An entomological study of onchocerciasis vectors, Simulium damnosum s.l., in Kinshasa, Democratic Republic of Congo

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Research Article

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Abstract

Background

Onchocerciasis, a parasitic disease caused by the filarial worm *Onchocerca volvulus* transmitted through the bite of *Simulium* (black flies), is a cause of global concern, with the African population being majorly affected. This study focused on the bite rates, bite cycle, and transmission potential of *Simulium damnosum* s.l. in two sites with river blindness outbreaks in Kinshasa, DRC: Gombe (S1) and Mont-Ngafula at Kimwenza (S2).

Methods

From August 1, 2019, to July 31, 2020, we captured adult female black flies near breeding sites along the Congo River at S1 and Lukaya Valley at S2. Collections using human baits at the two sites were conducted for five days/month.

Results

A total of 6082 black flies of species *Simulium squamosum* (classified based on other entomological surveys) were captured during the study period. The daily cycle of aggression revealed two peaks: one between 8 and 9 a.m. and the other between 4 and 5 p.m. Low bite rates were observed between 11 a.m. and 1 p.m. The annual biting rate/person reached 13,463 in S1 and 23,638 in S2, with a total of 37,101 bites/person. The average daily biting rate, 37 ± 10 and 69 ± 23 bites/day/person in S1 and S2, respectively, did not differ significantly (P = 0.8901). The high density of the host population can disrupt the transmission of vector-borne diseases by diluting the transmission indices. There was no evidence of onchocerciasis transmission at the study sites because of inadequate laboratory facilities in the DRC. Various larval supports have been identified: at Gombe: aquatic plants, plastic bags, dead leaves, and rocks; at Kimwenza: *Ledermaniella ledermanii* (the most abundant species at the site), plastic bags, artificial waste, and aquatic plants.

Conclusions

The study provides further evidence for the need for alternative strategies to eliminate the parasite in the formerly hyper-endemic foci.

1. Introduction

Onchocerciasis, also called "*river blindness*", is a parasitic disease caused by the filarial worm *Onchocerca volvulus* transmitted through the bite of infected black flies of the genus *Simulium* that breed in fast-flowing streams and rivers [1]. Michael et.al. (2020) reported WHO estimates for onchocerciasis: at least 25 million people are infected worldwide and 90 million people are at risk, with more than 99% of them living in Africa [2]. Therefore, onchocerciasis has been a public health problem for decades in the Democratic Republic of Congo (DRC) with all 26 provinces affected; in 2016, the estimated overall prevalence was 14.65 million people, including 12.22 and 1.03 million suffering from skin disease and loss of sight, respectively [3,4]. The city-province of Kinshasa, DRC, also suffers from this, despite various control programs established for more than twenty years: e.g., the African Programme for Onchocerciasis Control (APOC), National Programme for Onchocerciasis Control, and National Programme for Neglected Tropical Diseases Control (PNLMTN-CP) [3,4]. The disease epidemiology is defined by the presence of black fly breeding sites [1,5]. The larvae of this insect are aquatic and rheophilic: the larvae attach to submerged supports, in running water, at a shallow depth, for oxygen and food [6]. *S.damnosum s.i.* was first identified in 1903 in Kinshasa by an English expedition from the Liverpool School of Tropical Medicine; however, only in 1926, Blacklock and his team demonstrated that onchocerciasis was transmitted by black flies [7–9]. Several onchocerciasis sites have been identified in Kinshasa since then [4]. Wanson, Henrard, and Peel claimed *S.damnosum* as the only known vector in 1945, in Kinshasa villages, located on the bank of the Congo river, along the rapids [4]. In 1944 and 1945, Wanson and Henrard studied the behavior of *S.damnosum* in Kinshasa and located larval deposits mainly in the rapids, downstream of the city [4]. Other larval sites were also detected: along the Nsele river, 60 km from Kinshasa, in the north of Kisundu; along tributaries of the Congo River, about 100 and 130 km upstream from Kinshasa (the Lufimi River); Mfuti River near Sabuka in Mong Ngafula; along tributaries of the Lukunga, along Ndjili, and several other small rivers around Kinshasa, including Lukaya, the Binza River in Kinsuka, etc. [4]. A vector control program with the aerial spraying of dichlorodiphenyltrichloroethane was organized from 1948 to 1952 [10,11]. Brown, in 1962, reported that *S.damnosum* did not reappear in Kinshasa for 10 years after the eradication campaign. Kinshasa remained unscathed for several years until the flies reemerged in 1984 [12]. Till now, only three sites of onchocerciasis have been explored in the city: Kinsuka-pêcheur, N'sele, and Mont-Ngafula at Kimwenza [4,10–13]. Several authors have confirmed the presence of disease vectors in the three sites: *S.damnosum, S.squamosum* [14,15], and *S.albivirgulatum* have been identified in the sites [16].

A fourth site, Gombe, was reported after 1945, in the north of the capital along the Congo river but was never explored [4,17]. This study determines the presence of the vector, *S.damnosum s.l.*, in Kinshasa, particularly in the sites Mont-Ngafula at Kimwenza and Gombe.

2. Materials And Methods

2.1 Description of the study environment

Kinshasa, the capital of the DRC, is a large city (area: 9,965 Km^2), with 24 municipalities and four districts, and an estimated population: 11 million (population density: ±3,600 inhabitants/Km^2) [18].

Kinshasa has a tropical savannah climate, equatorial in nature (hot and humid), with a rainy season lasting 8 months and a dry season lasting 4 months—a dry winter. The average annual temperature and precipitation are 25.3°C and 1,273.9 mm, respectively [18].

The dry season (tropical winter), June to September, is characterized by rare and low rainfall and lowest temperatures [18]. The sky is generally overcast and vegetation suffers from a lack of water [18]. During the rainy season (tropical summer), March to May, the precipitation is between 1000 and 1500 mm [18]. Generally, in Congo, it is hot to very hot all year round. Maximum temperatures are 30–35ºC during rainy periods (peaks at 40°C); the temperature rarely drops to below 20°C in the dry season [18].

