

In Southern Nigeria *Loa loa* Blood Microfilaria Density is Very Low Even in Areas with High Prevalence of Loiasis: Results of a Survey Using the New LoaScope Technology

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Abstract. Ivermectin treatment can cause central nervous system adverse events (CNS-AEs) in persons with very high-density *Loa loa* microfilaria ($\geq 30,000$ mf/mL blood). Hypoendemic onchocerciasis areas where *L. loa* is endemic have been excluded from ivermectin mass drug administration programs (MDA) because of the concern for CNS AEs. The rapid assessment procedure for *L. loa* (RAPLOA) is a questionnaire survey to assess history of eye worm. If $\geq 40\%$ of respondents report eye worm, this correlates with $\geq 2\%$ prevalence of very high-density loiasis microfilaria, posing an unacceptable risk of CNS-AEs after MDA. In 2016, we conducted a *L. loa* study in 110 ivermectin-naïve, suspected onchocerciasis hypoendemic villages in southern Nigeria. In previous RAPLOA surveys these villages had prevalences between 10% and 67%. We examined 10,605 residents using the LoaScope, a cell phone-based imaging device for rapidly determining the microfilaria (mf) density of *L. loa* infections. The mean *L. loa* village mf prevalence was 6.3% (range 0–29%) and the mean individual mf count among positives was 326 mf/mL. The maximum individual mf count was only 11,429 mf/mL, and among 2,748 persons sampled from the 28 villages with $\geq 40\%$ RAPLOA, the $\geq 2\%$ threshold of very high *Loa* mf density could be excluded with high statistical confidence ($P < 0.01$). These findings indicate that ivermectin MDA can be delivered in this area with extremely low risk of *L. loa*-related CNS-AEs. We also concluded that in Nigeria the RAPLOA survey methodology is not predictive of $\geq 2\%$ prevalence of very high-density *L. loa* microfilaria.

INTRODUCTION

Onchocerciasis, commonly known as river blindness, is a filarial nematode infection caused by *Onchocerca volvulus*, transmitted by certain insect vector species of the genus *Simulium*.¹ This disease is of public health importance because of its associated visual impairment, blindness, stigmatizing skin disease, and debilitating itching. Human disease results from inflammation around microfilaria (mf) released from fertilized adult female worms residing in fibrous subcutaneous “nodules.” Disease is more severe in individuals who have high numbers (“intensities”) of mf. The *Simulium* black fly vectors breed in rapidly flowing rivers and streams and become infected when they ingest mf during a blood meal; mf develop into third stage larvae that can infect humans when the vector takes subsequent blood meals. The World Health Organization (WHO) estimates that about 198.2 million people are at risk of infection and that 99% of them reside in Africa.^{1–3} Nigeria is among the countries with the highest burden of onchocerciasis.⁴

Mass drug administration (MDA) with ivermectin (Mectizan[®], an oral medication donated by Merck, Kenilworth, NJ) kills the mf stage of the parasite and prevents onchocerciasis-associated eye and skin disease. A global partnership against river blindness, which includes the endemic countries, non-governmental organizations, the Mectizan[®] Donation Program, and the WHO, has given more than 1 billion treatments for onchocerciasis since 1987.⁵ If the coverage and duration of ivermectin MDA programs are sufficient, mf densities can be kept sufficiently low to prevent the vectors from transmitting the

infection. Because there are no important animal reservoirs of *O. volvulus* to maintain the transmission cycle independent of the human population, permanent elimination of transmission of onchocerciasis can be achieved, such as in four countries in the Americas and in some parts of Africa.^{6,7} In 2014 the African Program for Onchocerciasis Control called for a new goal of onchocerciasis transmission elimination for Africa. As part of that policy, an expansion of ivermectin MDA into previously untreated areas was proposed. These areas (the so-called “hypoendemic” areas) are those with sufficient *O. volvulus* transmission to maintain the adult parasite population but very little morbidity due to the near absence of high mf density infections. Untreated areas bordering ivermectin MDA programs are those most likely to be hypoendemic and therefore newly targeted for MDA.⁸

Loa loa, another filarial parasite prevalent in central Africa, is complicating the MDA expansion plan under the new onchocerciasis elimination paradigm. *Loa loa* is transmitted by deerflies (*Chrysops* species) that breed in high canopy-forested areas in Africa. Adult *L. loa* worms may migrate under the eye's conjunctiva and be recognized by the infected individual.^{9–11} Adult female *L. loa* worms produce mf that (unlike in onchocerciasis) enter the blood stream; circulating *L. loa* mf can reach extremely high densities in the blood. The abrupt death of mf after the administration of a microfilaricidal agent such as ivermectin can rarely result in central nervous system adverse events (CNS-AEs) shortly after treatment that include changes in consciousness and, rarely, coma. Deaths have resulted from complications arising from prolonged coma events.¹² Only individuals with very high *L. loa* mf densities ($\geq 30,000$ /mL of blood) are at risk of these CNS-AEs.^{13–15}

