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Analysis of the transmission level of onchocerciasis in a health area in Kinshasa, Democratic Republic of Congo (DRC)

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Abstract

The present study was designed to investigate the current level of onchocerciasis transmission in Mont-Ngafula I area, a health area of Kinshasa from October 7, 2014 and November 27, 2015. Pre-imaginal black flies were collected at breeding sites for adult fly collection on Lukaya River and in the most appropriate place for human-fly contacts. Entomological prospections showed that *Simulium squamosum* was the only vector species present in the focus. A total of 2,573 female flies were trapped (1,296 at S1 and 1,277 at S2). The daily aggressiveness cycle showed two peaks: the highest peak was registered between 7:00 and 8:00, while the second (lower) peak was observed between 17:00 and 18:00 at both trapping sites. Out of all dissected females, 5.4% were parous. The number of parous females varied according to the site (5% at S1 vs. 6% at S2), but not significantly (p=0.663). Only 27% of parous females were infectious, among them, 11% at S1 and 16% at S2. The annual biting rate per person reached 5,269 at S1 and 5,183 at S2. The females black flies density was not different in sites 1 and 2, with a mean of 14.4±4.3 and 14.2±3.6 bites per human per day. The Monthly Transmission Potential (MTP) was null during the period of study, when *Simulium* multiply and become more harmful. Various substrates were identified for larvae: aquatic plants, plastic bags, dead leaves and rocks.

Keywords: Onchocerciasis, transmission level, entomological study, *Simulium squamosum,* DRC

Introduction

Onchocerciasis is a parasitic disease caused by the filarial worm *Onchocerca volvulus*. It is transmitted through the bites of infected black flies of the *Simulium* genus, which breed in fast-flowing streams and rivers [1]. The larval stage of that insect is aquatic and rheophilic: larvae are found attached to submerged substrates, in running waters, at shallow depth, which provide oxygen and food [2]. Onchocerciasis occurs mainly in tropical areas, especially in Sub-Saharan Africa, but is also spread in Yemen and Latin America [3]. It is considered as a neglected tropical disease, affecting mainly low-income populations living in tropical and subtropical regions[4]. It is commonly referred to as « river blindness », because the black fly transmitting the disease is abundant along fertile river shores. In the human body, the adult female worm *O. volvulus* produces thousands of larvae (microfilaria), which migrate in the skin and eyes, causing visual deficiencies and blindness. Onchocerciasis is also responsible for a skin disease expressed through depigmentation and persisting itching unbearable for the patient [5]. Onchocerciasis is one of the main causes of blindness in the world (among them: cataract [46%], trachoma [12.5%], child blindness [3.3%], onchocerciasis [0.6%] and other causes [37.5%]. It is the second infectious cause of blindness in the world, after trachoma[6,7]. It is estimated that 120 million people are exposed to the risk of *O. volvulus* infection, while 37 million of people would be infected worldwide [7, 8]. Half a million people would be blind because of river blindness [9, 10]. In Africa, onchocerciasis occurs in a large area, limited by a northern border at 15° north latitude and a southern border at 13° south latitude, thus from Pre-Saharan Sahel to Angola and Tanzania [11]. The African continent is the most affected in the world. According to the World Health Organization (WHO), 99% of infected people live in 31 countries of Sub-Saharan Africa and the remaining 1% in Yemen and Latin America [12]. In the Democratic Republic of Congo (DRC), onchocerciasis is a public health concern, and is believed to be endemic in 11 provinces[13]. About 26 million people were exposed and 13 million were infected in 2013; blindness was reported in 70,000 people [16]. Several species of

