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Serological Evaluation of Onchocerciasis and Lymphatic Filariasis Elimination in the Bakoye and Falémé Foci, Mali

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Background. Ivermectin-based onchocerciasis elimination, reported in 2009–2012, for Bakoye and Falémé, Mali, supported policy-shifting from morbidity control to elimination of transmission (EOT). These foci are coendemic with lymphatic filariasis (LF). In 2007–2016 mass ivermectin plus albendazole administration was implemented. We report Ov16 (onchocerciasis) and Wb123 (LF) seroprevalence after 24–25 years of treatment to determine if onchocerciasis EOT and LF elimination as a public health problem (EPHP) have been achieved.

Methods. The SD Bioline Onchocerciasis/LF Ig[immunoglobulin]G4 biplex rapid diagnostic test (RDT) was used in 2186 children aged 3–10 years in 13 villages (plus 2 hamlets) in Bakoye and in 2270 children in 15 villages (plus 1 hamlet) in Falémé. In Bakoye, all-age serosurveys were conducted in 3 historically hyperendemic villages (1867 individuals aged 3 -78 years).

Results. In Bakoye, IgG4 seropositivity was 0.27% (95% confidence interval [CI] = .13%–.60%) for both Ov16 and Wb123 antigens. In Falémé, Ov16 and Wb123 seroprevalence was 0.04% (95% CI = .01%–.25%) and 0.09% (95% CI = .02%–.32%), respectively. Ov16-seropositive children were from historically meso/hyperendemic villages. Ov16 positivity was <2% in ≤14 year-olds, and 16% in ≥40 year-olds. Wb123 seropositivity was <2% in ≤39 year-olds, reaching 3% in ≥40 year-olds.

Conclusions. Notwithstanding uncertainty in the biplex RDT sensitivity, Ov16 and Wb123 seroprevalence among children in Bakoye and Falémé is consistent with EOT (onchocerciasis) and EPHP (LF) since stopping treatment in 2016. The few Ov16-seropositive children should be skin-snip polymerase chain reaction tested and followed up.

Keywords. onchocerciasis; lymphatic filariasis; serological monitoring; elimination; Mali.

Onchocerciasis and lymphatic filariasis (LF) are endemic in Mali [1], but large-scale interventions have progressed toward elimination of transmission (EOT) for onchocerciasis and elimination as a public health problem (EPHP) for LF. The Onchocerciasis Control Programme in West Africa (OCP) began vector control in Mali in 1977, identifying and larviciding *Simulium* (black fly) breeding sites [2]. Some endemic parts of Mali were included in the OCP's western extension, with ivermectin mass drug administration (MDA) starting in 1987. MDA was initially performed by mobile teams and later with community-directed treatment with ivermectin (CDTI) assisted by the African Programme for Onchocerciasis Control (APOC) [3]. The Global Programme to Eliminate Lymphatic Filariasis started in Mali in 2004, supporting ivermectin and albendazole distribution [1].

In 2010, APOC launched a conceptual and operational framework for onchocerciasis elimination with ivermectin treatment [4], spurred by promising findings in foci of Mali and Senegal using this strategy. In 2012, following the World Health Organization (WHO) road map on neglected tropical diseases [5], the target for onchocerciasis changed from morbidity control to EOT, contrasting with the LF goal of EPHP [6].

Mali became one of the first African countries to demonstrate the principle of onchocerciasis elimination by ivermectin MDA as the sole intervention when elimination was documented in the Bakoye and Falémé foci in 2009 and 2012 [7, 8], following 15 (Bakoye) and 16 (Falémé) years of annual treatment. Treatment

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duration corresponds to first–last year when all first-line villages were treated, 1992–2006 for Bakoye and 1991–2006 for Falémé [7]. Because the LF program started in 2007 in the same river basins, ivermectin distribution continued de facto for 9 years after 2006 (LF MDA stopped in 2016), bringing treatment duration to 24–25 years. Since 2011, a bednet distribution program for malaria (which would also impact *Anopheles*-transmitted LF [9], the type occurring in Mali) was implemented.

For onchocerciasis, a threshold of <0.1% seropositivity (by immunoglobulin (Ig)G4 enzyme-linked immunosorbent assay [ELISA]) to the *Onchocerca volvulus* Ov16 antigen in children aged <10 years is currently recommended by the WHO for stopping ivermectin MDA [10]. For *Anopheles*-transmitted LF, the WHO guidelines recommend a threshold of <2% seropositivity to *Wuchereria bancrofti* circulating filarial antigen (CFA) with the filariasis test strip (FTS; which replaced the immunochromatographic card test [ICT]) in children aged 6–7 years before ivermectin plus albendazole MDA may be stopped [11].

LF transmission assessment surveys (TAS) were conducted in 2016, and treatment stopped because all of the health districts of the 2 foci passed the TAS using FTS, that is, *W. bancrofti* antigenemia prevalence after 9 years of treatment was <2% at the health district level [12], where sampling followed a community-based (household) design according to the WHO TAS protocol [11].