A technique called the Rapid Assessment Procedure for *L. loa* (RAPLOA) was developed over a decade ago to quickly and noninvasively assess an area for the risk of *L. loa*-related

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CNS-AEs after ivermectin MDA. A sample of 80 residents aged 15 years and older are individually asked if they at some point in the past experienced a worm moving across the surface of their eye. During the interview the respondents are shown a photograph of a *L. loa* worm in the eye. A multi-country study showed a strong correlation with $\geq 40\%$ of residents answering “yes” (e.g., a RAPLOA prevalence of $\geq 40\%$), a village prevalence of *L. loa* microfilaremia $\geq 20\%$, and the village prevalence of very high-density *L. loa* $\geq 2\%$.^{16–20} These critical and correlated thresholds (RAPLOA $\geq 40\%$, *L. loa* microfilaremia prevalence $\geq 20\%$ and very high-density *L. loa* $\geq 2\%$) define an area at high risk for *L. loa* CNS-AEs. The magnitude of this risk is poorly defined.¹⁴

High RAPLOA determinations in onchocerciasis hypoendemic areas are roadblocks to the onchocerciasis elimination agenda in *L. loa*-endemic countries such as Nigeria. Expansion of MDA into these hypoendemic areas is difficult to justify because the benefit from MDA in reducing morbidity from onchocerciasis is low compared with the risk of CNS-AEs from *L. loa* treatment. We report a survey in just such an area in Nigeria where there is presumed hypoendemic onchocerciasis and hyperendemic *L. loa*. Our purpose was to reevaluate the relationships among RAPLOA, *L. loa* microfilaremia prevalence, and most importantly, very high-density *L. loa*. We also assessed for onchocerciasis endemicity using a rapid diagnostic test for OV16 IgG4 antibodies; the results of that study will be reported elsewhere.

MATERIALS AND METHODS

Study area. The survey was conducted in five Nigerian states assisted by The Carter Center in Abia, Anambra, Delta, Ebonyi, and Imo states, which are located in the forested

South-South and Southeast geopolitical zones of Nigeria. The economic activities in these states are mainly agricultural subsistence farming and fishing. The climate is characterized by a rainy season from March to October and a dry season from November to February.

Study design. The survey was built in a way to purposefully identify villages likely to have both (hypoendemic) onchocerciasis and individuals with very high-density *L. loa* infections. First, twenty ivermectin untreated districts (in Nigeria called local government areas, LGAs) were selected that bordered LGAs already under ivermectin MDA for meso- or hyperendemic onchocerciasis. It was assumed that due to this proximity the selected LGAs would likely have hypoendemic onchocerciasis. Second, villages within those LGAs were selected for survey that had the highest historical RAPLOA prevalence in studies conducted in 2012–2013 by The Carter Center or in 2014–2015 by the Federal Ministry of Health (FMOH) (Figure 1, Table 1). Villages were sorted by RAPLOA prevalence in descending order. All villages having $\geq 20\%$ RAPLOA were selected for survey, followed by a portion of villages with 10–19.9%, until the target of 110 villages was met. Five villages were excluded due to insecurity in their LGA and were replaced by five villages with the next highest RAPLOA prevalence. In surveyed villages, we determined *L. loa* mf prevalence and density using a revised version of the original LoaScope.²¹ This is an innovative cell phone-based microscope reader that gives a reading of *L. loa* mf density within 3 minutes of placing a special cubical capillary tube containing uncoagulated blood (obtained by finger stick) under the lens. From the same finger stick another 10 μ L of blood was obtained for a thin smear and 10 μ L for an OV16 RDT test for onchocerciasis IgG4 antibodies.

