

The interruption of transmission of onchocerciasis in Kaduna, Kebbi and Zamfara states, Nigeria: another milestone achievement

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Background: More than 40 million people live in onchocerciasis-endemic areas in Nigeria. For at least 19 y, mass drug administration (MDA) with ivermectin was implemented with at least 65% total population coverage in Kaduna, Kebbi and Zamfara states. Impact surveys done using skin biopsies yielded no infections. Serological and entomological assessments were undertaken to determine if onchocerciasis transmission had been interrupted and MDA could be stopped.

Methods: The presence of onchocerciasis-specific immunoglobulin G4 antibody was measured by enzyme-linked immunosorbent assay conducted on dried blood spots collected from 5- to 9-year-old children resident in each state. O-150 polymerase chain reaction testing of *Simulium damnosum* s.l. heads for *Onchocerca volvulus* DNA was done on black flies collected by human landing capture and Esperanza window traps.

Results: A total of 9078 children were surveyed across the three states. A total of 6139 vectors were collected from Kaduna state, 129 from Kebbi state and 2 from Zamfara state; all were negative. Kebbi and Zamfara states did thousands of hours of black fly catching and intensive river prospecting. The resulting low fly catch was due to a low fly population incapable of sustaining transmission.

Conclusion: Onchocerciasis transmission has been interrupted and the three states meet World Health Organization thresholds: seropositivity in children <0.1% and <1/2000 infective black flies with 95% confidence. The 2.2 million people in Kaduna state and 4 million in Kebbi and Zamfara states no longer need ivermectin for onchocerciasis.

Keywords: black flies, entomological assessment, onchocerciasis, Ov16 serological assessment, *Simulium*, stopping mass drug administration.

Introduction

Onchocerciasis is a neglected tropical disease (NTD) caused by *Onchocerca volvulus*, a parasitic worm that is transmitted to humans through the bites of infected black flies, with the most common vector being *Simulium damnosum* sensu lato (s.l.).¹ According to the World Health Organization (WHO), in 2018 an estimated 187 million people lived in areas at risk of onchocer-

ciasis.² This estimate does not include low-transmission areas that still need to be mapped.³ In addition to severe eye disease, onchocerciasis causes papular or hypopigmented skin lesions and intense itching. Annual mass drug administration (MDA) with ivermectin (Mectizan, donated by Merck & Co., Kenilworth, NJ, USA) is an effective strategy for controlling the disease as a public health problem.^{4,5}

Nigeria accounts for more than a third of the global prevalence of onchocerciasis⁵ due to the large size of the country and its population and the wide distribution of conditions that favour onchocerciasis transmission. In 2017–2018, an estimated 7–10 million Nigerians were infected with *O. volvulus* and approximately 50 million people required MDA for the disease,² with many thousands suffering from disabling complications of the disease.⁷

Across Africa, efforts to control onchocerciasis with MDA, together with sustained political commitment of governments, the African Programme for Onchocerciasis Control (APOC), non-governmental development organizations and endemic communities, has shown how the reality of onchocerciasis elimination can be achieved. Pioneering studies by Diawara et al.⁸ in Mali and Senegal showed evidence of onchocerciasis elimination after 15–17 y of mass ivermectin distribution, establishing the principle of onchocerciasis elimination using ivermectin. Results from other endemic African countries, including Nigeria, also showed that sustained high-coverage treatment with ivermectin interrupted onchocerciasis transmission.^{9–11}

Following these results, the WHO set new guidelines for countries on the journey to onchocerciasis elimination. A key starting point is the creation of National Onchocerciasis Elimination Committees (NOECs) who steer national programmes through the milestones and strategies needed to reach the end phase of the elimination process.¹² In Nigeria, the NOEC was inaugurated in 2015. The NOEC developed a strategic plan, specific to the context and epidemiological setting of Nigeria, that provides a road map for national onchocerciasis elimination by 2025. Prior to 2015 the Nigerian onchocerciasis control programme had considerable success controlling onchocerciasis, thus laying the foundation for elimination.

Baseline prevalence assessments in Kaduna, Kebbi and Zamfara states

In order to accurately establish the distribution of onchocerciasis in Nigeria a series of Rapid Epidemiological Mapping of Onchocerciasis (REMO) and Rapid Epidemiological Assessment (REA) surveys were conducted nationwide between 1990 and 1995 following standard protocols.^{13–15} Nodular prevalence of $\geq 20\%$ and leopard skin of $\geq 10\%$ qualified a local government area (LGA) for treatment. The nodular rates ranged from 1.9 to 92.4% in Kaduna state, 1.4 to 28.7% in Kebbi state and 1.1 to 66.0% in Zamfara state. A total of 16 of 23 LGAs in Kaduna state, 9 of 22 in Kebbi state and 5 of 14 in Zamfara state were classified as needing MDA.

History of the MDA programmes in Kaduna, Kebbi and Zamfara states

Onchocerciasis treatment began in 1989 in Kaduna state and in 1997 in Kebbi and Zamfara states, initially using a health worker-based campaign delivery mechanism. In 1997, the APOC supported a switch to delivering MDA through community directed treatment with ivermectin (CDTI). In 2010, onchocerciasis MDA in all states was integrated with lymphatic filariasis (LF) MDA; ivermectin treatment was consequently expanded to LF-endemic areas that were not previously being treated for onchocerciasis. Influenced by the remote nature of the three states, their dispersed

population, as well as occasional insecurity in northern Nigeria, the control programmes in each of the three states took time to expand to all endemic areas and reach the 65% epidemiological coverage (proportion of individuals in the implementation unit who have ingested the MDA drugs of the total population in the implementation unit) and 100% geographic coverage (proportion of communities that are implementing MDA of all those that require MDA) recommended by the APOC.¹⁶ Between the years 1997 to 2017, all three states attained 100% geographic coverage and the minimum threshold of 65% epidemiological coverage for at least 19 y (Figure 1).

