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# **Test-and-not-Treat strategy for onchocerciasis elimination in areas co-endemic with *Loa loa* infection: implications for annual re-testing based on an observational cohort study**

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# Abstract

## **Background**

A Test-and-not-Treat strategy has been developed to prevent post-ivermectin serious adverse events (SAEs) occurring in persons with high levels of circulating *Loa loa* microfilariae during mass drug administration to eliminate onchocerciasis. An important question related to cost and programmatic issues is whether annual re-testing is required for everyone.

## **Methods**

We have investigated the evolution of *L. loa* microfilarial density between two rounds of Test-and-not-Treat conducted 18 months apart in a population of about 20,000 people living in the Okola health district, central Cameroon.

## **Findings**

Totals of 16,182 and 18,697 individuals were examined in 2015 and 2017, respectively. Eighteen months after ivermectin treatment, the microfilarial reservoir was significantly reduced in the population, with the geometric mean of positive counts dropping from 2,825 to 1,485 microfilariae per mL. The proportion of participants at risk of SAEs, and therefore excluded from ivermectin treatment, decreased from 2.1% (344 of 16,182) to 1.5% (283 of 18,697). Follow-up data could be incontrovertibly ascertained for 6,983 individuals. In this cohort, in 2017, 99.97% (6,981 of 6,983) of individuals treated with ivermectin in 2015 had *L. loa* microfilariae levels below the level associated with neurologic SAEs.

## **Interpretation**

Individuals treated with ivermectin during the preceding round do not have to be retested for *L. loa* microfilaraemia prior to the next treatment, provided that they can be re-identified. This will lead to dramatic cost savings and facilitate reaching programmatic goals for elimination of onchocerciasis in areas coendemic for loiasis.

## **Funding**

This research was funded by the Bill & Melinda Gates Foundation and, in part, by the Division of Intramural Research, NIAID, NIH.

**Keywords** onchocerciasis elimination, loiasis, *Loa loa*, alternative treatment strategy, ivermectin

## **Research in context**

### **Evidence before this study**

In 2015, a large-scale evaluation of a Test-and-not-Treat strategy provided proof of concept that testing for high *L. loa* mf density before treatment prevents occurrence of post-ivermectin SAEs in areas coendemic for onchocerciasis and loiasis. Although the strategy is considered promising, there is concern about the increased cost of this strategy compared to normal Community Directed Treatment with Ivermectin. The cost difference over the programme life span depends strongly on whether or not people are re-tested every year.

The objective of this study was to evaluate whether the *L. loa* mf density in a person, who was not at risk of SAEs (mf density <20,000/mL) and received ivermectin, increased above the threshold level (mf density >20,000/mL) 18 months after treatment. We searched the PubMed and ISI Web of knowledge databases using the terms “(loiasis OR loase OR *Loa loa*) AND (ivermectin) AND (trial OR therapeuti)” for articles published in English or French, with no time limit. We also checked the references of all studies identified by the above methods. A recent meta-analysis of previous trials of ivermectin on *L. loa* mf density indicated that, one year after treatment, none of 238 individuals with an initial *L. loa* mf density <20,000 microfilariae/mL were at risk of post-ivermectin severe adverse events. This suggests that once eligible individuals are treated with ivermectin once, they can safely receive yearly ivermectin treatment without testing.

### **Added value of this study**

This is by far the largest study evaluating the evolution of individual *Loa loa* microfilarial density over two campaigns of community treatment with ivermectin for onchocerciasis. Our results suggest that individuals with an initial *L. loa* mf density <20,000 microfilariae/mL who have received standard treatment with ivermectin as part of onchocerciasis elimination

activities are unlikely (observed frequency: 0.03%) to have a microfilarial density associated with an increased risk of *Loa*-related SAEs if retreated within 18 months and could be treated again without being retested.