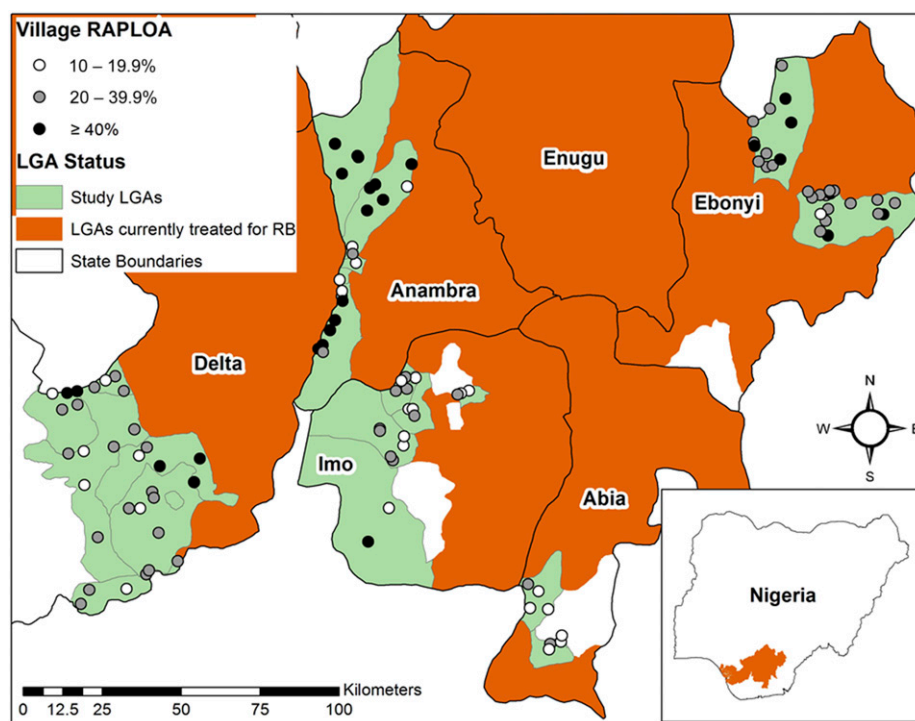


FIGURE 1. Map of study area: Location of surveyed villages and their historical Rapid Assessment Procedure for *Loa loa* (RAPLOA) prevalence. LGA = local government area. This figure appears in color at www.ajtmh.org.

TABLE 1

Village sample size, Rapid Assessment Procedure for *Loa loa* (RAPLOA) information, max and average cellScope counts, and LoaScope prevalence

State	LGA	Village	Number surveyed	Max of RAPLOA (%)	Source of max RAPLOA value	Year of RAPLOA survey	Max of LoaScope mf/mL	Average LoaScope mf/mL among positives	Prevalence of LoaScope positives (%)		
Abia	Osisioma	Amapu Ife	96	15	FMOH	2015	439	207	7		
		Umuakpara	99	14	FMOH	2015	592	229	9		
		Umule	101	19	FMOH	2015	282	179	7		
	Ugwunagbo	Umumba	77	23	FMOH	2015	526	209	6		
		Owerri Aba	100	14	FMOH	2015	1,049	602	2		
		Umule Osoamadi	100	11	FMOH	2015	92	92	1		
		Umuode	100	13	TCC	2013	461	370	4		
		Umuodo	87	24	FMOH	2015	921	271	8		
		Agbudu Nando	98	65	TCC	2012	921	264	14		
		Nneyi Umueri	98	67	TCC	2012	877	263	26		
Anambra	Anambra east	Ogwari Nsugbe	100	62	TCC	2012	680	237	21		
		Otuocha	100	49	TCC	2012	263	197	2		
		Ubaru Ugwuoji	104	55	TCC	2012	1,259	313	19		
		Mmiata Anam	101	46	TCC	2012	1,254	292	11		
		Nzam Assa	99	56	TCC	2012	197	121	5		
		Umuenwelum	100	47	TCC	2012	856	179	11		
	Anambra west	Umueze Anam	100	59	TCC	2012	461	197	18		
		Umuoba Abegbu	101	47	TCC	2012	1,930	301	15		
		Ogbaru	Atani	100	20	FMOH	2015	486	166	18	
			Isiolu Ugalo	97	61	TCC	2012	307	143	10	
			Odekpe	102	14	FMOH	2015	2,632	297	15	
			Ohita	99	13	FMOH	2015	614	298	6	
			Okpoko	99	18	FMOH	2015	1,290	208	22	
			Onyili/Ibelenta	101	67	TCC	2012	128	59	12	
			Umudashi/Esielle	100	65	TCC	2012	329	128	10	
			Umuezegbo	100	50	TCC	2012	1,100	182	20	
			Umunankwo	100	26	FMOH	2015	1,290	184	21	
			Umuokoloigbo	101	49	TCC	2012	351	120	13	
		Onitsha north	American Quarters	100	16	FMOH	2015	61	61	1	
			Onitsha south	Fegge	100	21	FMOH	2015	154	137	3
		Delta	Ethiophe east	Ekrejeta	69	28	TCC	2013	1,791	591	6
				Eku (Emure)	99	43	TCC	2012	2,610	569	7
Isoko north	Igun		100	48	TCC	2012	563	216	9		
	Okpara Inland		100	11	TCC	2013	11,429	4,047	3		
	Okurekpo		99	35	TCC	2012	1,177	405	6		
	Orhoakpo		100	25	TCC	2012	461	217	3		
	Oria Abraka		100	29	TCC	2013	921	368	3		
	Otorho Abraka		100	29	TCC	2013	307	165	5		
	Samagidi		100	34	TCC	2012	31	31	1		
	Urhuvie Igun		99	13	FMOH	2015	522	522	1		
	Ofagbe		100	40	TCC	2012	338	338	1		
	Okpe Isoko		100	60	TCC	2012	184	107	5		
Isoko south	Otor-Igho/Emevor		100	13	TCC	2013	7,568	1,281	8		
	Owhelogbo		100	20	TCC	2012	338	148	5		
	Ozoro		100	40	TCC	2012	706	231	9		
	Emede		101	15	TCC	2012	329	142	6		
	Emore		99	20	TCC	2012	746	172	7		
	Irri		100	21	TCC	2012	998	363	5		
	Olomoro		100	21	TCC	2012	526	287	4		
	Uzere		100	29	TCC	2012	307	149	4		
	Patani		Abari	97	36	TCC	2012	0	0	0	
			Bolu Angiama	97	16	TCC	2013	465	465	1	
Bulu Aperebiri		100	39	TCC	2012	154	92	2			
Odorubu		100	35	TCC	2012	369	138	4			
Patani II		100	34	TCC	2012	0	0	0			
Uduophri		100	35	TCC	2012	0	0	0			
Ugheli north		Odovie	100	13	TCC	2013	44	44	1		
		Oghara Agharha	101	20	TCC	2013	397	229	2		
	Onidjor Uwheru	100	25	TCC	2013	92	51	3			
	Orogun	97	21	TCC	2013	0	0	0			
Ebonyi	Abakaliki	Otovwodo	102	18	TCC	2013	0	0	0		
		Abofia (Unagbo Oke)	100	33	TCC	2012	439	126	5		
		Amachi Unuhu	100	25	FMOH	2015	491	321	2		
		Amagu Onicha	100	28	TCC	2012	526	396	5		
		Ametta Amachi	100	25	FMOH	2015	483	228	5		
		Azugwu	98	21	FMOH	2015	768	257	8		
		Azuiyokwu	100	28	FMOH	2015	230	99	4		
		Egwudinagu	100	39	FMOH	2015	0	0	0		