No onchocerciasis impact assessment surveys have been done in Kebbi or Zamfara state. Results of impact assessments in Kaduna state indicate little or no ongoing transmission for several years. To ascertain if continued MDA is needed, the FMOH, in partnership with Sightsavers, conducted stop-MDA assessments, following WHO requirements.¹²

Methods

Study area/sites

The NOEC selected 42 first-line (communities located closest to a black fly breeding site, without any other communities between it and the river) and second-line (communities located further away from the breeding site) communities to serve as sentinel sites for entomological and epidemiological assessments in Kaduna, Kebbi and Zamfara states, as shown in Figures 2–5. This was done after detailed evaluation of the river system, including places suspected for onchocerciasis and places where REMO was not carried out. Within each state, first-line communities were selected along the main river or rivers, close to suitable black fly breeding conditions and 30–50 km apart, until that river was exhausted or had crossed out of the state. Further from each first-line community, a second-line community was selected within a 15–20 km radius of the first-line community. This process was repeated on tributaries of the main rivers. Additional communities known as extrasentinel communities were also selected for entomological assessments to provide more evidence of the impact of MDA on the transmission of *O. volvulus*.

Kaduna state

Kaduna state has an estimated land area of about 46 063 km² and an estimated population of 7.7 million people in 2016 when the survey was conducted.¹⁷ The state lies between 10°36'33" N and 7°25'46" E and is bordered by Zamfara, Katsina and Kano states to the north; Bauchi and Plateau states to the east; Nasarawa state to the south; Niger state to the west and Abuja to the southwest. The study was carried out in 16 communities selected as mentioned above (Figure 2). Fourteen of the communities were in endemic LGAs under MDA and two were in non-endemic LGAs.

Kebbi state

Kebbi state is located in northwestern Nigeria (11°30'0" N and 4°0'0" E), had an estimated population of 4.1 million people in

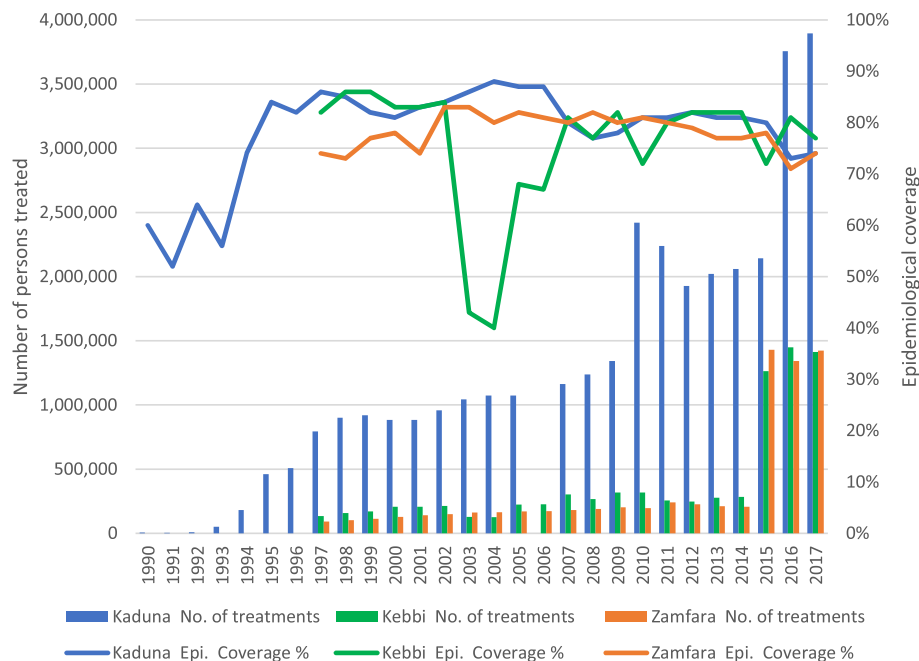


Figure 1. MDA coverage in Kaduna, Kebbi and Zamfara states (1989–2017).

2016 at the time of the survey¹⁷ and has a land area of about 36 800 km². The state shares boundaries with Sokoto state in the north, Zamfara state to the east, Niger republic to the west, Benin republic to the southwest and Niger state to the south. Thirteen communities were selected in Kebbi state (Figure 3). Ten of the communities were in endemic LGAs under MDA and three were in non-endemic LGAs.

Zamfara state

Zamfara state is located in northwestern Nigeria (12°10'0" N, 6°15'0" E), had an estimated population of 4.1 million people in 2016 when the survey was conducted¹⁷ and has an estimated land area of about 398 762 km² and shares boundaries with Niger republic in the north; Katsina state to the east; Sokoto, Kebbi and Niger states to the west and Kaduna state to the south. Thirteen communities were selected in Zamfara state (Figure 4). Nine of the communities were in endemic LGAs under MDA and four were in non-endemic LGAs.

Epidemiological evaluation

The surveys in all three states were conducted in September–October 2016.

Study population

Only children between 5 and 9 y of age, both male and female, were included in the surveys.

Determination of sample size

Based on WHO guidelines, at least 3000 children ages 5–9 y were randomly selected in each state. The sample from each community was proportionally based on the estimated total population of children in the community.

Inclusion criteria

Children ages 5–9 y from whom samples could be collected irrespective of any other status (ethnicity, gender etc.), for whom consent was obtained and who are normally resident in the selected community were included.

Exclusion criteria

Children from whom consent was not received were excluded from the study.

Consent

The sampled children provided oral assent before they were finger-pricked and blood obtained. Written informed consent was obtained from each child's parent or his/her representative. Individuals conducting human landing capture (HLC) of black flies were informed of the risks associated with the activity and the community benefits of their participation. They had the opportunity to opt out at any time during black fly collections without repercussions. The HLCs and trap attendants were given a 150 µg/kg dose of ivermectin at the end of the catching period.

