

Population dynamics of *Onchocerca volvulus* microfilariae in human host after six years of drug control

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Abstract

Background & objectives: Mass administration of ivermectin drug was carried out annually between 1995 and 2001 in three villages that were endemic for onchocerciasis in the Lower Cross River Basin, Nigeria. The aim of this study was to evaluate the population dynamics (dispersion patterns, distribution, prevalence and intensity) of *Onchocerca volvulus* microfilariae in their human host after six years of ivermectin treatment.

Methods: A total of 1014 subjects from three rural areas in Etung Local Government Area of Cross River State, Nigeria were screened for skin microfilariae using standard parasitological method of diagnosis.

Results: Ivermectin drug intervention had significantly reduced the prevalence of skin microfilariae (PMF) from 69.3% pre-control to 39.3% and community microfilarial load (CMFL) from 7.11 to 2.31 microfilariae per skin snip. Males (45%) were significantly ($p < 0.05$) more infected than females (34%). Both microfilarial prevalence and intensity increased with age. Pearson correlation test between intensity and age was not significant ($r = 0.37$; $p > 0.05$). The correlation between age-dependent parasite prevalence and mean abundance was also not significant ($r = 0.42$; $p > 0.05$). The degree of dispersion as measured by variance to mean ratio (VMR), coefficient of variation (CV) and exponent 'K' of the negative binomial model of distribution showed that the parasite aggregated, clumped and overdispersed in their human host. The relative index of potential infection of each age group showed that adults between the age of 21 and 50 yr accounted for 52.7% of microfilariae positive cases.

Interpretation & conclusion: Aggregated and overdispersion of *O. volvulus* observed in this study showed that active transmission could still be going on, because the tendency of the vector, *Simulium damnosum* ingesting more microfilariae was high due to the aggregated and overdispersed nature of the parasite with its host.

Key words Cross River State – epidemiology – Nigeria – onchocerciasis – population dynamics

Introduction

Onchocerciasis is a major public health problem in Nigeria. It is caused by infection with the filarial parasite *Onchocerca volvulus*. It is estimated to affect over 17 million people in 26 African countries with

3.3 million residing in Nigeria¹. There has been considerable interest in the way and manner by which parasites are distributed in their host. The pattern of spatial dispersion of a population and its descriptions are of considerable importance for understanding population dynamics and this is particularly true in

the field of epidemiology where aggregation of a parasite population with respect to their hosts appear to be ubiquitous^{2,3}. In human communities, it is typical for about 15% of individual to play host to about 80% of the helminth parasites⁴. When considering host-parasite relationship the host provides a convenient spatial sampling unit, count being made of the number of parasites concerned⁵.

In onchocerciasis studies, attempts have recently been made to relate the estimated number of adult worms in the body to the estimated total load of microfilariae⁶ and also to the estimated exposure to infective larvae⁷. Like most macroparasitic infections, onchocerciasis is an endemic, stable, resilient and chronic disease. In the affected communities, re-infection is the norm and parasite prevalence may reach high levels. These characteristics are probably due to the existence of density-dependent regulatory constraints on parasite population growth and on the other hand to the human host's inability to mount a strong protective immune response or to do so after many years of exposure⁸.

On the Lower Cross River Basin, Nigeria, *Simulium damnosum s.l.* is an important vector of onchocerciasis and it breeds in rivers, streams and waterfall in this basin⁹. Mass drug administration (MDA) through community directed treatment with ivermectin¹⁰ is used to control onchocerciasis in Nigeria. In the onchocerciasis endemic region of Lower Cross River Basin, ivermectin has been administered once a year over a six-year period (1995–2001). The aim of this report is to examine the degree of dispersion and nature of frequency distribution of *O. volvulus* in human host after six years of drug administration to assess its epidemiological implication.

