal saline as carried out by [19]. Standard entomological techniques was used for identification of morphological characteristics; its small size, feathery body, long hopping legs, terminalia, nature of stomach and raised wings above the body surface at about 60° were significant features observed using microscope [20]. Taxonomic identification is based on different morphological criteria described by [21].

Standard method of identification was used in the detection of amastigote form of *Leishmania* [22]. Thin blood film was prepared on a slide and fixed in methanol. Staining was done with geimsa stain for about 10mins and allowed to air dry. Washing was carried out on the slide with a running tap water and allowed to air dry. A drop of immersion oil was dropped on the prepared film and

mounted on a microscope for a view using x100 oil immersion objective.

### Statistical Analysis

The composition of Sandfly and occurrence of cutaneous leishmaniasis in Owerri North Local Government Area of Imo State was subjected to statistical analysis using Chi-square ( $X^2$ ). Percentage was used to represent abundance of sandfly species and prevalence of cutaneous leishmaniasis.

### Result

Table 1 show the distribution of Sandfly species in Owerri Noeth L.G.A. The result revealed that the 204 Sandflies were trapped from three locations in the study area. Sandfly of *Phlebotomus* genera accounted for 66.17% (135) while 33.82% (69) were of *Lutzomyia* genus. Umundula village recorded significantly higher number of the species followed by Emii village, while the least occurrence was recorded in Orji village ( $P \leq 0.05$ ).

**Table 1: The distribution of Sandfly species in Owerri North LGA**

Village <i>Phlebotomine</i> <i>Lutzomyia</i> all species points										
	No collected		Male Female		No collected		Male Female		No collected	
Orji	18(13.33)	6(33.33)	12(66.67)	12(17.39)	9(75.00)	3(25.00)	30(14.70)	15(50.00)	15(50.00)	
Emii	36(26.67)	15(41.17)	21(58.33)	15(21.74)	6(40.00)	9(60.00)	51(25.00)	21(41.18)	30(58.82)	
Umundula	81(60.00)	51(62.96)	30(37.04)	42(60.86)	24(57.14)	18(42.86)	123(60.30)	75(60.98)	48(39.02)	
Total	135(100)	72(35.30)	63(30.88)	69(100)	39(19.11)	30(14.70)	204(100)	111(54.41)	93(45.59)	

The sex related abundance of the sandfly revealed that more males (54.41%) were collected than females (45.59%). The sex profile reflected in the two species collected, however the sex related prevalence were not significantly ( $P \geq 0.05$ ).

Table 2 summarized the occurrence rate of cutaneous leishmaniasis in the study area. Of the 42 household respondents that their blood samples were examined for amastigote form of the Leishmania parasite, 3(7.14%) produced positive result. Male and female respondents were infected, while six persons assessed within 16-28 years were not infected. Respondents in all categories of the marital status captured in the study were infected while 4.76% of farmers and 2.38% of traders examined had the parasite in their blood.

**Table 2: Occurrence of cutaneous leishmaniasis in the study area**

Variables	No. examined	Number infected	Prevalence (%)
<b>Sex</b>			
Male	30	2	6.67
Female	12	1	8.33
	42	3	7.14
<b>Age</b>			
16-28	6	0	0.00
29-40	9	1	16.67
$\geq 40$	27	2	7.41
<b>Marital status</b>			
Married	21	1	4.76
Single	19	1	5.26
Divorce	0	0	0.00
Widow	2	1	50.00
<b>Occupation</b>			
Farmer	12	2	4.76
Trader	18	1	2.38
Civil servant	6	0	0.00
Student	6	0	0.00

<b>No. in Household</b>			
<5	22	1	4.54
6-8	15	0	0.00
10>	5	2	40.00

### Discussion

Cutaneous leishmaniasis is a neglected tropical disease of public health relevance, primarily transmitted by sandfly in the world. The result reveals two sandfly genera, *Phlebotomus* and *Lutzomyia* with a relative abundance of 66.17% and 33.82% respectively [23]. In a review paper reported that only 98 species of *Phlebotomus* and *Lutzomyia* genera are currently proven or suspected vectors of human leishmaniasis of 800 phlebotomine sandfly species estimated to exist. These species have been recovered by [1,7] in Northern parts of Nigeria, while identified *Sergentomyia* genus in addition However, the role of species belonging to the genus *Sergentomyia* in *Leishmania* spp. transmission among mammal hosts needs to be elucidated [12,16,24,25,26].

Reported that sufficient moisture in an environment is required for sand-fly egg survival [27]. This characteristic is shared by the three collection areas which are close to dense forest and stream. The result shows an even distribution of Sandfly in the community which may indicates even high risk of Sandfly bite and cutaneous leishmaniasis transmission. The area with highest number of trapped Sandflies (Umundula) is very much closer to a high denser forest than other areas. The relative abundance of Sandfly determined for the three sampled areas of the community implicates environmental and geographical conditions as a contributing factor favoring distribution and abundance of Sandfly [28].

The result showed that more males (54.41%) flies were collected than females (45.59%), though the difference was not significant. This is in conformity with the report of but differs from the report of who observed more females than males [29, 30]. The identification of adult female sand flies in the all the villages sampled remains a public health concern because they are the gender capable of transmitting cutaneous leishmaniasis to humans.

Parasitological analysis of blood samples of 42 household respondents revealed a 7.14% prevalence of leishmania amastigote revealing occurrence of cutaneous leishmaniasis in the study. This is the first report of the disease in Imo state despite recorded incidence of high rate of sandfly bites and the prevalence could have been higher if more sensitive PCR method was employed. There are reports on cutaneous leishmaniasis in Nigeria; however they are limited to the Northern region [13, 14, 15, 16].

The result revealed that the infection did not discriminate among sexes of different marital status assessed, however there was disparity in infection based on age group, household profile and occupation of respondents, which could be attributed to the sample size of the study. The researchers could not identify any skin lesion of leishmania etiology based on absence of typical skin signs. It is possible that the inhabitants are no longer reactive having developed immunity to the bites. However, the presence of proven vector of cutaneous leishmania and isolation of the leishmania amastigote in the blood of respondents could connote possible viable transmission of cutaneous leishmaniasis [31,32].

The public health significance of this result is magnified by the fact that leishmania infection suppresses the immunity of sufferers which will have dire consequences to the susceptible population in study area, already under pressure from of barrage endemic diseases like malaria. Consequently, this situation provides tremendous obstacles in achieving an improved quality of life. It could be possible that unreported cutaneous leishmaniasis infection factors in the perpetual occurrence of complicated malaria observed in the villages. Our results underscore the need for concerted efforts toward control of sand fly and shed light into the potential active transmission of cutaneous leishmaniasis and its public health implication in Owerri North L.G.A. of Imo State.

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