Documentation of onchocerciasis elimination in Bakoye and Falémé was based on skin-snip microscopy for detection and quantification of *O. volvulus* microfilaridermia and polymerase chain reaction (PCR)–based pool screening of black fly samples for detection of infective L3 larvae [7, 8]. These data, in addition to historical infection trends and treatment coverage information, were later modeled using EPIONCHO and ONCHOSIM to estimate the risk of resurgence in Bakoye and the neighboring River Gambia focus in Senegal [13] (data from Falémé were not available). Both models captured adequately the temporal prevalence trends and suggested a very low risk of resurgence in Bakoye, although EPIONCHO indicated a more substantive risk, particularly in historically hyperendemic communities [13]. Since the publication in 2016 of the WHO guidelines for stopping MDA and verifying onchocerciasis elimination [10], serological evaluation has become an important tool for transmission assessment.

Here, we use a rapid diagnostic test [14, 15] to assess onchocerciasis and LF seroprevalence in the Bakoye and Falémé foci. Our testing was done 10 years after the first evidence of onchocerciasis elimination in these foci [7] and 3 years after passing TAS for LF in an onchocerciasis–LF coendemic area in Mali.

METHODS

Ethical Approval

The Ethical Committee of the University of Sciences, Techniques and Technologies of Bamako, Faculty of Medicine, Pharmacy, and Odontostomatology approved the study protocol. The objectives, procedure, and methodology were explained to village elders and residents, and informed consent was obtained from parents/guardians of children and from participants aged ≥18 years.

Study Sites and Baseline Infection Indicators

The study was conducted between November 2017 and January 2018 in the Bakoye and Falémé former onchocerciasis foci [7, 8]. In Bakoye, 13 villages (plus 2 hamlets) that had had a baseline *O. volvulus* microfilarial (mf) prevalence of 31%–70%, were studied. In Falémé, 15 villages (plus 1 hamlet) whose baseline mf prevalence had been 20%–57% were studied. Figure 1 shows the locations of the foci and study villages in Mali. Tables 1 and 2 summarize precontrol parasitological data (mf prevalence and community microfilarial load [CMFL]) for Bakoye and Falémé, respectively. LF was endemic in the health district of Kita (Bakoye) and Kéniéba (Falémé) with a baseline antigenemia prevalence (in those aged ≥15 years) of 8.61% (13/151; 95% confidence interval [CI] = 5.1%–14.2%) using ICT to detect CFA in 2004 [12]. The Supplementary Materials provides an additional description of the study sites and onchocerciasis temporal trends under MDA.

Study Populations

Serum samples were collected from children aged 3–10 years born in/resident of Bakoye and Falémé in December 2017. Because modeling [13] suggested a potential risk of resurgence in previously hyperendemic villages in Bakoye, serosurveys were also performed in January 2018 in the remaining (long-term resident) population (aged 11–78 years) in Kantila, Nioumala, and Galé, which had had mf prevalences greater than 60% (Table 1). This was deemed not necessary in villages of lower baseline endemicity in both the Bakoye and Falémé foci.

Serology Test

The SD Bioline Onchocerciasis/LF IgG4 biplex rapid diagnostic test (RDT) [14, 15] was used at the point of care as recommended by the manufacturer (SD Diagnostics, Korea). The test result was read after 30 minutes.

Sample Sizes

Sample sizes were calculated based on updated census data obtained in 2016–2017, provided by the Kita and Kéniéba health districts. It was estimated that children aged 3–10 years comprised approximately 22% of the population based on empirical data from onchocerciasis-endemic communities in Africa used to inform EPIONCHO's demographic structure [19]; this is in agreement with the OCP reference population [16]. The proportional representation of different age groups in the 3 historically hyperendemic communities in the Bakoye focus was calculated in an analogous manner.