Material & Methods

Study site: Cross River State is situated within the Cross River Basin between latitude 5°32' and 4°27' N and longitudes 7°50' and 2°20' E. It occupies an

area of 23,074 km². The climate is tropical except for Obudu Plateau which is temperate (5200 ft above the mean sea level). There are two seasons, the rainy season from April to October and the dry season from November to March with a perennial rainfall of about 350 mm in the coastal areas and between 120 and 200 mm in the hinterland with maximum precipitation during July to September. Ambient temperature remains high throughout the year (22.4–33.2°C). Relative humidity is high (60–93%). Natural resources include petroleum, palm produce, timber, rubber, fish and livestock. The economy of Cross River State is agro-based with farming and fishing being the predominant occupations of the people.

A total of three villages (Ajassor, Agbokim and Bendeghe-Ekiem) from Etung Local Government Area of Cross River State, Nigeria were surveyed for onchocerciasis through random sampling. Pre-treatment (baseline) epidemiological data were collected by the National Onchocerciasis Control Programme (NOCP) in 1994, just before the commencement of treatment with ivermectin. The communities were selected on the basis of their hyperendemic status and were receiving treatment with ivermectin. A detailed description of the study site is reported elsewhere^{11,12}. Ethical clearance was obtained from the Cross River State Ministry of Health. Informed consents of the individuals and the communities were also obtained.

Methods: This study was conducted between August and November 2001. Two skin biopsies were obtained from the right and left iliac crests of all individuals who presented themselves for the survey. Holth corneoscleral punch (Storz instrument GMBH, Heidelberg, Germany) was used to obtain the skin biopsies as previously described^{13,14}. After each series of two skin snip was obtained from a subject, the scleral punch was sterilised by washing sequentially in glutaraldehyde, sodium hypochlorite solution, distilled water and alcohol^{15,16}. The entire process is to ensure that HIV and other blood-borne infections are not transferred.

The snips were incubated in buffered saline for 24 h at room temperature. Thereafter, emerged microfilariae were observed and counted. Microfilariae were fixed with 2% formalin and stained with Giemsa in order to confirm their specific identity as *O. volvulus* as described previously¹⁷. The arithmetic mean of the two skin snip counts was used as a measure of the level of infection.

Mass drug administration: Treatment of onchocerciasis with ivermectin started in Cross River State in July 1995 as a combined effort of State Ministry of Health and United Nations International Childrens Emergency Fund (UNICEF). Successive treatments were given between the months of February and March each year before evaluation in 2001. In 1998, Cross River State began receiving African Programme for Onchocerciasis Control (APOC's) support with the initiation of community directed treatment with ivermectin (CDTI). The CDTI approach empowers endemic communities to take full responsibility for the drug delivery process, decide how and when and by whom treatment should be administered and oversee its implementation and follow-up for upwards of 15 years, long after the cessation of direct APOC support¹⁸. In each of the communities examined, community directed distributors (CDDs) were incharge of ivermectin distribution. The heights of all the subjects were first determined and the exclusion criteria adopted in administering the drug¹. The drug was administered according to the following regimen, less than 90 cm, 0 mg; 90–119 cm, 3 mg or 1 tablet; 120–140 cm, 6 mg or 2 tablets; 141–158 cm, 9 mg or 3 tablets; and 159 cm and above, 12 mg or 4 tablets. For all those who accepted the drug, individual compliance was ensured as the subject was made to ingest the drug in the presence of the CDD. The full name, sex, age and height of every treated subject were recorded at every treatment period and the dose was noted.

Treatment coverage: We determined the total number of persons treated in each community during the

period of 1998–2001 by reviewing the community treatment register and annual summary treatment statistics maintained by the National Onchocerciasis Control Programme, Calabar Office. The Pre-CDTI period (1995–1997) could not be evaluated because treatment was at the local government level and there was no community specific treatment data. Ivermectin was made to the public primarily through mobile teams and static facilities, as such register was not properly kept.

$$\text{Annual therapeutic coverage (ATC)} = \frac{\text{No. of persons treated}}{\text{Total population determined each year of treatment}} \times 100$$

$$\text{Interval therapeutic coverage (ITC)} = \frac{\text{Sum of total of all treatment (in yr)}}{\text{Sum of total of population (in yr)}} \times 100$$

