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Impact of repeated mass ivermectin administration using a community directed approach on *L. loa* infection in *Chrysops silacea* of the rain forest and forest savanna of Cameroon

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ABSTRACT

Background: Loiasis is an endemic filarial infection in the rainforest zone of West and Central Africa. Repeated annual community-directed treatment with ivermectin (CDTI) delivered for several years to control onchocerciasis has been shown to reduce the prevalence and intensity of Loiasis in some *Loa loa-Onchocerca volvulus* co-endemic areas. However, the impact of these multiple rounds of CDTI on entomological indicators of loiasis transmission is not known, and was therefore assessed in this study in areas with contrasting histories of CDTI.

Methods: The study was conducted in the East, North-west and South-west 1 CDTI project sites of Cameroon. Two communities per CDTI project were selected for fly collection and dissection.

Abbreviations: ALB, Albendazole; APOC, African Programme for Onchocerciasis Control; CDC, Center for Disease Control and Prevention; CDTI, Community Directed Treatment with ivermectin; DEC, Diethylcarbamazine; IR, Infection rate; L1, Larval stage one; L3, Larval stage three (infective larvae); LF, Lymphatic filariasis; MDA, Mass Drug Administration; MHL3, Mean Head L3 load; Mf/mf, Microfilaria(e); MIBD, Monthly Infective Biting Density; MTP, Monthly Transmission Potential; PIR, Potential Infective Rate; PR, Parous rate; SAE, Severe adverse events; SPSS, Statistical Package for the Social Sciences; WHO, World Health Organization.

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Ivermectin treatment coverage was documented in these areas, and this was correlated to *Chrysops* infection and infective rates. A total of 7029 female *Chrysops* were collected from 6 communities of the 3 CDTI projects (East, North-west, and South-west 1) and from 2 communities in a non-CDTI district (East).

Results: Chrysops biting densities and parous rates were significantly reduced in the North-west and South-west sites post-CDTI, while in the East, biting densities were similar in non-CDTI and CDTI sites, with higher parous rates observed in the non-CDTI site. Infection and infective rates in the East non-CDTI site were 4.4% and 1.8% respectively, as compared to 3.3% and 1.3% in the CDTI site after 10 ivermectin rounds (there were no baseline data for the latter). In the North-west site, significant reductions in *Chrysops* infection and infective rates from 10.2% and 4.2% respectively, to 3.5% and 1.2 (after 9 rounds of ivermectin treatment), were recorded following CDTI. In the South-west, infection rate significantly increased from 1.74% to 2.8% and infective rate remained statistically unchanged after 14 rounds of CDTI (0.45% - 0.40%). Similar trends in Mean Head L3 were observed except in the East site where this indicator was similar in both CDTI and control sites. Only in the North-west site did monthly transmission potentials decrease significantly.

Conclusion: This study demonstrated that the impact of repeated annual treatment with ivermectin for the control of onchocerciasis using community directed delivery approach on the entomological indicators of loiasis varies with bioecological zones. Community directed treatment with ivermectin induced a significant reduction in the entomological indicators of loiasis in the North-West project site which lies in forest savanna area. A non-significant decrease was observed in the East project site and in contrast, a significant increase was observed in the South-West 1 project site which both lies in the rainforest zones.

1. Introduction

Loiasis is a filarial infection that occurs only in Africa, primarily in rainforest areas of Central and West Africa (Zouré et al., 2011), though isolated cases have been reported in West Africa from Ghana to Guinea, and in Uganda, Malawi, Ethiopia, and Zambia (Kouam et al., 2013). Chronic infection may occur in 3–13 million residents of these endemic areas (Wanji et al., 2018). The distribution of disease is predefined by the predilection of the vector flies, *Chrysops silaceae* and *C. dimidiate,* to reside in the forest canopy and lay their eggs in swamps and at river edges. The flies are attracted by movement, dark colours, and wood smoke (Wanji et al., 2002). Rain forests, with relatively low canopies and scant undergrowth, seem to constitute a particularly desirable habitat for these vectors. The prevalence of the disease in the human population is higher in adult males than in children and varies depending on vector abundance (Esum et al., 2001; Takougang et al., 2007). Seasonal fluctuations in the entomological indicators such as infection rate, infective rate, and mean head L3s have also been reported (Esum et al., 2001; Wanji et al., 2003; Takougang et al., 2002; *Climate and Relief of Cameroon*, n.d.).

In West and Central Africa, *Loa loa* infection remains the most significant impediment to Lymphatic Filariasis (LF) and Onchocerciasis control and elimination (Zouré et al., 2011). It has hampered LF mapping in areas of co-endemicity due to reported cross-reactivity of the rapid diagnostic tests (ICT, FTS) used for LF mapping in *Loa loa* affected areas (Wanji et al., 2015; Wanji et al., 2016; Wanji et al., 2019). Further, in onchocerciasis co-endemic areas, cases of severe adverse events (SAEs) including fatal cases of encephalopathy, were registered post ivermectin mass treatment in the early 1990s (Twum-Danso and Meredith, 2003). The primary determinant of this situation was found to be the level of *L. loa* microfilaraemia and the inflammatory response to the dying worms (Boussinesq et al., 1998, 2001; Gardon et al., 1997). These SAEs hampered the success of community-directed treatment with ivermectin (CDTI), the main strategy adopted by the African Programme for Onchocerciasis Control (APOC) (Twum-Danso and Meredith, 2003; Duke, 2003). There was therefore a wide-spread concern about the sustainability of CDTI (Addiss, 2010; Amazigo et al., 2002), as a consequence of the risk of observing an encephalopathic syndrome in some individuals in communities while distributing ivermectin for the control of onchocerciasis in *Loa* co-endemic areas. However, the Mectizan Expert Committee/Technical Consultative Committee (MEC/TCC) focused the mass treatment decision on the health benefits of preventing blindness due to Onchocerciasis rather than on the risk of *L. loa* encephalopathy following Ivermectin mass treatment (MEC/TCC, 2004), which therefore allowed CDTI activities to continue in some *L. loa* co-endemic areas.

