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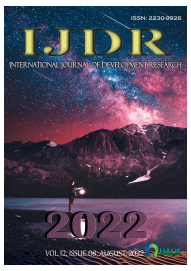

RESEARCH ARTICLE **OPEN ACCESS**

ABOUT THE ELIMINATION OF ONCHOCERCIASIS IN CÔTE D'IVOIRE: Potential frontline localities in the Aboisso region

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ABSTRACT

Objective: To highlight the agglomerations where the risk of transmission of onchocerciasis is highest in the region of Aboisso, with a view to eliminating this endemic in Côte d'Ivoire. **Methods:** Entomological surveys were carried out in the Aboisso region in the south-east of Côte d'Ivoire, in the forest zone, during the last semester of 2013 (from 2 July to 27 December). These surveys are part of a better knowledge of the status of onchocerciasis transmission in Côte d'Ivoire. The investigations took place at a time when the distribution of ivermectin had just been severely disrupted throughout the country, after six years of application. Thus, breeding places of *Simulium damnosum* s.l were prospected in the rivers of the main watersheds, the aggressiveness parameters of the populations of this complex were determined and the transmission of onchocerciasis was evaluated in the Aboisso region. **Finding:** Only 23.33% of the potential breeding places hosted preimaginal stages of *Simulium damnosum* s.l. These preimaginal stages were mostly collected in the Bia river and its tributaries (49.20%), as well as in the Noé river (15.11%). The monthly bite rate (MBR) for females fluctuates from 4.86 p/H/h to 124.24 p/H/h and the annual bite rate (ABR) varies from 58.27 p/H/year to 1,490.86 p/H/year. In the experimental area, each person receives approximately 1 to 17 bites every hour from 7 a.m. to 6 p.m. The highest rate of aggressiveness of *Simulium damnosum* s.l. (1490.86 p/H/year) was recorded in Bianouan. The annual potential for transmission (APT) is zero in the experimental area as none of the blackfly females dissected carried an infective stage of *Ochocerca volvulus*. **Conclusion:** Thus, the risk of transmission of onchocerciasis is non-existent at Aboisso, providing in this additional evidence of the efficacy of the control of this infection through treatment with ivermectin under community guidelines (CDTI). In addition, the annual bites rate (ABR) of females of *Simulium damnosum* s.l determined, argues in favor of a focus of the fight towards the elimination of onchocerciasis in the region. In the latter case, the village of Biaka, the N'guessan Assamoikro Camp in Bianouan and the locality of Kotoka, which are located near *Simulium damnosum* s.l. breeding places, appear as localities that can constitute first-line villages. To be able to easily establish the phase of elimination of onchocerciasis in Côte d'Ivoire, such a study should be undertaken on the whole of ivorian territory in order to updat the data on the preimaginal breeding places of *S. damnosum* s.l. which indeed date from the period of the OCP.

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INTRODUCTION

Onchocerciasis is a parasitic disease, a filariasis caused by *Onchocerca volvulus* (Leuckart, 1893; Gentilini and Richard-Lenoble, 2012). The disease is transmitted to humans by the blackfly, a blood-sucking dipterous insect of the genus *Simulium* (Philippon, 1978; Boatin and Richards, 2006). It is the leading cause of blindness in infested areas and the second leading cause of infectious blindness worldwide. Onchocerciasis occurs mainly in tropical areas. In Africa, it occurs in 31 countries in the western and central zones. Of the more than 6.5 million people who suffer from severe itching or dermatitis and almost 270000 who have lost their sight due to onchocerciasis, more than 90% of the cases are from Sub-Saharan Africa (Gentilini *et al.*, 2012). The endemic is also present in 6 Latin American countries and in Yemen in the Arabian Peninsula, where it is thought to have been introduced through the slave trade (Boussinesq, 1997; Boatin and Richards, 2006). In West Africa, onchocerciasis has been eradicated as a disease of public health importance through the operations of the Onchocerciasis Control Programme (OCP), implemented from 1974 to 2002 (WHO, 1999). As a result, nearly 600,000 cases of blindness were prevented, 18 million children were spared the risk of blindness due to onchocerciasis, and 25 million hectares of fertile arable land that had been idle due to the disease were able to be recovered for agricultural production (WHO, 2006; Hodgkin *et al.*, 2007). The initial and unique intervention strategy of the OCP was vector control directed against the aquatic larvae of the blackfly (Agoua *et al.*, 1991). To this end, the OCP mapped the preimaginal breeding places of *Simulium damnosum*s.l., excluding from the treatment perimeter the forest areas where ocular complications of the disease are less frequent, cases of blindness due

