Seroprevalence of onchocerciasis in Ogun State, Nigeria after ten years of mass drug administration with ivermectin[‡]

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Background: This serological study conducted between March and July 2015 investigated the status of onchocerciasis in Ogun State, Nigeria after a decade of mass drug administration (MDA) with ivermectin. Baseline information from the rapid epidemiological mapping of onchocerciasis (REMO) prior to MDA had indicated that Ogun State was meso-endemic to onchocerciasis. Following years of treatment with ivermectin, it has become important to investigate the current status of the disease using more sensitive diagnostic methods.

Methods: The study was conducted in 32 communities in eight onchocerciasis-endemic local government areas (LGAs). Using the Ov16 rapid diagnostic test (Ov16 RDT), finger-prick whole blood was obtained from 3 895 consented participants from age five and above. Skin snips were obtained from a subset of 481 corresponding participants as the majority of community members did not consent to skin snipping.

Results: Study revealed a cumulative seroprevalence of 9.3% (CI 9.29–9.31%) by RDT and 17.3% (CI 16.73–18.34%) microfilaridermia prevalence by the skin snip subset. Seroprevalence among children between the ages of five and nine was 2.9% (CI 1.74–3.53%) across all LGAs. A community microfilaria load (CMFL) between 0 and 1.21 MF/skin snip was recorded in the different communities studied. Among the studied LGAs, children in Odeda LGA recorded the highest seroprevalence by RDT (14.9%) followed by Abeokuta North (5.1%), Abeokuta South (4.8%) and Imeko-Afon (0.6%), while Ewekoro, Ifo, Obafemi-Owode and Yewa North LGAs recorded zero prevalence.

Conclusion: It appears that the elimination of onchocerciasis in some LGAs is possible considering the lack of new infection among children and the hypo-endemicity among the adult population. However, the microfilaria prevalence observed among adults is of concern as it may imply that mass treatment has not been effective. Increased therapeutic coverage is advised to fully maximise the potential of ivermectin treatment to achieve the disease elimination.

Keywords: CMFL, ivermectin, Nigeria, Onchocerciasis, Ov16 RDT, Ogun State, seroprevalence

Background

Onchocerciasis is an irreversible blinding disease associated with infection with the parasite *Onchocerca volvulus*, which is transmitted by insect vectors of the *Simulium* species. It is distributed mostly along communities with fast-flowing rivers with rocky vegetation, which serves as suitable breeding ecology for the vectors.[1–3] The disease is of global concern as it is found in 31 African countries, Yemen and South America with confirmed elimination in Mexico, Ecuador, Guatemala and Colombia.[4–8] Nigeria ranks among those countries with the highest burden of the disease in the world, accounting for about a third of the global prevalence.[9–12]

Control of onchocerciasis is anchored on annual mass drug administration (MDA) of ivermectin (Mectizan®, donated by Merck & Co., Inc., NJ, USA) a freely distributed potent microfilaricide which kills the *O. volvulus* microfilaria in the human host and reduces greatly the disease burden. In the past, computer-based models had projected 25 years' long-term continuous treatment with ivermectin to achieve the suppression and interruption of *O. volvulus* transmission.[13] While this model is exhaustive, factors such as baseline infection rate, endemicity (intensity of

transmission) and numerous programmatic issues e.g. (85% therapeutic coverage and adherence) have suggested that 5–15 years of mass treatment with ivermectin could interrupt transmission.[4–15] As a result of long-term treatment with ivermectin, elimination of onchocerciasis in certain foci in Africa now appears to be possible.[16–18] Successes recorded in MDA and increased global efforts to eliminate onchocerciasis and other neglected tropical diseases (NTDs) as a public health concern by the World Health Organization has called for a need for field-deployable sensitive diagnosis and disease surveillance tools.[19,20]

Skin biopsy is the standard diagnosis of onchocerciasis and it involves detection of microfilaria in the subcutaneous area of the upper iliac crest of an individual.[13] However, the inability to sufficiently detect early infections in persons with low microfilaria density and the associated invasive procedure are among the numerous challenges encountered in the quantification of onchocerciasis prevalence in endemic areas.[18] Sensitive diagnostic tools to evaluate treatment progress in regions with low prevalence, continuous MDA and where control intervention has stopped are important in decision-making in any control programme. In recent times, Programme for Appropriate