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the genus *Simulium* play the role of vector all around the country: *Simulium damnosum*, *S. neavei* and *S. albivirgulatum*[14, 15]. The city of Kinshasa, capital of the DRC, was also affected by river blindness; three foci of onchocerciasis were reported in the city: Kinsuka-fishers, Nsele and Mont-Ngafula. Several authors reported the presence of the disease vector in Kinshasa: *Simulium* sp. belonging to the *damnosum* complex (*S. Squamosum*) and *S.albivirgulatum* have been identified [14, 15, 17]. The National Programme for Onchocerciasis Control (NPOC), with the help of the African Programme for Onchocerciasis Control (APOC), implemented onchocerciasis control 15 years ago, through the Bas- Congo/Kinshasa Community-Directed Treatment using Ivermectin (CDTI) project [13]. The presence of *S. damnosum s.I.* in Kinshasa was described for the first time in 1903 by an English mission of the Liverpool School of Tropical Medicine; nevertheless, it is only in 1926 that a scientific team conducted by Blacklock, demonstrated that onchocerciasis was transmitted by black flies [18-20]. In 1948, the biting nuisance caused by *S. damnosum s.I* became more and more significant. Meanwhile, onchocerciasis prevalence was very high, up to the point that 100% of shoreline inhabitants of the river, and 45% of Europeans living in the western neighbourhood of the city, were infected by *O. volvulus* [21]. In 1941, the exploration of all rivers pointed out rapids as the breeding sites; a campaign vector control was implemented, leading to the disappearance of biting nuisance for a long period. The left shore of Congo River (Kinsuka area, Kinshasa) was prospected for pre-imaginal breeding sites, but none was discovered despite the abundance of larval and nymph substrates. The tributaries of the river located upstream of Kinsuka village (until 10 km: Lukunga, Binza, Mampeza, Basoko and Yolo Rivers) had low stream and were quite contaminated [21]. Our survey targeted Mont-Ngafula I health area (HA), following the Rapid Epidemiological Mapping of Onchocerciasis (REMO) performed in 2015, and included seven rural Health Area (HA) covering a population of 95,355 inhabitants [17]. In 2012, the therapeutic coverage of the Bas- Congo/Kinshasa CDTI project reached 75.7%, but remained below the 80% required to eradicate onchocerciasis and interrupt its transmission [21]. As no study has been performed so far in Mont-Ngafula, the present study was designed to assess the current transmission level of onchocerciasis in that focus.

Materials and Methods
Study framework
The urban-rural HA of Mont-Ngafula I was targeted in the present survey, as its rural part is well known as an onchocerciasis focus; nevertheless, entomological data have not been assessed lately. In its southern part, Mont-Ngafula I HA is crossed by Lukaya River which flows into Ndjili River, forming the limit with N'sele HA. Lukaya River is 15 m wide and flows over 70 km in the city of Kinshasa: some sections include rapids, which are suitable locations for the development of black fly larval sites. The water level is modulated by seasonal rainfalls. The main flood occurs in November and December [17]. Savanna partly covers that area above the hills, and a forest gallery runs along the watercourses and Lukaya River. Three vegetal formations are identified at adult flies catching sites and larvae breeding sites along Lukaya River, namely: semi-aquatic grassland, ruderal formation and forest gallery (Picture 1 and 2).
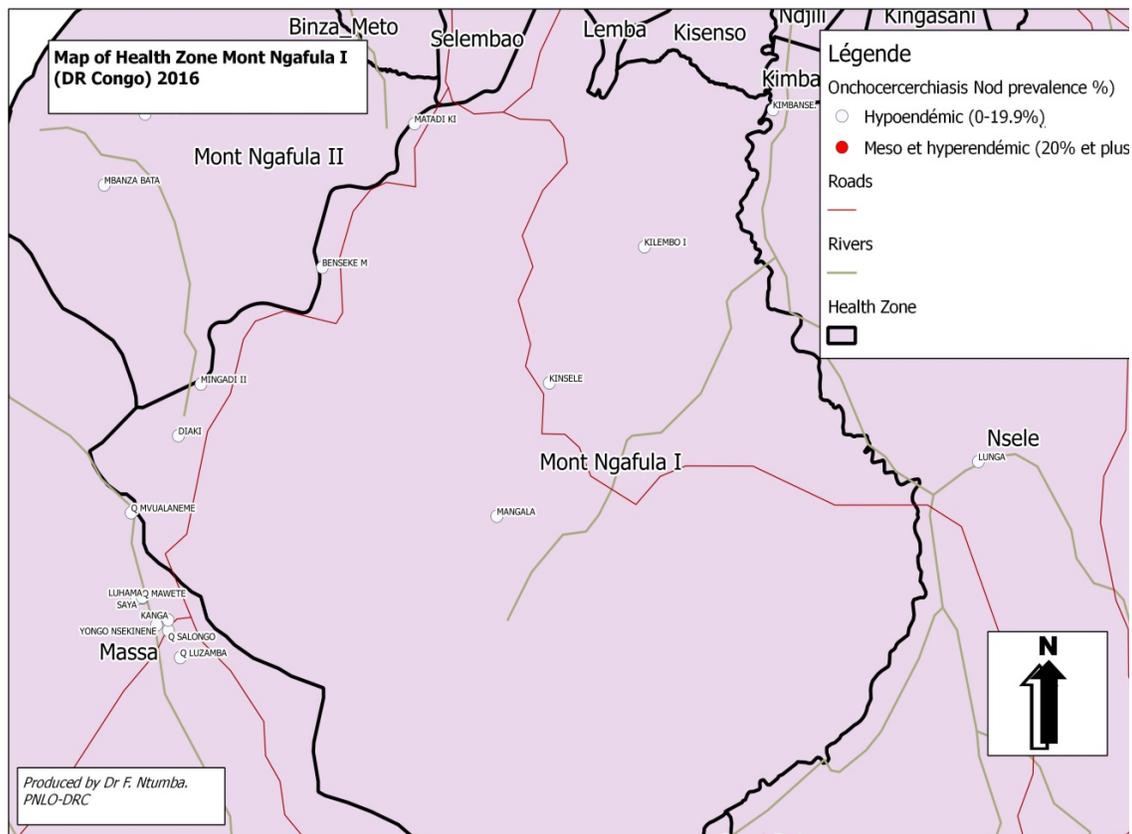