to onchocerciasis are very rare, and where there was no abandonment of riparian land due to the endemic (Prost *et al.*, 1980). In order to prevent a re-emergence of onchocerciasis above the threshold where it constitutes a public health problem, a new chemotherapy control strategy, based on community-directed treatment with ivermectin (CDTI), has been implemented (Boussinesq, 2005). It reduces microfilarial burdens and controls onchocerciasis morbidity (APOC, 2010). From 1989 to 2002, CDTI was applied in association with anti-vectorial treatments to continue control in the savannah and forest areas of the 11 countries covered by the OCP (Agoua *et al.*, 1991; Hodgkin *et al.*, 2007; Cupp *et al.*, 2011). Thus, with the combined application of disease vector control and mass treatment with ivermectin, onchocerciasis was almost no longer a public health problem and an obstacle to socio-economic development in the area covered by the OCP (ANONYME, 2014). After the closure of the OCP in 2002, ivermectin was used alone within the framework of the national health services.

Subsequently, the onchocerciasis control programmes of the 11 former countries covered by the OCP were integrated into APOC (African Program for Onchocerciasis Control). This programme was set up in 1975 to cover the 19 other countries in Africa outside the OCP. Moreover, already in the early 2000s, APOC's strategy was progressively oriented towards the objective of eliminating the disease through CDTI in all the 31 onchocerciasis-endemic countries in West and Central Africa. This approach consists of the elimination of transmission, which means according to Hopkins (2015), that the infection can no longer be transmitted in a given geographical area and that the population in that area is no longer at risk of being infected or re-infected, even if there are still a few sick people. After APOC the Expanded Special Project for Elimination of Neglected Tropical Diseases of the World Health Organization (WHO/ESPEN) was set up at the regional office of the World Health Organization (WHO) for Africa. The mission of ESPEN is to accelerate the elimination of the five most prevalent neglected tropical diseases (NTDs) on the African continent: lymphatic filariasis, onchocerciasis, schistosomiasis, helminthiasis and trachoma. ESPEN was established in 2016 to maintain the momentum towards the control and elimination targets set by WHO and endorsed in the London Declaration on NTDs in January 2012 (ANONYME, 2012) In Côte d'Ivoire, the distribution of ivermectin began in 1988 with the operations of the OCP. It was applied on its own from 1990 and was generalised throughout the country from 2007. From 2007 to 2010, the data transmitted to the WHO indicated that, on ivorian territory, the prevalence of pathogenic agents of onchocerciasis (microfilariae) had fallen sharply in the villages of the forest zone; it was practically zero in the localities of the pre-forest sector and the Sudanese zone. On the other hand, in the period 2010-2013, due to the socio-political unrest that severely disrupted control activities, the onchocerciasis situation deteriorated (ANONYME, 2016).

Fortunately, with the support of its partners, the national progrmme for onchocerciasis control succeeded in gradually rebuilding the distribution network and and took over CDTI. The required rates of geographic and therapeutic coverage were achieved and maintained over a 12-year period. For example, in 2019, CDTI in Côte d'Ivoire had a geographic coverage rate of 88.2% and a national therapeutic coverage rate of 95.5%. At these rates, in fact, the conventional regional target (treatment of at least 85% of the eligible population) is reached to move on to the onchocerciasis elimination phase. To achieve the goal of elimination, the first step is to do a mapping of the exclusion of non-aendemic areas (Rebollo et *al*., 2018). The approach requires a prior localization or an update of the data on the preimaginal breeding places of *S. damnosum* s.l. They will make it possible to identify the communities most at risk (1st line villages) (WHO, 2019) from which sentinel localities will be selected for monitoring and evaluation of the effectiveness of the control (Hopkins, 2015). In the forest areas of Côte d'Ivoire where OCP vector control activities have not been carried out, little or no data is available on the distribution of blackfly breeding places. Thus, this study aims to highlight the frontline villages (communities where the risk of onchocerciasis transmission is highest) in the Aboisso region located in the forest zone of south-eastern Côte d'Ivoire. The data used come from an entomological survey based on the geolocation and prospection of larval breeding of *S. damanosum*s.l., as well as on the evaluation of onchocerciasis transmission, organised in the region. The investigations took place during the last half of 2013 (from 2 July to 27 December), at a time when onchocerciasis control had just experienced a major disruption in Côte d'Ivoire.