A considerable proportion of CDTI projects in the APOC are situated in areas where onchocerciasis and loiasis are co-endemic. This is the case in countries such as Nigeria, Cameroon, the DRC and Angola. In Cameroon, amongst the 15 CDTI projects, 13 are partially or entirely situated in areas of onchocerciasis-loiasis co-endemicity. For those Cameroon CDTI projects situated in areas of co-endemicity, eight of them had been distributing ivermectin for >10 rounds (11−13) and 5 of them for <10 rounds (6–8) by 2014. Although some of these CDTI projects are situated in areas of co-endemicity with historical data and even more recent data on L. *loa* transmission before the introduction of the programme, studies evaluating the impact of CDTI on entomological indicators of *L. loa* transmission have been very few and spotted (Kouam et al., 2013). Nevertheless, recent studies on parasitological indicators provided evidence that CDTI reduces the prevalence and intensity of L. *loa* microfilaraemia (Wanji et al., 2018), We hypothesized that Mass Drug Adminstration (MDA) with Ivermectin in these areas would have altered the *L. loa* transmission in the CDTI project sites. This study aimed to assess the effect of long-term treatment with Ivermectin on the entomological indices of Loiasis.

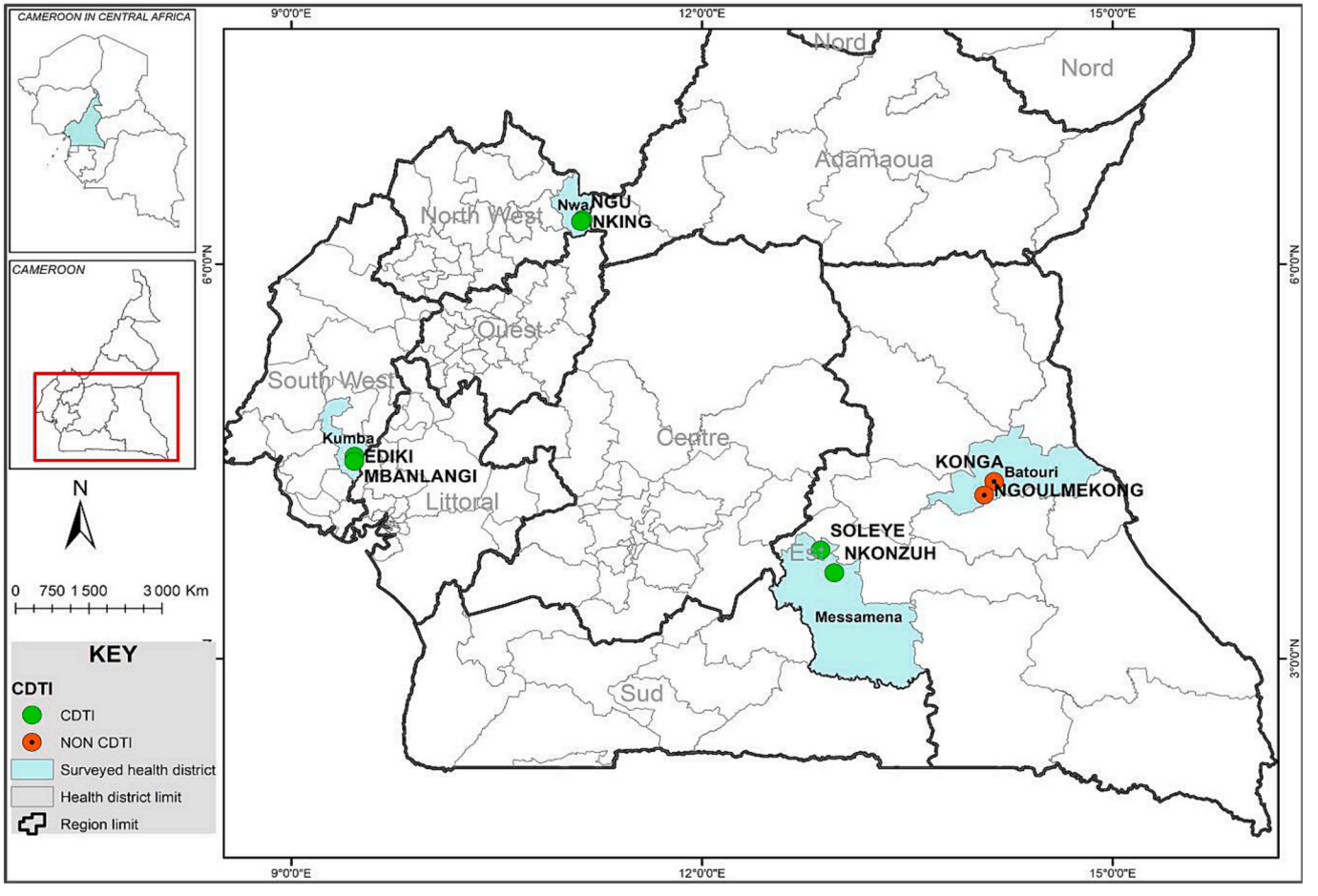

Fig. 1. Study sites with fly collection points.