Data analysis: The prevalence was measured as the percentage of persons found to be infected, while intensity was determined as the arithmetic mean and geometric mean of the microfilarial counts among those infected. Differences between proportions were tested using chi-square (χ^2). Variations in mean values between groups were tested using student's *t*-tests. The community microfilariae load (CMFL) is the geometric mean number of microfilariae per skin snip among adults aged 20 yr or above in the community including those with negative counts. This mean was calculated using a log ($x+1$) transformation¹⁹. Two measures of the degree of parasite clumping in the host population were calculated; the variance to mean ratio (VMR) $\frac{S^2}{\bar{x}}$ of parasite numbers per host measures the degree of aggregation or contagious distribution²⁰. The negative binomial distribution $[k = \frac{\bar{x}^2}{S^2 - \bar{x}}]$ is a discrete unimodal distribution defined by a positive exponent (*k*) and the population mean (\bar{x}) where *k* varies inversely with respect to the degree of overdispersion. The coefficient of variation (CV%) was determined according to Gregory and Woolhouse⁴.

$$CV = \frac{SD}{\bar{x}} \times \frac{100}{1}$$

Pearson correlation analysis was carried out between the indices of dispersion and mean load, or host age. Index of potential infection (IPI) and relative index potential infection (RIPI) were calculated as:

$$\text{IPI} = \frac{\text{No. of infected} \times \% \text{ infected} \times \text{geometric mean}}{100}$$

$$\text{RIPI} = \frac{\text{No. infected} \times \% \text{ infected} \times \text{geometric mean}}{\sum \text{IPI}} \times 100$$

All statistical analyses were performed using SPSS for windows version 10 (SPSS Inc., Chicago, IL, USA).

Results

The interval therapeutic coverage (ITC) for the study area over the six years period was 73.5% (range 72–74%). The annual therapeutic coverage (ATC) for the communities ranged from 39–97% (Table 1).

To assess the impact of this treatment on the disease, changes in prevalence and intensity were monitored in 1014 subjects of the three endemic villages in 2001. In 1994, 69.3% of the 440 adults examined were parasitologically positive for *O. volvulus* microfilariae in their skin snip. In 2001, microfilariae could only be detected in 39.3% of the 1014 subjects examined representing 43.3% reduction. The CMFL in all the treated communities was also reduced during the period of investigation. Overall CMFL for the

study area was 7.11 microfilariae/skin snip in 1994, and this reduced to 2.31 microfilariae/skin snip in 2001, representing a 67.5% reduction (Table 2). Males (45%) had a significantly ($\chi^2 = 12.44$; $p < 0.05$) higher infection rate than females (34%) (Table 3). The mean microfilarial burden was 3.51/skin snip. Both microfilarial prevalence and intensity increased with age. Pearson correlation test between intensity and age was not significant ($r = 0.37$; $p > 0.05$). The correlation between age dependent parasite prevalence and mean abundance was also not significant ($r = 0.42$; $p > 0.05$). High variance to mean ratio (6.8–23.46) was significantly ($p < 0.001$) greater than unity suggesting an aggregated or contagious model of dispersion (Table 4). The values of the exponent (k) of the negative binomial distribution were all less than one (–1.0 to 0.87) indicating over dispersion (Table 4). The coefficients of variation were also higher than 100%, exhibiting a clumped distribution. The values ranged from 115.81 to 210.58%. The RIPI of different age groups infected with *O. volvulus* microfilariae showed that children between 1 and 20 yr age accounted for 12.56% of positive cases while the active age group (21–50 yr) accounted for 52.7% of positive cases. The older age group (>50 yr) contributed 34.7% of the *O. volvulus* positive cases (Table 5).