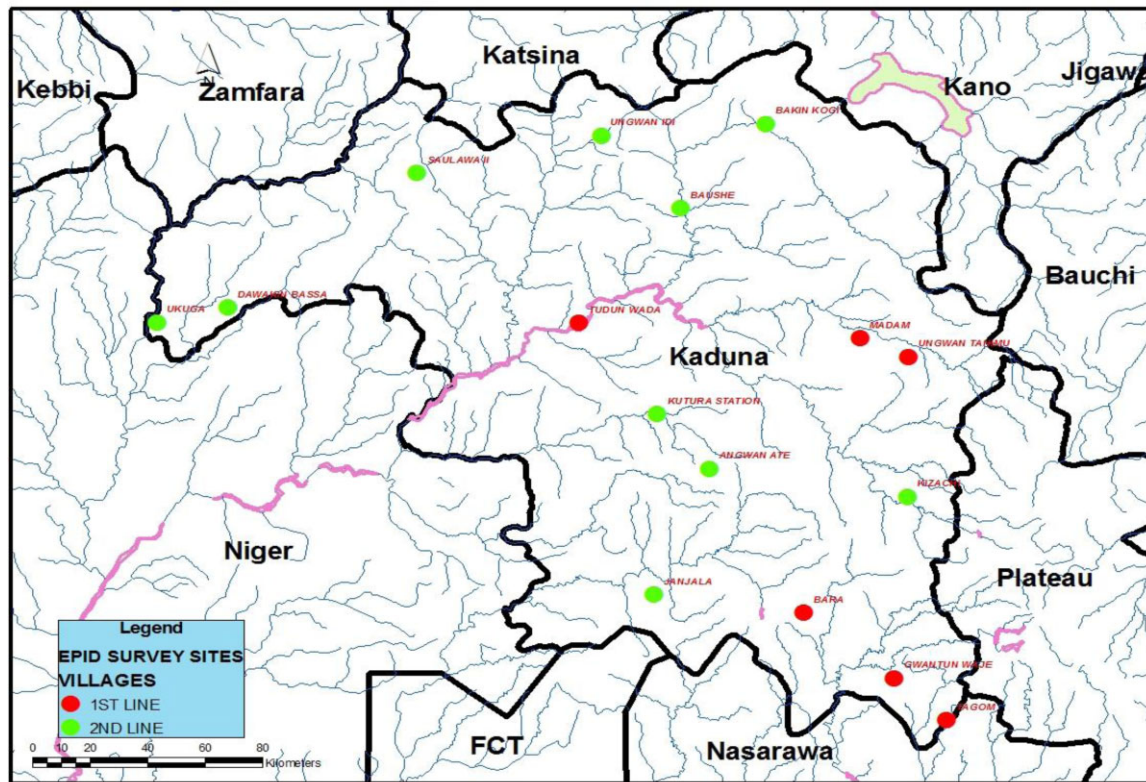


Figure 2. Selected first- and second-line villages for epidemiological surveys, Kaduna state.

Specimen collection

In line with federal and state regulations, certified laboratory scientists collected finger-prick blood from all verbally assenting children. Alcohol swabs were used to cleanse the finger and then a sterile lancet was used to prick it, after which 100 μ L of blood was collected into capillary tubes and used to make blood spots on Whatman number 2 filter paper (Sigma-Aldrich, St. Louis, MO, USA). After the filter papers were air dried they were then stored in small Ziploc plastic bags, one sample per bag, which were then placed into a separate larger Ziploc bag (one large bag for each community) and labelled. These were transported to The Carter Center (TCC) laboratory in Jos, Plateau state for enzyme-linked immunosorbent assay (ELISA) analysis.

Entomological evaluation

Community leaders were asked to identify two persons who would serve as HLCs of *Simulium* vectors. The HLCs were trained and deployed in all 42 selected communities for collection of *Simulium* vectors; there were two HLCs per site and two sites per community. The HLC teams were supervised by the front-line health facility staff, LGA, Federal Ministry of Health (FMOH) personnel and Sightsavers staff. Fly catches took place during the peak black fly breeding season, June–November 2017 and June–November 2018 in Kaduna, Kebbi and Zamfara states, and

a year-round collection from August 2019 and July 2020 in Kebbi and Zamfara states, respectively. Each site had 3 capture days per week consisting of hourly collections between 07:00 h and 18:00 h. The two HLCs worked in alternate fashion. The HLCs exposed their legs below the knee and sat quietly with catching tubes waiting for black flies to land. Flies were collected just as they landed but before they could bite by placing a tube over the fly, mildly agitating it for the fly to take flight and fly into the tube, and then the tube was removed from the leg and closed. Flies were collected together by transferring into a larger bottle containing 100% isopropanol. These bottles were labelled with the date, method of capture (HLC), number of flies the tube contained and community name.

A total of 19 Esperanza window traps (EWTs) were placed within 3 km of known or potential local breeding sites in 15 selected communities, as per guidance from Loum et al.¹⁸ (Table 1). The traps were made of 1 m \times 33 cm blue-coloured vinyl tarpaulin stripes flanking a 1 m \times 33 cm black tarpaulin stripe attached to a 1 m² wooden frame. The surfaces were coated with Tangle-Trap insect trap coating paste (Contech, Victoria, BC, Canada).¹⁸ The traps were mounted perpendicular to the river on a stand elevated a few centimetres off the ground. Carbon dioxide (CO₂) was generated from a 5 L plastic bottle containing 2 L of water, yeast and sugar and was replaced every 48 h. CO₂ was released from the bottle to diffuse at the top of the trap via plastic tubing. Dirty, worn clothes were also hung at the top of the trap to