MATERIALS AND METHODS

Study area: The department of Aboisso has an area of 4201 km2 (ANONYME, 2015a). It is part of the administrative region of South-Comoé, bounded to the West by the districts of Grand-Bassam and Alépé, to the North by that of Abengourou, to the East by Tiapoum and to the South by the department of Adiaké. The town of Aboisso (5°40 N, 3°10 E), the department head, is located 116 km from Abidjan and 60 km from the border with Ghana. Climatological data from the last five years collected by the services of the Ministry of Agriculture, show that the climate of the region is characterized by the four successive seasons usually described in the forest zone of West Africa(Eldin, 1971) : a great dry season from December to the end of March, a great rainy season with a peak in June, a short dry season from mid-July to mid-August and a short rainy season with a peak in September. However, the recorded rainfall is around 1300 mm per year, contrary to what is generally reported (1500 mm per year) for this type of climate (Avenard *et al*., 1971). The dense rainforest characteristic of the region (Guillaumet and Adjanohoun, 1971), now only exists in the form of two classified forests : the isolated forests of Soumié (725 ha) and Nèguè (350 ha). The relief is made up of sedimentary plateaus slightly inclined towards the south, with an average altitude of 35 metres. In the sub-prefectures of Aboisso, Ayamé, Yaou and Bianouan, in particular, it is very hilly in general with steep slopes. In the lagoon area, the relief is flat. The region is watered by the watersheds of the Bia and Eholié rivers, and those of the Noé and Ehania rivers. The Bia River is the main watercourse. It originates in Ghana and flows into the Aby Lagoon. The Eholié is a coastal river located further east and also flows into the Aby Lagoon. The Noé and Ehania rivers are two tributaries of the Tanoé or Tano River, a Ghanaian watercourse that also flows into the Aby Lagoon. It partly runs along the border between Côte d'Ivoire and Ghana, on the last few kilometres of land leading to its mouth (Zadou *et al.*, 2011). In the region of Ayamé, the Bia has two dams 4 km apart, the first (Ayamé I) built in 1959 and the second (Ayamé II) downstream in 1964 (Bossche and Bernacsh, 1990). The population of the department of Aboisso increased from 222,053 inhabitants in 1998 (Zanou and Yéo, 2001) to 307,852 inhabitants in 2014 (ANONYME, 2015b). It is spread over 62 villages and is made up mostly of Agni population from the Sanwi Kingdom, but also of allochthonous Baoulés and allogens Burkinabè, Ghanaians, Togolese, Nigeriens and Nigerians. The main economic activity of the department is the exploitation of cash crops (cocoa, coffee, oil palm, rubber) or food crops (cassava, plantain, pineapple, mango, coconut), as well as fisheries production, livestock farming and trade.

Method and techniques: Prospections took place from July 2 to 16, 2013, during the great rainy season. The waterways were previously identified on the tourist map at 1/800,000 of Côte d'Ivoire (Michelin map, 2012 Edition). Surveys of fishermen in the city of Aboisso, administrative and customary authorities, as well as local health officials living in riparian areas, have made it possible to locate the rapids in the watersheds of the four exploited rivers. The survey areas were also selected on the basis of the level of prevalence of *O. volvulus* microfilaria carriers determined in 2013 among the populations of the localities of the Bia watershed, by the services of the National Programme for Onchocerciasis Control (PNSO-LO : *Programme National de Santé Oculaire et de Lutte contre l'Onchocercose*). The surveys were conducted at various accessiblelocations. From the pre-selected starting points, each river was visited upstream and downstream, up to the limit of reach. No paths were openedin order to go further, but in some cases, the team members had to use perspicacity (travelling by canoe for example) to

reach areas of breeding places that were practically inaccessible. The places identified as prospecting sites were visited and referenced using a GPS (Global Positioning System). Sites that did indeed present the characteristics of breeding places for blackflies (fast current and presence of supports for the preimaginal stages such as vegetation or rocks), were surveyed. The survey technique adopted was that used by the OCP, described in the training modulemanual for entomologist technicians (OCP, 2002). At the end of a day of prospecting, all the collections obtained were transported to the study station erected in Aboisso. At the Aboisso station, the collected supports are immersed in clear water contained in a tray. Larvae that detach on contact with the water are immediately spotted and recovered one by one from the bottom of the tray using a flexible pliers. The nymphs are directly detached from the supports. Blackfly species are then identified using a key (Crosskey, 1960). At the end of the survey period, all information concerning the larvae was listed on a sheet. All of the larvae samples packed in CARNOY liquid, together with the prepared information sheets, were then sent to the WHO/MDSC (World Health Organisation Multi-Disease Surveillance Centre) laboratory. In this laboratory, the twin species of the *S. damnosum* complex present in the experimental area were identified from the cytotaxinomic analysis of the larvae (Boakye, 1993) contained in the samples sent. Captures on human bait were carried out from August to December 2013, in four localities: Biaka, Kotoka, Bianouan and Bolébakankro. According to the National Programme for Onchocerciasis Control, the populations of these localities are subject to CDTI, except in Bolébakankro. In all cases, each bait man (capturer) was placed on ivermectin treatment during the period of the operations. The capture sites were chosen in well-cleared and shaded areas outside the villages and at the edge of the nearest watercourse.