Fig 1: Mont-Ngafula I Health Area in Kinshasa city, DRC

Type and population of study

The present longitudinal entomological study was designed to determine the transmission potential of onchocerciasis in Mont-Ngafula I focus. The study covered a period of 59 weeks, from October, 7 2014 to November, 27 2015, corresponding to the two main seasons in Kinshasa, the rainy and dry seasons. In the rainy season, waters were high, which was suitable for a significant increase of black fly populations. The population of study included all black flies caught at two sites (S1 and S2) of Mont-Ngafula I focus. Human landing collections were performed two days in a week during 416 days (around 59 weeks).

Sampling of the study population (black flies)

In order to sample black flies, larval sites on Lukaya River were first prospected by land: plants and rocky substrates were carefully inspected for larval sites, and for the collection of pre-imaginal flies. All samples were conserved for further lab analysis.

Description of black flies trapping sites
Criteria for selecting trapping sites

The following criteria determined the selection of trapping sites: the presence of human habitations or other human activity in the neighbourhood, the proximity of a breeding site in the river and an accessible shadowy place sheltered from the wind.

Prospection of black flies breeding sites in Mont-Ngafula I focus

The focus was explored two days earlier, in order to locate sites of pre-imaginal forms, to identify their fixation substrates and to select the vector-human contact points. The two trapping sites for adult flies, S1 and S2, were selected among the most appropriate vector-human contact points.

Collection and conservation of larvae

Pre-imaginal forms were collected by hand, with a wooden spatula, at larvae breeding sites. They were further transported to the lab, in clean plastic pots containing river water, for immediate microscope observation.

Trapping and conservation of female flies
Selection of trapping site

The selection of adult trapping sites depended on samples collected at breeding sites and vector-human contact points. Trapping sites were located next to places where human beings reside, (and close to larvae sites), in the shade and sheltered from the wind.

Trapping of female flies

Samples were collected twice a week, two days in a row, during 59 weeks. Adult flies were caught on human baits, as described by Le Berre in 1966 [14, 15, 28]. Hydrological parameters were recorded as well (thermometer and pH meter) [14, 27].

Variables of the study
Dependent variable

Transmission of onchocerciasis was considered as the dependent variable in the study.

Independent variables
a) Variables related to the vector
- Black fly density: average number of bites per human, per day

- Nulliparous female: female which has never layed eggs
- Parous female: female fly which has layed eggs at least once
- Infected parous female: fly hosting evolutionary larvae (stage L1, L2 or L3), regardless of their location
- Infective parous female: fly hosting infective larvae (L3) in the head capsule
- Infection rate: number of infective flies per 1,000 parous flies
- Fly parasite load: number of *O. volvulus* L3 per 1,000 parous flies
- Black fly species acting as a vector in the focus: scientific and entomologic nomenclature of the fly responsible for onchocerciasis transmission

b) Variables linked to the environment
- Breeding site: aquatic substrate likely to host pre-imaginal stages in running waters
- River flow: water flow speed, expressed in cubic meters per second (m^3/sec)
- Depth : water height of a river
- Physico-chemical characteristics of water: pH, turbidity, conductivity and temperature
- Atmospheric conditions: temperature, relative humidity and wind speed.