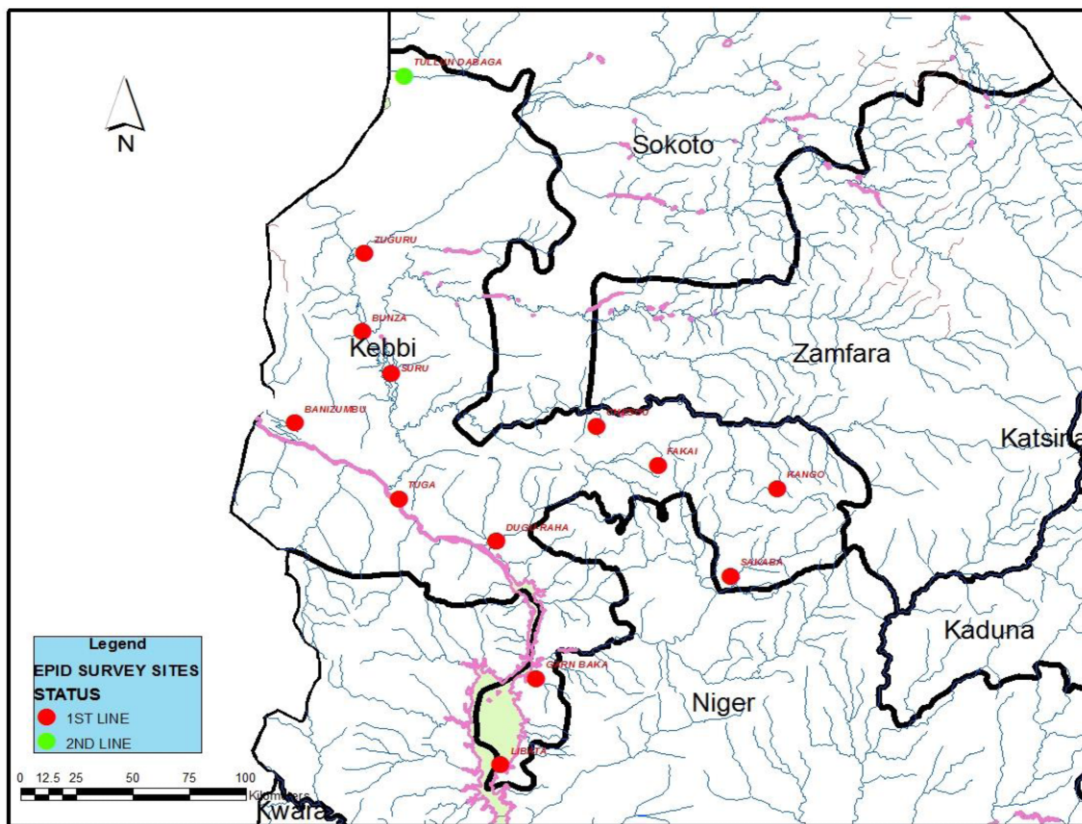


Figure 3. Selected first- and second-line villages for epidemiological surveys, Kebbi state.

attract black flies. All black flies caught on the trap were removed from the Tangle-Trap adhesive using a spot application of kerosene. The flies were collected into separate labelled tubes indicating the site name, catch date range and the number of flies the tube contained. They were preserved in 100% isopropanol and sent to the TCC laboratory in Jos, Plateau state, for sorting and polymerase chain reaction (PCR) analysis.

Breeding site prospection

In Kebbi and Zamfara states, confirmatory breeding site prospection was required due to a dearth of adult black flies caught. Between August and October 2018 and August 2019, a team of two entomologists carried out the prospection in the two states. Prior to this, a desk review was done and potential breeding sites were identified using satellite maps.

Rocks and plants around potential breeding sites were inspected for larvae and pupae of black flies. Where found, a pair of forceps was used to collect in situ samples or the complete plant and/or rock debris supporting the breeding site was moved to the bank of the river where the samples were collected. Larvae were preserved (in groups of up to 50) in 30 ml of cold Carnoy's fixative (three parts absolute alcohol mixed with one part glacial acetic acid) for later identification. Information associated with the location of the prospection was also recorded; this included the name of the river, river system or basin; nearest community; coordinates of the site; water flow and date of collection.

Data collection

Laboratory analytical methods

Blood samples were analysed for immunoglobulin G4 antibody against the Ov16 recombinant antigen using the standard Onchocerciasis Elimination Program for the Americas ELISA methodology.^{9,12,19,20}

The flies were sent to the TCC laboratory in Jos, where the *S. damnosum* s.l. flies were morphologically identified under a dissecting microscope. Flies identified were grouped by site of capture in maximum pool sizes of 100. The heads were removed from the bodies by freezing the flies in liquid nitrogen vapor, agitating to shear off the heads and then sieving them out in a 25 mm mesh. The DNA was extracted from head pools and an O-150 PCR analysis was conducted as described by Yameogo et al.²¹ The OVS2fl probe was used to detect PCR products with an alkaline phosphatase-labelled anti-fluorescein antibody and read using ELISA.²² Concentrations above the highest standard were diluted further and retested.

Statistical data analysis

An Ov16 antibody prevalence in children of <0.1% at a 95% upper confidence limit (UCL) and a rate of infective blackflies of <0.05% at a 95% UCL were the criteria for successful interruption of onchocerciasis, in line with NOEC and WHO recommendations.¹² In any state where positive samples from children were detected