Technology in Health (PATH) and Standard Diagnostics (Yongin-si, Gyeonggi-do, South Korea) made commercially available point-of-care RDT, which detects antibodies to a parasite antigen called Ov16 by checking for these antibodies in a single drop of blood from a finger prick.[21]

Ogun State, located in Southwest Nigeria, has eight local government areas (LGAs) in which onchocerciasis is endemic. Some of the communities in this LGAs are traversed by the Ogun and Osun River systems, which have rocky substratum and vegetation suitable for breeding of vector species.[22,23] Control efforts using community-directed treatment with ivermectin (CDTI) have been ongoing since 2004 following rapid epidemiological mapping of onchocerciasis (REMO), which provided a cumulative nodule prevalence of 22.7%.[24] Sam-Wobo *et al.*[16] conducted epidemiological studies in a few sentinel communities along the Ogun River to measure the impact of mass treatment and microfilaria status in the human population and reported the first evidence of the possibility of onchocerciasis elimination in Ogun State.

To further determine the impact of MDA and the possibility of elimination of onchocerciasis in endemic LGAs of Ogun State, epidemiological studies using the Ov-16 RDT and skin snip microscopy was conducted in 32 onchocerciasis endemic communities in Ogun State, Nigeria.

Materials and methods

Study location and design
The study was conducted between March and July 2015. Using a grid sampling method, 32 first-line onchocerciasis-endemic communities from eight LGAs in Ogun State were selected for the study seven months after the last round of ivermectin mass treatment. The LGAs included Abeokuta North, Abeokuta South, Ewekoro, Ifo, Imeko Afon, Obafemi Owode, Odeda and Yewa North (Figure 1). The study communities fall within the derived savannah and forest bio-climatic belt of the state. Data obtained from the State Ministry of Health revealed an annual therapeutic coverage between 65% and 85% employing the CDTi strategy. A GPS device was employed to take the coordinates of each of the community sampled for geo-referencing.

Study population
Communities with a population of not more than 300 were selected for ease of assessment and logistic coordination. Information on the community population was obtained from treatment records detailed in the annual ivermectin distribution by community directed distributors (CDDs). With the aid of photographs and posters, community members were addressed on the importance of the study using the Yoruba, Hausa or French language as some communities were host to immigrants from the neighbouring French-speaking Benin Republic.

Ethical clearance and permission
Ethical approval for the study was obtained from the Ethics Review Committee (ERC) of Ogun State Hospital Management Board, Abeokuta (trial registration number: SHA/RES/VOL.2/153). Informed consent forms were duly signed by voluntary participants and by parents or guardians of child participants during the skin snip and Ov16 RDT study. Consent to participate in either skin snips, finger prick or both was contained in a questionnaire administered to each participant prior to the study. Participants were allowed to freely decide their preference without coercion.
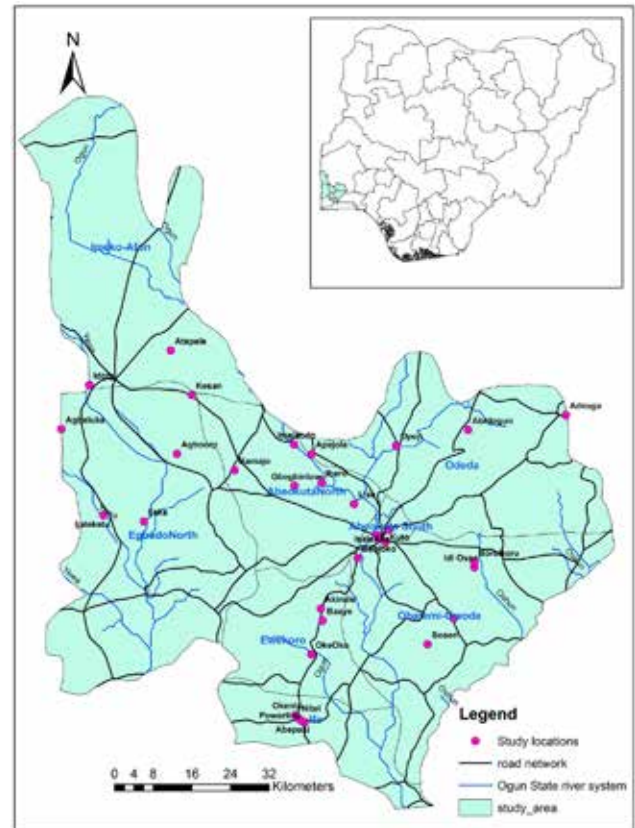

Figure 1: Map showing the study locations and LGAs in Ogun State.