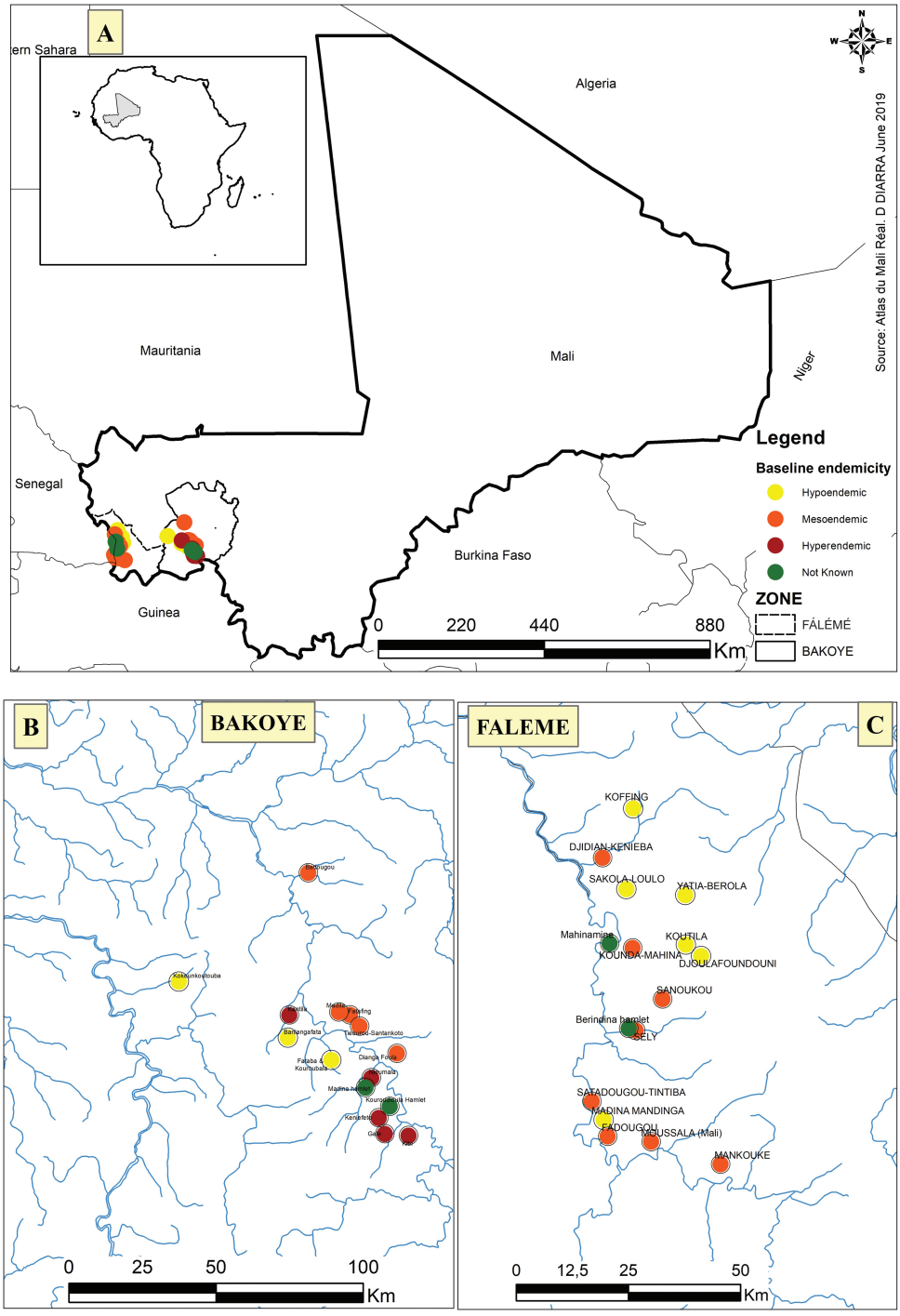

Figure 1. Map of the study area and the location of the study villages in the Kayes Region of Mali. *A*, Map of Mali showing the location of the Bakoye (solid contour) and Falémé (dashed contour) foci; inset indicates location of Mali within Africa. *B*, The Bakoye focus is in the Kita cercle, with most the 15 study communities (13 villages and 2 hamlets) located along the Bakoye River. *C*, The Falémé focus is in the Kéniéba cercle, with the 16 study communities (15 villages and 1 hamlet) located along the Falémé River (bordering Senegal). Cercles are the second administrative unit in Mali (the first being regions). Villages (indicated by solid circles) are colored by their baseline endemicity level: yellow (hypoendemic), orange (mesoendemic), and red (hyperendemic). Green circles denote locations for which no baseline data (collected in 1985–1989) were available and therefore their initial endemicity status is unknown.

The study was powered on the basis of our a priori expectation that onchocerciasis EOT had taken place in Bakoye and Falémé since the last rounds of CDTI for onchocerciasis were undertaken in 2006–2007 [7, 8] and for an additional 9 years for LF. Therefore, the "true" underlying seroprevalence in children aged 3–10 years would be 0%. We further assumed that the specificity of the RDT was approximately 97.5% [14, 15]; therefore, we calculated sample sizes required to measure an RDT

Table 1. Prevalence and Intensity of *Onchocerca volvulus* Microfilariae in the Pre-Ivermectin Mass Drug Administration Period (1985–1988) for 13 Villages in Bakoye, Mali

Village	Year of Survey	Census (% Examined)	Positive/ Examined	Prevalence (%)[a] (95% Confidence Interval)[b]	Community Microfilarial Load (Microfilariae/Skin Snip)[c]	Endemicity Level[d]
Fataba-Kouroubala	1988	284 (75.4)	57/214	30.6 (24.5–36.7)	3.22	Hypoendemic
Kokounkoutouba	1987	173 (70.5)	28/122	30.7 (22.6–38.8)	2.29	Hypoendemic
Baniangafata	1988	208 (75.5)	57/157	39.7 (32.1–47.3)	4.36	Hypoendemic
Dianga-Foula	1985	214 (47.2)	48/101	43.5 (34.1–53.3)	16.91	Mesoendemic
Fatafing	1987	335 (78.5)	106/263	45.5 (39.7–51.7)	6.17	Mesoendemic
Badougou	1988	160 (68.1)	45/109	48.4 (39.4–58.0)	4.37	Mesoendemic
Tieourou-Santankoto	1988	340 (86.2)	142/293	48.6 (42.8–54.2)	10.20	Mesoendemic
Madila	1987	204 (83.3)	82/170	50.1 (42.5–57.5)	8.17	Mesoendemic
Kantila	1985	171 (83.6)	84/143	60.1 (52.0–67.9)	17.25	Hyperendemic
Keniefeto	1988	127 (78.0)	51/99	62.4 (52.9–71.7)	14.92	Hyperendemic
Nioumala	1985	98 (72.5)	52/71	64.9 (53.3–75.2)	33.94	Hyperendemic
Kibi	1988	205 (72.2)	90/148	67.5 (59.8–74.8)	21.62	Hyperendemic
Galé	1985	410 (62.9)	173/258	70.0 (64.4–75.5)	31.13	Hyperendemic