(continued)

TABLE 1
Continued

State	LGA	Village	Number surveyed	Max of RAPLOA (%)	Source of max RAPLOA value	Year of RAPLOA survey	Max of LoaScope mf/mL	Average LoaScope mf/mL among positives	Prevalence of LoaScope positives (%)	
Imo	Ohaukwu	Enyigba	100	40	FMOH	2015	263	175	2	
		Igbegu Unuhu	100	14	TCC	2012	276	146	4	
		Ndiaja ndiagu	100	44	FMOH	2015	483	285	3	
		Ndigboke	100	24	FMOH	2015	197	137	3	
		Nkaliki	100	25	FMOH	2015	307	307	1	
		Onuebonyi	100	44	TCC	2012	0	0	0	
		Orizor ndiuruku	100	24	FMOH	2015	0	0	0	
		Uburu Amachi	100	38	TCC	2012	505	505	1	
		Ugwuachara	100	26	FMOH	2015	0	0	0	
		Unagboke	100	31	FMOH	2015	51	51	1	
		Agodo	100	43	FMOH	2015	2,874	487	9	
		Enyimagu	100	36	TCC	2012	1,259	334	7	
		Ezzamgbo	100	25	FMOH	2015	246	127	4	
		Iniki ri	87	33	FMOH	2015	7,875	1,559	8	
		Ishi-izhia	89	35	FMOH	2015	829	829	1	
	Ndi agu obu okposhi ehaku	89	40	FMOH	2015	505	505	1		
	Ndiaguonwe	82	38	TCC	2012	154	153	4		
	Ndiakparata Ugwuagba	84	31	FMOH	2015	614	399	6		
	Obodo Ogbani	88	39	TCC	2012	505	188	5		
	Omocha	76	48	FMOH	2015	1,750	721	9		
	Otaku Umuagara	84	40	TCC	2012	1,228	595	7		
	Ufoboto	77	33	TCC	2012	215	138	5		
	Nkwere	Ezemeneha	100	30	TCC	2013	395	395	1	
		Umuakuma	100	18	TCC	2013	706	263	5	
		Umunaga	100	19	TCC	2013	790	246	4	
		Oguta	Abosi Izombe	100	20	TCC	2013	276	88	7
			Ejemekwuru	97	19	TCC	2013	486	293	3
			Obujuju Akabor	99	10	TCC	2013	92	92	1
			Ugbele Izombe	100	28	TCC	2013	491	204	7
		Umuoba Agwa	100	30	TCC	2013	102	102	1	
		Umuofeke Agwa	96	25	TCC	2013	0	0	0	
		Ohaji/Egbema	Obitti	100	41	TCC	2013	2,815	1,076	4
	Umuogologo		100	19	TCC	2013	31	31	1	
Oru east	Abia Omuma	63	30	TCC	2013	1,106	614	5		
	Amagu Awo	54	15	TCC	2013	435	182	7		
	Awo-omama	71	13	FMOH	2015	592	167	27		
Oru west	Eziawo	81	14	TCC	2013	5,042	953	21		
	Ihitte Akata	120	14	TCC	2013	4,212	795	6		
	Ubachima Awo	92	29	TCC	2012	150	150	2		
	Ihite Oha	91	15	TCC	2013	150	150	2		
	Nempi	66	21	TCC	2013	4,936	640	29		
	Umuduru Ezigbidi	101	20	TCC	2013	972	972	1		
Total/Max/Average/Overall			10,605	NA	NA	NA	11,429	326	6	