### **Implications of all the available evidence**

Since 95% of participants can be safely treated with ivermectin during any given Test-and-not-Treat campaign, not having to redo microfilarial quantification the following year would result in significant savings in time and money.

## Introduction

Loiasis, often referred to as “the eye worm disease” because of the spectacular subconjunctival migration of the adult worm seen in some infected individuals, is a vector-borne parasitic infection endemic to Africa, from southeastern Benin in the West to the Republic of South Sudan in the East and south to Angola and the northern border of Zambia.<sup>1</sup> *Loa loa*, the filarial parasite responsible for loiasis, is transmitted between humans through the painful bites of female tabanids (deerflies), *Chrysops silacea* and *C. dimidiata*. In endemic areas, loiasis transmission occurs all year long, with peaks during the rainy seasons,<sup>2,3</sup> and reinfection is common. Together with the long lifespan of adult worms – a female worm was removed from the eyelid of a patient 21 years after visiting an endemic area<sup>4</sup> – this contributes to the chronicity of infection. Adult worms live in the upper layer of the epidermis and in the conjunctival tissues. After mating with males, females produce embryos – or microfilariae (mf) – that circulate in the peripheral bloodstream with a diurnal periodicity. Some infected individuals have the ability to clear mf from their bloodstream; whereas others harbor tens to hundreds of thousands of mf per milliliter of blood – a condition sometimes termed “hypermicrofilaraemia”. The reasons for this variability in response are not entirely understood, but may be due in part to a genetic predisposition.<sup>5,6</sup> However, the major problem associated with high grade *L. loa* microfilaraemia first emerged in the context of the large-scale treatment with ivermectin to combat onchocerciasis in the central African region during the early 1990s. In 1997, data emerged that demonstrated that severe adverse events (SAEs), with sometime fatal outcome, could occur in people with high levels of circulating *L. loa* mf after a standard dose (150 µg/kg) of ivermectin.<sup>7</sup> Those SAEs appeared to be triggered by the rapid and massive death of the ivermectin-sensitive *L. loa* mf. Since then, implementation of ivermectin-based community treatment for onchocerciasis elimination has been halted or delayed in some foci of Central Africa.

Between August and October 2015, we conducted a campaign of selective treatment with ivermectin in an area of Cameroon where onchocerciasis and loiasis are co-endemic.<sup>8</sup> To prevent the occurrence of SAEs, we used a “Test-and-not treat” (TaNT) strategy: point-of-care quantification of *L. loa* microfilaraemia was performed in all consenting residents of the Okola health district aged 5 years or older [N=16,182]. All individuals with >20,000 *L. loa* mf/mL, deemed at-risk for SAEs, and those with contraindications to ivermectin (pregnant or breastfeeding women or with a serious acute or chronic concomitant illness) were excluded from ivermectin treatment but were offered a single oral dose of albendazole 400 mg (unless the latter was also contraindicated) for intestinal deworming. In addition, individuals with >20,000 *L. loa* mf/mL were revisited after the campaign to ascertain their onchocerciasis status using the standard skin-snip method. Those who were infected with *Onchocerca volvulus* received a 5-week daily treatment regimen with doxycycline (100 mg) (unless contraindicated).

During this ivermectin-based TaNT campaign for onchocerciasis elimination, 95.5% of participants received ivermectin, and only 2.1% of participants were excluded for *L. loa* densities above the risk threshold.<sup>8</sup> No SAEs occurred in the entire treated population. Because multiple ivermectin treatment campaigns are needed to achieve onchocerciasis elimination, an important question related to cost and programmatic issues is whether annual testing of the whole population is required. More specifically, do previously treated individuals require repeat testing? Data collected previously from 238 individuals with an initial *L. loa* mf density <20,000 mf/mL indicated that none was at risk of SAEs one year after receiving ivermectin.<sup>9</sup>

We took advantage of a second TaNT campaign conducted in Okola between March and May 2017 (~18 months after the initial campaign) to investigate changes in *L. loa* microfilarial densities after the first treatment in 2015 at the individual and community levels.

