Evaluation of Onchocerciasis Transmission in Tanzania: Preliminary Rapid Field Results in the Tukuyu Focus, 2015

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Abstract. To compare diagnostic tests for onchocerciasis in a setting that has suppressed transmission, a randomized, age-stratified study was implemented in an area in Tanzania that had received 15 rounds of annual mass drug administration (MDA) with ivermectin. Study participants ($N = 948$) from 11 villages underwent a questionnaire, skin examination, skin snips, and blood draw. The burden of symptomatic disease was low. Ov-16 antibody rapid diagnostic test (RDT) results were positive in 38 (5.5%) participants, with 1 (0.5%), 1 (0.4%), and 2 (0.8%) in children aged 0–5, 6–10, and 11–15 years, respectively. Despite significant impact of MDA on transmission, the area would have failed to meet World Health Organization serologic criteria for stopping MDA if a full evaluation had been conducted. The specificity of the RDT, which is 97–98%, may result in the identification of a number of false positives that would exceed the current stop MDA threshold.

INTRODUCTION

More than 186 million people live at risk for infection with the parasite *Onchocerca volvulus*, and over 26 million people are infected with the parasite.^{1,2} *Onchocerca microfilariae* migrate through the skin and the eye where the body's inflammatory response to dying microfilariae causes symptomatic disease.³ More than 1 million people have visual impairment or blindness; millions more have debilitating skin itching or other skin disease. Ivermectin kills the microfilariae and reduces or eliminates symptoms.⁴ Evidence from the Americas and Africa suggests that the elimination of onchocerciasis through mass drug administration (MDA) with ivermectin is feasible.^{5–10}

Onchocerciasis control programs have transitioned to elimination programs, which required a reassessment of the diagnostic techniques available. Skin snips are inadequately sensitive for determining when treatment programs can be safely stopped.¹¹ The Ov-16 serologic test is more sensitive, but positive results may not necessarily represent current infection. The new World Health Organization (WHO) verification of elimination guidelines require the use of an Ov-16 serologic test in children less than 10 years old for deciding when to stop MDA, as positive results in children most likely represent current infection.¹² The serologic criterion requires demonstration of a seroprevalence $< 0.1\%$ with a 95% confidence interval (CI) that excludes 0.1%. The Ov-16 rapid diagnostic test (RDT) is not yet validated for this determination. This study examined the proportion of Ov-16 antibody positivity in different age groups to identify which age group provides an accurate indication of transmission status in a focus that is nearing elimination.

METHODS

The Tukuyu onchocerciasis focus in southwestern Tanzania had a population of 732,445 people in 2015. Annual ivermectin MDA began in the project area around 2000, though there was

some treatment as early as 1994. A pilot vector elimination project took place from 2003 to 2005. Reported annual ivermectin coverage has been 69–86% since 2009. The national program performed a skin snip assessment of 3,599 people in 20 villages in 2012, and no microfilariae were found.

Eleven villages in proximity of breeding sites, where *Simulium* (black flies) will be collected in a parallel study, were chosen. From these villages, households were randomly selected and one resident in each of five age groups was randomly selected to participate from each selected household. Selected residents had to have lived in the village for ≥ 10 years or since birth for children < 10 years old. Persons who were too sick to participate in the study or who were unable to give informed consent were excluded. Age groups were categorized as: ≤ 5 years, > 5 to ≤ 10 years, > 10 to ≤ 15 years, > 15 to ≤ 20 years, and > 20 years. The study took place July to August 2015, around 9 months after the last ivermectin distribution.

Participant data were recorded on a smart phone-based standard questionnaire that included the following domains: demographic information; prior antifilarial medication use; self-reported skin, lymph, and eye symptoms; and results of skin and lymph node examinations that were conducted by trained medical personnel. Venipuncture and two skin snips from the iliac crest were performed on participants older than 5 years old; capillary blood collection alone was performed on younger participants. Skin snips and daytime blood smears were examined by microscopy for filarial parasites, whole blood samples were tested by the SD Bioline Ov-16 RDT for onchocerciasis.

A sample of 1,000 individuals, with 200 per age group, provided 80% power to detect an Ov-16 antibody population prevalence of 1% and an age-stratified prevalence of 2% with a 95% CI that excluded zero. Sampling weights were calculated by inverting the household sampling probability and adjusted for refusals and oversampling based on the total population of the 11 villages. SAS software version 9.3 (SAS Institute Inc., Cary, NC) was used for all analyses. Clustering at the village level was accounted for by Taylor series linearization.¹³

The study protocol was approved by Institutional Review Boards at the Centers for Disease Control and Prevention and the Tanzanian Medical Research Coordination Committee.