FMOH = federal ministry of health surveys; LGA = local government area; TCC = the Carter Center surveys.

Procedures. In each village, we aimed to test 50 adults (more than 18 years of age) and 50 children (≥ 5 and < 10 years of age). We excluded anyone who was ill or who might not tolerate finger-stick blood collection. Village leaders met with the research team who explained the study and then obtained verbal permission for the study to take place. Village leaders selected one of their common meeting areas as the survey venue for adults. For children, the venue was the village primary school. On the day of the study, written consent was obtained from each individual (assent from children) and a unique ID number was assigned. The ID and general information (name, age, gender, occupation, and duration of time living in the village) was recorded in tablets and on paper forms.

Sampling took place from 10:00 AM to 4:00 PM when the maximum number of *L. loa* mf circulate.²² Whole blood was obtained using standard finger-lancing procedures by gloved technicians working in an enclosed private area. After

cleaning the puncture site with isopropyl alcohol, a sterile single-use lancet was used to obtain blood from the index finger of the subject. A glass capillary tube with a rectangular cross section (Vitrocom Inc., Mountain Lakes, NJ) was filled first and immediately passed on to another technician who operated the LoaScope and read the number of *L. loa* mf in 15 µL of whole blood. A 10-µL capillary tube was used to make a thin smear blood slide. A final sample of 10 µL of blood was obtained by capillary tube and immediately placed on a Bioline Ov16 rapid test card (Standard Diagnostics, Inc., Gyeonggi-do, Republic of Korea) and then read per directions. LoaScope and RDT results were recorded in the tablets and on paper. The LoaScope automatically recorded in its memory the mf results along with the participant's ID number; the team was later able to verify the LoaScope results uploaded by the tablets using the stored data uploaded directly from the Loascope. The entire registration, blood draw and testing procedure took approximately 10–15 minutes for each

participant; the LoaScope portion of this process took about 5 minutes. It should be noted that the LoaScope is calibrated to capture high-density infections accurately, and as a result, a single capillary per person in the standard settings for number of fields of view per capillary is accurate to within 150 mf/mL.

The 10 μ L blood smears were labeled with the participant's ID, air-dried and returned to The Carter Center laboratory in Owerri. All individuals with LoaScope readings $\geq 20,000$ mf/mL and a random selection of 10% of slides from those with readings $< 20,000$ ("negatives") were stained with Giemsa and examined under light microscopy by an experienced technician. All *L. loa* mf on the slide were counted and recorded with their corresponding ID on paper forms.

Data analysis. Paper data forms with personal identifiers were kept locked in a file cabinet at The Carter Center Owerri office. Data were uploaded from the tablets and LoasCopes using only ID number identifiers, combined in a Microsoft Excel spreadsheet, cleaned, and then analyzed using IBM SPSS 24, Excel pivot tables, Epiinfo7, and OpenEpi statistical packages. Crude (age unadjusted) prevalence of *L. loa* mf, overall and by village, were calculated by dividing the number of mf positive persons by the number of persons examined. Density among LoaScope-positive readings was expressed as arithmetic means and medians. Very high-density *L. loa* infection was defined as $\geq 20,000$ mf/mL because the lower 95% confidence intervals from LoaScope readings of $\geq 30,000$ included 20,000 mf/mL.²³ Prevalence of very high-density infection was calculated as the number of persons with $\geq 20,000$ mf/mL divided by the total number of persons examined overall, by age group (adult or children), and in the subset of villages having RAPLOA prevalence $\geq 40\%$. Two percent very high-density infection was assumed to be at a CNS-AE risk threshold at or above which ivermectin MDA should not be given. The categorical χ^2 test was used to determine if the observed percent positive with high-density infection differed from the expected result of 2%. We used the

Spearman's rho to test the correlation between RAPLOA versus *L. loa* village mf prevalence, and RAPLOA versus peak village *L. loa* mf density.