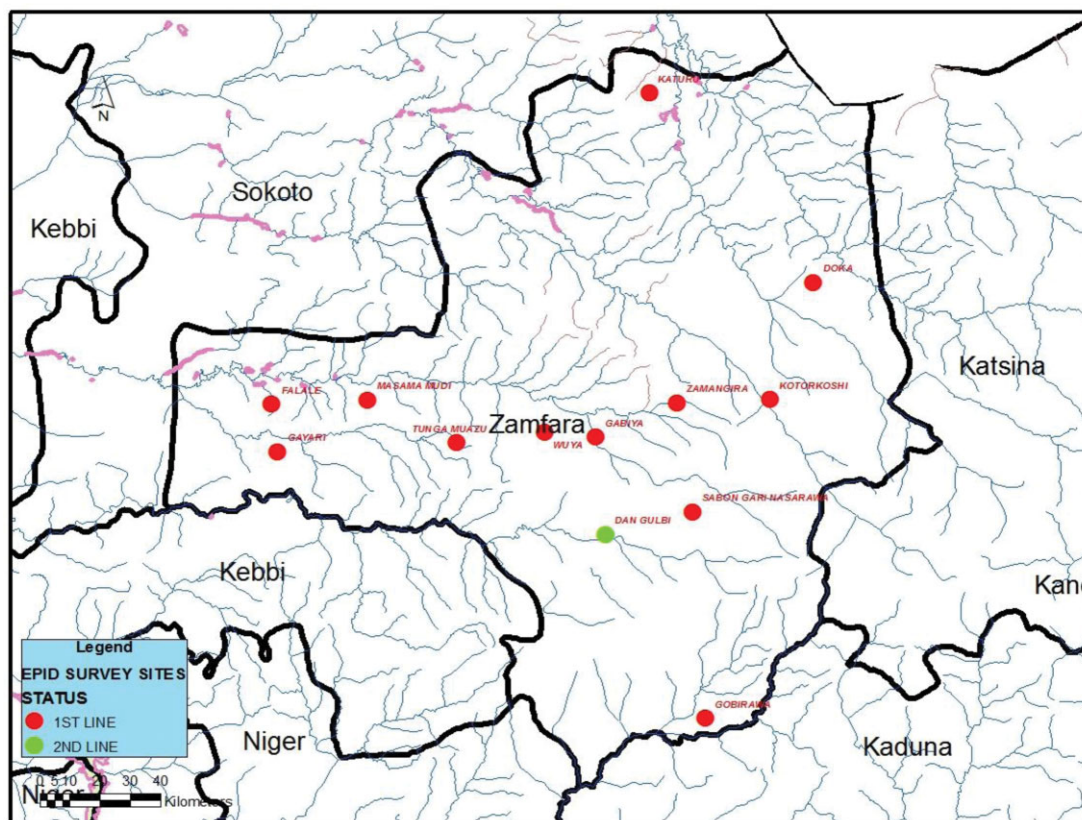


Figure 4. Selected first- and second-line villages for epidemiological surveys, Zamfara state.

the 95% confidence intervals (CIs) for antibody prevalence were determined using the following standard formula:

$$95\% \text{ CI} - \text{proportion positive } (p) \pm 1.96 \frac{\sqrt{p(1-p)}}{n}$$

In states where positive children were absent, the 95% UCL was calculated using the Bayesian algorithm in PoolScreen 2.1.^{21,23,24} PoolScreen was also used to calculate the prevalence and 95% CIs for the black fly O-150 PCR infectivity rate. The annual biting rate (ABR), which represents the number of bites received by a person in a year, and the annual transmission potential (ATP), which represents the approximate number of infective larvae any one individual may be exposed to in a year, were calculated as key entomological indicators.^{21,23,24} The ABR was calculated as the arithmetic sum of the monthly biting rates for 12 months and the calculation for monthly biting rate is:

$$\text{MBR} = (\text{number of flies caught} \times \text{days in the month}) / \text{number of catching days}$$

$$\text{MTP} = (\text{number of days in the month} \times \text{number of infective [L3] larvae}) / \text{number of catching days} \times \text{number of flies tested.}$$

The ATP was calculated as the arithmetic sum of the MTP.

Ethical clearance

The survey protocol was approved by both the FMOH and the ministries of health of Kaduna, Kebbi and Zamfara states.

Results

Entomological assessments

Table 2 presents a combined summary of the entomological results from HLCs and EWTs in each of the states. In Kaduna state, a total of 6139 *S. damnosum* s.l. flies were collected and all pools were negative for *O. volvulus* DNA in O-150 PCR, giving a prevalence of 0% (95% CI 0–0.003 12). The ABRs for Kaduna state could not be computed, as the flies collected were not disaggregated per collection method (HLC and EWT). Despite this, in the absence of any infective fly pools, the ATP was zero.

However, for Kebbi and Zamfara states, the number of black flies caught was very low; two *Simulium* in Zamfara state and a total of 129 *Simulium* in Kebbi state. In both states, no pools were positive for *O. volvulus* DNA in O-150 PCR, giving a prevalence of 0% (95% CI 0–0.0135). Other species of flies were caught and sent to the lab but were identified as non-*Simulium* and excluded from further analysis. Kebbi and Zamfara states completed 11 056 and 12 825 person-hours of black fly catching, respectively, from August 2019 to July 2020. All the flies had negative PCR results in all pools for *O. volvulus* DNA. Fly catching across the three states occurred in the dry and rainy seasons. In Kaduna state, fly catching was done in 2018 and in Kebbi and Zamfara states, fly catching was done in 2019–2020, as there was an additional year-round catch in both states. ABRs of 25.0 and 0.3 were recorded in Kebbi and Zamfara states, respectively, despite the