[a]Standardized prevalence according to the Onchocerciasis Control Programme in West Africa reference population [16].

[b]Wilson 95% confidence intervals [17].

[c]Community microfilarial load: geometric mean number of microfilariae per skin snip in those aged ≥20 years according to Remme et al [18].

[d]Endemicity levels are defined as follows: hypoendemic = microfilarial (mf) prevalence <40%, mesoendemic = mf prevalence ≥40% but <60%, and hyperendemic = mf prevalence ≥60% following [13].

seroprevalence of 2.5% with a ±0.5% precision. For the first phase of the study, this yielded sample sizes of 3508 in Bakoye and of 2739 in Falémé. According to the WHO/Department of Control of Neglected Tropical Diseases [10], a sample size of 2000 children is needed to detect an Ov16 seroprevalence <0.1% (upper 95% confidence limit).

For the second phase (all-age serosurvey) of the study, sample sizes for all other age groups (≥11 years) in the 3 selected historically hyperendemic communities in Bakoye were based on estimating 50% seroprevalence (by RDT) with a precision of ±5%. The value of 50% provided the most conservative sample size estimate of 1257. In all cases, sample size calculations included a finite population size correction (ie, sampling without replacement).

Data Analyses

The standardized mf prevalences at baseline reported in Tables 1 and 2 are accompanied by 95% CIs calculated according to the Wilson score interval [17]. Point seroprevalence estimates and associated 95% CIs were calculated using the same method by focus, village, and age group.

RESULTS

Study Population Characteristics

In the Bakoye focus, 2186 children (aged 3–10 years) were tested for Ov16 and Wb123 IgG4 positivity (62% of the target sample size). The median age was 7 years and 53.6% were boys. For the 3 villages in which all-age serological surveys were conducted, 825 individuals aged ≤10 years and 1042 individuals aged ≥11 years were tested (93% of the target); of the latter, the median age was 19 years and 47.6% were men. In the Falémé focus, 2270 children aged 3–10 years were included in the study (83% of the target). Village-level sample sizes for Bakoye and Falémé are given in Supplementary Table 1. The median age was 6 years and 51.9% were boys. Table 3 describes the population tested.

Onchocerciasis mf Prevalence and CMFL Trends in Bakoye and Falémé

Figures 2 and 3 depict the decrease in mf prevalence and CMFL for the 2 foci, respectively, during 15–16 years of annual ivermectin MDA [7]. In Bakoye, levels of initial endemicity were higher than in Falémé. By 2010 (the last skin-snip–based parasitological evaluation following the last ivermectin MDA round for onchocerciasis in 2006), most reported values were zero in both foci, with 95% CIs reflecting uncertainty due to sampling.

Ov16 and Wb123 Seroprevalence Among Children in Bakoye and Falémé

In Bakoye, 6/2186 children aged 3–10 years were positive for Ov16 IgG4 (0.27%, 95% CI = .13%–.60%), and 6 children were also positive for Wb123 IgG4 antibodies (same prevalence and 95% CIs but not necessarily the same children). The children who were Ov16-positive were from the initially mesoendemic village of Badougou (a girl aged 10 years), the hyperendemic village of Kibi (a boy aged 10 years who was also positive for Wb123), and the highly hyperendemic village of Galé, where 4 children were seropositive (1 girl aged 4 years and another aged 8 years, positive for Ov16 only, plus 1 boy aged 7 years and 1 girl aged 10 years, positive for both Ov16 and Wb123). The children who were only positive for Wb123 came from the villages of Kokounkoutouba (a boy aged 3 years), Kantila (a girl aged 9 years), and Galé (a boy aged 4 years). Supplementary

Table 2. Prevalence and Intensity of *Onchocerca volvulus* Microfilariae in the Pre-Ivermectin Mass Drug Administration Period (1986–1990) for 14 Villages in Falémé, Mali