Discussion

Mass drug administration using ivermectin for the treatment of onchocerciasis brings about a reduction in the transmission of the parasite. The effect has been

Table 1. Annual therapeutic coverage in the study areas from 1998–2001

Village	1998			1999			2000			2001			Total		
	Pop.	Treated	% cov	Pop.	Treated	% cov	Pop.	Treated	% cov	Pop.	Treated	% cov	Pop.	Treated	% cov
Ajassor	2454	1828	74	3806	3543	93	3806	2050	54	3820	2634	69	13,886	10,055	72
Agbokim	2289	1072	47	2110	1678	79	3491	2822	81	3873	3174	82	11,763	8,746	74
Bendeghe-Ekiem	2893	1960	68	3110	1212	39	4227	4116	97	4259	3411	80	14,489	10,699	74
Total	7636	4860	64	9026	6433	71	11524	8988	78	11952	9219	77	40,138	29,500	74

Pop – Population; Cov – Coverage.

Table 2. Prevalence of skin microfilariae (PMF) and community microfilarial load (CMFL) in eight communities receiving ivermectin

Village	Prevalence of skin microfilariae ¹²			Community microfilarial load (mf/ss) ¹²			Total number examined	
	1994	2001	% Reduction	1994	2001	% Reduction	1994	2001
Ajassor	66.5	27.2	59.1	5.63	1.56	72.29	142	368
Agbokim	75.5	56.3	25.4	8.44	3.22	61.85	144	238
Bendeghe-Ekiem	66.0	40.2	39.1	7.25	2.15	70.35	154	408
Total	69.3	39.3	43.5	7.11	2.31	67.50	440	1014
p-value	p<0.05			p<0.05				

Table 3. Prevalence of *Onchocerca volvulus* microfilariae by village and sex

Village	Male		Female		Total		Prevalence ratio	P-value (95%)
	No. examined	No. infected (%)	No. examined	No. infected (%)	No. examined	No. infected (%)		
Ajassor	108	44 (40.74)	260	56 (21.54)	368	100 (27.17)	1.89	p < 0.05
Agbokim	118	60 (50.84)	120	74 (61.19)	238	134 (56.3)	0.83	p < 0.05
Bendeghe-Ekiem	252	111 (44.05)	156	53 (33.97)	408	164 (40.19)	1.10	p < 0.05
Total	478	215 (45)	546	183 (34)	1014	398 (39.3)	1.15	p < 0.05

Table 4. *Onchocerca volvulus* prevalence, intensity and measures of dispersion by age

Age	No. examined	No. infected (%)	Arithmetic mean intensity	VMR	K	CV (%)
1 – 10	62	5 (8.06)	1.06 ± 0.24	20.0	-1.00	22.64
11 – 20	172	6 (34.88)	4.19 ± 5.34	6.8	0.72	127.45
21 – 30	194	7 (38.14)	4.59 ± 6.87	10.28	0.49	149.67
31 – 40	188	70 (37.14)	5.25 ± 6.08	7.04	0.87	115.81
41 – 50	116	44 (37.23)	6.23 ± 9.13	13.38	0.50	146.55
51 – 60	108	28 (37.93)	5.29 ± 11.14	23.46	0.23	210.58
61+	174	97 (44.44)	3.82 ± 7.32	14.03	0.29	191.62

demonstrated in this study and also in previous investigations^{12,21,22}. The study has shown that after six-years period of annual ivermectin treatment, hyperendemicity status of the study area had been reduced to mesoendemicity. There was a remarkable reduction in PMF and CMFL in the study area. The varied percentage reduction observed in PMF and CMFL in all the communities might be due to the different

treatment coverage patterns. The high treatment coverage (>70%) recorded in the study area indicates acceptance, compliance, efficacy and safety with annual ivermectin treatment.