The 10 μ L-thin blood films from all persons with LoaScope readings of $\geq 20,000$ mf/mL were examined by light microscopy to confirm those results. In addition, 10% of negatives (e.g., $< 20,000$ mf/mL) were also examined to determine if high-density carriers might have been missed by LoaScope. Results however were not used to check the range of LoaScope density readings because the volume of blood used in the thin smear was 50% less than that examined by the LoaScope, and so less sensitive.

RESULTS

One of the twenty selected ivermectin-naïve LGAs could not be visited due to insecurity. In the remaining 19 LGAs, 110 villages were surveyed: 82 of these (75%) had RAPLOA prevalence of $\geq 20\%$ and 28 villages (25%) were $\geq 40\%$ (range 40–67%). A total of 10,605 persons were tested by LoaScope, an average of 96 persons per village; 5,776 (54.5%) were females and 5,282 (49.8%) were children. The sample included 2,748 residents of the highest risk villages (RAPLOA $\geq 40\%$).

Only 661 (6.2%) persons were positive for mf by LoaScope. The mean village *L. loa* prevalence was 6.3% (median 4.9%, range 0–28.8%). The mean mf count among positive individuals was 326 mf/mL (median 150 mf/mL) and the maximum individual mf count was 11,429 mf/mL. Results for villages with RAPLOA $\geq 40\%$ showed a mean village prevalence of 7.1% (median 5.0%, range 0.0–25.5%), a mean mf count among positives of 267 mf/mL (median 150 mf/mL), and the highest individual mf count 2,874 mf/mL. There was no correlation between *L. loa* mf prevalence and RAPLOA results (Figure 2). Peak village microfilaremia also did not correlate with RAPLOA results (Figure 3).

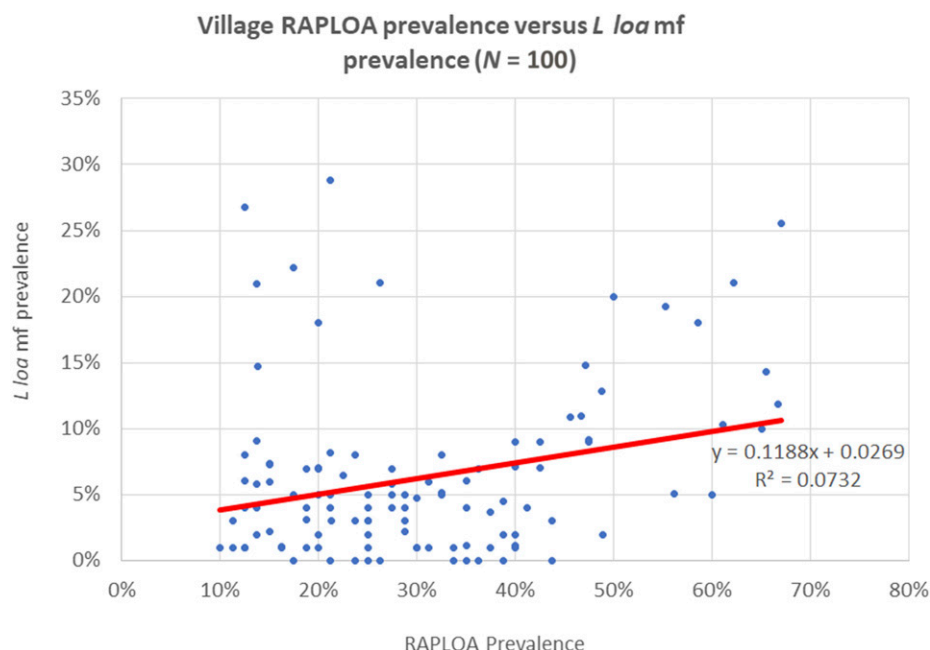


FIGURE 2. Village Rapid Assessment Procedure for *Loa loa* (RAPLOA) prevalence vs. *L. loa* microfilaria (mf) prevalence (N = 110). This figure appears in color at www.ajtmh.org.