Village	Year of Survey	Census (% Examined)	Positive/ Examined	Prevalence (%)[a] (95% Confidence Interval)[b]	Community Microfilarial Load (Microfilariae/Skin Snip)[c]	Endemicity Level[d]
Madina-Mandinga	1990	414 (81.9)	53/339	19.9 (15.9–24.3)	2.30	Hypoendemic
Koffing	1987	244 (84.4)	46/206	23.3 (18.1–29.5)	1.55	Hypoendemic
Yatia-Berola	1986	217 (81.6)	45/177	24.3 (18.6–31.1)	1.29	Hypoendemic
Sakola-Loulo	1986	343 (70.3)	52/241	26.6 (21.4–32.5)	1.68	Hypoendemic
Djoulafoundouni	1986	220 (74.6)	43/164	30.3 (24.0–37.9)	2.88	Hypoendemic
Koutila	1986	227 (77.1)	55/175	33.0 (26.6–40.4)	5.37	Hypoendemic
Djidian-Kenieba	1986	133 (89.5)	48/119	40.1 (32.0–49.3)	6.23	Mesoendemic
Moussala	1989	214 (80.4)	65/172	42.2 (35.3–49.9)	5.18	Mesoendemic
Sanoukou	1986	164 (82.9)	55/136	42.2 (34.0–50.3)	5.18	Mesoendemic
Satadougou-Tintiba	1986	170 (82.4)	55/140	43.3 (35.6–51.9)	4.36	Mesoendemic
Sely	1986	231 (71.4)	61/165	43.5 (36.3–51.3)	6.20	Mesoendemic
Fadougou	1986	200 (76.0)	65/152	47.5 (39.6–55.3)	6.41	Mesoendemic
Kounda-Mahina	1986	271 (80.1)	72/217	47.7 (41.4–54.6)	7.02	Mesoendemic
Mankouke	1986	290 (74.8)	125/217	56.8 (50.0–63.1)	20.93	Mesoendemic

[a]Standardized prevalence according to the Onchocerciasis Control Programme in West Africa reference population [16].

[b]Wilson 95% confidence intervals [17].

[c]Community microfilarial load: geometric mean number of microfilariae per skin snip in those aged ≥20 years according to Remme et al [18].

[d]Endemicity levels are as defined in Table 1 [13].

Table 1 presents Ov16 and Wb123 seropositivity per village in Bakoye.

In Falémé, Ov16 positivity was 1/2270 (0.04%, 95%CI = .01%–.25%) in children aged 3–10 years, the positive child originated from the mesoendemic community of Sely (1 boy aged 7 years). Wb123 seropositivity was 2/2270 (0.09%, 95% CI = .02%–.32%), the positive children were from the villages of Madina-Mandinga (a girl aged 7 years) and Mahinamine (a boy aged 10 years). Supplementary Table 2 presents Ov16 and Wb123 seropositivity per village in Falémé.

Age-specific Seroprevalence of Ov16 and Wb123 in Bakoye

In the 3 historically hyperendemic villages selected for full age-range serological sampling, Ov16 IgG4 antibodies were detected in all 3 villages. Overall seroprevalence estimates (for individuals aged 3–78 years) were 0.63% (95% CI = .17%–2.26%) in Kantila (2 positives/319 tested), 7.0% (95% CI = 4.22%–11.41%) in Nioumala (14 positives/200 tested), and 2.97% (95% CI = 2.19%–4.02%) in Galé (40 positives/1348 tested).

Wb123 IgG4 antibodies were also detected in all 3 villages. Overall seroprevalence estimates (for individuals aged

Table 3. Description of the Study Population Tested With Onchocerciasis/Lymphatic Filariasis Immunoglobulin G4 Rapid Diagnostic Test in Bakoye and Falémé, Mali, 2017–2018

Characteristic	Bakoye		Falémé	
	N (% Target)	% or [Range]	N (% Target)	% or [Range]
Age group, y				
≤10	2186 (62.3%)		2270 (82.9%)	
3–6	1003	45.9	1415	62.3
7–10	1183	54.1	855	37.7
Median age (y)	7	[3–10]	6	[3–10]
Male/Female	1172/1014	53.6/46.4	1178/1092	51.9/48.1
Children aged ≤10 years (Kantila, Nioumala, Galé)	825 (110.4%)	…	–	–
Persons aged >10 years (Kantila, Nioumala, Galé)	1042 (82.9%)	…	–	–
11–14 y	309 (149.3%)	29.7	–	–
15–19 y	232 (124.7%)	22.3	–	–
20–24 y	114 (69.1%)	10.9	–	–
25–29 y	70 (47.6%)	6.7	–	–
30–39 y	134 (72.0%)	12.9	–	–
40–49 y	71 (48.6%)	6.8	–	–
≥50 y	112 (50.9%)	10.8	–	–
Median age (y)	19	[11–78]	–	–
Male/Female	496/546	47.6/52.4	–	–