## Methods

### *Study area and population*

TaNT campaigns for onchocerciasis were conducted in six health areas (89 communities) in Okola health district (Cameroon) in August-October 2015<sup>8</sup> and March-May 2017. Total population was 26,415 in 2015 and 29,587 in 2017. All individuals aged 5 years old and older (N=22 842 in 2015 and N=25421 in 2017) without other contraindications to treatment were invited to be screened for *L. loa* microfilaraemia before being offered ivermectin.

### *Assessment of *L. loa* microfilarial density and decision to treat*

*L. loa* microfilarial density was measured at the point-of-care (POC) using the LoaScope.<sup>10</sup> All those with a *L. loa* microfilarial density  $\leq 20,000$  mf/mL were offered treatment; in the first two weeks of the 2015 campaign a higher exclusion threshold of  $\leq 26,000$  mf/mL was used as explained previously.<sup>8</sup>

### *Data entry, data matching and statistical analysis*

The TaNT studies were not initially designed to provide longitudinal data. Nonetheless, at both rounds of intervention, participants were registered using a paper form. Personal information collected on this form included name, surname, age, gender, phone number and a household number assigned during an exhaustive census conducted a few weeks before the campaign. In 2017, we also recorded whether each individual reported participation in the



2015 campaign. All data collected on paper forms were entered into an electronic database using double-entry for quality control. An automated script was developed using the Stata® statistical software (StataCorp, College Station, TX USA) to identify discrepancies between the two series of data entries from a given year, and all discrepancies were resolved by reference to the paper form. The 2015 data were matched to those from 2017 based on the following criteria: name, surname, age, village of residence and, when available, the individual barcode assigned in 2015. We assumed that some of the names and surnames of individuals who took part in both campaigns may have been spelled differently and used a semi-automated algorithm to generate lists of likely matches. All matches with a high degree of certainty were validated manually. When the data forms were inconclusive, we contacted participants by phone for confirmation of their participation in the TaNT campaigns. This allowed us to define a cohort of individuals tested in both 2015 and 2017.

Unless otherwise stated, geometric means (GM) were used as a measure of central tendency. The prevalence and intensity of *L. loa* microfilarial infection in all individuals tested in 2015 were compared to those tested in 2017 using Chi-square or Wilcoxon-Mann-Whitney test, respectively. For those participating in both campaigns, the McNemar and Student t tests for paired samples were used to compare the prevalence and intensity of *L. loa* microfilarial infection between 2015 and 2017. In addition, transition matrices were constructed to represent the evolution of microfilarial densities based on a semi-quantitative scale using the following groups of densities: 0, 1–100, 101–500, 501–2,000, 2,001–10,000, 10,001–20,000 and >20,000 mf/mL. All statistical tests were performed using the Stata® statistical software V15.1.

### **Role of the funding source**

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study, and had final responsibility for the decision to submit the publication.

## Results

The therapeutic coverage (persons treated/total population) in the six health areas varied between 50.8% and 65.5% in 2015 and between 60.5% and 78.0% in 2017. In 2015, 16,182 individuals were examined with the LoaScope. In 2017, 18,697 were examined in the same communities. Records from 2017 could be matched to those from 2015 for 6,983 individuals, that is 43.2% of the 2015 participants. Demographics of the participants are presented in Table 1.

Based on the LoaScope results, overall *L. loa* microfilarial prevalence decreased from 17.9% to 15.9% ( $p < 0.0001$ ). The GM of positive *L. loa* microfilarial densities decreased from 2,825.00 mf/mL in 2015 (95% confidence interval (95% CI): 2,660.80 – 2,999.22) to 1,484.72 mf/mL (95%CI: 1,394.95 – 1,580.27) in 2017 ( $p < 0.0001$ ). This drop in intensity is reflective of a significant shift toward lower values in the frequency distribution of *L. loa* microfilarial density (Wilcoxon-Mann-Whitney test,  $p < 0.0001$ , Figure 1).