Captures were made on a weekly basis, generally during the first three days of the week (Monday, Tuesday, Wednesday). Two teams of two catchers each took turns every hour at each study site. The capture session lasts from 7 a.m. to 6 p.m. Two types of capture were carried out in different stages: one day of standardised capture and two days of bulk capture. The most accessible capture sites of Biaka and Bolébakankro, in the sub-prefecture of Aboisso, were used for both types of collection (standardised captures and bulk captures). As for the catch points of Bianouan (Sub-prefecture of Bianouan) and Kotoka (Sub-prefecture of Kouakro), they only accommodated bulk captures because these localities were difficult to access (difficult roads) and quite far from the Aboisso study station (at least 80 km). At the end of the standardized capture session, the day's collections are kept by time slot and immediately transported to the Aboisso study station.

As regards bulk captures, the day's collections are immediately preserved in alcohol at 80°and recovered at the end of each month to be transported to the Aboisso study station. In the field, species of *S. damnosum* s.l. were identified by observing the pigmentation of morphological characters, including antennae, procoxa, pair of forelegs, arculus, scutellum setae, 9th tergite setae and wing tufts located on the main vein of the insect wing. For each character examined, a code is assigned which varies according to the extent of pigmentation. This method makes it possible to separate blackflies into savannah females and forest females (Quillévéré *et al.*, 1977; Dang and Peterson, 1980; Kurtak *et al.*, 1981; Garms and Zillmann, 1984; Baker *et al.*, 1990). Bulk captures kept in 80° alcohol were sent to the WHO/MDSC laboratory for biomolecular analyses based on PCR (Polymerase Chain Transferase). This process, which consists of the extraction and purification of *O. volvulus* DNA from batches of blackfly heads, aims to verify the infestation of these vectors by infective larvae (third stage larvae or L3Head) of the parasite (Toé *et al.*, 1998). All blackflies from the standardized captures were dissected under a binocular magnifying glass (WILD brand, models M3A and M5A).

The physiological age of the populations was determined by observation of the ovaries to distinguish parous from nulliparous females, to look for residual eggs in parous females and to identify gravid females. For each blackfly dissected from the ovaries, the head was decapitated and the thorax detached from the abdomen. The insect tagms thus obtained were in turn dissected one after the other under a binocular magnifying glass, then observed. We then looked for the infestation of females by larvae of *O. volvulus* at different stages of its development: first stage (L1), second stage (L2) or third stage (L3 or L3Head) (Lewis, 1957; Le Berre, 1966; Philippon, 1977). Two parameters were used to measure the population density of *S. damnosum*s. l. : the Monthly Bite Rate (MBR) and the Annual Bite Rate (ABR). The MBR is the theoretical number of bites suffered by a human placed permanently at the point of capture for a month and the ABR is the sum of the MBR recorded during the year.

Table 1. Importance of blackflies species collected in the preimaginal breeding places of prospected streams in the Aboisso region

Watercourse	*S. damnosum*	*S. adersi*	*S. unicornutum*	*S. hagreavesi*	TOTAL
BIA, *coastal river and itstributaries*	1257 *(49,2%)*	491 *(19,22%)*	202 (-7,90%)	3 (-0,12%)	1953 *(76,44%)*
NOE, tributary of Tanoé	386 *(15,11%)*	179 *(7,00%)*	13 (-0,51%)	0 (0,00%)	578 *(22,62%)*
EHOLIE, *coastalriver*	7 (-0,27%)	14 *(0,55%)*	2 (-0,08%)	0 (0,00%)	23 *(0,90%)*
EHANIA, *tributary of Tanoé*	0 (0,00%)	1 *(0,04%)*	0 (0,00%)	0 (0,00%)	1 *(0,04%)*
TOTAL	1650 *(64,58%)*	685 *(26,81%)*	217 (-8,49%)	3 (-0,12%)	2555 *(100,0%)*

Table 2. Numbers of *S. damnosum* s.l. females collected on human bait in the Aboisso region

	August	September	October	November	December	Total
Biaka	710	477	291	183	418	2079
Bolébakankro	76	50	107	62	57	352
Bianouan	24	91	4281	2097	940	7433
Kotoka	92	18	37	49	31	227
Total	902	636	4716	2391	1446	10091

The transmission parameters standardized under the OCP programme are the Monthly Transmission Potential (MTP) and the Annual Transmission Potential (ATP). They are used to assess the intensity of transmission of the *O. volvulus* parasite by *S. damnosum* s.l. at a given site (Duke, 1968; Walsh *et al.*, 1978). For statistical analyses, proportions and percentages were compared using the KI-TWO test ($\chi 2$), at the first species risk of 5% (Schwartz, 1963), on Statistica (Version 12.24, 1975).

RESULTS

Larval breeding places and blackfly species in the Aboisso region: A total of 70 sites were visited and geo-referenced. Of these, 60 sites were found to have the characteristics of preimaginal breeding places of *S. damnosum* s.l. In the end, only 30 of them proved to be potential breeding places for blackflies (Figure 1).