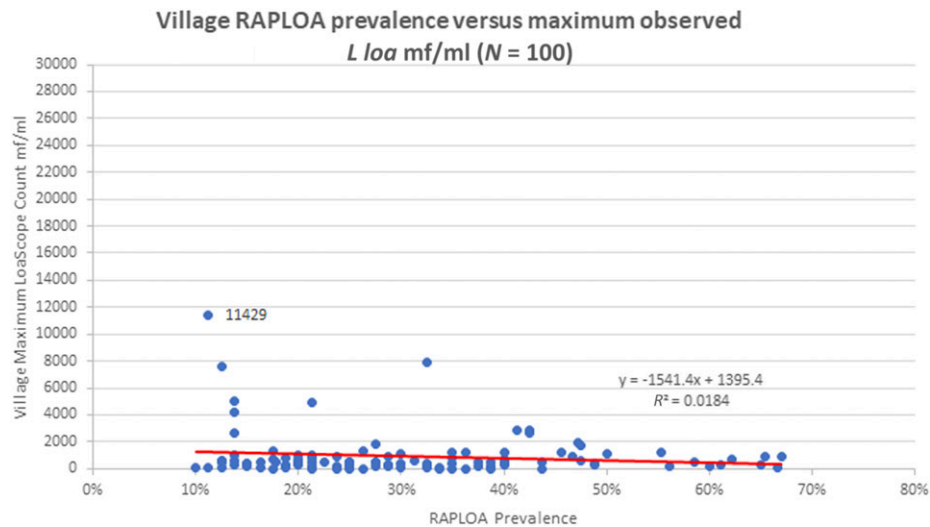


FIGURE 3. Village Rapid Assessment Procedure for *Loa loa* (RAPLOA) prevalence vs. maximum observed *L. loa* microfilaremia. This figure appears in color at www.ajtmh.org.

No participants were detected with high-density *L. loa* microfilaremia. The highest count in the study (11,429 mf/mL) was in a resident of the low risk RAPLOA village of Okpara Inland (RAPLOA 11%) of Ethiope East LGA in Delta State. The second (7,875 mf/mL) and third highest (7,568 mf/mL) mf densities were in residents of villages with RAPLOAs of 33% and 13%, respectively.

The 2% prevalence of very high-density microfilaremia did not occur in the overall sample ($P < 0.01$) and the subsample of 2,748 persons resident in $\geq 40\%$ RAPLOA villages ($P < 0.01$). Similarly, the 2% threshold was not exceeded when only adults were considered in the analysis for all villages ($N = 5,323$, $P < 0.01$) or villages with RAPLOA $\geq 40\%$ ($N = 1,380$, $P < 0.05$).

A random selection of 1,342 (12.7%) of the 10- μ L blood slides were stained and read by light microscopy. The maximum *L. loa* density was 11,800 mf/mL, from the same individual with a LoaScope reading of 11,429. No very high-density *L. loa* infections were observed in the blood slide reading.

DISCUSSION

In 2013 the FMOH of Nigeria released a master plan for neglected tropical diseases that included a new policy for using ivermectin MDA to interrupt onchocerciasis transmission throughout the country. Soon thereafter the minister of health launched the Nigeria Onchocerciasis Elimination Committee (NOEC) to provide technical advice to the FMOH on how to accomplish this goal by 2025. In 2016 the NOEC completed a “National Guideline for Onchocerciasis Elimination in Nigeria” that called for the MDA program to expand treatment into hypoendemic areas.²⁴ That expansion had to address concerns about possible CNS-AEs in *L. loa* endemic states, especially where there were villages with RAPLOA survey results $\geq 40\%$. The purpose of our study was to assess whether MDA expansion into hypoendemic areas in *L. loa* endemic states assisted by The Carter Center could be done with minimal risk. Using the new LoaScope technology we sampled residents from all villages in these states with $> 20\%$ RAPLOA prevalence located in ivermectin-naïve and likely

onchocerciasis hypoendemic LGAs. Our purpose was to determine the prevalence of very high-density *L. loa* microfilaremia. A key outcome was to identify how many of these villages had $\geq 2\%$ residents with microfilaremia $\geq 30,000$ mf/mL.

In 110 villages sampled we found no LoaScope readings more than 20,000 mf/mL in 10,605 LoaScope examinations. In residents of villages with RAPLOA $\geq 40\%$, where the prevalence of very high-density microfilaremia should equal or exceed 2%, we could exclude the 2% threshold with $> 99\%$ statistical confidence. We concluded that ivermectin MDA for treatment of hypoendemic onchocerciasis could be deployed in these areas with very low risk of CNS-AEs.

In our study *L. loa* prevalence was lower than expected based on the RAPLOA findings. We found only 661 participants (6.2%) to be positive for mf and a mean village prevalence of 6.3% (median 4.9%, 0–28.8% range). Only eight villages had a *L. loa* prevalence $\geq 20\%$, compared with 28 villages having RAPLOA $> 40\%$. *Loa loa* prevalence did not increase with RAPLOA as other studies has shown.²⁵ We also were unable to demonstrate a correlation between RAPLOA and *L. loa* mf density, in contrast to Takougang et al.’s study that showed this relationship in Cameroon’s Southwest and Northwest provinces. Although Takougang et al.²⁵ detected no persons with very high-density microfilaremia in Nigeria’s Cross River State, the RAPLOA prevalence of all villages in his study was below 40%. An important contribution of our study is that we were able to assess residents of 28 Nigerian villages where RAPLOA results were above the 40% threshold, and found no very high-density infections there. Our results are consistent with a report by Hassan et al.,²⁶ who studied a village in southwest Nigeria (Ikpakodo) with 54% eye worm history where the highest *L. loa* mf count was only 420 mf/mL. Our village sample included an expanded range of RAPLOA prevalence (10–67%) that mitigated our risk of missing very high-density infections if they occurred in villages with RAPLOA $< 40\%$ that are not believed to be at risk of CNS-AEs.