Rivers of various sizes drain the plains of Kinshasa; they generally originate in the hills, flow from south to north, and empty into the Congo river [18,20]. Lakes (smaller waterbodies than rivers), such as Lac Ma Vallée and Lac Vert, are also located here [18,20]. Some of these waterbodies exhibit geographical characteristics that enable rapid waterfalls compatible with the survival of *Simulium*, particularly, the Lukaya river in rural Kimwenza and Congo river in the urban part of the bay of Gombe [18,20].

2.2 Specific characteristics of the municipalities chosen for the capture of black flies

Two of 24 municipalities of the city of Kinshasa (Kimwenza: 4° 27' 33" South, 15° 17' 20" East; Gombe: 4° 18' 24" South, 15° 18' 12" East), with contrasting environments, served as study sites for this work [20]:

Site 1. Gombe, formerly Kalina, is a municipality located in the north of Kinshasa, with the Congo river acting as its natural border. It includes a residential area and renowned business district with many trees and is located opposite Brazzaville. It is also home to the main organs of power of the DRC (Fig. 1) [20].

Site 2. The Mont-Ngafula commune is a municipality located in the south of Kinshasa, in the hilly areas of rural Kimwenza occupied by the Lukaya valley. It is a recent settlement, there are highland plateau dominating the city, at an altitude of 493 m, near the Lukaya Little Waterfalls and tourist site "Lola Ya Bonobo" (Fig. 1) [20].

The sites were explored two days before the study to locate the roosts of pre-imaginal forms of black flies, identify their fixation supports, and select the vector-human contact points. The best contact points were defined as the two capture points of anthropophilic adult black flies for determining entomological indices (S1 and S2).

2.3 Capture and dissection of adult black flies

Human baits were used to capture adult black flies based on Le Berre's method [13,14,21]. Capture points at each study site were selected following Enyong's criteria, including, proximal to black fly-breeding grounds (along the river), accessible throughout the year, located in shade, sheltered from the wind, and distal from regular human activity [22]. Four staff, two per site, were trained in standard methods of capturing adult flies, "human landing collection [23]". Test samples were collected over five days, between 7 a.m. and 6 p.m., to identify sites with high black fly activity and assess staff performance in capturing black flies. The actual collection was done five times a month, for 24 weeks, over 12 months. The collections by human baits were simultaneously performed at the two sites, between 7 a.m. and 6 p.m. The two staff capturing black flies were at least 500 m apart. The teams were exchanged regularly, every hour, to minimize any potential bias relating to the efficiency of staff capturing black flies. The hourly capture rates were noted to determine the daily bite rate (the cycle of aggression) [14].

The study was conducted in a randomly chosen 24-week period, from August 1, 2019, to July 31, 2020, including the two main seasons in Kinshasa: rainy and dry seasons. During the rainy season, the water levels in the rivers were high, and so were the black fly populations. All black flies captured at S1 and S2 were analyzed.

2.4 Determination of entomological indices and data analysis

The cycle and level of aggressiveness, as well as parity and transmission potential, were used to determine entomological indices, following WHO guidelines [24]. The cycle of aggression refers to the daily biting pattern of black flies. From an epidemiological and entomological viewpoint, this criterion determines the time of the day when the risks of bites and transmission are the greatest [14]. The average number of flies captured per hour (estimated using Microsoft Excel) determines the cycle. The aggressiveness rate is represented by the daily biting rate (DBR; the number of black fly bites/day), monthly biting rate (MBR; the average number of bites per person per month), or annual biting rate (ABR; the sum of MBRs calculated for 12 months) [14]. Determining parity requires dissecting the black flies to distinguish between nulliparous and parous black flies. The parous black flies were then opened up by cutting the abdomen and thorax in a drop of physiological water and carefully examined for *O.volvulus* larvae. The team carefully noted the number, stage of development (L1, L2, or L3), and location of the larvae. The black flies harboring developing larvae (L1, L2, or L3), whatever be their location, were considered to be infected, while those harboring infective larvae were categorized as infectious [14,25]. Larvae of the *S.damnosum* complex were identified morphologically by the presence of tubers and dorsal abdominal scales on the prothoracic prolegs [26]. These dissections were conducted in the laboratory of the Kinshasa School of Public Health (ESP).

To assess the degree of onchocerciasis transmission by black flies, we calculated the monthly transmission potential (MTP) using the formula proposed by WHO [10]: MTP = MBR x (total number of L3 *O.volvulus* larvae observed in the head during black fly dissection/ number of dissected black flies). MTP is the number of infective *O.volvulus* larvae received per person per month. The annual transmission potential (ATP; the number of infective *O.volvulus* larvae received per person per year) is obtained by summing up 12 subsequent MTPs. The potential for onchocerciasis transmission in an outbreak is expressed as the number of infective larvae per infectious black fly. The hourly catch of black flies was recorded, and the black flies were preserved in absolute ethanol before analysis at the bioecology laboratory for preliminary morphology, according to dichotomous keys proposed by Crosskey [26], and physiological age [26].

Furthermore, the entomological data were recorded daily on collection cards. Consistency of the information declared and potential omissions were first assessed by the field collectors. Later, the whole team (fly catchers, supervisors, and main researchers) reviewed all the collection tools to identify possible inconsistencies. Two-sample t-tests with unequal variances were used to compare the mean values of study parameters at the two capture sites (S1 vs. S2) and capture period; the χ^2 or Fisher's exact tests were used to compare the proportions of parous females at the two capture sites. $P < 0.05$ was considered significant. The data were analyzed using Excel ™ and SPSS 20 ™ Data Analysis Software -.

3. Results

3.1 Entomological indices

The results of black fly capture, dissections, and onchocerciasis transmission indices are summarized in Tables 1 and 2. A total of 6082 black flies were captured during the study period, 2207 at S1 and 3875 at S2. The density of female black flies was different between S1 and S2 but not statistically significant (P = 0.2198); the average bites per day and per person were 37 ± 10 and 69 ± 23, respectively (Fig. 2 **and Table 4**).