In an onchocerciasis focus, vector control is performed through two main activities: campaign of larvicide treatments and entomological assessment of their efficiency. These activities require the commitment of human, material and financial resources, among others:
- Coordination of control activities
- Training of entomological technicians
- Weekly larvicide treatment against black flies in the water system
- Long-term weekly entomological surveillance of the water system
- Reporting of data and results of control activities
- Hydro-biological surveillance of aquatic fauna
- Management of logistic and maintenance of equipment

Identification of black fly species caught

Observation through stereo-microscope allowed identifying species by considering morphological criteria and their comparison with morphological criteria described by Traore *et al* [21], but also by following dichotomic keys suggested by Crosskey [14, 17].

Determination of entomological indices

Agressiveness cycle and level, as well as parity and transmission potential, were selected to determine entomological indices, according to WHO directives [23,27].

Agressiveness cycle and rate

Agressiveness cycle is the daily rhythm of fly bites. From epidemiological and entomological points of view, such key determines the times of day when risks of bites and transmission are the most important [14]. The mean number of flies caught per hour (estimated by Excel software) determined the cycle. The agressiveness rate is represented by the Daily Biting Rate (DBR) or daily number of fly bites. It can also be expressed as the mean number of bites per person per month or per year [14]. The monthly agressiveness rate, or Monthly Biting-Rate (MBR), is determined through the

following formula [14, 27]:

MBR = (NbBF x NbDM) / NbDC

MBR = Monthly Biting-Rate

NbBF = Number of black flies

NbDM = Number of days during the month of interest

NbDC = Number of days of capture

The cumulation of MBRs results in the Annual Biting-Rate (ABR).

Parity and infection

All flies were dissected at the School of Public Health laboratory to determine their parity (nulliparous vs. parous). Parous flies were further dilacerated in a drop of physiological water by opening abdomen and thorax, then carefully examined for *O. volvulus* larvae. Larvae number, evolution stage (L1, L2 or L3) and location were registered. Black flies hosting evolutionary larvae (L1, L2 or L3), whatever their location, were considered as infected; black flies hosting infective larvae in head capsule were called infective [14, 21]. The following materials and reagents were used: a VWR™ stereo-microscope (Stemi DVA model), at 10x magnification, a dissection kit including entomological needles, thin clamps, microscope slides, dropper and physiological water.

- Parous females presented the following characteristics: presence of egg-laying relics, possible residual eggs, elastic ovaries and clear or semi-clear Malpighian tubules
- Nulliparous females were characterised by: absence of egg-laying relics and residual eggs, breakable ovaries, opaque Malpighian tubules and abundant abdominal fats

After dissection, parity was estimated by the following formula [14]:

Parity = (NbPBF / NbDBF) x 100

NbPBF = Number of Parous Black Flies

NbDBF = Number of Dissected Black Flies

The determination of parity is crucial, as only parous flies are able to transmit *O. volvulus*. The proportion of parous flies influences the importance of parasite transmission in humans. Furthermore, the parous/nulliparous ratio is often used to estimate the fly survival rate. Indeed, the abundance of parous flies is proportional to their survival time, which directly influences the risk of parasite transmission. Infection rates, parasite loads and transmission potential were estimated in the study area [14, 24].

Transmission potential

In order to assess the importance of onchocerciasis transmission by the vector, we estimated the Monthly Transmission Potential (MTP), according to the WHO formula [14]:

MTP = MBR x (NbL$_3$ / NbDBF)

MTP = Monthly Transmission Potential

MBR = Monthly Biting-Rate

NbL$_3$ = Total number of *O. volvulus* L3 observed in fly head capsules

NbDBF = Number of Dissected Black Flies

The MTP is expressed as the number of *O. volvulus* infective larvae received per person and per month. The Annual Transmission Potential (ATP) is estimated by summing the 12 corresponding MTP. It is expressed as the number of *O. volvulus* infective larvae received per person and per year. The transmission potential of onchocerciasis is expressed through the number of infective larvae per fly.

Statistical analysis

Entomological data were registered daily on collection cards. A first assessment of consistency of reported information and of potential omissions was performed by catchers in the field. Afterwards, the whole team (catchers, supervisor and main investigator) reviewed all collection tools to identify any possible incoherence. The student test (t test) was used to compare the means of blackflies at two trapping sites (S1 vs S2) and the trapping period; chi-square tests or Fisher's exact tests were used to compare proportions of parous females at both trapping sites. The p-value < 0.05 was considered significant.

Data were analysed with Excel™ and SPSS 20™ software.