2. Materials and methods

2.1. Study design

This cross-sectional entomological survey was conducted during a period after the annual CDTI distribution that is, from August to October 2014 in 3 CDTI projects and aimed to evaluate the impact of CDTI on L. *loa* transmission, in areas of co-endemicity with onchocerciasis in Cameroon (Fig. 1). Two CDTI project sites were purposively selected based on pre-control data on loiasis transmission and these were the North-west and South-west 1 project sites (Wanji et al., 2002, 2018). However, there was no baseline data available for the East, another CDTI project site, and therefore, a non-CDTI health district, also from the East and adjacent to one of the East CDTI sites, was selected. Although no formal comparison is truly possible between the CDTI and the non-CDTI areas in the East, the inclusion of the latter provides useful information of the loiasis endemicity situation in an area of geographical proximity (Fig. 1). Two geo-referenced communities were selected per project site in which, 5 trained fly collectors were assigned to collect wild *Chrysops* for 5 days using sweep nets. In each community, ivermectin mass treatment was documented at the individual level based on oral declarations of participants following an interview on the number of times they had received ivermectin. We relied on oral information due to inconsistency in the availability of treatment registers in study communities (Wanji et al., 2018). CDTI coverage rounds per site were later related to some L. *loa* transmission indices such as *Chrysops* infection and infective rates. The following entomological indicators of L. *loa* were generated: infection rate, infective rate, intensity of infectivity, biting density, parous rates, and monthly transmission potential. Indicators generated within CDTI projects were compared to pre-control indicators where baseline data were available (North-west and South-west 1 projects) and to a geographically close study site with no on-going CDTI in the case of the East study site.

2.2. Study sites

This survey was carried out in three CDTI project sites (East, North-West and South-West 1) and a non CDTI control site (Batouri Health District in the East region) belonging to the rain forest (East and South-West 1) and forest-savanna (North-West) bioecological zones in Cameroon. The South-west 1 CDTI project, situated in an area of L. *loa* mild endemicity, started in the year 2000 and, by the time of the study, had been under CDTI for 14 years (Esum et al., 2001). The East and North-West CDTI projects, situated in areas of high *L. loa* endemicity, started much later (in 2004 and 2005, respectively) and so had, respectively, been under CDTI for 10 and 9 years prior to the study (Takougang et al., 2002, 2007; Wanji et al., 2003).

The climate in the South-west and North-west is tropical with two seasons, one wet season of about 9 months, lasting from March to November, and a short dry season from mid-November to mid-March. The mean annual rainfall in these areas varies from 2500 to 4000 mm. The ambient temperature ranges from 20 °C to 40 °C depending on the seasons. The climate of the East region is a Type A wet equatorial climate (*Climate and Relief of Cameroon*, n.d.), and an average temperature of about 24 °C and four seasons (a long dry season from December to May, a light wet season from May to June, a short dry season from July to October, and a heavy wet season from October to November). Humidity and cloud cover are relatively high, and precipitation averages 1500–2000 mm per year except in the extreme eastern and northern parts of the region, where it is slightly less. Fly collection sites were either in farming or fishing camps based on information from community members on where *Chrysops* flies were the most abundant. Two communities were selected for surveys per CDTI project site. The characteristics of the fly collection points are described in Table 1 and Fig. 1.

2.3. Ivermectin treatment in the study sites

The study areas are situated in areas with varying CDTI coverage profiles. Prior to our study, CDTI was 14, 9, and 10 years old in the South-west 1, North-west, and East project sites, respectively. From 2001 to 2014, the South-west CDTI Project I, which covers the Meme and Mungo drainage basins, achieved 100% geographic coverage, except in 2008 (97%) and 2009 (98.33%). Therapeutic coverage increased from 32.56% in 2001 to 82.83% in 2010, and has remained at or above 81.4% as of 2014 (Duamor et al., 2017). Furthermore, data on the CDTI therapeutic coverage obtained from the national onchocerciasis control programme of Cameroon indicates an 81.3% therapeutic coverage in the North-west and an 80.87% coverage for the East CDTI Project, with geographic coverage being 100 and 97.79%, respectively.

Table 1
Summary description of the study sites.

CDTI project site (years of treatment)	Fly collection point/village	GPS coordinates (latitude and longitude)
North-west (9)	Nking	11.12858, 6.33909
	Ngu	11.11629, 6.32479
South-west 1 (14)	Ediki	9.4614, 4.5377
	Mbalangi	9.4603, 4.4994
East CDTI (10)	Soleye	12.86596, 3.82495
	Nkonzul	12.96753, 3.65252
East non- CDTI	Konga	14.13458, 4.34694
	Ngoulmekong	14.06095, 4.2437

2.4. Collection and identification of Chrysops

Flies were captured daily for 5 days in each community by 5 fly collectors stationed near wood fires from August to October 2014 (rainy season). The survey took place in the same sites under similar conditions as during baseline entomological surveys (Wanji et al., 2002). Attracted by wood fires, blood-seeking female flies were collected using sweep nets when they were attempting to get a blood meal on the collectors (Duke, 1955). Catches were made from 7 a.m. to 6 p.m. The caught flies were stored individually in labelled plastic tubes designed to provide suitable conditions for their survival. The number of flies caught during each hour interval was recorded. At the end of each session, flies were transported in a cooler box to the field laboratory for identification and dissection. *Chrysops* were identified by morphological features (Duke, 1958; Williams, 1961; Oldroyd, 1957).