The two-sample t-tests with unequal variances showed no statistically significant difference in the mean number of black flies captured between S1 and S2. All captured black flies were dissected. The highest number of parous flies were obtained in January (S1, 63%; S2, 57%), February (S1, 62%; S2, 63%), March (S1, 77%; S2, 71%), and April 2020 (S1, 70%; S2, 56%) (Figs. 3 and 4; **Tables 3 and 4**)

The daily cycle of aggression showed two high peaks: one recorded between 8 and 9 a.m. and another between 4 and 5 p.m.; low bite rates were observed in the middle of the day between 11 a.m. and 1 p.m. (Fig. 5).

The ABR reached 37,101 bites per person. Of all dissected black flies, none carried *O. volvulus*, and no molecular test was performed due to the lack of laboratories in Kinshasa. There is no evidence of onchocerciasis transmission at the study sites.

3.2 Fixation supports of pre-imaginal forms in the capture sites

Different larval fixation supports were identified in the study area, from which pre-imaginal flies were collected: artificial waste (used clothing and plastic bags), aquatic plants (*Ledermaniella ledermannii, Tristicha trifaria, Pennisetum divisum, Phragmites mauritianus, Mimosa pigra, Cyperus distans, Ipomoea aquatica, Ludwigia adscendens, Echinochloa pyramidalis, Eichhornia crassipes, and Pistia stratiotes*), and plant remains (pieces of wood, tree branches, and palm leaves) wedged between rocks and trees.

These supports were found in abundance around the rapids of the Lukaya Valley and along the Congo River, especially in places frequented by humans from the surrounding areas. The pre-imaginal population was more prominent on *Ledermanniella ledermannii* (the main support) > artificial supports > plant remains.

3.3 Black fly species at the capture sites

The identified black fly species, *S.Squamosum*, belonging to the *S.damnosum s.l.* complex, was the same as in Kinshasa. Its morphological characteristics are the color of wing tufts (tufts of hair at the base of the wings) and relative coloring of antennae, procoxa, and prosternum (antenna parts) [25].

3.4 Entomological parameters

This study identified the following entomological parameters for onchocerciasis transmission: MBR, ABR, parity, black fly infection rate (percentage of infected and infectious parous females), and transmission potential.

As shown in Fig. 2, the DBR reached acceptable levels of 164 and 425 from August to September 2019 and 59 and 63 from June to July 2020, in the entire study period. The DBR was low in February, March, and April. With an average of 1216 bites in the entire study period, the DBR peaked in September (10th day of collection), with 329 bites per person, and dropped to six bites per person in February 2020 (35th day of collection), at S1 and S2 (Figs. 2 and 3).

The average physiological age of female black flies was expressed against the proportion of parous females (Fig. 4). The parous/nulliparous ratio helped estimate the survival rate of black flies, which was directly linked to the risk of disease transmission. In S1 (Table 1) and S2 (Table 2), the dissection of 2,207 and 3875 female flies revealed an average parity rate of 43% (N = 952) and 28% (N = 1075), respectively.

Table 1

Summary of entomological indices of O.volvulus by S.squamosum from August 2019 to July 2020 in the Gombe capture site

Month of capture	Aug 2019	Sept 2019	Oct 2019	Nov 2019	Dec 2019	Jan 2020	Feb 2020	Mar 2020	Apr 2020	May 2020	June 2020	Jul 2020	TOTAL
No. of capture days	5	5	5	5	5	5	5	5	5	5	5	5	60
No. of females caught	237	586	513	107	142	121	21	26	30	125	134	162	2204
Daily biting rate	47	117	103	21	29	24	4	5	6	25	27	32	441
Monthly biting rate	1469	3516	3181	642	893	756	122	161	180	775	804	1004	13463
Annual biting rate													13463
No. of females dissected	237	586	513	107	142	121	21	26	30	125	134	162	2207
No. of parous females	119	242	183	26	83	76	13	20	21	53	52	64	952
% of parous females	50%	41%	36%	24%	58%	63%	62%	77%	70%	42%	39%	56%	43%
No. of parous females dissected	119	242	183	26	83	76	13	20	21	53	52	64	952
No. of infected parous females	0	0	0	0	0	0	0	0	0	0	0	0	0
% of infected parous females	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
No. of infected females/1000 parous females	0	0	0	0	0	0	0	0	0	0	0	0	0
No. of L3 at peak	0	0	0	0	0	0	0	0	0	0	0	0	0
L3 at peak /1000 parous females	0	0	0	0	0	0	0	0	0	0	0	0	0
PMT (L3/person/month)	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2

Summary of entomological indices of O.volvulus by S.squamosum from August 2019 to July 2020 in the Kimwenza capture site

Capture month	**Aug 2019**	**Sept 2019**	**Oct 2019**	**Nov 2019**	**Dec 2019**	**Jan 2020**	**Feb 2020**	**Mar 2020**	**Apr 2020**	**May 2020**	**June 2020**	**Jul 2020**	**TOTAL**
No. of capture days	5	5	5	5	5	5	5	5	5	5	5	5	60
No. of females caught	1014	1057	769	223	222	138	8	7	36	25	140	176	3875
Daily biting rate	214	211	154	45	44	28	2	1	7	5	28	35	775
Monthly biting rate	6646	6342	4768	1338	1376	856	46	43	216	155	852	1091	23638
Annual biting rate													23638
No. of females dissected	1014	1057	769	223	222	138	8	7	36	25	140	176	3815
No. of parous females	224	337	148	38	94	78	5	5	20	15	54	57	1075
% of parous females	22%	32%	19%	17%	42%	57%	63%	71%	56%	60%	39%	32%	28%
No. of parous females dissected	224	337	148	38	94	78	5	5	20	15	54	57	1075
No. of infected parous females	0	0	0	0	0	0	0	0	0	0	0	0	0
% of infected parous females	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
No. of infected females/1000 parous females	0	0	0	0	0	0	0	0	0	0	0	0	0
No. of L3 at peak	0	0	0	0	0	0	0	0	0	0	0	0	0
L3 at peak /1000 parous females	0	0	0	0	0	0	0	0	0	0	0	0	0
PMT (L3/person/month)	0	0	0	0	0	0	0	0	0	0	0	0	0

However, when studying these parous females, we could not validate infection with *O.volvulus* larvae (regardless of the stage). We preserved the samples for a thorough laboratory examination (molecular test: pool screening) abroad since the necessary laboratory facility was unavailable at DRC. The MTPs were 0 at S1 and S2 during the study period, with no parous females carrying *O.volvulus* (Tables 1 and 2). The daily bite cycle was diurnal; two peaks were observed: a moderately major peak between 8 and 9 a.m. and a major peak between 4 and 5 p.m. (Figs. 5). The lowest biting rate was between 11 a.m. and 1 p.m. The morning peak may be because human activity took place near the larval breeding sites at that time (Fig. 5).