Inclusion criteria
Males and females aged five years of age and above of all ethnic groups residing in first-line communities were included for the serological and parasitological assessment according to standard protocols.

Rapid diagnostic test (RDT)
Participants were serially arranged in a census form and the corresponding number was written on each Alere™ SD Bioline IgG4 test kit (Abbott Laboratories, Abbott Park, IL, USA) cassette using a permanent marker for easy identification. The kit was used according to the manufacturer's instruction. In sum, the alcohol swab included in the pack was opened and used to clean the finger to be pricked using a circular motion for 10 s; the swabbed finger was allowed to air dry. The participant's finger was pricked with a lancet to obtain a drop of blood and the lancet was discarded into the biohazard-labelled sharps container. The first drop of blood was used for the RDT. Using the capillary pipette provided in the test kit, 10 μl of blood was collected to fill up to the marked line on the pipette. A clean piece of gauze was used to apply pressure on the pricked finger to stop bleeding following blood collection.

The 10 μL collected blood was added to the round sample well on the test kit cassettes and absorbed by the sample pad of the test. The capillary pipette was also discarded in the sharps container. Four drops of assay diluent were then added into the square buffer well on the test kit cassette and the timer was started. After 30 min the result was read. The area marked 'C' on the test cassette stands for the control line, while the area marked 'T' designates the test line. Valid results include areas on the result window on the cassette showing either two lines of the

control 'C' and test 'T' implying 'positive', or the area marked control 'C' alone implying 'negative'. Invalid results include result window panel with 'T' line alone appearing without 'C'.

Skin snip examination

Two bloodless skin biopsies of each participant were obtained from the left and right posterior iliac crest with the aid of a 2 mm corneoscleral punch (Holth and Modified Walser) and placed on a glass slide with a drop of saline water. The tissues in the slides were examined under a x40 binocular microscope after 30 min

for micro-filarial manifestation. After examination, each skin tissue was incubated in a 96-well microtitration plate containing a normal saline solution and when the column was full the wells were covered with a transparent adhesive film and kept for re-examination within 24 h. After 24 h, the biopsies were removed with curved tweezers and the fluid from each well was examined separately on a slide for microfilaria. After each skin snip in the field, the scleral punch was thoroughly sterilised in hot water for 10 min followed by sequential washing with sodium hypochlorite solution, distilled water and alcohol before re-used.[16] Microfilaria

Table 1: Seroprevalence of onchocerciasis in selected communities

Local government area	Community	Number examined by Ov 16 RDT	No. (+)ve (%)	Number of children (5–9) years examined	Prevalence in children aged 5–9 years (%)
Abeokuta North	Gbegbinlawo	80	15 (19)	21	(0)
	Ibaro	125	43 (34)	19	1 (5.3)
	Imala odo	199	10 (6)	36	2 (5.6)
	Lisa	36	14 (39)	3	1 (33.3)
Sub-total		440	82 (19)	79	4 (5.1)
Abeokuta South	Isale Ake	123	8 (7)	20	2 (10)
	Itori Odo	116	7 (6)	13	0
	Kuto	239	1 (0.4)	19	0
	Oke Iganna	109	5 (5)	11	1 (9.1)
Sub-total		587	21 (4)	63	3 (4.8)
Ewekoro	Akinale	147	12 (8)	22	0
	Baaye	78	0	29	0
	Banjoko	239	7 (3)	57	0
	Oke Oko	37	0	5	0
Sub-total		501	19 (4)	113	0
Ifo	Abepaki	98	0	15	0
	Anuoluwapo	134	1 (0.7)	23	0
	Nitel/powerline	144	0	24	0
	Oke nla	123	1 (0.8)	18	0
Sub-total		499	2 (0.4)	80	0
Imeko Afon	Agbaluka	133	5 (3.7)	73	0
	Atapele	107	24 (22.4)	27	0
	Idofa	230	14 (6.1)	45	0
	Kesan	164	23 (14)	36	1 (2.8)
Sub-total		634	66 (10.4)	181	1 (0.6)
Obafemi Owode	Boru Boru	31	1 (3.2)	3	0
	Idi Osan	60	4 (7)	10	0
	Ogbe Eruku	95	17 (18)	10	0
	Soseri	147	13 (9)	24	0
Sub-total		333	35 (10.5)	47	0
Odeda	Abidogun	136	51 (38)	28	4 (14.3)
	Adeaga	76	9 (12)	14	(0)
	Apejola	75	4 (5.3)	21	(0)
	Opeji	155	74 (48)	25	9 (36)
Sub-total		442	138 (31.2)	88	13 (14.8)
Yewa North	Agbooro	65	0	8	0
	Bamajo	38	1 (2.6)	6	0
	Ijaka Isale	181	0	27	0
	Ijale ketu	175	0	27	0
Sub-total		459	1 (0.2)	68	0
Grand total		3895	364 (9.3)	719	21 (2.9)