2.5. Dissection of Chrysops

Chrysops were dissected alive after a slight knockdown using a needle tip. Fly dissection was performed in the field laboratory immediately after collection in physiological saline (0.9% NaCl) under a dissecting microscope. The head, thorax, and abdomen of each fly were separated and placed on slides containing a drop of dissecting medium. The abdomen was teased gently to pull out the ovarioles and spread out to determine the presence (parous) or absence (nulliparous) of follicular relicts on the pedicel as described by Duke (Duke, 1960). Parous flies were further dissected for the presence or absence of L. *loa* larvae. Larvae were classified into three stages: sausage (L1), larval stage 2 (L2) and infective larvae (L3). Larvae were identified following methods by Duke and Orihel (Duke, 1958; Orihel and Lowrie, 1975).

2.6. Entomological indices of loiasis transmission

Flies caught in the morning and in the afternoon were dissected separately and the parous rate (PR), infection rate, infective rate (IR), mean head L3 loads (MHL3), and monthly transmission potential (MTP) were generated as previously described (Wanji et al., 2002; Noireau et al., 1990).

2.7. Statistical analysis

Captured flies were dissected, and the results were entered into a userform created in Epi Info 3.5.3 (CDC, Atlanta, USA), which was then exported to SPSS version 20 (IBM SPSS Statistics 20; Armonk, NY) and Graph Prism 5 (GraphPad Software, Inc., La Jolla, USA) for analysis and graphical illustrations. The parous rate was estimated as the proportion of parous flies amongst the total number of dissected flies. The infection rate was determined as the proportion of infected flies to the total number of flies dissected. The Infective Rate (IR_3) was estimated as the proportion of *Chrysops* with L3s in the head. The mean head L3 load (MHL3) was defined as the arithmetic mean of L3s collected per *Chrysops* head harbouring L3s. The monthly biting density (MBD) was defined as the number of flies visiting a collector per month and was a theoretical expression deduced from the daily biting density. The monthly transmission potential (MTP) was calculated as the product of the MHL3 load, the IR, and the MBD. The Man Whitney *U* rank test or unpaired *t*-test was used to compare the distribution or average of MBD, MHL3, MIBD, and MTP before and after several rounds of CDTI where applicable (or between East non-CDTI and East CDTI sites). Contingency tables with chi-square test and Fischer's Exact test (where theoretical size <5) were used to assess the association between indicators (PR, IR_{3H}, and IR) and both study periods (or sites for the East). The significance level was set at 0.05 in all cases.

3. Results

3.1. Number of flies collected

A total of 7029 female *Chrysops* flies were collected from 3 CDTI project sites and 1 non-CDTI site using sweep nets. Of these, 1861 were collected from the East, 485 from the North-west, 2318 from South-west 1, and 2365 from the East non-CDTI (Table 2).

Table 2
Summary of the total number of *Chrysops* collected in the different study sites in the morning and afternoon periods.

Project site	Collection period		Total
	Morning	Afternoon	
East	1019	842	1861
NW	224	261	485
SW1	1162	1156	2318
Non-CDTI	1343	1022	2365
Total	3748	3281	7029

NW = North West; SW = South West 1; Non-CDTI = Non-Community Directed Treatment with Ivermectin.