The average number of black flies captured per hour during the study period was higher in the morning (before noon) and evening. However, the hourly changes were not statistically significant; $P > 0.05$ **(Table 3).**

Table 3 *Average number of black flies captured per hour, in the Gombe and Kimwenza capture sites, from August 2019 to July 2020, according to the two-sample t-tests with unequal variance*

HOUR	Site 1: GOMBE Mean ± SD	Site 2: KIMWENZA Mean ± SD	P-Value	Significance
7-8H	13 ± 7	25 ± 9	0.2068	NS
8-9H	15 ± 5	36 ± 16	0.2242	NS
9-10H	16 ± 5	30 ± 12	0.2977	NS
10-11H	13 ± 5	29 ± 11	0.2008	NS
11-12H	12 ± 8	20 ± 8	0.3650	NS
12-13H	12 ± 4	19 ± 7	0.4633	NS
13-14H	15 ± 5	19 ± 6	0.5521	NS
14-15H	19 ± 6	31 ± 10	0.3270	NS
15-16H	22 ± 6	37 ± 14	0.3212	NS
16-17H	26 ± 6	42 ± 17	0.3775	NS
17-18H	23 ± 7	36 ± 16	0.4671	NS
H = Hour; SD = Standard deviation; NS = Non-significant				

Since the calculated P-values were > 0.05, the averages of none of the variables showed statistical significance **(Table 4)**.

Table 4 The comparison of the averages of all variables calculated based on data collected from August 2019 to July 2020, in the Gombe and Kimwenza capture sites, according to two-sample t-tests with unequal variance

	Site 1: GOMBE Mean ± SD	Site 2: KIMWENZA Mean ± SD	P-Value	Significance
No. of black flies (captured and dissected)	184 ± 53	323 ± 116	0.2920	NS
No. of parous females	79 ± 20	90 ± 29	0.7764	NS
Proportion of daily bites	37 ± 10	69 ± 23	0.2198	NS
Proportion of monthly bites	1125 ± 320	1977 ± 710	0.2911	NS
SD = Standard deviation; NS = Non-significant				

4. Discussion

4.1 Larval supports of pre-imaginal forms at capture sites

This study identified a diversity of larval breeding supports, such as waste, aquatic plants, and marine rocks, in and along the Lukaya and Congo rivers. Similar supports have been reported by Henry et al. (1984) [13, Mansiangi et al. (2014) [14], and Makenga et al. (2015) [27]. Considering the supports identified at S1 and S2, this study confirms the breeding of *S.damnosum* at these sites; the flies lay their eggs in the river rapids, where the larvae hatch and develop into adults within eight to twelve days [28]. The pre-imaginal forms (eggs, larvae, and nymphs) are all aquatic and strongly rheophilic [28]. After hatching, the young larvae either remain attached to aquatic supports or drift with the current [28]. They feed with their rigid mandibular soles, which they use to randomly catch particles suspended in running water, including the nutrients they need [28].

4.2 Black fly species at capture sites

Morphological examination of the collected larvae and adult females revealed that they all belonged to the species *S.squamosum* of the *S.damnosum s.l.* complex; this was in coherence with results from previous surveys by Mansiangi (2014) [14] and Makenga (2015) [25] in Kinshasa. Henry et al. had also identified the vectors of *O.volvulus* to belong to the *S.damnosum s.l.* complex during the 1984 Kinsuka outbreak [13].

However, Traoré et al. reported species of the *S.neavei* complex to be the only vectors at the Sankuru basin in DRC from July 1998 to January 1999 [25]. The credibility of onchocerciasis control programs depends on thoroughly evaluating the effectiveness of treatments. The entomological evaluation includes verifying the effectiveness of the treatment during an outbreak, by estimating larval mortality and determining the bite rate and onchocerciasis transmission parameters [29]. All species reported (*S.squamosum* of *S.damnosum* s.l. complex) exhibit the phenomenon of "limitation", a factor that favors disease transmission [30–34]. "Limitation" refers to vectors being effective even at very low parasite densities [35]; the greater the number of microfilariae ingested, the lower the percentage that reach the hemocoel [32]. Flies infected with a low microfilaria load have a greater chance of surviving and transmitting parasites [33,36]. The peritrophic membrane facilitates "limitation" by reducing the number of ingested microfilariae that reach the hemocoel. This percentage of reduction is lower in tropical forests [37,38] than in savannahs [31]. Therefore, *Simulium sp.* found in forests transmit more infectious larvae than those in savannahs.

4.3 Entomological parameters

This study shows a diurnal fly bite cycle; two peaks, a minor peak between 8 and 9 a.m. and a major peak between 4 and 5 p.m. (Fig. 5), were observed. Our results confirm the conclusions of Henry et al. (1984) who observed the same two peaks in the Kinsuka study [13]. Mansiangi et al. also observed two peaks at Kinsuka: a major peak between 4 and 5 p.m. and minor peak between 9 and 10 a.m. [14]. Makenga et al. also observed two peaks: a major peak in the morning and minor peak at around 5 p.m. [27]. This difference may have resulted from an increased fly activity when people were concentrated around the breeding sites, namely in the morning and at sunset, as in our case. Our results corroborate with those of Nascimento-Carvalho in Brazil or Homoxi, where the biting activity of female Simuliidae showed a bimodal pattern, with peaks in activities early morning (between 7 and 8.50 a.m.) and afternoon (between 4 and 5.50 p.m.) [39]. In Thirei, the biting activity was evenly distributed throughout the day [40]. Black flies were captured simultaneously in Homoxi and Thirei using systematic methods to compare the traditional capture method using human bait (Human Landing Catch, HLC) with HLC protected by MosqTent® [39]. Comparing the anthropophilic profiles of black flies captured using MosqTent® and HLC, the percentage of species caught fluctuated seasonally in both Homoxi and Thirei, except between 11 h–11 h 50 min in Homoxi and between 4 and 4.50 p.m. in Thirei, where the difference was based on the method of capture [39].