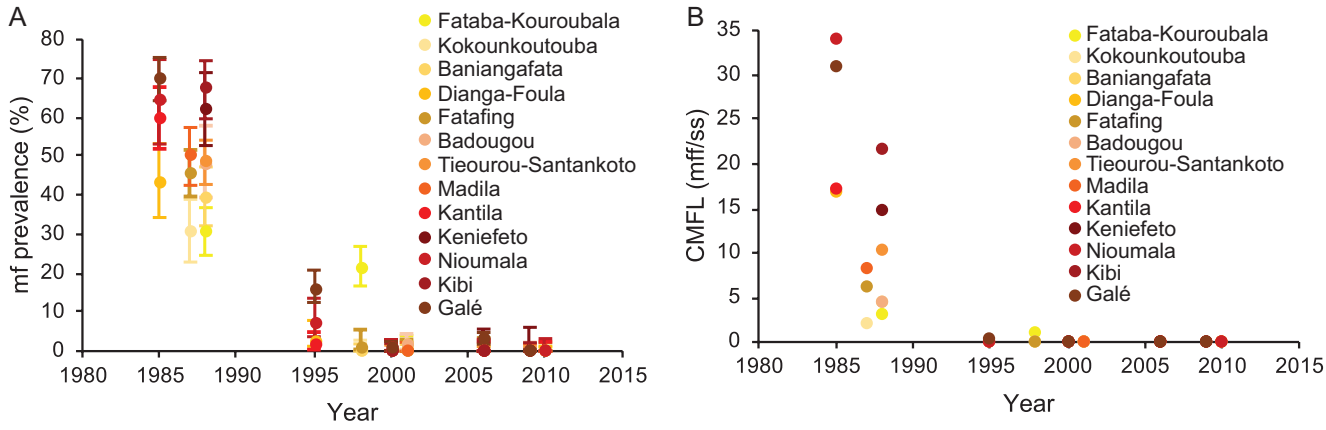

Figure 2. Trends in *Onchocerca volvulus* microfilarial infection in Bakoye from 1985 to 2010. *A*, The mf prevalence. *B*, CMFL, as defined in Table 1 [18]. Error bars for prevalence denote (Wilson score interval) 95% confidence intervals [17]. The baseline data correspond to 1985–1989. In 1989, annual ivermectin mass drug administration started with an initial coverage of 59%–62%, which improved to 73%–83% in 1998–2006 [13]. From 1992 onward, all first-line villages were treated [7]. Abbreviations: CMFL, community microfilarial load; mff/ss, microfilariae/skin snip; mf, microfilarial.

3–78 years) were 0.31% (95% CI = .06%–1.75%) in Kantila (1 positive/319 tested), 1.0% (95% CI = .27%–3.57%) in Nioumala (2 positives/200 tested), and 1.19% (95% CI = .73%–1.92%) in Galé (16 positives/1348 tested). Figure 4 presents the age profiles of Ov16 and Wb123 seropositivity in the 3 villages combined. Supplementary Table 3 presents the results of Ov16 and Wb123 serology by age group in each of the 3 villages.

DISCUSSION

The proof-of-principle onchocerciasis elimination from the Bakoye, Falémé, and River Gambia foci in Mali and Senegal [7, 8] provided seminal evidence that elimination in Africa could be achieved using ivermectin alone. This was pivotal in driving change in the WHO/APOC onchocerciasis goals from control to elimination [4]. Elimination was assessed based on epidemiological (skin snipping to detect/enumerate microfilariae) and entomological (PCR to detect *O. volvulus* L3 larvae in black flies) evidence [7, 8]. Since 2016, the WHO has advocated more stringent serological criteria for the safe stopping of MDA. These include the (statistically significant) demonstration of an Ov16 (by IgG4 ELISA) seroprevalence of <0.1% in children aged <10 years [10]. For Bancroftian (*Anopheles*-transmitted) LF, the FTS test is recommended for TAS and postelimination surveillance, with a threshold of <2% [11]. The serological data presented here were obtained using the SD Bioline Onchocerciasis/LF IgG4 RDT test and so cannot be used for direct comparison with recommended thresholds indicative of elimination. However, these data do provide important empirical information on filariases transmission status in Mali.

Robust interpretation of serological data critically depends on the assay's diagnostic performance. The SD Bioline biplex test has a manufacturer-reported sensitivity of 92%–98% for onchocerciasis and 81%–95% for LF. Specificity estimates are,