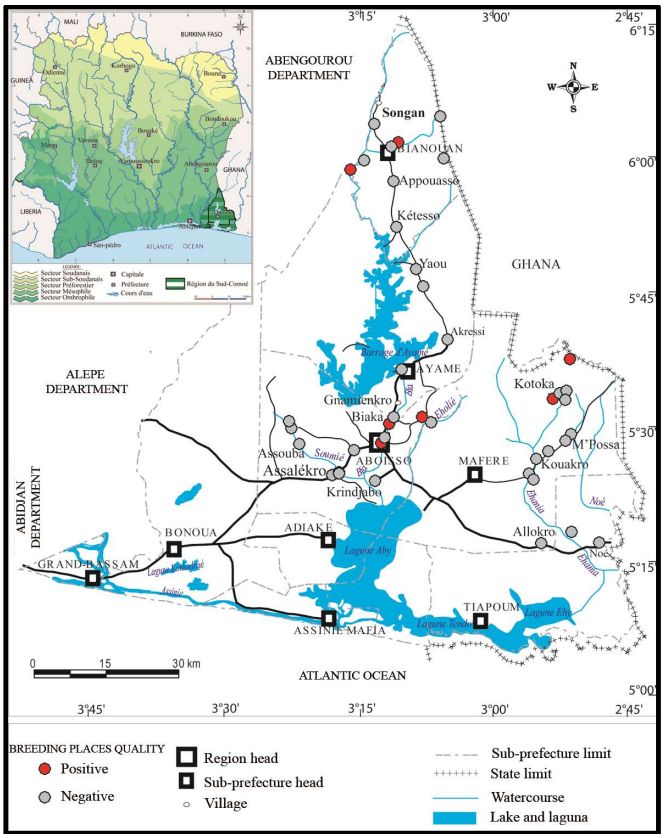

Figure 1. Location of the preimaginal breeding places of the blackflies according to the category, on the water ways prospected in the department of Aboisso (Côte d'Ivoire)

Among these 30 potential breeding places, we distinguish:

- Seven (7), or 23% positive breeding places, those with larvae of S. damnosum s.l.,
- Twenty-three (23), or 77% negative breeding places, of which:
  - Seven (7) (23%) breeding places containing larvae of blackfly species other than *S. damnosum* s.l. one,
  - Eight (8) (27%) potential breeding places with no larvae,
  - Eight (8) (27%) drowned potential breeding places.

At the end of the surveys, 2555 preimaginal stages of blackflies (2076 larvae, 479 pupae) were collected. *S. damnosum* s.l. and three other species (*S. adersi*, *S. agreavesi*, *S. unicornutum*) were identified in the collections. The comparison of the numbers recorded in Table I, show significant differences (P-value<0,001). Thus, the *S. damnosum* s.l. constitutes the majority species (64.58%). In particular, specimens of this complex are abundant at the level of the Bia deposits (population density evaluated at more than 50 individuals on the supports) in the localities of Biaka (1000/1650 pre-imaginal stages of *S. damnosum* s.l.) and Bianouan at Campement N'guessan Assamoikro (256/1650 preimaginal stages of *S. damnosum* s.l.), as well as at Noé in Kotoka (386/1650 preimaginal stages of *S. damnosum* s.l.). In particular, specimens of this complex abound at the level of the Bia breeding places (population density estimated at more than 50 individuals on the supports) in the localities of Biaka (1000/1650 preimaginary stages of *S. damnosum* s.l.) and Bianouan at the level of Campement N'guessan Assamoikro (256/1650 preimaginary stages of *S. damnosum* s.l.), as well as in those of Noé at Kotoka (386/1650 preimaginary stages of *S. damnosum* s.l.). They are rare in the breeding laces of the Eholié river (7/1650 preimaginary stages of S. damnosum s.l.), with less than 10 individuals on the supports of the breeding places considered. In addition, one breeding place on the Bia river was found positive with a single larva of *S. damnosum* s.l. in the town of Aboisso. Finally, *S. damnnosum* s.l. seems to be non-existent at the level of the Ehania river where only one larva of *S. adersi* was collected. In particular, the *S. damnosum* complex is represented at the larval level by four twin species: *S. damnosum*s.s.,*S. sirbanum*, *S. sanctipoli* s.s. and *S. yahense*.