The MBR was relatively high in this study, with an average of 3,103 monthly bites per person per month. Our results differ from those of Makenga et al. (2015) in Kimwenza, where the harm was insignificant [27]. We observed an MBR above the tolerable threshold of 30 bites per person per month, in line with Henry et al. in Kinsuka in the 1980s (MBR: 101–330) [13]. Significant variation in TPM was reported by Mansiangi in Kinsuka (2008: 8,463–10,070; 2009: 6,840–6,099; 2011: 1,798–2,750) [14]. Changes in ecological conditions may explain this difference. Besides, although the Lukaya River is a small tributary and offers few breeding sites, just like the Congo River at Gombe, the fast-flowing freshwater may be conducive for black fly production (larval and pupal stages) and support the main ecological characteristics of black flies.

In our study, we captured 952 i.e. 43 % of parous females at S1 in Gombe and 1075 i.e. 28% of parous females S2 in Kimwenza, respectively (Fig. 4, Tables 1 and 2). Our observations differ from those of Makenga et al.(2015), where only 5.4% of the captured flies were parous [27]. The black fly survival rate, in our study, was not as low as that reported by Makenga et al. (2015) [27]. Contrarily, Enyong et al. (2006) observed a relatively higher percentage at Zinga in the Central African Republic, with an estimated parity rate of 5.3% [40], than Kahn, Cameroon (parity rate: 7.2%) [22]. The higher parity rate in the Cameroonian study was because it was conducted for 12 months.

Nulliparous females play no role in the transmission of *O.volvulus*. As no infected parous females were not identified in our study, the intensity of transmission (0 Li / 1000 pares) and ATP recorded in both Kimwenza and Gombe were supposed zero. This may be because of the significant ivermectin coverage in Kinshasa (around 91% in 2018), according to WHO and APOC, which may interrupt the chain of transmission during an onchocerciasis outbreak [20]. Our observations corroborated with Makenga et al. (2015) in Mont-Ngafula, Kimwenza [27] and Enyong et al. (2006) in Zinga (Central African Republic), where no infected parous females were captured [40]. In 2014, Mansiangi found that 0.7% of parous females were infected but not infectious; therefore, no ATP was determined in Kinsuka due to the implemented Community-directed treatment with ivermectin (CDTI) measures [14]. Considering our entomological results, the black fly density should be monitored and the efficacy of CDTI should be assessed in the study area. Comparing the average number of black flies between the capture site and period in the bivariate analysis did not show statistically significant differences. Our results matched with those of Mansiangi et al. in Kinshasa; despite a high black fly density at S2, the average number of black flies captured did not differ significantly between S1 and S2 [14].

5. Conclusion

This study concluded that larval supports and vectors for onchocerciasis exist at both sites, Mont-Ngafula in Kimwenza and Gombe. *S.squamosum* is responsible for disease transmission at these sites. However, entomological indices of onchocerciasis transmission were low at the target sites during the study period. The high density of the host population can disrupt the transmission of vector-borne diseases by diluting the transmission indices.

Following the results of this study, we recommend the following to the Ministry of Public Health through PNLMTN-CP:

1. To provide DRC with a laboratory capable of molecular testing (pool screening).
2. To organize an epidemiological and entomological assessment at the four sites in Kinshasa according to the WHO/APOC protocol to enable the PNLMTN-CP to demonstrate the elimination of onchocerciasis from Kinshasa and monitor its re-emergence at these sites. These sites, located in Kinshasa city, have high population densities that may dilute the entomological indices and disrupt onchocerciasis transmission.
3. To perform entomological monitoring in Kinshasa to study the black fly biting rates. This will enable PNLMTN-CP to collect the data necessary to advocate vector control.

To the Scientific Community, we recommend promoting research in the field of medical entomology, in general, and onchocerciasis entomology, in particular, to support the Ministry of Health in research and entomological evaluations of onchocerciasis across the country.

6. Abbreviations

ABR, annual biting rate;

APOC, African Programme for Onchocerciasis Control;

ATP, annual transmission potential;

CDTI, Community Directed Treatment with Ivermectin

DRC, Democratic Republic of Congo;

HLC, Human Landing Catch;

ISTM; Institut Supérieur des Techniques Médicales

MBR, monthly biting rate;

MTP, monthly transmission potential;

NS, Non-significant;

PNLMTN-CP, National Programme for Neglected Tropical Diseases Control;

SD, Standard deviation;

ULB Université Libre de Bruxelles ;

UNIKIN, Université de Kinshasa ;

WHO, World Health Organization; DBR, daily biting rate;

7. Declarations

7.1 Ethics approval and consent to participate

The study was approved by the ethics committee of the Kinshasa School of Public Health (approval number ESP / CE / 139/2019). The data were collected following the ethical principles defined in the Declaration of Helsinki. Informed and written consent were obtained from the four black fly catchers used as human bait in this study. They did not benefit financially from this initiative. They underwent medical examinations after participating in the study but were not subjected to preventive Ivermectin treatment to avoid possible side effects. The two fly catchers employed per site, alternated after each hour, which reduced their exposure times.

7.2 Consent for publication

Not applicable

7.3 Availability of data and materials

“The dataset(s) supporting the conclusions of this article is (are) included in the article (and its additional file(s): Tables and figures)”.

7.4 Competing interests

The authors declare that they have no competing interests.

7.5 Funding

No funding sources.

7.6 Authors’ contributions

MBJC is the main author: he designed the study, participated to data collection and analysis. MBJC, MP, ZJ and YC participated to the writing of the manuscript, data analysis and marking of the final version. IF took part to data analysis and marking of the final version. YC participated to the writing of the manuscript, methodology and read-through. All authors were involved in the preparation of the manuscript, edition and finalization of the version to be published and agreed to be accountable for all aspects related to the integrity of the work.