Results

Entomological indices

Results of trappings, dissections and indices of onchocerciasis transmission are summarised in Tables 1 and 2. A total of 2,573 black flies were trapped during the period of study, 1,296 at trapping site n#1 (S1) and 1,277 at trapping site n#2 (S2). Densities of female black flies were similar at both trapping sites, with an average of 14.4 ± 4.3 and 14.2 ± 3.6 monthly bites per person, at S1 and S2, respectively. Student t test highlighted no significant difference between both sites for what the mean number of trapped black flies was concerned. All black flies captured at S1 and S2 were dissected. The highest parities were obtained in July (10%) and September (10%) and November (9%). The daily aggressiveness cycle showed two peaks at both trapping sites; the highest peak occurred between 7:00 and 8:00 and the second one between 17:00 and 18:00 (Figure 3). The annual biting rate (ABR) reached 5,269 bites per person. Among all dissected black flies, none was carrying larvae of *O. volvulus*. The transmission potential was thus estimated as null at both sites.

Substrates of pre-imaginal sites in Mont-Ngafula I focus

Different larvae substrates were identified in the study area, on which pre-imaginal stages were collected: artificial waste (used clothes and plastic bags), aquatic plants (*Echinochloa pyramidalis*, *Eichhornia*, *colocasia esculanta* and *commelina diffusa*), plant remains (pieces of wood, tree branches and palm leaves) and rocks. These substrates were more numerous around Lukaya River rapids, especially in places frequented by the surrounding human population. Pre-imaginal population was more important on artificial substrates compared to plant remains.

Identification of fly species in Mont-Ngafula I focus

The species of flies identified, namely *S. squamosum* belonging to the *S. damnosum s.l.* complex, was the same as in Kinshasa area. Colour of tuft wings (tufts of bristles located at the wing bases) and relative colour of antenna, procoxa and prosternum (aspect of antenna), were the morphological characteristics that allowed identification [23].

Determination of entomologic parameters for onchocerciasis transmission

The present study identified entomologic parameters of onchocerciasis transmission: DBR, MBR, parity, fly infection rate (percentage of infected parous females and percentage of infective parous females) and transmission potential.

Daily biting rate of black flies
As shown in Fig 1, the DBR was up to the acceptable level of 30 from December to January and between March and April. It was low during approximately eight months of the study period. The nuisance associated with bites peaked at 39 on the 48th day of collection (April), while only one bite was recorded on the 6th day of collection (October). With a mean of 18 for the whole period of study, the DBR reached 9 in October and peaked at 18 in November (mean for the whole period of study). The MBR peaked in November, with 544 bites per human, and reached 270 in October 2015 in Mont-Ngafula I site) (Fig 1).

Parity (fly 'physiological age')
The mean physiological age of female flies was expressed through the proportion of parous females. The parous/nulliparous ratio estimates the fly survival rate, directly linked to the risk of disease transmission. In S1, the dissection of 1,296 female flies allowed estimating a mean parity rate of 5% (N=67) (Table 1). Out of the 1,277 dissected female flies at S2, only 71 were parous (6%-mean parity rate), as illustrated in Table 2.

Black fly Infection rate
When studying the parous females dissected at both sites (N=67 at S1 and N=71 at S2), none was infected by *O. volvulus* larvae (whatever the stage). The infection rate was null during the period of study, as well as the rate of infective females.

Transmission potential of onchocerciasis during the study
Table 1 and 2 illustrate the null monthly transmission potential estimated during the period of study in Mont-Ngafula I focus. As mentioned above, no parous female was carrying *O. volvulus* larvae (Table 1 and 2).
The biting daily cycle was diurnal, as shown in Fig 3. Two peaks were noticed: one at the beginning and the other at the end of the day. The major peak was observed in the morning, i.e. between 7:00 and 8:00, while a minor peak was registered in the evening, between 17:00 and 18:00. The morning peak could be related with the moment human activities take place at larvae breeding sites (fig 3).
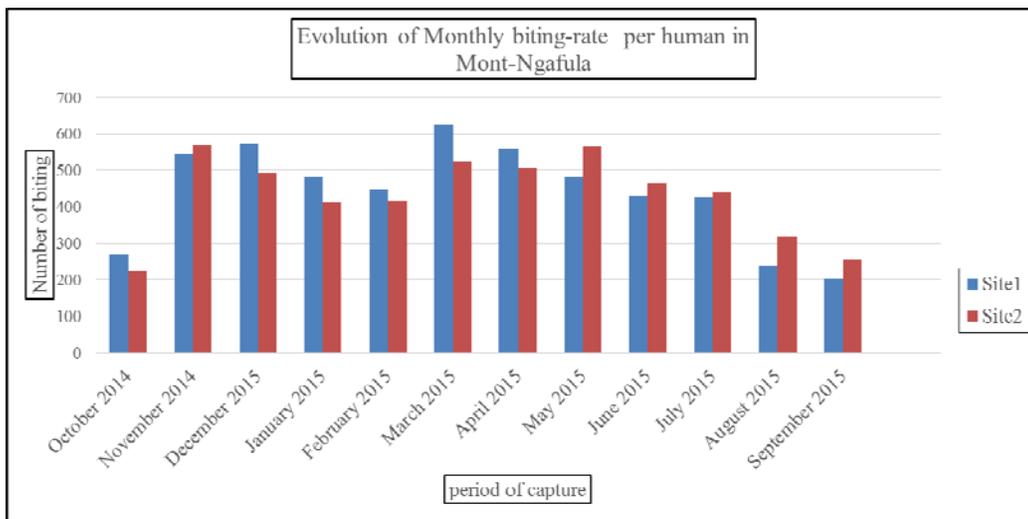