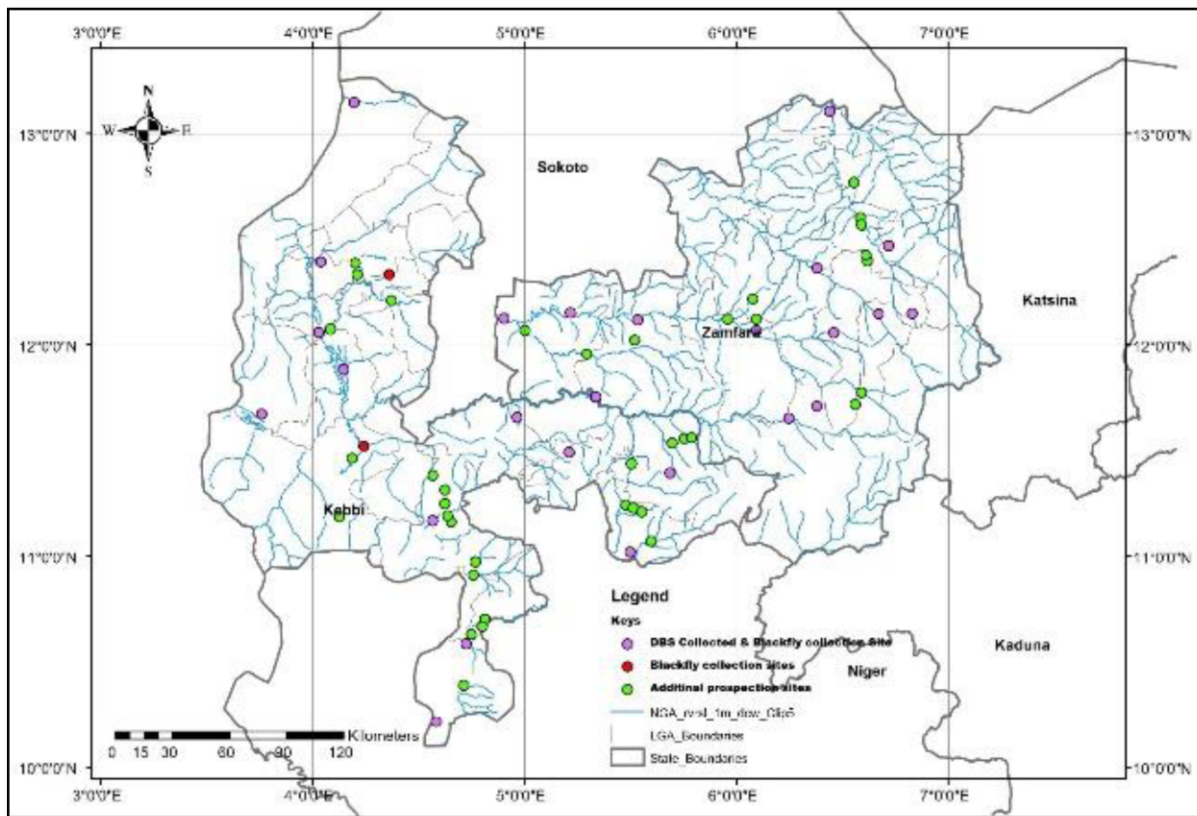


Figure 5. Black fly prospection and collection sites in Kebbi and Zamfara states.

huge working efforts of fly collectors (Table 3). In the absence of any infective fly pools, the ATP was zero.

The results of the breeding site prospections done in Kebbi and Zamfara states revealed few additional sites with potentially suitable conditions for black fly breeding. Seventy sites were prospected or reprospected, including the original 26 sites. Black fly larvae were found at seven sites. The original 26 sites were observed to be suitable for black fly breeding, so HLC continued at these sites. A map of prospection sites overlaid with the 26 sentinel communities where samples were collected in each state is presented in Figure 5.

Epidemiological assessments

A total of 3021, 3049 and 3008 finger-prick blood samples were collected from randomly selected children in Kaduna, Kebbi and Zamfara states, respectively (Tables 4–6). None of the children were found to be Ov16 antibody positive and in each of the states the 95% UCL for prevalence was <0.004.

Discussion

These results indicate that Kaduna, Kebbi and Zamfara states have achieved interruption of onchocerciasis transmission, reaching both the entomological and epidemiological thresholds set out by the WHO. All three states are therefore qualified to stop

onchocerciasis MDA. The FMOH approved halting 2.2 million ivermectin treatments for onchocerciasis in 2018 for Kaduna state and 4 million ivermectin treatments for onchocerciasis in 2019 in Kebbi and Zamfara states. This is the second and third stop-MDA decision for onchocerciasis in Nigeria and among the largest reported to date. The first stop-MDA decision for a population of 2 million was in Plateau and Nasarawa states in December 2017.⁹ In total, there are 8.2 million people across five Nigerian states who no longer require onchocerciasis MDA with ivermectin, although LF treatment is ongoing in some areas pending the outcome of pre-transmission assessment surveys (pre-TASs) and TASs. This is about 16% of the 2017–2018 estimate of 50 million people requiring treatment in Nigeria, and nearly 4% of the global total of 217.4 million people in that same year. This is incredible progress towards onchocerciasis elimination in Nigeria, and globally towards meeting the onchocerciasis elimination goals set out in the WHO 2030 NTD roadmap.²⁵ The results reported here provide a strong evidence base to support the conclusion that transmission has been interrupted.

Kaduna state provided ivermectin MDA for 28 y (1989–2017) and attained 65% epidemiological coverage for 20 y (1997–2017), Kebbi state has 21 y (1997–2017) of ivermectin MDA and accomplished epidemiological coverage of >65% for 19 y (1997–2002 and 2005–2017) and Zamfara state has 21 y (1997–2017) of ivermectin MDA and epidemiological coverage of at least 65% has been sustained for 21 y. All three states attained 100% geographic coverage from 1997 to 2017. The treatment duration

Table 1. Sites for EWTs

State	LGA	Community	Latitude	Longitude
Zamfara	Gummi	Falale	12.151 377	5.218 606
Zamfara	Bukkuyum	Masamar Mudi site 2	12.118 327	5.534 730
Zamfara	Bukkuyum	Masamar Mudi site 1	12.118 693	5.533 550
Zamfara	Bukkuyum	Gadan Zaima 1	11.752 450	5.337 519
Zamfara	Bukkuyum	Gadan Zaima 2	11.749 783	5.337 618
Zamfara	Anka	Wuya site 1	12.066 517	6.095 378
Zamfara	Anka	Wuya site 2	12.066 870	6.095 010
Kebbi	Aleiro	Gomozo	12.332 383	4.362 078
Kebbi	Fakai	Chesgu	11.656 863	4.965 686
Kebbi	Fakai	Fakai	11.490 960	5.210 784
Kebbi	Bagudo	Kurgu	11.519 660	4.242 870
Kebbi	Sakaba	Sakaba	11.019 080	5.500 070
Kaduna	Kagarko	Kuratam	9.601 010	7.816 029
Kaduna	Kagarko	Gurara	9.666 240	7.883 630
Kaduna	Lere	Kudaru	10.639 110	8.470 410
Kaduna	Birnin Gwari	Saulawa	11.008 300	6.573 120
Kaduna	Birnin Gwari	Dawakin Bassa	10.561 120	6.324 850
Kaduna	Birnin Gwari	Ukuga	10.538 010	6.114 650