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Figures

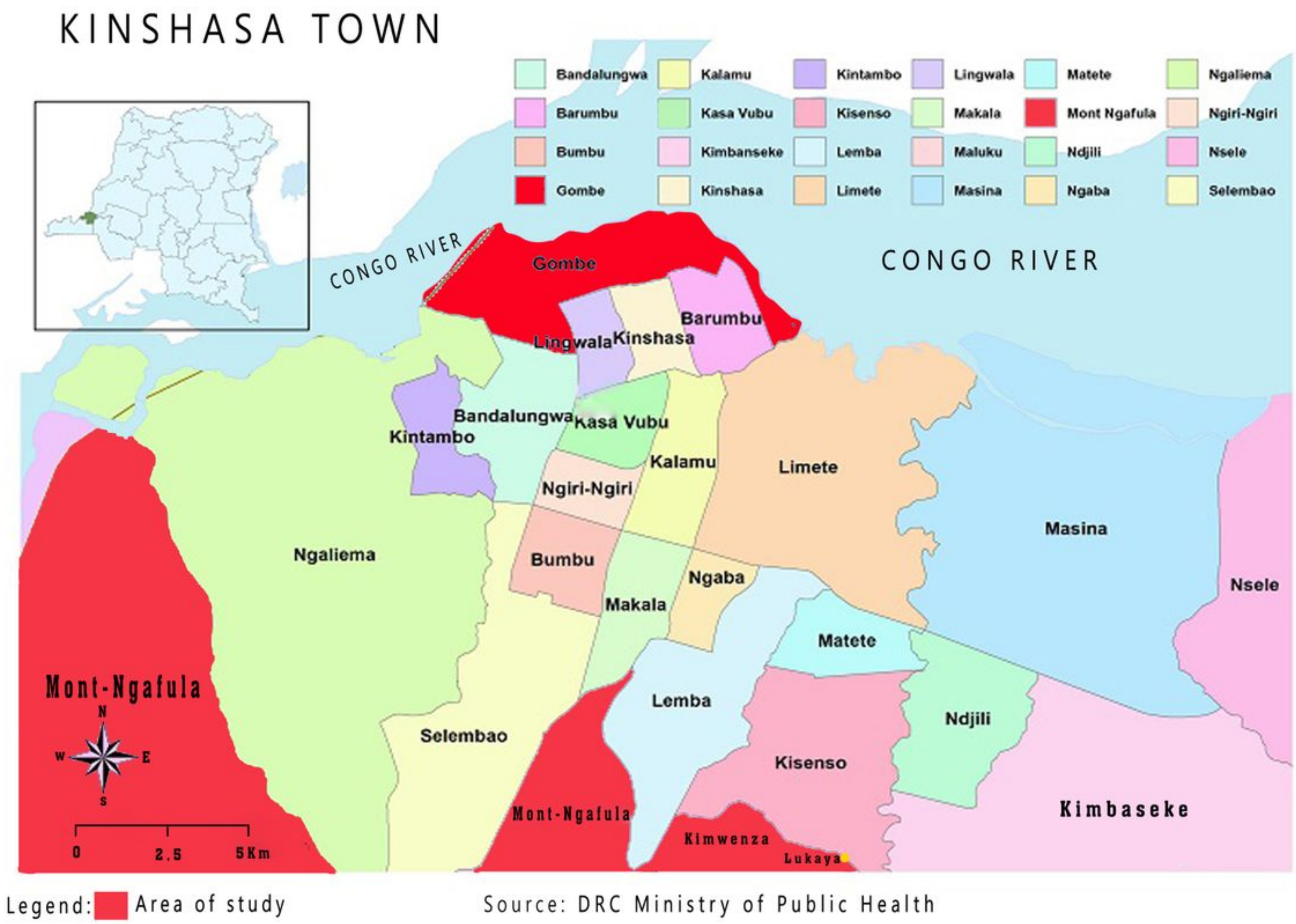

Figure 1

Map of the city-province of Kinshasa showing the two sites under study: Gombe (Site 1) and Kimwenza (Site 2) [inspired by the Atlas of the administrative organization of the DRC; Ministry of Public Health] [19]. DRC, Democratic Republic of Congo. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