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Informed consent, parental permission, and assent were obtained from participants as appropriate.

RESULTS AND DISCUSSION

Over 23 days, 695 households were approached; 617 (88.8%) households had at least one household member participate yielding a total of 948 participants; median household size was four persons. Basic demographics, survey responses, and physical findings of the enrollees are shown in Table 1. The target sample size was nearly achieved or achieved for each of five age groups except for the 16- to 20-year-old group. Among 795 eligible to receive treatment during MDA, 522 (65.7%) reported taking ivermectin in the past year. Itchy skin was reported by 207 (21.9%) participants and skin nodules by 91 (9.6%). Onchocercal skin findings were infrequent, with 12 (1.3%) participants having a nodule (median count 1, range: 1–3) and four (0.4%) having a form of onchodermatitis. Atrophy, depigmentation, and adenopathy were each identified in < 1% of participants.

All skin snips ($N = 727$) and blood smears ($N = 917$) were negative. Table 2 shows the Ov-16 RDT-positive results,

TABLE 1

Demographic characteristics and laboratory, Tukuyu focus, Tanzania, 2015, $N = 948^{*†}$

	<i>n</i>	% Or IQR or range
Male, sex	499	52.7%
Median age of respondent (years)	12	6–26 (IQR)
Age group in years		
0–5	201	21.2%
6–10	227	23.9%
11–15	179	18.9%
16–20	88	9.3%
> 20	253	26.7%
Occupation		
Student	391	41.3%
Farmer	287	30.3%
Preschool	242	25.6%
Other	27	2.9%
Spends most of the day		
Near the river	675	71.3%
Near cultivated land	890	94.0%
Ivermectin†		
Eligible for MDA (age 5 years and above)	795	83.9%
Of those eligible, MDA taken in the past year	522	65.7%
Symptoms		
Itchy skin past year	207	21.9%
Skin nodules	91	9.6%
Examination results		
Skin nodules	12	1.3%
Median nodule count	1 nodule	1–3 (range)
Acute papular onchocercal dermatitis	2	0.2%
Chronic papular onchocercal dermatitis	0	0.0%
Lichenified onchocercal dermatitis	2	0.2%
Atrophy	1	0.1%
Depigmentation	5	0.5%
Lymphadenopathy	3	0.3%
Hanging groin	0	0.0%

IQR = interquartile range; MDA = mass drug administration; RDT = rapid diagnostic test.

* $N = 947$ participants provided data on sex, age, occupation, spend most of day, and symptoms.

† $N = 938$ participants had examination results.

‡ Seven participants noneligible for MDA (children aged 0–4 years) reported taking ivermectin in the past year and were not included in response.

which varied by age group from 0.5% to 10.5%. Table 3 summarizes the baseline endemicity of each study village and the weighted proportion of participants who tested positive for Ov-16-specific antibody in each village (overall 0.0–14.3%). No association was apparent between baseline endemicity and proportion RDT positive.

This rapid evaluation of the Tukuyu focus in Tanzania demonstrated that the program has had significant impact on the burden of disease. There was a low burden of reported symptoms and disease detected on examination. No participant had detectable parasites by microscopy. The proportion of the study population with positive Ov-16 RDT results was 5.5% (95% CI: 2.2–8.8) overall and 10.5% (95% CI: 4.8–16.2) among people > 20 years old. These findings are consistent with a setting where transmission has been suppressed for some time. Ov-16 positivity in adults would approximate baseline microfiladermia if the Ov-16 response was lifelong. The finding of 10% antibody positivity in participants > 20 years old, which was below what one would expect in a hyper- or meso-endemic area, is consistent with the growing body of evidence that the Ov-16 response at the community level declines over time after sufficient treatment.^{14,15}

Despite the demonstrated impact of the elimination program, the RDT results in children would have resulted in the area failing the current WHO serologic criteria for stopping MDA of 0.1%. The original Ov-16 test was developed to be a highly sensitive and specific assay¹⁶ to detect early and light infections, when skin snips are likely to be negative. The new RDT has a 97–98% specificity,^{17,18} so a false-positive rate of 2–3% is expected. It is possible that the few positive RDT results we found in children could be false positives. The fact that we did not see an age-dependent increase in prevalence in children through 15 years of age suggests that the positive results we found are background noise and not indicative of ongoing transmission. However, we cannot assume that the RDT-positive results were false-positive results. Unfortunately, the specificity of the RDT renders it incapable of measuring the 0.1% threshold.