Aggressiveness of S. damnosum s.l. and transmission of Onchocerciasis: A total of 10091 females of S. damnosum s.l. were caught on human bait. Table II shows the catch numbers by locality and by month. Their comparison shows significant differences (P-value<0,001). Thus, the greatest number of simulies was collected at the Bianoua site (7433 simulies), followed by that of Biaka (2079 simulies). In Bolébakankro (352 simulies) and Kotoka (227 simulies) the numbers are low. However, there is a slight predominance of collections in Bolébakankro, except in October where it is the opposite (92 simulies in Kotoka, 76 simulies in Bolébakankro). The savannah species identified are *S. damnosum* s.s. and *S. sirbanum* described as the SAV group. At the level of forest species, we distinguished *S. yahense* (YAH) and *S. soubrense*/*S. sanctipauli*/*S. squamosum* s.s. group (SUS/SQUA), group whose species remain inseparable by morphology. During the 5 months of capture on human bait, the average MBR per locality, expressed in the number of blackfly bites per man per hour (p/H/h), varied from 4.86 p/H/h to 124.24 p/H/h. It is in Bianouan (124.24 p/h) and in Biaka (38.75 p/h) that the aggressiveness of the blackflies is very strong. In Bolebakankro (8.19 p/h) and in Kotoka (4.86 p/h), this aggressiveness is low (P-value ≤ 0.001). Thus, the average ABR varies, depending on the locality, from 58.27 p/H/year to 1490.86 p/H/year: Bianouan, 1490.86 p/H/year; Biaka, 467.02 p/H/year; Bolébakankro, 98.28 p/H/year; Kokota, 58.27 p/H/year. Whatever the locality, the daily aggressiveness of the blackflies seems to present two peaks: one in the morning and another in the afternoon. However, in Bolebakankro in particular, the second peak of blackflies bites seems to be at noon. In general, the bitting rate appears more or less significant throughout the day in each of the localities visited (Figure 2).

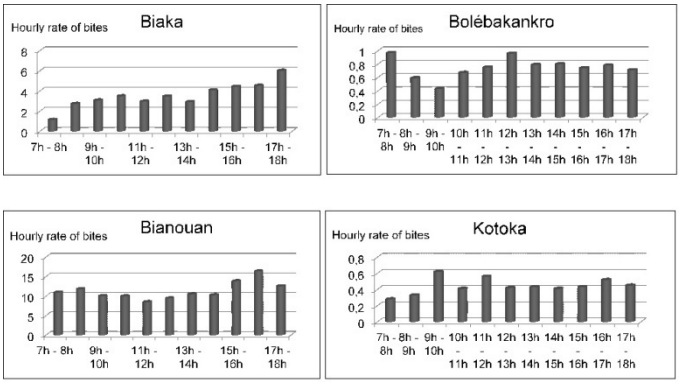

Figure 2. Variations in the hourly rate of blackflies bites in the Aboisso region

Thus, in the experimental area, the population seems to be regularly bitten by blackflies during the day. Indeed, depending on the locality, each person receives about 1 to 17 bites every hour, between 7 am and 6 pm. The entomological parameters of transmission that could be determined are the percentage of parous females and the number of evolving larvae of *O. volvulus* by infected female (Table III (A & B)). Indeed, the larvae observed in the dissected parous females were sausage stages, espacially thoracic L1 (first-stage larvae) and L2 (second-stage larvae). No infective larvae (third-stage larvae or L3Tête) were observed at the head level, neither under the microscope nor by PCR analyses. Moreover, it is in Biaka that we were able to note the presence of evolving larvae in the females dissected each month, the greatest number of these larvae per female having been recorded in September, but also in November. In Bolebakankro, there were only 2 parous females in December, each being infected on average by a microfilariae larva at the first-stage of development. Finally, parous females were also loaded with microfilariae, on the one hand, in August and December in Biaka and, on the other hand, only in December in Bolebakankro.

Table 3. Transmission parameters in females of *S. damnosum*s. l. in the Aboissoregion A. Biaka

	Numbers of females dissected	Number of parous females	Infectedparous females (%)	Parousinfective females (%)	Number of evolvinglarvae per infected female	Number of infectinglarvae per infectious female	Monthly Transmission Potential (MTP)
August	174	100	7	0	2,14	0	0
September	115	54	3,70	0	5,5	0	0
October	115	77	2,60	0	2,5	0	0
November	65	23	4,35	0	5	0	0
December	116	65	10,77	0	1,43	0	0
TOTAL	585	319	5,96	0	2,42	0	0

B. **Bolébakankro**

	Numbers of females dissected	Number of parous females	Infectedparous females (%)	Parousinfective females (%)	Number of evolvinglarvae per infected female	Number of infectinglarvae per infectious female	Monthly Transmission Potential (MTP)
August	30	14	0	0	0	0	0
September	24	14	0	0	0	0	0
October	53	33	0	0	0	0	0
November	40	13	0	0	0	0	0
December	27	16	12,50	0	1	0	0
TOTAL	174	90	2,22	0	1	0	0