Table 2. Pool screen prevalence of *O. volvulus* infection in *S. damnosum* s.l. flies collected in Kaduna state in 2018 and in Kebbi and Zamfara states in 2019–2020

State	Total number of flies collected	Number identified as <i>S. damnosum</i> s.l.	Pools of <50 flies	Pools of 50 flies	Pools of 100 flies	Pools positive	PoolScreen 95% CI
Kaduna	6139	6139	1	0	61	0	0 to 0.00312
Kebbi	2394	129	1	2	0	0	0 to 0.0135
Zamfara	3475	2	1	0	0	0	NA

NA: not available.

Table 3. Monthly and annual biting rates (MBR, ABR) of *Simulium* flies collected in Kebbi and Zamfara states (August 2019–July 2020)

State	MBR												ABR
	Aug 2019	Sep 2019	Oct 2019	Nov 2019	Dec 2019	Jan 2020	Feb 2020	Mar 2020	Apr 2020	May 2020	Jun 2020	Jul 2020	
Kebbi	0	25.0	0	0	0	0	0	0	0	0	0	0	25.0
Zamfara	0	0.3	0	0	0	0	0	0	0	0	0	0	0.3

in all states is long enough to outlast the adult female worm's 12–15 y lifespan; death of all adult female worm leads to the permanent interruption of local transmission.⁸

Previous epidemiological impact assessment surveys in Kaduna state showed zero infection rate using skin biopsy,

most probably due to the microfilaricidal effect of ivermectin and sustained high MDA coverage, which agrees with studies conducted in other parts of sub-Saharan Africa (SSA) and the Americas.^{9,10,20,26} Results from this epidemiological survey demonstrate no evidence of recent transmission or exposure,

Table 4. Kaduna state results of Ov16 analysis on dried blood spots (DBSs), 2017

Sample no.	LGA	Community	DBSs collected, n	DBSs processed, n	Spots positive, n	Spots negative, n	Spots invalid, n
1	Birnin Gwari	Saulawa	300	300	0	300	0
2	Birnin Gwari	Dawakin Bassa	300	300	0	300	0
3	Birnin Gwari	Ukuga	79	79	0	79	0
4	Giwa	Anguwan Idi	136	136	0	136	0
5	Igabi	Baushe	242	242	0	242	0
6	Jama'a	Bara	200	200	0	200	0
7	Kachia	Anguwan Ate	206	206	0	206	0
8	Kaduna North	Tudun/Wada	50	50	0	50	0
9	Kagarko	Janjala	161	161	0	161	0
10	Kajuru	Kutura Station	290	290	0	290	0
11	Kauru	Madam	100	100	0	100	0
12	Kauru	Kizachi	240	240	0	240	0
13	Lere	Anguwan Taremu	212	212	0	212	0
14	Makarfi	Bakin Kogi	237	237	0	237	0
15	Sanga	Gwantu Waje	149	149	0	149	0
16	Sanga	Tigon	119	119	0	119	0
Total			3021	3021	0	3021	0

Table 5. Kebbi state results of Ov16 analysis on dried blood spots (DBSs), 2017

Sample no.	LGA	Community	DBSs collected, n	DBSs processed, n	Spots positive, n	Spots negative, n	Spots invalid, n
1	Arewa	Tulun Dabaga	128	128	0	128	0
2	Bagudo	Tuga	240	240	0	240	0
3	Bunza	Bunza	230	230	0	230	0
4	Dandi	Banizumbu	274	274	0	274	0
5	Danko/Wasagu	Kango	345	345	0	345	0
6	Fakai	Fakai	202	202	0	202	0
7	Fakai	Chesgu	230	230	0	230	0
8	Kalgo	Zuguru	250	250	0	250	0
9	Ngaski	Libata	234	234	0	234	0
10	Ngaski	Garin Baka	141	141	0	141	0
11	Sakaba	Sakaba	243	243	0	243	0
12	Shanga	Dugu Raha	312	312	0	312	0
13	Suru	Suru	220	220	0	220	0
Total			3049	3049	0	3049	0

as our results show that of the 9078 children (5–9 y of age) tested in the three states, none were positive. This implies that no new infections are occurring in the area. The survey results concur with those in other SSA countries with similar disease transmission profiles.^{5,8,10,26}

Another strong indicator demonstrating the interruption of transmission was the absence of infection in black flies. The presence of infective larvae in the head of the vector fly is an unequivocal signal of current *O. volvulus* transmission. Here, entomologi-

cal evaluations carried out in the sentinel and extrasentinel communities of all foci showed the absence of infective-stage (L3) larvae of *O. volvulus* in the black fly biting populations and, in many communities in Kebbi and Zamfara states, the absence of or very low numbers of black flies. This indicates parasite–vector contact has not taken place recently and transmission is not currently taking place. Additionally, the very low ABRs recorded potentially demonstrate the absence of ongoing transmission, as an ABR of <1000 flies is considered satisfactory in terms of onchocerciasis

Table 6. Zamfara state results of Ov16 analysis on dried blood spots (DBSs), 2017