The lack of reported CNS-AEs after more than 70 million ivermectin MDA treatments in known *L. loa* areas in Nigeria is

further evidence that very high-density *L. loa* infections are exceedingly rare in the country. Of 758 CNS-AEs reported to the Mectizan Donation Program between 1990 and 2014, 99% of these were from Democratic Republic of Congo and Cameroon (Y. Sodahlon, personal email communication). Only one case (in 1991) in this series (0.1%) was reported from Nigeria.¹⁵ The NOEC has concluded that the lack of CNS-AEs despite millions of ivermectin treatments is evidence that the risk of these events in Nigeria is essentially nonexistent.²⁷

The reasons that *L. loa* mf densities rarely, if ever, reach very high levels in Nigeria are unknown but could include the following: 1) The Nigerian strain of the *L. loa* parasite is not prone to produce very high-density microfilaremia; 2) the Nigerian human population may have genetic or other host factors that limit mf circulation; 3) deforestation or other recent environmental changes may have reduced *Chrysops* breeding and biting rates, leading to fewer adult *L. loa* adult parasites resulting in a decreasing mf prevalence and density; 4) unrecognized ivermectin treatment in the study population that has lowered mf prevalence and density. This last possibility is unlikely considering that in a questionnaire survey administered to 5,910 (56%) of our study participants, 98% stated that they had not taken ivermectin.

In Cameroon, the LoaScope has been successfully used to determine each individual's treatment eligibility based on his/her *L. loa* density, excluding those with microfilaremia exceeding 20,000 mf/mL.^{21,23} This strategy, called "Test and Treat," is proposed as a possible way forward for onchocerciasis programs in *L. loa*-endemic areas. In this report we used the LoaScope as a survey technology to judge village (rather than individual) risk for CNS-AEs. We demonstrated that the LoaScope can be used to rapidly delineate areas where high-density *L. loa* infections do not occur and so allow ivermectin MDA using approaches that can be implemented more quickly and cheaply than "Test and not Treat." This same survey approach could be used for lymphatic filariasis (LF) elimination programs in areas in Africa coendemic for *L. loa*. In this case, LF MDA programs could use a strategy of ivermectin and albendazole once per year rather than the costlier WHO-recommended strategy of albendazole alone every 6 months.²⁸ Our recommendation, therefore, is that the LoaScope should be the primary tool in surveys needed to rapidly expand ivermectin use in onchocerciasis and LF elimination programs operating in Africa where RAPLOA findings suggest that *L. Loa* microfilaremia densities could be dangerously high.

This study had a number of limitations: 1) We selected study villages based on historical RAPLOA results from surveys conducted between 2012 and 2015. This was justified because these villages were not receiving ivermectin treatment so that the prevalence and density of *L. loa* infections were unlikely to change dramatically in the 1–4 years between the RAPLOA assessment and the LoaScope survey; 2) We assumed that a convenience sample of 50 adults (aged ≥ 18 years) and 50 children (aged ≥ 5 and < 10 years) accurately reflected village *L. loa* prevalence. The sample was skewed toward younger children to assess OV16 antibody in an age group where positive results would indicate recent transmission of *O. volvulus* in the area. It is likely that having 50% of our overall sample consisting of children under 10 years of age resulted in an underestimate of the village *L. loa* prevalence and density, assuming adults are more likely than children to have very high-density *L. loa* infections. However, in a subanalysis of

our data we could still statistically rule out the $\geq 2\%$ very high-density prevalence threshold among all adults in the study, and among adults resident in high risk (RAPLOA $\geq 40\%$) villages.

CONCLUSION

We conducted a LoaScope-based blood survey in 10,605 residents of 110 ivermectin-naïve villages located in *L. loa*-endemic areas in southern Nigeria. The villages likely have hypoendemic onchocerciasis transmission and need to be treated with ivermectin MDA. Twenty-five percent of the villages surveyed had RAPLOA values of $\geq 40\%$. We determined that there is minimum risk for CNS-AEs because no very high-density *L. loa* infections could be detected, and the LoaScope prevalence of very high-density infections was significantly below 2%. The LoaScope is a useful tool for use in village surveys designed to assess risk of ivermectin MDA in *L. loa*-endemic areas.

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