DISCUSSION

In the Aboisso region, the number of breeding places that have been found to be conductive to the proliferation of S. damnosum s.l. appears to be reduced in comparison to the overall number of potential blackfly breeding places listed. In general, from this point of view, the river waters of the region are exposed to pollution due to human activities. Agricultural practices based on cash crops (oil palm, banana, cocoa, pineapple, etc.) require the use of chemical fertilizers. Rivers also constitute for the riparian populations, places to do landry and a dumping ground for domestic waste (Ouattara *et al.*, 2001; Niamien-Ebrottié *et al.*, 2008). All these anthropogenic inputs constitute a risk of water eutrophication, unfavourable to the survival of *S. damnosum* s.l. larvae. Various authors (Niamien-Ebrottié *et al.*, 2008) have reported that phytoplankton species indicative of eutrophy, were observed in the Bia and Tanoé rivers, as well as in the Ehania and Soumié rivers. Shortcomings in the methodological approach during the surveys may also have been an obstacle. Of the three watersheds investigated, the Bia river was the one that was best covered, particularly in the Aboisso and Bianouan areas. In Aboisso, the river was followed downstream of the city as far as the Ayamé dams and the tributaries (Soumié, Lingué) were surveyed in several places. In Bianouan, the river was also followed, but here upstream and downstream and the tributaries were visited. Canoes were even used to access and prospect productive breeding places. On the other hand, on the route from Ayamé to Bianouan, the team was forced to limit visits to the most easily accessible parts of the Bia River and its tributaries. It was the same for the Ehania and Noé rivers, but also for the Eholié river. At the level of these rivers, very few places were visited because their watersheds were difficult to access.

This could justify the fact that the largest number of the breeding places listed are from the Bia. On this point, in addition to the methodological approach, the one characteristics of the Bia river may have been a favourable factor. Indeed, the Bia River is the most important of the watercourses surveyed, due to its flow rate (59.7 m2/s in flood, 1.68 m2/s in low-water period) and the extend of its watershed (2534 km² in ivorian territory over 10,200 km²) (Reizer, 1967; Girard *et al.*, 1971; Wango *et al.*, 2013; Eblin *et al.*, 2014 ; Wango *et al.*, 2014 ; N'cho *et al.*, 2020).The course of the river also crosses several areas with drop of 4 to 25 m and, as a result, presents a series of rapids, spread over several kilometres (Girard *et al.*, 1971). The twin species of *S. damnosum* s.l. collected in the Aboisso region (*S. damnosum*s.s., *S. sirbanum*, *S. soubrense*, *S. sanctipauli* s.s., *S. squamosum* s.s., *S. yahense*), are the important taxa of the complex described in West Africa (Vajime and Dunbar, 1975; Quillévéré *et al.*, 1977; Philippon, 1978). In Côte d'Ivoire, S. *damnosum* s.s., *S. sirbanum* and *S. squamosum* occur in the savannah zone: the first two species are found in streams in general and the last one (*S. squamosum*) is found mainly in small rivers. *S. soubrense* and *S. yahense* are forest species. *S. soubrense* lives in large rivers, while *S. yahense* is found in small rivers (Quillévéré, 1979). It is these species that constitute the vectors of human onchocerciasis in the initial OCP area. *S. damnosum* s.s and *S. sirbanum* transmit the severe blinding form of onchocerciasis in the savannah zone, unlike the other four species (*S. soubrense*, *S. sanctipauli*, *S. squamosum*s.s., *S. yahense*) which are responsible for the less severe form of the disease in the forest zone (Agoua *et al.*, 1991). Among the twin species of *S. damnosum* s.l. caught on human bait in the Aboisso region, four were also collected at the larval stage in the region's streams. These are the savannah species *S. damnosum* s.s. and *S. sirbanum* on the one hand, and *S. sanctipauli* s.s. and *S. yahense*, which are forest species, on the other. As far as the savannah blackfly species vectors of onchocerciasis are concerned, *S. damnosum* s.s. and *S. sirbanum*, their presence in the Biaka and Bolebakankro areas could be explained by their adaptation in this place to an ecological environment that has become favorable. Indeed, several authors (Adjami, 2006; Houévoganwa *et al.*, 2014) have shown that in forest areas, the destruction of forest cover and its replacement by crops and fallow lands, plantations and agglomerations, favors the establishment of savanna species of the *S. damnosum* complex. This is certainly the case in Bouaflé in the Marahoué region of central-western Côte d'Ivoire, where species of *S. damnosum* s.l. including *S. damnosum*s.s., *S. sirbanum*, have also been encountered (Yapi *et al.*, 2014). Moreover, Biaka is a locality bordering the Bia river and Bolébakankro is served by the Soumié river, a tributary of Bia river. The total length of the Bia river is 300 km, including 120 km in Côte d'Ivoire, and its average width is around 150 m. It has a catchment area of 10200 km2, including 2534 km² in Côte d'Ivoire and 7666 km² in Ghana. In Côte d'Ivoire, the department of Aboisso alone occupies 1940 km² of this watershed (Reizer, 1967; Girard *et al.*, 1971; N'cho *et al.*, 2020). In addition, Ghana is along with Côte d'Ivoire, one of the countries covered by the OCP programme. Moreover, the Aboisso region is contiguous in its northern part with the onchocerciasis focus of the lower Comoé, which was once part of the OCP extension areas of onchocerciasis in Côte d'Ivoire (WHO, 1999).