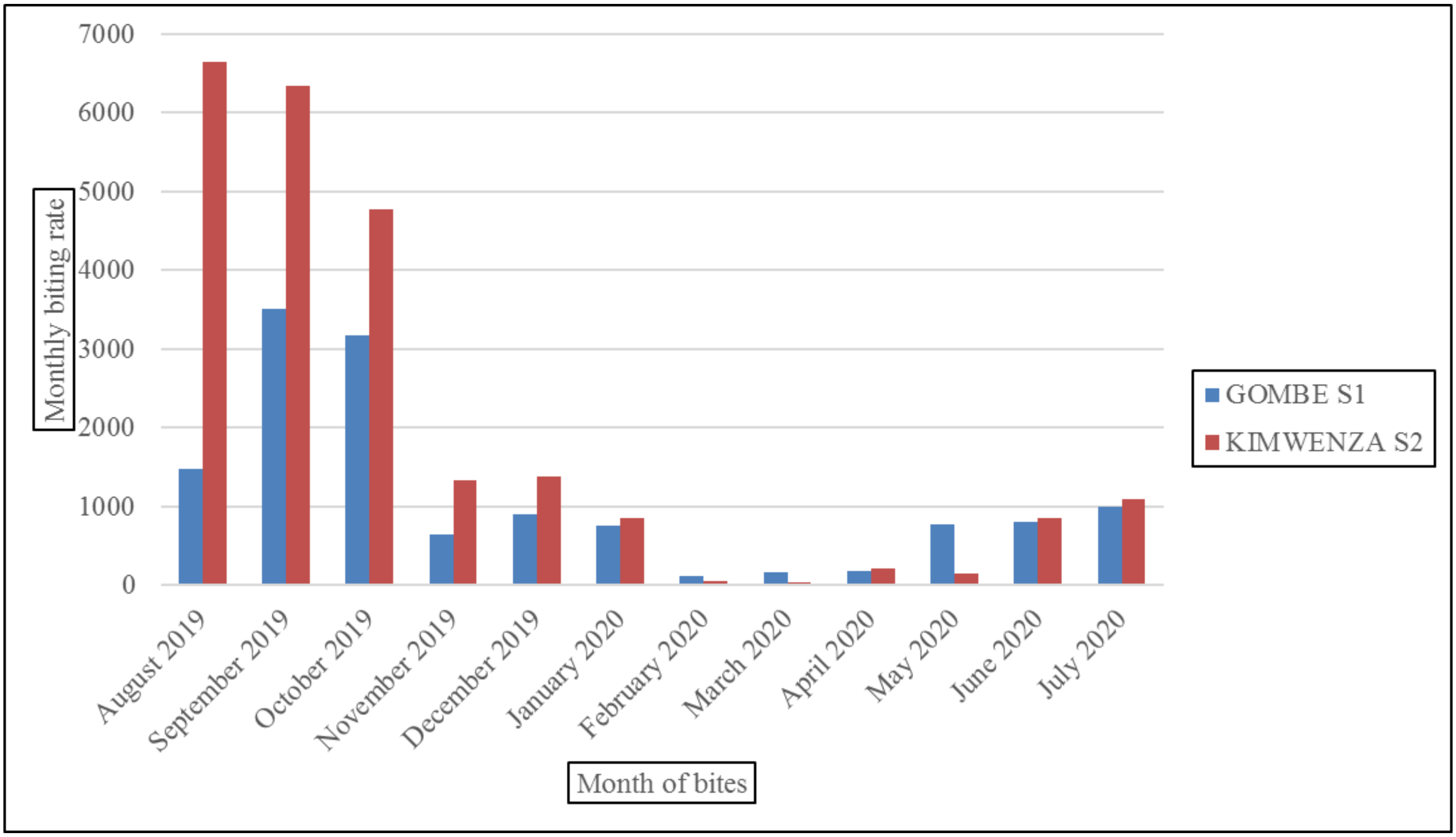

Figure 2

Monthly biting rate per person at the Mont-Ngafula site in Kimwenza and Gombe site, in the city-province of Kinshasa, from August 2019 to July 2020.

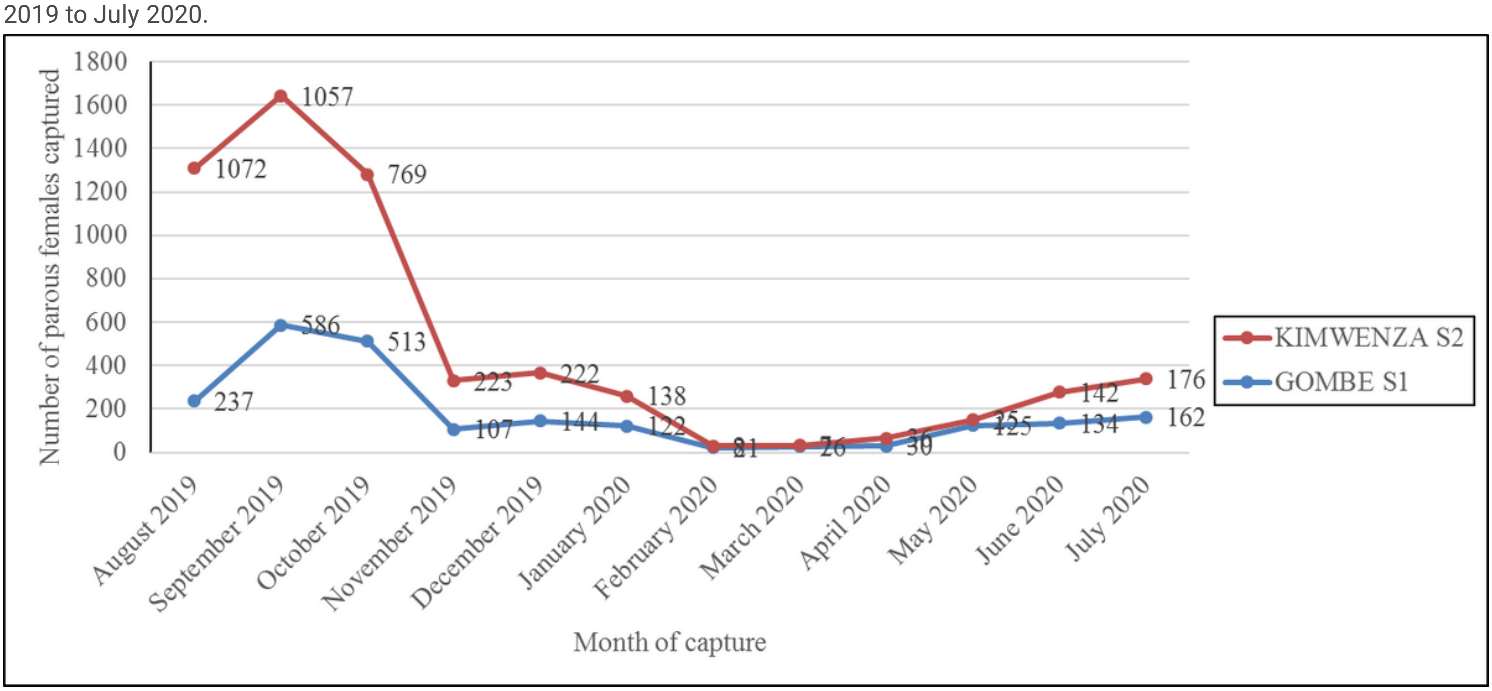

Figure 3

Variation in the number of parous females at the two capture sites, Gombe and Kimwenza, from August 2019 to July 2020, in the city-province of Kinshasa.