results were expressed as positive (mf present) or negative (mf absent); mf prevalence was expressed as number of persons positive divided by the total number of persons examined.[16]

Data analysis

The community microfilarial load (CMFL) was defined as the geometric mean number of microfilariae per skin snip (mf/ss) among adults aged 20 years or more. It was calculated using a log (x+1) transformation CMFL = $(e^{\frac{1}{N} \sum \ln(x+1)} - 1)$ where x is the individual microfilarial density, and N the total number of individuals aged 20 years and above. Individual data were entered and coded in Windows 8.1 Microsoft Excel® (Microsoft Corp, Redmond, WA, USA) and analysed statistically using SPSS® version 20 (IBM Corp, Armonk, NY, USA). Frequencies and percentages were used to group variables while variation and association in (sero)prevalence were analysed using Pearson's chi-square test.

Results

Demographic information of study participants

A total of 3 895 participants with age ranging from 5 to 98 years enrolled for the Ov16 RDT study. A subset (*n* = 481) of study participants consented to provide skin snips for microscopic examination of microfilaria (MF) emergence. Analysis of demographic data showed that 58% were females and 42% were males. The ages of study participants were grouped into the following class intervals: 5–9, 10–14, 15–19, 20–29, 30–49 and ≥ 50 respectively with each representing 719 (18.5%), 693 (17.8%), 254 (6.5%), 483 (12.4%), 959 (24.6%) and 787 (20.2%).

Cumulative seroprevalence of onchocerciasis by RDT in each community and LGA

An overall seroprevalence of onchocerciasis recorded for the eight endemic LGAs studied by RDT was 9.3% (CI 9.29–9.31%) (Table 1). There were variations in the distribution of seroprevalence by Ov16 RDT across the different communities studied. Communities in Odeda LGA reported the highest *O. volvulus* exposure (31.2%) while communities in Yewa North LGA recorded the lowest (0.2%) (Figure 2). Seroprevalence of onchocerciasis was not statistically significant among the different LGAs studied (chi-square = 2.301, df = 1 and *p* = 0.129).

Age, and Gender Seroprevalence by RDT

There was a significant relationship between age and seroprevalence (chi-square = 86.589, df = 3, *p* = 0.000) (Figure 3).
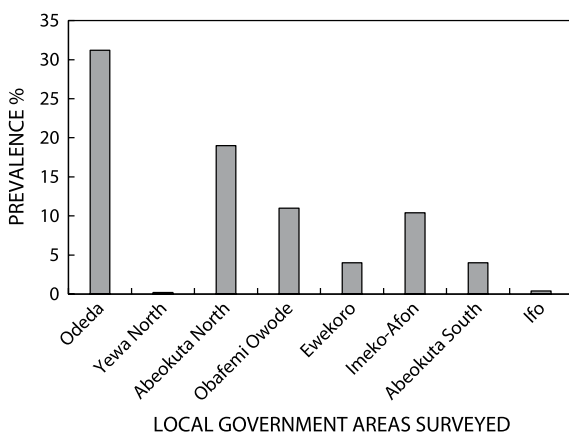
Figure 2: Seroprevalence of *Onchocerca volvulus* across the different LGAs.
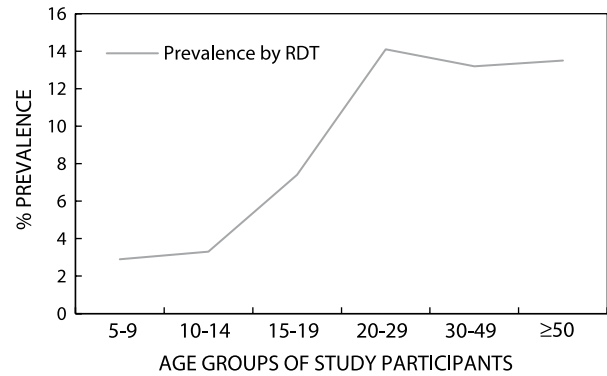
Figure 3: Cumulative seroprevalence by age across all LGAs.