The proportion of individuals that were excluded from ivermectin treatment due to a high *L. loa* microfilarial density decreased from 2.1% in 2015 to 1.5% in 2017 ( $p < 0.0001$ , Table 1). Of the 6,983 individuals whose *L. loa* microfilarial density was assessed with the LoaScope in both 2015 and 2017, 6,692 (95.8%) had received ivermectin in 2015. The prevalence of *L. loa* microfilaraemia in these treated individuals decreased from 17.3% to 13.0% ( $p < 0.0001$ ), and the GM *L. loa* microfilarial densities decreased from 2,550.30 mf/mL (95% CI: 2,336.56 – 2,783.59) to 1,123.82 mf/mL (95% CI: 1,017.06 – 1,241.78) ( $p < 0.0001$ ). In contrast, the prevalence of microfilaraemia remained unchanged (59.5%) in

the 291 individuals who were not treated with ivermectin in 2015, although the GM mf density decreased from 16,516.4 (95% CI: 13,170.0 – 20,713.1) in 2015 to 12,121.7 (95% CI: 9,810.2 – 14,977.9) ( $p < 0.00001$ ).

Based on the transition matrix (Table 2), among the 5531 individuals without detectable *L. loa* microfilaraemia in 2015 who received ivermectin, 5274 (95.4%) were still negative in 2017 and 257 (4.6%) became microfilaraemic in 2017 with all densities below 10,000 mf/mL. Among the 1161 microfilaraemic individuals in 2015 who received ivermectin, 957 (82.4%), 160 (13.8%) and 44 (3.8%) had a reduced, similar and increased density in 2017, respectively.

In the ivermectin-untreated group (Table 3), among the 118 individuals without detectable *L. loa* microfilaraemia in 2015, 109 (92.4%) were still amicrofilaraemic in 2015, and the remaining nine (7.6%) became positive in 2017 but, again, with densities below 10,000 mf/mL. Among the 173 microfilaraemic individuals untreated in 2015, 67 (38.7%), 94 (54.3%) and 12 (7.0%) had a reduced, similar and increased density in 2017, respectively. When focusing on those 134 individuals who did not receive ivermectin in 2015 due to *L. loa* microfilarial density >20,000 mf/mL, 83 (61.9%) had a *L. loa* microfilarial density >20,000 mf/mL in 2017. Fifty-one (38.1%) had microfilarial densities below the risk-threshold (Figure 2, Table 4) and received ivermectin without incident. Two individuals (0.03% of ivermectin-treated individuals in 2015) had a microfilarial density slightly over 20,000 mf/mL in 2017 despite treatment: a 75-year old man whose *L. loa* density increased from 7,294 to 23,208 mf/mL and a 45-year old man whose *L. loa* density increased from 8,051 to 20,499 mf/mL.

Two individuals who did not receive ivermectin treatment in 2015 due to pregnancy or bad state of health (14,105 and 12,569 mf/mL, respectively) had a microfilarial density above the threshold 18 months later (28,999 and 29,109 mf/mL, respectively).

## Discussion

The TaNT campaign conducted in 2015 in the Okola health district was successful in the sense that more than 15,000 inhabitants of an area co-endemic for both onchocerciasis and loiasis were treated with ivermectin without SAEs. A second round of TaNT, with systematic testing for *L. loa* microfilaraemia, was initially planned to take place one year later to concur with the usual schedule of repeated community treatments with ivermectin against onchocerciasis. However, field activities were delayed by six months because of logistical reasons, and the second round was therefore conducted 18 months after the first.