Sample no.	LGA	Community	DBSs collected, n	DBSs processed, n	Spots positive, n	Spots negative, n	Spots invalid, n
1	Bungudu	Zamangira	150	150	0	150	0
2	Gusau	Sabongari Nasarawa	236	236	0	236	0
3	Maru	Gabiya/Bingi Maru	120	120	0	120	0
4	Birnin Magaji	Gora	300	300	0	300	0
5	Bukkuyum	Tungar Mu'azu	120	120	0	120	0
6	Maru	Gobirwa	300	300	0	300	0
7	Bukkuyum	Masama Mudi	260	260	0	260	0
8	Shinkafi	Katuru	300	300	0	300	0
9	Gummi	Falale	280	280	0	280	0
10	Gummi	Gayari	300	300	0	300	0
11	Maru	Dangulbi	230	230	0	230	0
12	Bungudu	Kotokorshi	202	202	0	202	0
13	Anka	Wuya	210	210	0	210	0
Total			3008	3008	0	3008	0

control.²⁷ The absence of any infective black flies also means that the ATP across all three states was zero during the survey period.

A major challenge in the entomological survey in the Kebbi and Zamfara state transmission zones was the absence of black flies, evidenced by the low numbers of black flies caught in each state. Even with the intensive follow-up prospection that was done in 2019, it was not possible to locate many adult or larval black flies in either state. In the absence of vector control, this suggests that changing patterns in land use, changing climate, river flow rates, water quality and seasonality could have changed the suitability of Kebbi and Zamfara states for black fly breeding, and thus for onchocerciasis transmission. Further research is needed to assess these individual factors, but it greatly reduces the chance of any recrudescence of transmission when MDA is stopped. The challenge in collecting sufficient flies was also noted in impact studies in Nigeria conducted by the APOC in neighbouring Kaduna state and has been an issue for other countries as well.^{24,28} Given that to align with WHO guidelines for entomological surveys at least 6000 flies need to be collected and processed for O-150 PCR to demonstrate transmission interruption, or an ATP of <20, the NOEC recommended a year-long fly collection in these two zones to confirm there were no black flies. Consequently, 8432 and 8509 person-hours of black fly catching efforts were conducted in Kebbi and Zamfara states, respectively. Despite these intensive efforts, few black flies were found.

Other states in Nigeria and other onchocerciasis-endemic countries should take note of the changing dynamics of black fly abundance observed here over the duration of the MDA programme. What were identified as highly productive, high-risk breeding and black fly catching sites early in the control programme did not yield any larval or adult black flies at the end of the programme. This highlights the need for continued entomological monitoring and evaluation over the long treatment period needed for onchocerciasis. Year to year and longer-term changes in black fly habitat suitability can complicate stop-MDA surveys if not known about in advance. Recent, detailed entomological

data are a strong advantage when planning onchocerciasis stop-MDA surveys. To make the best use of often limited resources, we recommend inspections take place before stop-MDA entomology surveys begin.^{24,29}

Post-treatment surveillance (PTS) is now recommended for the three states. However, in large parts of Kaduna, Kebbi and Zamfara states LF is co-endemic and ivermectin treatment will continue as part of the LF elimination programme. LF and onchocerciasis are co-endemic in 13 of 16 onchocerciasis-endemic LGAs in Kaduna state, all 9 onchocerciasis-endemic LGAs in Kebbi state and the 6 LGAs in Zamfara state. Monitoring and evaluation of the LF treatment programme provides an opportunity to integrate continued onchocerciasis surveillance. When ivermectin treatment stops for all diseases in each LGA and state, then the PTS period can formally begin.

Given the slow evolution of the Ov16 response to microfilariae in the skin, the earliest signal for reintroduction of onchocerciasis transmission is positive PCR pools from vector black flies.^{12,30} Therefore PTS strategies will have a strong focus on vector monitoring, in accordance with Nigerian and WHO guidance on PTS.^{12,13,27} Since other states in Nigeria have ongoing onchocerciasis transmission, there is the risk of reintroduction of onchocerciasis from neighbouring states and/or countries through movement of infected humans or vectors. The plan is for PCR monitoring of pools of *S. damnosum* s.l. captured from new HLC and EWT sites on or very close to the borders, especially in states with ongoing transmission. The border states to Kaduna (Kano, Niger and Federal Capital Territory) had good previous skin snip evaluation results, while Bauchi state is already suspected to have interrupted transmission and Katsina state, which also borders Zamfara state, is hypo-endemic. Plateau and Nasarawa states have interrupted transmission. Sokoto state, which together with Niger state borders Kebbi and Zamfara states, is hypo-endemic. In the absence of black flies, PTS strategies in Kebbi and Zamfara states need to be carefully considered. Some monitoring is likely required, however, without local black fly

populations there can be no local onchocerciasis transmission, so the two states are in a very favourable position.

These results provide robust evidence that onchocerciasis transmission has been interrupted. This is a significant achievement for Nigeria, where the national goal is to eliminate onchocerciasis by 2025, and also a huge contribution to meeting the WHO 2030 NTD roadmap goals. The roadmap target is that 12 countries will achieve verification of interruption of onchocerciasis transmission by 2030.²⁵ Nigeria is making excellent progress towards nationwide interruption of transmission and the WHO verification milestone.

Conclusions

We report here evidence of the local interruption of *O. volvulus* transmission by *S. damnosum* in endemic LGAs of Kaduna, Kebbi and Zamfara states. This comes after 20, 19 and 21 y of sustained MDA with at least 65% epidemiological coverage in Kaduna, Kebbi and Zamfara states, respectively. The absence of parasite infective larvae in the black fly vectors and of Ov16 in children ages 5–9 y show these three states have comfortably met the WHO criteria for stopping MDA. Together, the results presented here provide evidence that onchocerciasis infection no longer poses a significant public health risk in these three states of Nigeria. Treatment with ivermectin will completely cease when LF pre-TASs and TASs are passed. At this point, post-treatment surveillance will formally begin.

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