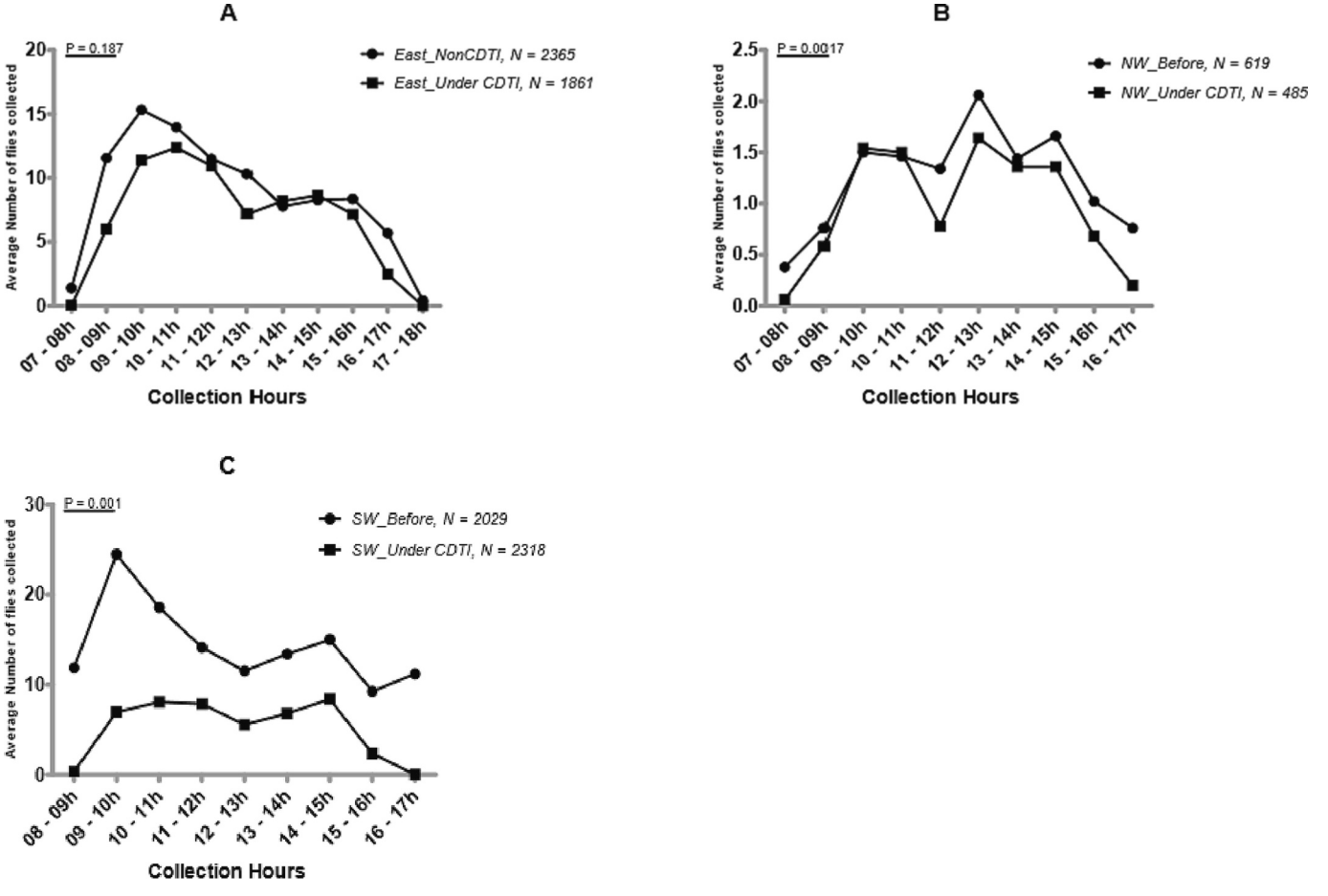

Fig. 2. *Chrysops* biting densities before and after (A) 10 years of CDTI in the East, (B) 9 years of CDTI in the North-West and (C) 14 years of CDTI in the South-West 1.

3.2. Entomological indices generated before and after several rounds of CDTI East project site

In the East region, where baseline data was not available, data from the non-CDTI site were used for comparisons because of the non-availability of the baseline data. The occurrence of biting *Chrysops* in the two sites was observed throughout the day with two biting peaks (Fig. 2A): one higher peak in the morning (09:00–11:00 am) and a less prolonged peak in the afternoon (14:00–16:00 pm). The biting density in the non-CDTI site was not significantly different from that in the CDTI site ($p = 0.187$, $t = 3.21$, $df = 10$). The earlier peaks on both sites accounted for >25% of the total *Chrysops* collections. The two daily peaks in both sites accounted for >45% of total collections in either location. The parous rate was significantly higher in the CDTI site ($p = 0.012$, $X^2 = 6.27$, $df = 1$). The similar number of flies collected in the two sites was also translated into similar infection and infective rates generally and between the morning and afternoon periods specifically (Table 3).

3.3. North-west Project site

This project's fly collection site had baseline entomological data, so pre-control data were compared to those generated after 9 rounds of CDTI. The total number of flies collected during both study periods was similar. As depicted in Fig. 2B, *Chrysops* biting density followed the same pattern daily with a peak biting hour during the early afternoon hours (12:00–13:00). The biting density before CDTI was significantly higher than after CDTI ($p = 0.0017$, $t = 4.26$, $df = 10$). Over 50% of flies were collected in the afternoon period during both study periods. The number of *Chrysops* collected during the morning and afternoon periods was statistically similar during both study periods ($p = 0.457$, $X^2 = 0.553$, $df = 1$). The parous rate was significantly lower post CDTI ($P < 0.0001$, $X^2 = 47.69$, $df = 1$). Infection ($P < 0.0001$, $X^2 = 18.01$, $df = 1$) and infective rates ($p = 0.004$, $X^2 = 8.484$, $df = 1$) were found to be significantly decreased after 9 rounds of CDTI, as shown in Table 4.

3.4. South-west 1 project site

Entomological indices of L. *loa* transmission before CDTI were compared to those generated 14 years after CDTI in the South-west region, where baseline data was also available. The biting density (Fig. 2C) and parous rate were significantly lower than at baseline ($p < 0.0001$, $t = 5.42$, $df = 18$ and $p < 0.0001$, $X^2 = 18.56$, $df = 1$ respectively). The L. *loa* infection rate in *Chrysops* significantly increased post CDTI ($p = 0.010$, $X^2 = 6.608$, $df = 1$) while the infective rate remained unchanged ($p = 0.952$, $X^2 = 0.004$, $df = 1$). As depicted in Table 5, the total number of flies collected and dissected during both study periods in the morning and afternoon was significantly different ($p = 0.001$, $X^2 = 11.815$, $df = 1$).

3.5. Mean head infective larvae, monthly infective biting densities and monthly transmission potentials

The MHL3 in *Chrysops* of the East CDTI site was slightly higher than that observed in the non-CDTI, although the difference was not significant ($p = 0.9754$, $t = 0.031$, $df = 22$). There was likewise, an insignificant increased ($p = 0.5386$, $t = 0.6256$, $df = 20$) observed in the South-west site, while a decreased was observed in the North-west site which was also not significant ($p = 0.0883$, $t = 1.792$, $df =$

Table 3
Loa loa transmission indices in the East CDTI project after 10 years of CDTI compared to the non-CDTI site.