Although seroprevalence varied among different age groups in all the LGAs, the highest prevalence of 14.1% (CI 12.9%–15.6%) was recorded among participants in the age group 20–29 years and the lowest at 2.9% among age class 5–9 (CI 1.74–3.53%). Conversely, there was no significant relationship (chi-square = 2.301, df = 1, *p* = 0.129) between prevalence and gender. Of the 1 646 males 170 were seropositive, representing 10.3%, and of 2 249 females 194, representing 8.6%. Among children between the ages of 5 and 9 years who are markers to indicate ongoing transmission, children in Yewa North, Obafemi-Owode, Ewekoro and Ifo LGA recorded a zero prevalence compared with Odeda (14.8%), Abeokuta North (5.1%), Abeokuta South (4.8) and Imeko-Afon (0.6%).

Microfilaria prevalence across age groups in all LGAs

The study revealed no significant relationship between age and prevalence by skin snip (chi-square = 3.835, df = 1, *p* = 0.0502). Overall MF prevalence was 17.3% (CI 16.73–18.34%) (Table 2). All communities were surveyed with the exception of communities in Ifo and Abeokuta South LGA whose members did not consent to be skin snipped. CMFL was less than one MF per skin snip in all communities sampled with the exception of Idofa in Imeko Afon LGA who recorded 1.21 MF per skin snip. Prevalence by age showed that participants within the age group 5–9 years recorded the least infection (4.8%) while those in the age class 15–19 years recorded the highest (28.4%) (Table 3).

Discussion

Onchocerciasis seroprevalence ranging between 0% and 31.2% was observed in the different LGAs indicating that individuals in communities may be reservoirs of past infections. This would have been corroborated by the comparative corresponding microfilaria study but, regrettably, 88% of the study population refused to be skin snipped owing to past experience of the painful and invasive nature of this method, which reinforced their unwillingness to cooperate. Despite this limitation, a cumulative seroprevalence of 9.3% (CI 9.29–9.31%) was observed across all age groups and a 2.9% (CI 1.74–3.53%) seroprevalence among children < 10 years of age in all LGAs.

Our findings among children have severe epidemiological implications since they are considered 'serological markers' of recent transmission of *O. volvulus*.[25] this is likely to indicate that MDA has not been that effective in affected LGAs. The serological exposure observed among the adult population ought to have been of little consequence in the elimination of onchocerciasis as it may imply that they had been infected prior to initiation of MDA activities and therefore could still be having a historic sero-

Table 2: Community microfilaria load of *Onchocerca volvulus* in the study communities

Local government area	Community	Number examined	Number infected	Prevalence	CMFL
Abeokuta North	Gbegbinlawo	23	3	13	0.2
	Ibaro	34	4	11.7	0.3
	Imala odo	19	4	21.1	0.4
	Lisa	15	3	20	0.2
Ewekoro	Akinale	17	0	0	0
	Baaye	12	0	0	0
	Banjoko	20	0	0	0
	Oke Oko	22	0	0	0
Imeko Afon	Agbaluka	30	1	3.3	0.2
	Atapele	14	3	21.4	0.3
	Idofa	19	7	36.8	1.2
	Kesan	19	4	21.1	0.2
Obafemi Owode	Boru Boru	14	3	21.4	0.5
	Idi Osan	17	0	0	0
	Ogbe Eruku	21	4	19.1	0.7
	Soseri	15	5	26.7	0.6
Odeda	Abidogun	11	4	36.4	0.4
	Adeaga	23	6	26.1	0.3
	Apejola	7	2	28.6	0.9
	Opeji	57	17	29.8	0.4
Yewa North	Agbooro	16	2	12.5	0.3
	Bamajo	17	4	23.5	0.7
	Ijaka Isale	18	3	16.7	0.8
	Ijale ketu	21	4	19.1	0.3