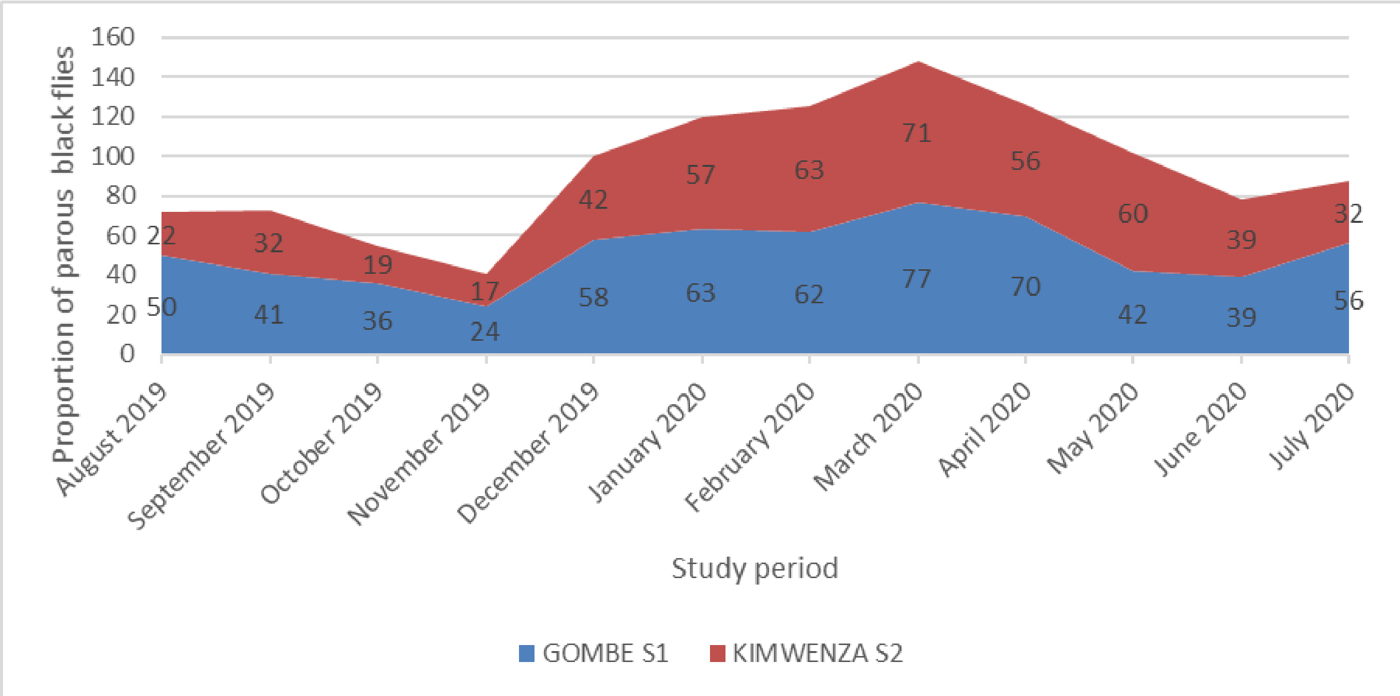

Figure 4

Proportion of parous black flies captured at the two sites, Gombe and Kimwenza, from August 2019 to July 2020, in the city-province of Kinshasa.

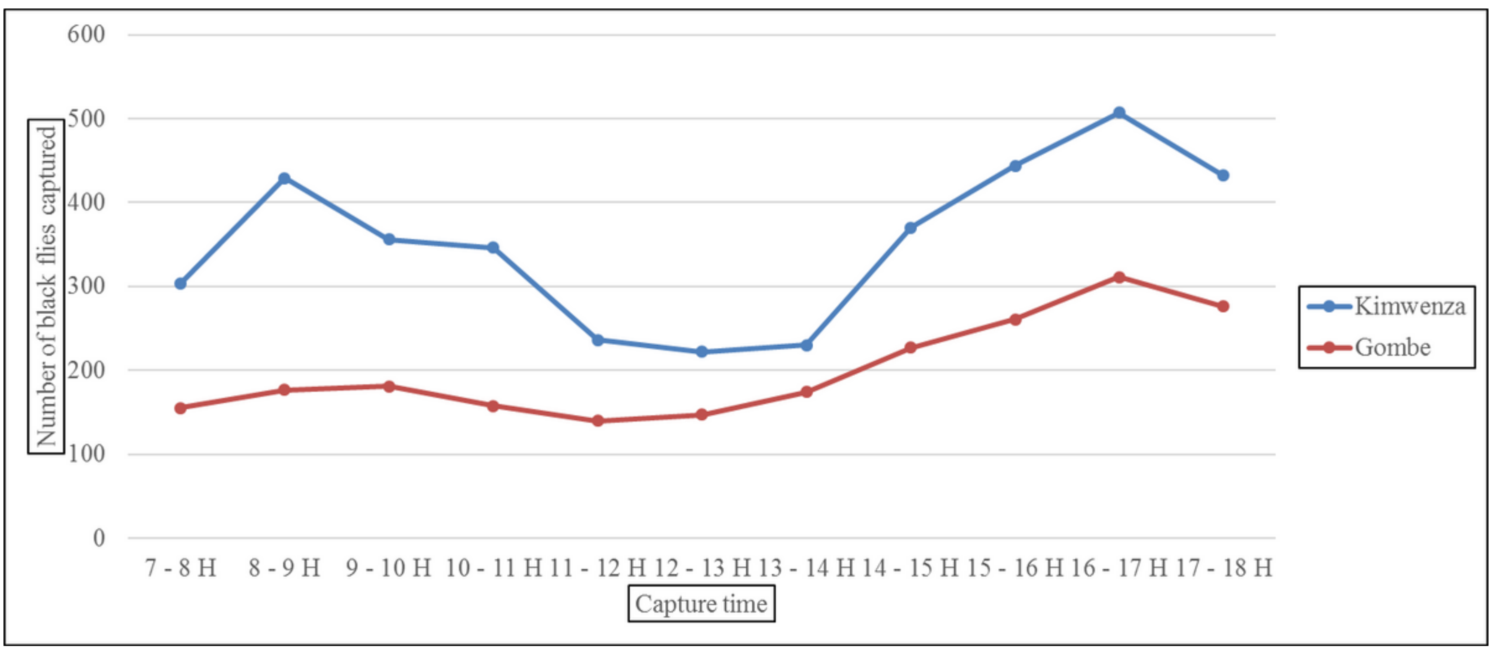

Figure 5

Daily cycle of aggression of female black flies in Gombe and Kimwenza during the study period, August 2019 to July 2020. H, hour.

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