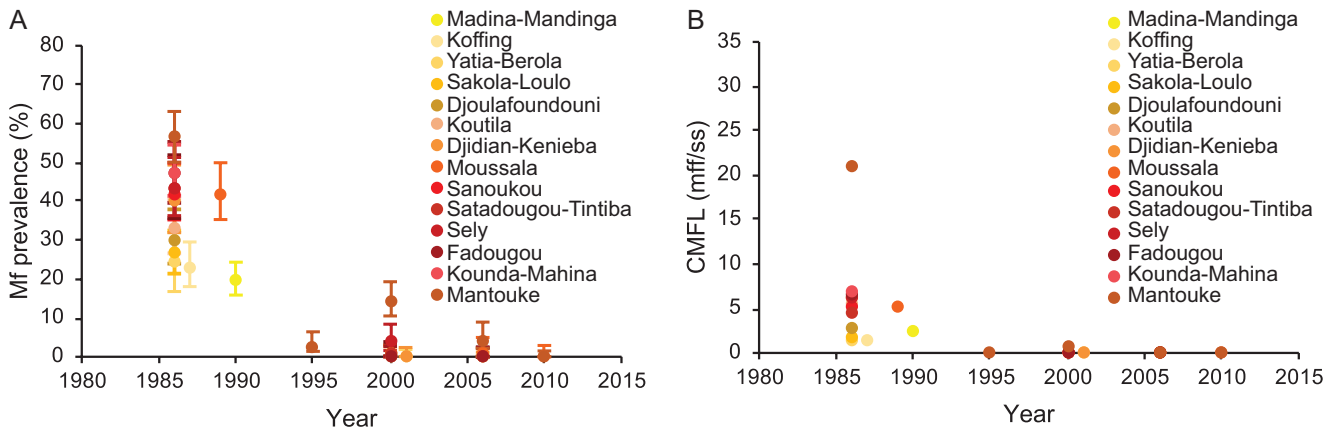

Figure 3. Trends in *Onchocerca volvulus* microfilarial infection in Falémé from 1986 to 2010. *A*, The mf prevalence. *B*, CMFL, as defined in Table 1 [18]. Error bars for prevalence denote (Wilson score interval) 95% confidence intervals [17]. The baseline data correspond to 1986–1990. In 1990, annual ivermectin mass drug administration started with an initial coverage of 63%, which improved to 75%–82% in 2000–2006. From 1991 onward, all first-line villages were treated [7]. Abbreviations: CMFL, community microfilarial load; mff/ss, microfilariae/skin snip; mf, microfilarial.

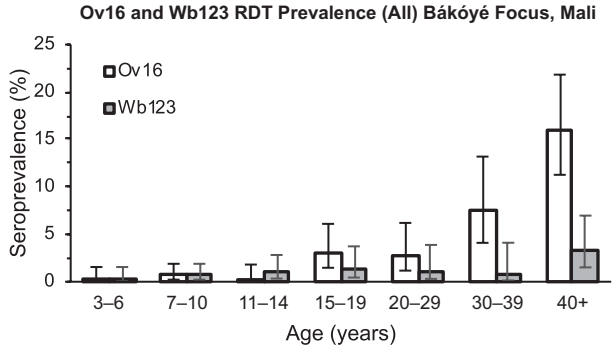

Figure 4. Age-specific Ov16 and Wb123 seroprevalence profiles in the villages of Kantila, Nioumala, and Galé combined. Ov16 (white bars) and Wb123 (gray bars) seropositivity by age group with 95% (Wilson score) confidence intervals. Abbreviations: Ov16, onchocerciasis; RDT, rapid diagnostic test; Wb123, lymphatic filariasis.

respectively, 97%–100% and 96%–99% [20]. Notwithstanding uncertainty in the field performance of the biplex RDT [14, 15, 21] and the need for field-based studies to confirm the utility of the test for verification of elimination [22, 23], the Ov16 seroprevalence in children aged 3–10 years in Bakoye and Falémé of 0.27% and 0.04%, respectively, is broadly consistent with EOT. The Wb123 seroprevalence of 0.27% in Bakoye and 0.09% in Falémé is also likely consistent with EPHP for LF, although there is no operational guidance on the interpretation of Wb123 serology in the context of LF transmission (however, see [21]). Therefore, at the focus level and as of 2017–2018, we have found no substantive evidence of onchocerciasis or LF resurgence since MDA cessation in 2016. However, the few Ov16-seropositive children identified here should be PCR tested on skin snips to distinguish between parasite exposure and infection. If found negative, they could be omitted from the seroprevalence calculation but be reexamined 1–1.5 years later to determine if they have become patent and should be treated [10].

The serological results in Bakoye contribute to the validation of previous modeling projections, indicating sustained elimination when EPIONCHO was fitted to the entire longitudinal data series (Figure 3C of [13]). By contrast, likely resurgence was predicted by EPIONCHO (but not ONCHOSIM) in the neighboring and more highly endemic River Gambia focus in Senegal (which was treated biannually; Figure 3G of [13]). Indeed, ivermectin MDA was resumed by the Senegalese National Onchocerciasis Control Program in the River Gambia focus in 2013 [24]. In 2014, an Ov16/Wb123 serological evaluation (using Luminex multiplex-bead assay) across 3 river basins of the Kédougou Region (containing areas and some communities described by Diawara et al and Traore et al [7, 8]) found an Ov16 seroprevalence of 2.5% (7/279) in children aged <10 years, including 3 positives in the River Gambia focus and 4 positives in the Senegalese part of the Falémé focus [24]. It is unclear whether these children were found in the same communities previously reported free of onchocerciasis transmission [7, 8], but concerns were raised that transmission had either not been interrupted or had resurged [24]. All-age Ov16 serological profiles in the Bakoye focus of Mali (this work) are compared with those of the Kédougou Region of Senegal [24] in Supplementary Figure 1. Resurgence of onchocerciasis has been reported elsewhere in West Africa [25, 26], highlighting the importance of robust epidemiological and entomological surveillance following cessation of interventions [27].