Fig 2: Monthly biting rate per human in Mont-Ngafula I health area, October 2014 to November 2015
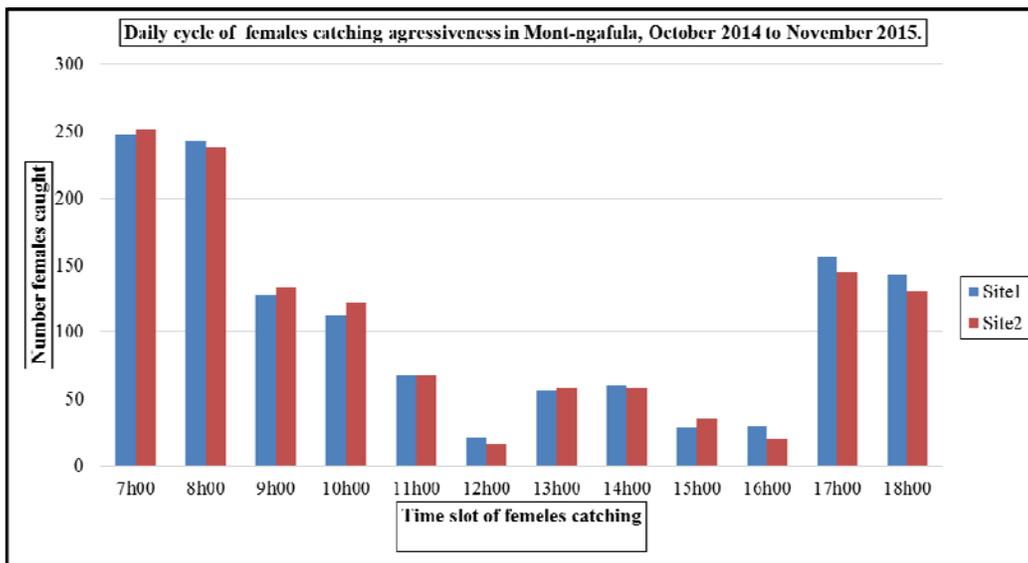

Fig 3: Daily cycle of females black fly agressiveness in Mont-Ngafula focus during the period of study
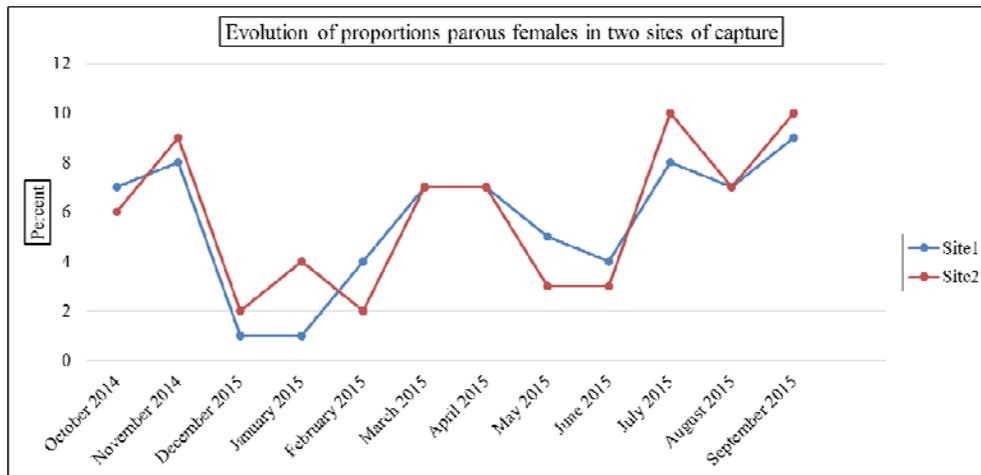
Fig 4: Evolution and Proportion of parous females in two sites of capture, October 2014 to November 2015, in Mont-Ngafula I focus

Picture 1: view of the semi-aquatic *Echinochloa* grassland in the middle of Lukaya River (site n#4), next to an artisanal quarry of cinder blocks

Picture 2: Relic of the forrest gallery close to the quarry (site #4) Location: site #4 (SO42752.3 : EO 151927.2 Alt. 213m).