Project	Period	No. flies caught	No. of collector	No. of hours worked/ collector	Total No. of hours worked	Biting density (f/m/h)	Monthly biting density	No. dissected	PR (%)	IR (%)	IR_{3H} (%)
East non-CDTI	Morning (7-12 h)	1365	5	50	250	5.46	805.8	1365	313 (22.9)	55 (4.0)	24 (1.8)
	Afternoon (12-18 h)	1000	5	60	300	3.33	613.2	1000	299 (29.9)	50 (5.0)	18 (1.8)
	Total	2365	10	110	550	4.30	1419	2365	612 (25.9)	104 (4.4)	42 (1.8)
				p-value: morning vs afternoon					0.001	0.351	0.962
East CDTI	Morning (7-12 h)	1019	5	50	250	4.08	611.40	1025	317 (30.9)	30 (2.9)	13 (1.3)
	Afternoon (12-18 h)	842	5	60	300	2.81	505.20	836	229 (27.4)	32 (3.8)	12 (1.4)
	Total	1861	10	110	550	3.39	1116.6	1861	546 (29.3)	62 (3.3)	25 (1.3)
	p-value: morning vs *afternoon*								0.065	0.306	0.780
p-value: East CDTI vs non-CDTI	0.0094								0.012	0.066	0.264
Percentage of variation (%)	−21.3								−13.1	−25.0	−27.8

PR = Parous rate; IR = Infection rate; IR_{3H} = Infective rate; CDTI = Community Directed Treatment with Ivermectin.

Table 4
Loa loa transmission indices in the North-west CDTI project after 9 years of CDTI compared to baseline.

Project	Period	No. flies caught	No. of collector	No. of hours worked/ collector	Total No. of hours worked	Biting density (f/ m/h)	Monthly biting density	No. Dis-sected	PR	IR	IR_{3H}
NW Baseline	Morning (7-12 h)	280	5	50	250	1.12	133.8	280	139 (49.6)	24 (8.6)	10 (3.8)
	Afternoon (12-17 h)	339	5	50	250	1.36	157.2	339	206 (60.8)	39 (11.5)	16 (4.7)
	Total	619	5	100	500	1.24	291	619	345 (55.7)	63 (10.2)	26 (4.2)
	p-value: morning vs afternoon								0.007	0.285	0.549
NW after 9 rounds CDTI	Morning (7-12 h)	225	5	50	250	0.90	163.2	225	70 (31.1)	5 (2.2)	2 (0.9)
	Afternoon (12-18 h)	260	5	50	250	1.04	208.2	260	99 (38.1)	12 (4.6)	4 (1.5)
	Total	485	10	100	500	0.97	371.4	485	169 (34.8)	17 (3.5)	6 (1.2)
	p-value: morning vs afternoon								0.131	0.237	0.691
p-value before vs after CDTI							0.0017		<0.001	<0.001	0.004
Percentage of variation (%)	−21.8						27.6		−37.5	−65.7	−71.4

PR = Parous rate; IR = Infection rate; IR_{3H} = Infective rate; CDTI = Community Directed Treatment with Ivermectin.

Table 5
Loa loa transmission indices in the South-west 1 CDTI project after 14 years of CDTI compared to baseline.

Project	Period	No. flies caught	No. of collector	No. of hours worked/ collector	Total No. of hours worked	Biting density (f/ m/h)	Monthly biting density	No. dissected	PR	IR	IR_{3H}
SW1 baseline	Morning collections (8-12 h)	1123	20	3	65	17.28	2073.23	1084	319 (29.4)	19 (1.75)	4 (0.37)
	Afternoon collections (12-17 h)	906	19	4	75	12.08	1812.00	891	203 (22.8)	15 (1.68)	5 (0.56)
	Total	2029	39	7	140	14.49	3913.07	1975	522 (26.43)	34 (1.72)	9 (0.46)
	p-value: morning vs afternoon						0.0823		0.001	0.950	0.739
SW1 after 14 rounds CDTI	Morning collections (7-12 h)	1163	5	40	200	5.82	697.80	1163	223 (19.2)	30 (2.6)	3 (0.3)
	Afternoon collections (12-18 h)	1155	5	50	250	4.62	693.00	1155	246 (21.3)	36 (3.1)	7 (0.6)
	Total	2318	5	90	450	5.15	1390.80	2318	469 (20.2)	66 (2.8)	10 (0.4)
	p-value: morning vs afternoon						0.4286		0.222	0.441	0.224
p-value before vs after CDTI							0.002		<0.001	0.010	0.952
Percentage of variation (%)						−64.5	−64.5		−23.6	62.8	−13.0

PR = Parous rate; IR = Infection rate; IR_{3H} = Infective rate; CDTI = Community Directed Treatment with Ivermectin.

20). Furthermore, the MIBD was lower, although not significantly, in the East ($p = 0.2223$, $U = 50.50$) and reduced in the South-west ($p = 0.3697$, $U = 47$). The North-west was the only site to register a significant reduction in MIBD ($p = 0.0163$, $U = 31$) as observed in Fig. 3B. As with the other entomological indicators, the reduction in MTPs was significant only in the North-west ($p = 0.0327$, $U = 28$), while the values in the East ($p = 0.5057$, $U = 60$) and South-west ($p = 0.5580$, $U = 42$) remained comparable (Fig. 3C).

4. Discussion

This study aimed to assess entomological indicators of loiasis after 9, 10 and 14 years post-ivermectin mass distribution in the North-west, East and South-west 1 CDTI projects, respectively. Previous studies have demonstrated that ivermectin, the drug of choice for Onchocerciasis and Lymphatic filariasis, has a measurable impact on L. *loa* prevalence, intensity and transmission when used in large-scale administrations (Kouam et al., 2013; Wanji et al., 2018). Because of the co-occurrence of the two filarial species in the study area, we seized this unique opportunity to assess for the first time, the effect of CDTI on the entomological indicators of loiasis. Prior to the launching of CDTI in these areas, entomological data on the transmission of loiasis was generated during the peak transmission periods coinciding with the rainy seasons (Uttah and Ibeh, 2011). Ivermectin distribution was also conducted annually in these areas, with various therapeutic coverage rates. Data generated from the current study served to evaluate the impact of MDA on disease transmission as we compared it to pre CDTI transmission data.