The 3 villages in Bakoye and 1 in Falémé that had children aged ≤10 years who were Ov16 IgG4 antibody positive were meso- or hyperendemic at baseline. The village of Galé, one of the largest and most hyperendemic villages before MDA (Figure 2), had 4 seropositive (out of 6 in Bakoye) children (0.6%, Supplementary Table 1). Although, arguably, these may all be false positives, variation in seropositivity among villages highlights the importance of spatial scale when designing protocols for monitoring, evaluation, and surveillance sampling. No explicit guidance currently exists on how onchocerciasis elimination surveys should be implemented nor on the appropriate spatial unit. For LF, an online survey-design tool can be used for school-based or community-based cluster-randomized surveys within "evaluation units" [11]. More guidance is needed on how best to implement onchocerciasis serological (and entomological) surveys. Spatially explicit protocols, which have been developed for onchocerciasis elimination mapping (to identify previously untreated hypoendemic areas) [21], may prove useful.

The certainty of evidence indicative of onchocerciasis elimination for the <0.1% serological threshold in children aged <10 years is low [10]. Recent modeling work has suggested that a higher threshold of 2% may be safe for stopping MDA and that children aged 5–14 years may be more informative [28]. Yet, considerable uncertainty remains on both the technical threshold for elimination, which is confounded by limited understanding of *O. volvulus* transmission dynamics and population biology at low transmission levels [29], and whether a particular threshold can be measured using current diagnostic tools [30], particularly RDTs [21, 22]. The WHO currently recommends Ov16 ELISA serology, but different ELISA protocols vary in performance characteristics, and laboratory capacity in Africa must be strengthened [31, 32]. The development and manufacture of standardized and quality-assured ELISA kits with sensitivity/specificity that is compatible with measuring (revised) serological thresholds are a priority.

Inspection of all-age serological profiles in the previously hyperendemic villages of Kantila, Nioumala, and Galé analyzed together provides an overview of historical exposure trends in Bakoye. Seroprevalence was very low in children who were aged ≤14 years, with upper 95% confidence limits of less than 2% in those aged 3–6, 7–10, and 11–14 years (Figure 4), suggesting pronounced transmission suppression. Despite the majority of young adults in the 15–19 and 20–24 age groups having lived

their lives under MDA with ivermectin, the Ov16 seroprevalence of between approximately 3% and 5% indicates some exposure to *O. volvulus*, probably before the interruption of transmission (ie, not necessarily indicative of current transmission). From the age of 15 years to 19 years, Ov16 seroprevalence increases slowly, only reaching 16% in those aged ≥40 years. These individuals would have been aged ≥15 to 20 years when ivermectin distribution began and, under intense pretreatment transmission, their age-specific mf prevalence would already have reached >60% [33]. These results are consistent with those of Paulin et al [34], indicating that Ov16 seropositivity likely declines over time after prolonged treatment (although mortality rates in these populations would also have to be considered).

The all-age seroprevalence profiles for Wb123 also increased somewhat with age, reaching 3% in those aged ≥40 years but remaining below 2% in all those aged <40 years. A recent study in Mali found a strong correlation between ICT and Wb123 seropositivity in children aged 6–7 years, suggesting that evaluating IgG4 to Wb123 might in the future (pending further (sero) epidemiological investigations) be useful in stop-MDA decisions or in cessation of TAS following MDA, as a measure of recent exposure rather than patent infection [35]. The study in Mali also reported Ov16 seropositivity in children from previously onchocerciasis meso- and hyperendemic communities and none from hypoendemic villages. These findings, together with those presented here and reported in Senegal for children aged <10 years where the 7 seropositive individuals were found in the River Gambia and Fálémé foci [24], indicate that not all Ov16-seropositive results should be dismissed as false positives, as a trend is clearly emerging associating Ov16 seropositivity with pre-MDA endemicity status. It is hoped that current LF antigenemia and mf prevalence thresholds for EPHP will be sufficient to lead to EOT, albeit empirical evidence for this is limited [23] and LF may persist despite passing TAS [36]. In reality, thresholds indicative of interrupted transmission will vary with transmission conditions and local vector biting rates [37]. Hence, while mathematical modeling [28, 37] can help decision-makers define thresholds based on acceptable levels of risk, elimination will ultimately be demonstrated using robustly sampled surveillance data. Surveillance will come at a cost that the global health community must recognize and prepare for to sustain the great progress already been made along the path to eliminating onchocerciasis and LF.

CONCLUSIONS

The antibody seroprevalence against Ov16 and Wb123 antigens among children in the Bakoye and Falémé foci in Mali is very low and consistent with both onchocerciasis and LF having been likely eliminated since stopping MDA in 2016 (pending skin-snip PCR testing of the few Ov16-seropositive children identified, and with the proviso that new studies are needed to confirm the field performance of the biplex RDT). Periodic (including entomological) surveillance should be continued in this region to detect and respond to any early signs of resurgence/reintroduction. This will help to sustain elimination and maintain the Bakoye and Falémé foci as an example of a global health intervention success.

Notes

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Potential conflicts of interest. T. B. N. is among the patent holders for Ov16 and Wb123 (no longer under patent) and, through the National Institutes of Health, has received licensing/royalty fees for Wb123. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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