Table 1: Synthesis of entomological indexes of *O. Volvulus* by *S. squamosum* from October 2014 to November 2015 at the first capture site in Mont-Ngafula I health areas.

Months of capture	October 2014	November 2014	December 2014	January 2015	February 2015	March 2015	April 2015	May 2015	June 2015	July 2015	August 2015	September 2015	TOTAL
Number days of catching	7	7	8	8	7	7	8	8	7	7	8	8	90
Number females caught	61	127	148	124	111	141	149	124	100	96	61	54	1296
Daily biting-rate	9	18	19	16	16	20	19	16	14	14	8	7	173
Monthly biting-rate	270	544	574	481	444	624	559	481	429	425	236	203	5269
Annual biting-rate													5269
Number femalesdissected	61	127	148	124	111	141	149	124	100	96	61	54	1296
Number parous females	4	10	1	1	4	10	10	6	4	8	4	5	67
Percentage parous females	7%	8%	1%	1%	4%	7%	7%	5%	4%	8%	7%	9%	5%
Number parous females dissected	4	10	1	1	4	10	10	6	4	8	4	5	67
Number infected parous females	2	2	0	0	1	1	2	1	0	1	0	1	11
Percentage infected parous females	50%	20%	0%	0%	25%	10%	20%	17%	0%	13%	0%	20%	16%
Number of parous females infected	0	0	0	0	0	0	0	0	0	0	0	0	0
Percentage of parous females infected	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of females infected/1000 parous	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of L3 at the top	0	0	0	0	0	0	0	0	0	0	0	0	0
L3 at the top/1000 parous	0	0	0	0	0	0	0	0	0	0	0	0	0
PMT (L3/man/mounth)	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2: Synthesis of entomological indexes of *O. Volvulus* by *S. squamosum* from October 2014 to November 2015 at the second capture site in the Mont-Ngafula I health areas.

Months of capture	October 2014	November 2014	December 2014	January 2015	February 2015	March 2015	April 2015	May 2015	June 2015	July 2015	August 2015	September 2015	TOTAL
Number days of catching	7	7	8	8	7	7	8	8	7	7	8	8	90
Number females caught	51	133	127	106	104	118	135	146	108	99	82	68	1277
Daily biting-rate	7	19	16	13	15	17	17	18	15	14	10	9	171
Monthly biting-rate	226	570	492	411	416	523	506	566	463	438	318	255	5183
Annual biting-rate													5183
Number femalesdissected	51	133	127	106	104	118	135	146	108	99	82	68	1277
Number parous females	3	12	2	4	2	8	9	5	3	10	6	7	71
Percentage parous females	6%	9%	2%	4%	2%	7%	7%	3%	3%	10%	7%	10%	6%
Number parous females dissected	3	12	2	4	2	8	9	5	3	10	6	7	71
Number infected parous females	1	2	0	2	1	2	2	2	0	2	0	2	16
Percentage infected parous females	33%	17%	0%	50%	50%	25%	22%	40%	0%	20%	0%	29%	23%
Number of parous females infected	0	0	0	0	0	0	0	0	0	0	0	0	0
Percentage of parous females infected	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of females infected/1000 parous	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of L3 at the top	0	0	0	0	0	0	0	0	0	0	0	0	0
L3 at the top/1000 parous	0	0	0	0	0	0	0	0	0	0	0	0	0
PMT (L3/man/mounth)	0	0	0	0	0	0	0	0	0	0	0	0	0

Discussion

The present study was designed to assess the current transmission level of onchocerciasis in Mont-Ngafula I HA focus.