Assessment of *L. loa* microfilarial density during this second round showed an impressive impact of the first large-scale treatment on the microfilarial reservoir of *L. loa*. The dramatic drop in *L. loa* microfilarial density was expected to some degree, based on data from a recent literature review and meta-analysis of the effect of ivermectin on *L. loa* mf up to one year after treatment.<sup>9</sup> The present study, however, demonstrates that ivermectin's effect is still highly significant 18 months after the initial dose. A similar effect was seen in a community trial conducted in an area neighboring the Okola health district<sup>11</sup> with *L. loa* microfilarial reduction rates one year after ivermectin comparable to those measured in the present study 18 months after ivermectin distribution (Appendix, page 1).

The major finding of the present study is that 99.97% of individuals treated with ivermectin in 2015 had a *L. loa* microfilarial density below the exclusion threshold of 20,000 mf/mL 18 months later. Together with the observations that no individuals with less than 20,000 mf/mL treated with ivermectin as part of all previous trials<sup>9</sup> [N=238 individuals] presented with a higher microfilarial count one year later, these findings suggest that individuals treated once with ivermectin could be safely retreated within 18 months without re-testing. Although two individuals in the current study with microfilarial densities below 20,000 mf/mL in 2015 had densities slightly above this level in 2017, it is important to

recognize that the LoaScope threshold was deliberately chosen to be extremely conservative based on the currently available SAE data<sup>7,12-15</sup> documenting post-ivermectin neurological adverse events only in individuals with  $\geq 50,000$  mf/mL. Thus, the two individuals whose counts increased slightly above 20,000 mf/mL in 2017 likely could have been safely treated with ivermectin without re-testing.

The present study is unique in its scale and documentation of the stability/variability of *L. loa* microfilarial density over time. *L. loa* microfilarial density is generally considered to be very stable over time in untreated individuals.<sup>16-18</sup> This general trend was confirmed in the present study in which approximately 70% of the participants (including amicrofilaraemic and microfilaraemic individuals) who did not receive ivermectin in 2015 had similar microfilarial densities 18 months later (i.e. remained in the same category in the transition matrix - see Table 3). Despite this, our data suggest that re-testing of individuals who did not receive ivermectin in the prior round is important, since 51/134 (38.1%) individuals excluded from ivermectin treatment in 2015 because of high microfilarial densities had  $\leq 20,000$  mf/mL in 2017 and could receive ivermectin and two individuals, whose microfilarial density was below the threshold in 2015 but who were excluded because of pregnancy or bad health, had microfilarial densities that precluded ivermectin treatment in 2017. Among the ivermectin-treated microfilaraemic individuals in 2015, most people had decreased counts (82.4%) in 2017; only 3.8% moved into a higher category in the transition matrix and only two had counts  $>20,000$  mf/mL as described above (see Table 2). Potential reasons for the variability in microfilarial densities include: (a) the diurnal periodicity of *L. loa* mf in the blood and resultant variation depending on the exact time of the blood draws, (b) acquisition or attrition of fertile female worms, and (c) alterations in host response following ivermectin treatment.<sup>19</sup>

The main limitation of this study was the relatively low number of individuals (only 43.2%) who could be definitively matched between the 2015 and 2017 campaigns. The main

source of the problem was uncertainty surrounding the identity of the participants, due to the lack of standardized spelling of names and inaccurate recording of age in the elderly population. In addition, there was a high proportion of individuals who participated in the initial TaNT campaign while visiting neighboring villages (and thus could not be matched on the basis of village in 2017). Although every participant was given an individual card containing their name, phone number, result of *L. loa* microfilarial density assessment, and treatment received, few people brought this with them when they presented for re-treatment in 2017. Despite this limitation, it is unlikely that individuals who actually participated in both rounds but whose records could not be definitively matched had a different response to ivermectin treatment than those who could be matched unequivocally, and the number of actual matched records (N=6,983) was sufficient for statistical power and accuracy around the estimates.