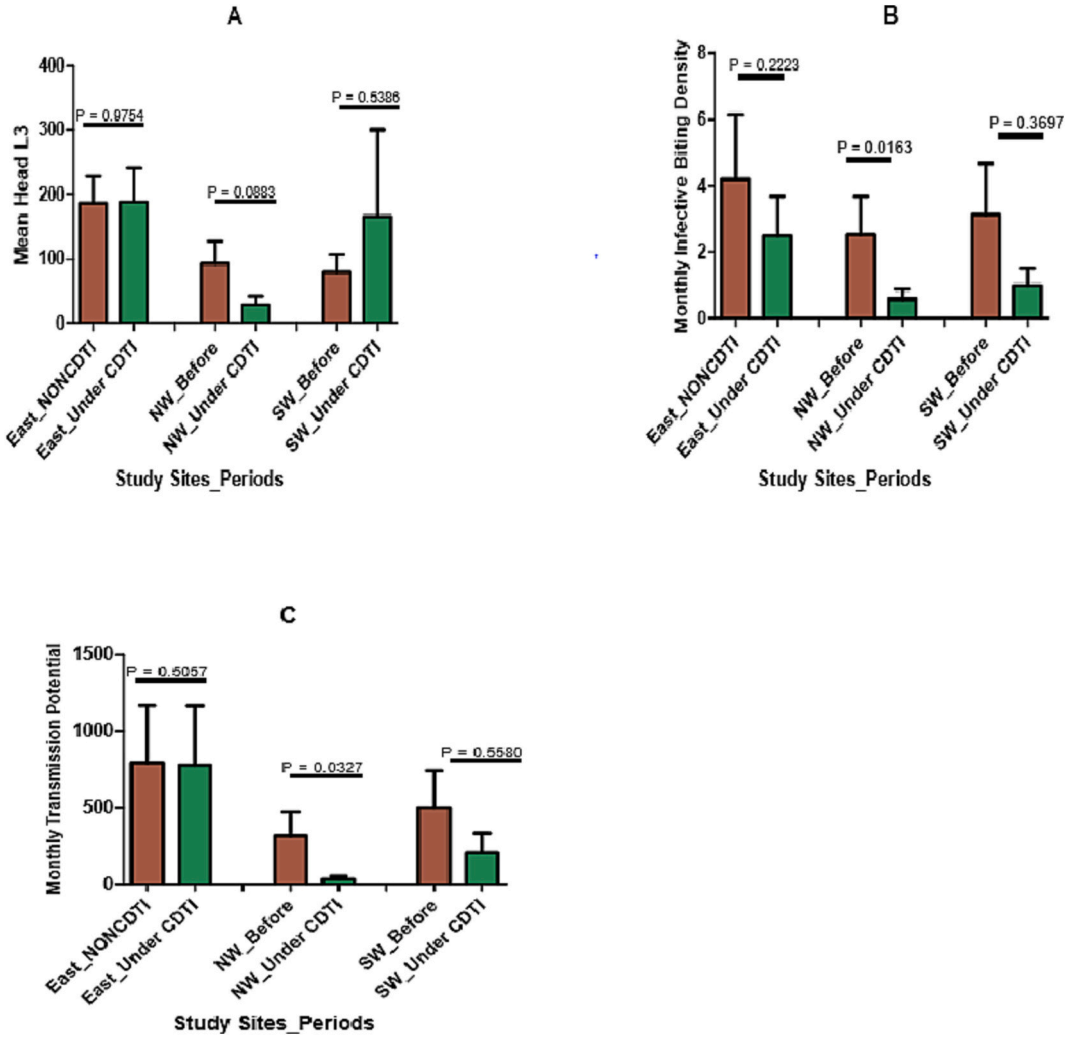

Fig. 3. *Chrysops* infection rate in the East, North-West and South-West 1 CDTI project sites. For the East project, baseline data were not available, but data from a geographically close non-CDTI district are shown. (A) In the East and South-West 1 sites, MHL3 increased after 10 and 14 rounds of CDTI respectively while the contrary occurred in the North-west after 9 rounds of CDTI. (B) Interestingly, the MIBD reduced after varying rounds of CDTI in the East, North-West and South-West 1 sites with a significant difference recorded only in the North-West. (C) The same trend was observed with MTPs in the same study sites with a significant difference still in the North-West only. The Man-Whitney U rank test was used to compare the MBD, MHL3, MIBD and MTP before and after several rounds of CDTI.

This study is the first to evaluate the impact of mass treatment with Ivermectin in a programmatic context on the entomological indicators of loiasis at large-scale. Studies have demonstrated that large scale treatment reduces the infection rate in *Chrysops* and thus reduces parasite transmission after several years of intervention (Kouam et al., 2013; Chippaux et al., 1998). These studies were, however, limited either due to different mass treatment strategies (Chippaux et al., 1998) or due to the size and focal nature of the study (Kouam et al., 2013). The present cross-sectional design addresses most of these limitations.