A quantitative understanding of the population dynamics of parasites and their impact on individual hosts and populations requires a good estimation of

Table 5. Relative index of potential infection (RIPI) of difference age group infected with *Onchocerca volvulus* microfilariae

Age (yr)	No. examined	No. infected	% infected	Geometric mean	Geometric mean transformed $\log_{10}(x + 1)$	Index of potential infection (IPI)	Relative index of potential infection (RIPI)	
1 – 10	62	5	8.06	1.07	0.0294	0.41	0.07	} 12.56%
11 – 20	172	60	34.88	3.79	0.5786	79.31	12.49	
21 – 30	194	74	38.14	4.43	0.6464	125.03	19.70	} 52.72%
31 – 40	188	70	37.14	4.46	0.6493	115.95	18.27	
41 – 50	116	44	37.23	5.71	0.7566	93.54	14.75	} 34.73%
51 – 60	108	48	37.93	4.44	0.6473	80.84	12.73	
61+	174	97	44.44	3.24	0.5105	139.67	22.00	
Total	1014	398	39.25	3.51	0.5453	634.75		

the intensity of infection in each host and how the parasites are distributed through the host population²³. The overall prevalence of *O. volvulus* infection by skin snip in the three communities was 39.3%. Adopting the endemic rates classification as defined²⁴, these communities may be regarded as mesoendemic for onchocerciasis. This result is consistent with the reports of other workers^{25–27} working in different parts of Nigeria, where the control programme is being carried out. The sex related prevalence shows that male subjects were significantly more infected than the females. The result is in consonance with other findings^{28,29}. The frequency duration and degree of exposure to infective flies may have accounted for this difference among males and females.

In the area surveyed, both microfilarial prevalence and intensity increased with age, while exhibiting the plateau effect reported in other studies and typically reached in the 31–50 yr old age group^{13,14,26,30}. It is possible that this pattern of increasing age-dependent *O. volvulus* infection may be due to the fact that the rate of parasite immigration is greater than the rate of worm death^{3,8,31}.

The frequency distribution of parasites among host is used as the basis of the quantitative assessment of the

nature of parasitism³². This work on population dynamics of *O. volvulus* has revealed the distribution, aggregation and intensity of this parasite on infected human host after six annual treatments with ivermectin. This infection pattern of *O. volvulus* might have been influenced by the availability of blackfly vectors harbouring the infective stages (L_3) of the parasite and constant exposure of human host to bites of these infective flies. The interaction of these factors frequently result in an external variability in the prevalence, intensity and overdispersion of the parasite in host population^{33–35}. This study did suggest that a very efficient and active process is in operation in the host-parasite interaction, probably due to the presence of appropriate water bodies for the growth and development of the blackfly pre-imaginal stages and human activities such as farming, fishing, swimming, hunting and wild fruit collection which increases the chances of vector-host contact¹¹.

The distribution of the counts of parasite per host was adequately described by the empirical negative binomial model of distribution and variance to mean ratio. These indices indicate that the population of *O. volvulus* is overdispersed and aggregated within their human host. This is evident by the apparent lack of a process effectively looping off the upper tails of microfilarial distribution per age group. Similar re-

sults have been obtained³⁶. According to Casie³⁷, helminth parasites of any one species are rarely randomly distributed within their host population. The overdispersion observed in this study might be due to the fact that individuals are exposed throughout their lives to severe transmission intensity and perhaps become unable to mount an efficient protective immune response^{38,39}. In addition, factors such as the abundance of infective blackfly vector, appropriate water bodies and human activities that bring vector-host contact, acting singly or collectively might be contributory to the aggregated distribution observed in this study.

The analysis of index of *O. volvulus* infection among positive subjects revealed that persons within the age bracket of 21–50 yr contributed 52.7% of the positive cases. It is possible that this active age group might be acting as a reservoir by which infection could be acquired and/or transmitted. This is likely because most of the activities that bring about man-vector contact are practiced by these age group in most rural communities, thereby exposing them to continuous bite of infective fly. It is suggested that control measure should be targeted at this age group, if meaningful reduction in transmission potential is to be achieved.

According to WHO²¹, ivermectin is known to cause a reduction in number of microfilariae, but it does not stop the transmission. The epidemiological implication is that active transmission could still be going on, because the tendency of the vector ingesting more microfilariae is high due to the aggregated and over dispersed nature of the parasite within its host.

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