Although these results are compelling, current WHO guidelines do not permit the RDT to be used for stopping MDA. The results we obtained with the RDT need to be compared with other tests, such as our planned Ov-16 enzyme-linked immunosorbent assay (ELISA), skin snip polymerase chain reaction (PCR), and blackfly PCR. If these studies provide evidence that transmission may have been interrupted in Tukuyu (i.e., skin snip PCR results are negative, and no infection is detected in the vector) and the ELISA similarly shows a low prevalence that exceeds 0.1% and no increase in seroprevalence in children 15 years old and younger, then consideration of additional studies to evaluate the appropriateness of a higher serologic threshold for stopping MDA, perhaps 1–2%, might be

TABLE 2

Ov-16 IgG4 RDT positivity overall and by age group, Tukuyu focus, Tanzania, 2015, $N = 948$

	<i>n</i>	Weighted proportion % (95% CI)
Overall	38	5.5 (2.2, 8.8)
Age group in years		
0–5	1	0.5 (0.0, 1.8)
6–10	1	0.4 (0.0, 1.4)
11–15	2	0.8 (0.0, 2.2)
16–20	2	2.2 (0.0, 5.7)
> 20	32	10.5 (4.8, 16.2)

CI = confidence interval; RDT = rapid diagnostic test.

TABLE 3
Tukuyu focus, Tanzania, baseline endemicity of study villages and 2015 Ov-16 RDT antibody positivity of study villages

Village	Pre-MDA village baseline endemicity type	Ov-16 IgG4 RDT positive	
		n	Weighted proportion % (95% CI)
Kilugu, N = 70	Hyper*	8	13.8 (4.7, 23.0)
Kambesegela, N = 82	Hyper	2	5.9 (0.0, 13.9)
Kasumulu, N = 121	Hyper	1	2.2 (0.0, 6.4)
Kibole, N = 76	Meso†	7	12.6 (3.9, 21.4)
Kifunda, N = 80	Meso	6	10.6 (2.5, 18.7)
Kisegese, N = 105	Meso	4	5.0 (0.1, 10.0)
Kapula Mpunguti, N = 106	Meso	3	4.3 (0.0, 9.1)
Masakulu, N = 48	Hypo‡	5	14.3 (2.8, 25.8)
Kasaybone, N = 62	Hypo	1	3.8 (0.0, 11.2)
Mbambo, N = 78	Hypo	1	3.1 (0.0, 9.1)
Busale, N = 120	Unknown	0	0.0

CI = confidence interval; MDA = mass drug administration; RDT = rapid diagnostic test.

* Hyperendemic; baseline nodule rate $\geq 40\%$ or microfilaria $\geq 60\%$.

† Meso-endemic; baseline nodule rate $\geq 20\text{--}39\%$ or microfilaria $\geq 30\text{--}59\%$.

‡ Hypo-endemic; baseline nodule rate $< 20\%$ or microfilaria $< 30\%$.

warranted. Modeling suggests that these thresholds could be consistent with interruption of transmission.¹⁹

The Ov-16 RDT results were similar in children aged 0–15 years, all of whom were born after MDA started, suggesting that it might be reasonable to include children ≤ 15 years old in transmission assessments. The upper limit of the age group will likely depend on the number of years of good MDA coverage that have been attained at the time of the assessment. Our results cannot confirm the lower limit of the age group, though a previous study found antibody positivity in 3- to 4-year-olds.²⁰ Future assessments should consider identifying the optimal lower limit of the age group for evaluation.

The study was designed to yield a representative sample of the selected villages' populations. However, 16- to 20-year-olds were often not present in the villages so they are underrepresented. The median household size was four persons, less than anticipated and the primary reason the target enrollment was not achieved; sampling methods for future studies should be adjusted to account for the low number of 16- to 20-year-olds found in the villages and potential biases associated with those present.

In conclusion, this survey in the Tukuyu focus of Tanzania revealed a low burden of clinical disease in the population and a low proportion of Ov-16 RDT-positive results in children. However, the point prevalence of infection in children born after the start of MDA was greater than 0.1% despite some parasitologic and clinical indications that transmission of infection may have been interrupted. In the absence of a confirmatory test that can be used to boost RDT specificity or absence of data that support raising the serologic threshold, the RDT does not appear to have the specificity required for its use in stopping MDA.

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