Table 3: Overall age microfilarial prevalence of *Onchocerca volvulus* in the study communities

Age groups (years)	Number examined	Number infected
5–9	84	4 (4.8)
10–14	69	7 (10.1)
15–19	67	19 (28.4)
20–29	86	13 (15.1)
30–49	83	10 (12.1)
≥ 50	92	30 (12)
Total	481	83 (17.3)

response to the Ov16 antigen.[26,27] This does not appear to be the case as the study outcome among the child population implies that they were recently exposed to the disease. Similarly, the high MF intensities and prevalence estimates among adults after seven months of prior treatment is suggestive that transmission is ongoing in affected LGAs.

Following a 10-year period of MDA with ivermectin, infection or exposure among children under recurrent treatment is expected to be < 0.1%.[26,28] This was the case in four LGAs with the exception of communities in Odeda, Imeko Afon, Abeokuta North and South LGAs, which calls for attention. On the other hand, the zero exposure in Yewa North, Obafemi-Owode, Ewekoro and Ifo LGAs should be a good measurement of the MDA activities in Ogun State. This ought hypothetically to indicate interruption of MF

densities in the adult population and a break in transmission of new infection to children below the age of five or individuals who recently crossed the age five threshold being considered safe for the uptake of ivermectin.[29] Unfortunately, the data show that MF densities in adults have not been eliminated in many LGAs and therefore transmission is still possible.

Interestingly, the CMFL observed in the study appeared to be very low owing to the intensity of infection, since the aim of controlling onchocerciasis with ivermectin is focused on reducing the vectorial potential of the *Simulium* vectors to pick up microfilariae during a blood meal for onward transmission. The overall CMFL of < 5 mf/skin snip observed may potentially reduce the risk of new infection, although some mathematical modelling has shown that under certain circumstances transmission can persist at very low parasite densities.[30] The declining community participation in this skin snip was a limitation to reaching a definite conclusion.

Importantly, the exposure to *O. volvulus* and MF prevalence observed among adults and children in Odeda LGA, a semi-urban settlement, is worrisome. The variation in this pattern of seroprevalence compared with other LGAs could be attributed to the proximity of communities in Odeda LGA to the Ogun River, a major focus in the state's onchocerciasis control programme. The river is a potent breeding site responsible for consistent bites by the *S. damnosum* sl vectors. Studies have shown that communities along these river systems are at higher risk owing to the preponderance of the *Simulium* vectors in these areas.[23]

Considering the 10 years of MDA in the state, the increase in prevalence and past exposure with the corresponding increase in age observed in the study is an epidemiological flashpoint that should be taken seriously. The disease persistence in Ogun State despite the treatment history may be attributed to the reservoirs of a high level of transmission before MDA implementation, rural–urban human migration due to the state's proximity to the neighbouring Benin Republic and four adjoining states, poor geographic and therapeutic treatment coverage, and difficult in reaching endemic areas during MDA implementation.[3,31,32]

Conclusion

The serological data obtained in this study serve as baseline information for future studies and epidemiological planning in Ogun State. It appears that the elimination of onchocerciasis in Yewa North, Obafemi-Owode, Ewekoro and Ifo LGAs is possible considering the serological information, which has shown the lack of new infection among children. This is also supported by the value of CMFL observed, which is also in agreement with similar studies on CMFL by Sam-Wobo *et al.*[16] This seeming success may be short-lived if appropriate measures are not put in place for increased therapeutic coverage.

List of abbreviations

CDA	Community development association
CDTI	Community directed treatment with ivermectin
CMFL	Community microfilaria load
ERC	Ethics Review Committee
GPS	Geographical positioning system
IgG4	Immunoglobulin 4
MDA	Mass drug administration
MF	Microfilaria
PATH	Programme for Appropriate Technology in Health
RDT	Rapid diagnostic test
REMO	Rapid epidemiological mapping of onchocerciasis
WHO	World Health Organization

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