Nature of substrates for pre-imaginal stages

The present study identified a diversity of substrates for larvae breeding sites in Lukaya River, such as waste, aquatic plants and marine rocks. Similar substrates were found by Henry *et al* and Mansiangi. Henry and collaborators reported the same diversity of larvae substrates in 1984 in Kinsuka focus (Kinshasa): *Vetiveria nigritiana,* rocks, floating plants (*Echinochloa, Eichhornia crassipes*) and some shrubs [15]. Mansiangi also mentioned a diversity of substrates such as aquatic plants (*Echinochloa pyramidalis*, *Eichhornia crassipes*), tree branches, artificial substrates (plastic bags) and domestic waste, as reported in Kinsuka focus in 2014 [14].

Vectors species in Mont-Ngafula I focus

The morphological examination of collected larvae and adult females caught on human baits revealed they all belonged to the genus *S. squamosum*, *S. damnosum s.l.* complex, confirming the previous 2014-survey performed by Mansiangi in Kinshasa [14]. Henry and collaborators had previously identified the vectors of *O. volvulus* as belonging to the *S. damnosum s.l.* complex, in 1984 in Kinsuka focus [15]. The same conclusions were drawn by Traore and collaborators in 2006 in Inga focus, Bas-Congo: nuisance was caused by biting females of *S. squamosum* and *S. soubrense* genus. Inga focus was further considered as the extension of Kinshasa focus [27].

Entomological indices of transmission

Daily cycle of fly bites (vector agressiveness cycle)

In the present study, the biting cycle of flies was diurnal; as mentioned above, two peaks were observed: a major peak between 7:00 and 8:00 and a minor peak between 17:00 and 18:00. Our results confirm the conclusions of Henry and collaborators in 1984 in Kinsuka focus: two peaks were observed, at the same moments [15]. In Kinsuka focus, Mansiangi also mentioned two peaks, the major peak being observed between 16:00 and 17:00, and the minor between 9:00 and 10:00 [14]. Such difference could be explained by an increased fly activity when people are concentrated around breeding sites, which was in the morning in our case.

Density of female adult flies (biting rate)

Nuisance was not important in the current study. The MBR we measured was above the tolerable threshold of 30 bites per human per month, similarly to the observations of Henry and collaborators in Kinsuka in the 1980's (range 101-330). An important variation of MBR was reported by Mansiangi in Kinsuka in 2008 (8,463-10,070), 2009 (6,840-6,099) and 2011 (1,798-2,750). Changes in ecological conditions could explain such difference. Furthermore, Lukaya River is a small affluent and offers less breeding sites.

Parity (longevity of female adults)

Only 5.4 % parous females were caught during our survey, thus our collection was mainly constituted by nulliparous young females. Black fly survival rate was low. Similar percentage were found in 2006 by Enyong and collaborators who had estimated a 5.3% parity rate in Zinga site, Central African Republic (CAR), [22] *vs.* 7.2% in Kahn, Cameroon [24] . The Cameroonian study was performed over a 12 month-period, which could explain a higher parity rate.

Parasitism of females by *O. volvulus* and transmission potential

Nulliparous females do not play any role in the transmission of *O. volvulus*. As no infected parous female was caught in our study, the transmission intensity (O Li/1,000 parous) and MTP registered in Mont-Ngafula I were null. Such observation could be consecutive to the important ivermectin coverage in Mont-Ngafula I HA (around 81% in 2014), likely to interrupt the transmission chain in an onchocerciasis focus, according to WHO and APOC [17]. Our results also confirm the observations of Enyong and collaborators in Zinga (Central African Republic), where no infectious parous female had been caught in 2006 [22]. In 2014, Mansiangi found 0.7% infected parous flies but no infective female, and thus came to the conclusion of a null ATP in Kinsuka, Binza Ozone HA; that area also implemented CDTI activities [14]. In view of our entomological results, surveillance was necessary to follow the fly density and to assess the efficiency of CDTI in the area of study.

The results of the bivariate analysis; comparing the average number of black flies according to trapping site and period, did not show any statistically significant difference. Our results corroborated those of Mansiangi and al in Kinshasa; despite a high black fly density at S2, the average number of captured black flies did not differ significantly between both trapping points [14].

Limitation to the study

Black fly trapping (human-vector contact) and prospection of

breeding sites were performed on a 4 km-section of Lukaya River only, and not the entire 70 km-distance the river flows in Kinshasa. All fly breeding sites, as well as all ideal locations for human-vector contacts, were not explored on Lukaya River.

Conclusion
The present study concluded that substrates for larvae sites and onchocerciasis vectors exist in Mont-Ngafula I focus. *Simulium squamosum* is responsible for onchocerciasis transmission. Entomological indices of onchocerciasis transmission were low in the HA targeted during the perid of study.

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