This survey demonstrated a reduction and difference in the *Chrysops* infection and infective rates in the North-west and East CDTI projects, respectively, despite relatively high baseline indicators. These reductions should be influenced by CDTI, since a relationship between Ivermectin intake and the prevalence/intensity of *L. loa* infection had been reported in the same study communities (Wanji et al., 2018). In a treated human population, *Chrysops* would progressively pick lower quantities of microfilaria during their blood meals, therefore reducing transmission potential. A $>$ 40% reduction in L. *loa* prevalence in the human population was registered in the East and North-west, which could have concomitantly reduced *Chrysops* infection and infective rates by 25% and 27.8% in the East and 65.7% and 71.4% in the North-West, respectively. These reductions were significant in the North-west site, while no significant decrease was observed in the East site.

In the South-west 1 CDTI project, a 62.8% significant increase in *Chrysops* infection rate was experienced while the infective rate remained unchanged. These unexpected findings could be attributed to a lukewarm attitude towards ivermectin intake in the study area (Wanji et al., 2015a, 2015b). This low adherence to ivermectin results from the fear of severe side effects and possibly death, associated with ivermectin treatment in L. *loa*-infected patients. (Wanji et al., 2015). This constitutes an inbuilt design that may prevent ivermectin to ever have full impact on L. *loa* transmission in endemic areas. This goes further to confirm findings by Kouam et al., (Kouam et al., 2013) who attributed this stability to the level of exposure of *L. loa* that hasn't changed after $>$10 years of treatment. Moreover, the intensity of infection also remained high in the South-west 1. In a similar vein, in Cameroon, many areas are now adopting the test and not treat (TNT) strategy to prevent severe adverse events from ivermectin treatment. This involves measuring microfilarial blood densities and excluding individuals with very high *L. loa* parasite burdens from treatment. Of course, such individuals may disproportionately contribute to transmission, thereby diluting CDTI's effectiveness in reducing transmission. The MHL3 experienced a significant drop and a difference in the North-west and East, respectively. For reasons discussed earlier, we could have expected that with more than a decade of repeated annual ivermectin MDA, the entomological indicators of infection would be near zero in the South-west 1, but that was not the case.

Chrysops biting densities post CDTI were generally lower than at baseline. Biting by filariasis vectors correlates with distance from the vector breeding site, and varies from one part of a locality to another (Lehane, 1991). Biting frequencies are higher when people are closer to vector breeding sites and lower when they are further away (Rwegoshora et al., 2007). The current lower biting density could be attributed to the massive deforestation by timber companies and farming activities by community members. Population growth and deforestation have altered fly ecology and breeding sites: *Chrysops* breed in mud covered by shallow running water, densely shaded by vegetation from which much debris falls to decay in the water (muddy detritus). Massive deforestation and farming must have reduced such close-to-human-habitation environments.

The surprisingly increase in MHL3 can be explained by the fact that since SAEs have been registered in situations of high mf loads in humans, individuals residing in communities who do not partake in CDTI constitute a reservoir of high mf loads that can be picked up and transmitted by *Chrysops* during blood meals. Therefore, *Chrysops* infective rates might decrease with time, but individuals who are heavy mf load reservoirs and don't participate in CDTI will continuously supply flies with high mf loads. Monthly biting rates also correlated positively with MTP, indicating that intense vector biting led to high transmission intensity since the probability of infected *Chrysops* to bite was increased.

Higher parous rates were recorded in the East CDTI site. The parity rate is likely influenced by environmental variables such as temperature, rainfall, relative humidity, and availability of breeding sites (Adeleke et al., 2010). Parous rates decreased in the North-west and South-west sites, probably due to human activity, which resulted in changes in insect ecology that influenced breeding and survival.

Overall, our data indicate that the forest savanna nature in the North-West site, could possibly influence the decrease in entomological indicators. Nevertheless, the unchanged and increase in entomological indices in the East and South-West site, respectively emphasizes the conserved nature of these rain forest ecological zones, making transmission very possible between the human host and the vectors.

5. Conclusion

This study demonstrated that the impact of repeated annual treatment with ivermectin for the control of onchocerciasis using community directed delivery approach on the entomological indicators of loiasis varies with bioecological zones. Community directed treatment with ivermectin induced a significant reduction in the entomological indicators of loiasis in the North-West project site which lies in forest savanna area. A non-significant decrease was observed in the East project site and in contrast, a significant increase was observed in the South-West 1 project site which both belongs to the rainforest zones. Also, adherence to ivermectin repeated annual treatment is a key determinant for its full impact on *L. loa* entomological indicators. However, the fear of SAEs by community members has negatively affected adherence to Ivermectin. These observations reinforce the urgent need of implementing alternative strategies to accelerate the elimination of onchocerciasis and LF in areas co-endemic with *L. loa* and motivate the call for a research agenda to develop safe drugs to combat *L. loa*.

Ethics approval and consent to participate

The study protocol, as a work package attached to the following studies (Wanji et al., 2015; Duke, 1955), received ethical approval from the Cameroon National Ethics Committee. The objective of the study was explained to the trained collectors, and informed consent was obtained from all participants. Measures were taken to minimize the health risk of microfilaraemic volunteers, workers, and neighbouring inhabitants as the flies were stored in properly closed tubes.

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Authors' contributions

SW conceived the work and designed the protocol with assistance of CBP, AH, KP, RAA, and GNA. GNA, PWCN, FFF, MR, and RAA performed formal analysis supervised by SW, KP, CBP and PWCN, GNA, AJN, RAA, TMN, AAB, FFF, PIE, SW and KD performed data curation and analysis. PWCN, AJN, GNA, FFF, RAA, SW drafted the manuscript that was reviewed and edited and approved by all authors.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

All data generated or analysed during this study are included within the paper and/or supplementary information files.

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