It is therefore also likely that the presence of the savannah species *S. damnosum* s.s. and *S. sirbanum* in the forest region of Aboisso, being the result of the dispersive movements of these species, following the watercourses of the watersheds areas in the onchocerciasis foci of the lower Comoé and Ghana of the OCP zone (Le Berre, 1966; Grams *et al.*, 1979). The situation is all the more worrying since it has recently been reported that entomological surveys carried out in seven villages in Ghana, show that the transmission of onchocerciasis still persists there, despite periods of treatment of the populations with ivermectin, ranging from 3 to 24 years (Lamberton *et al.*, 2015). In the Aboisso

region, the population seems to be regularly bitten by blackflies during the day. Populations of *S. damnosum* s.l. seem to be a nuisance problem only in Bianouan. The estimated ABR, which is the highest in the region, is higher than the minimum value (1000 p/h/year) considered as an indicator of nuisance by the WHO (OCP, 2002). The strong aggressiveness of blackflies recorded in the locality of Bianouan upstream of the Ayamé dams can be attributed to the flooding of the Bia caused by the waters of the rainy season (Le Berre, 1966). Because, it is usually in times of high waters that strong nuisances of blackfly are observed in a given locality (Traoré *et al.*, 2006). However, downstream of the Ayamé I and II dams, the situation appears different. Flooding was expected to occur normally during the normalization of flows during the rainy seasons, in order to limit the amplitude of seasonal and daily variations in the water level in the hydroelectric structures (Barbazan *et al.*, 1998). However, during the period 2011-2013, the water in the Ayamé dams did not reach the critical level that would have led to a regulation of the flows in these structures.

There is no risk of transmission of onchocerciasis in the Aboisso region, given that none of the blackfly females collected was found to carry the infective stage of *O. volvulus* and therefore the ABR is zero in the region. The same is true for the ABR determined by locality, which is well below the threshold value ordinarily considered to establish eliminating onchocerciasis in a given locality (Duerr and Eichner, 2010). These results, obtained after six years of CDTI application, are therefore further evidence of the efficacy of ivermectin against onchocerciasis (Prod'hon *et al.*, 1991; Boussinesq, 2005; Duerr *et al.*, 2011; Yaya *et al.*, 2014). Within the framework of the elimination of onchocerciasis in Côte d'Ivoire, among the localities that hosted the captures on human bait in Aboisso, the three centres (Biaka, Campement N'guessan Assamoikro in Bianouan and Kotoka) that are located near larval breeding places of *S. damnosum*s. l., seem to meet the criteria of first-line villages as defined by the WHO (2019). Indeed, the village Biaka, with 293 inhabitants, is built on the shore of the river Bia near a positive breeding place. The N'guessan Assamoikro camp in Bianouan is built about 500 metres from the Bia. Kotoka is an agglomeration of 3,466 inhabitants, found about one kilometre walk from the location of the positive breeding places on the Noé river.

CONCLUSION

In the Aboisso region, the greatest number of larval breeding places for *S. damnosum* s. l. were found in the Bia river, which is the most important river in the region. The twins species of S. *damnosum* s.l. encountered are *S. damnosum*s.s., *S. sirbanum*, *S. soubrense*, *S. sanctipauli*, *S. squamosum* s.s. and *S. yahense*. The presence of the savannah species (*S. damnosum* s.s. and *S. sirbanum*) in this forest area seems to be the result of the combined effects of the degradation of the vegetation cover by human activities and the dispersal phenomena of blackflies from the area of onchocercian foci of the lower Comoé and Ghana. The results of six years of CDTI application in the study area have shown once again the effecacy of ivermectin in a mass treatment against onchocerciasis. The risk of transmission of the disease is non-existent in Aboisso and the annual rate of bites of *S. damnosum* s.l. females determined, argues for the implementation of the phase of the elimination of onchocerciasis in the region. In the latter case, the village of Biaka, N'guessan Assamoikro camp in Bianouan and Kotoka, which are located close to larval breeding places of *S. damnosum* s.l., appear as localities that can constitute first-line villages. In any case, in order to be able to easily establish the phase of elimination of onchocerciasis in Côte d'Ivoire, such a study should be undertaken on the whole of ivorian territory in order to updat the data on the preimaginal breeding places of *S. damnosum* s.l. which indeed date from the period of the OCP.

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