Not having to re-test everyone yearly could have important implications for the costs and practical implementation of the strategy. In a neighboring area of Cameroon, where the TaNT strategy was implemented in a pilot study by local health personnel and community volunteers (with oversight by the research team), the costs were estimated at 9.2 US\$ per person tested. Importantly, it was projected that, under programmatic implementation scenarios, these costs could be reduced to about 5 US\$ per person treated.<sup>20,21</sup> If the participation rate is high during the first round, far fewer people will need to be tested during the subsequent round. Indeed, about 95% of the participants from round N would not have to be tested during round N+1. The extra cost of the TaNT strategy during subsequent years is likely to be much smaller than in the first round. After several years of TaNT, and this duration has to be determined, ivermectin-naïve people could be tested and treated in a central health structure (health area or health district) whereas the rest of the population could be

treated using the classic CDTI strategy. This would lead to dramatic cost savings (e.g. less time spent by health personnel for testing, lower costs for LoaScopes and capillaries).

In addition, previously-treated individuals could be re-treated even in the absence of a “LoaScopist” (the volunteer in charge of testing the *L. loa* mf density with a LoaScope), providing more flexibility in the implementation (how and when people are treated) and possibly an increase in the number of people treated per day. These benefits require that people treated in the prior round can be re-identified easily and reliably. Although this could theoretically be done using the individual card (containing their name, phone number, result of *L. loa* microfilarial density assessment, and treatment received), most people in our study did not present the card during the second campaign. Treatment registers specifically designed for a 5-year follow-up, possibly including an identification key (picture, signature) could be used to improve the liability of current treatment registers used during CDTI campaigns. Alternatively, biometrics (fingerprint or iris scan) could be used, as has been demonstrated for other public health applications.<sup>22</sup>

The populations living in areas in need of an alternative treatment strategy for elimination of onchocerciasis has been estimated, using mathematical projections, to be about 14 million in 2015, with the greatest number of individuals located mainly in the Democratic Republic of the Congo but also in Cameroon, Central African Republic and Gabon.<sup>23</sup> Additional onchocerciasis surveys should be conducted to refine those estimates. In addition, strategies other than TaNT (e.g. selective treatment of onchocerciasis-infected subjects with doxycycline) could be considered in small communities with a very low level of onchocerciasis prevalence.

Assessment of *L. loa* microfilaraemia in an endemic population 18 months after a large-scale treatment with ivermectin showed an impressive reduction in the reservoir of *L. loa* microfilariae with microfilarial counts in all previously treated individuals below the level

previously associated with neurologic SAEs. Based on the results of the current study, we would argue that individuals treated with ivermectin during the preceding 18 months would not have to be retested for *L. loa* microfilaraemia prior to receiving ivermectin. This would lead to huge cost savings and facilitate reaching programmatic goals for elimination of onchocerciasis in areas coendemic for loiasis.

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### **Competing interests**

The authors declare that they have no conflict of interest.

### **Authors' contributions**

Conceived and designed the work: SP, HND, CBC, CM, AK, TN, MB & JK

Conceived the analysis: SP, HND, CBC, AK, TN, MB & JK

Collected the data: SP, HND, CBC, AK, TN, MB & JK

Contributed data or analysis tools: SP, YNE, HD

Performed the analyses: SP, HD

All authors contributed to the preparation of the manuscript.

### **Ethical agreement**



This study was authorized by the National Ethics Committee of Cameroon (ethical clearance n° 2013/11/370/L/CNERSH/SP) and approved by the Division of Operational Research at the Ministry of Health (Administrative authorization n° D30-571/L/MINSANTE/SG/DROS/CRSPE/BBM). All volunteers provided written signed consent (or parental consent in the case of minors) before undergoing blood sampling and again before receiving treatment.

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	General population		Cohort	
	2015	2017	2015	2017
Participants	16,182	18,697		6,983
Sex ratio (M/F)	0.93	1.00		0.97
Age (inter-quartile range)	18 (11–42)	19 (11–40)	17 (10–47)	19 (12–49)
<i>L. loa</i> microfilarial density				
Arithmetic mean	1,465.90 (1,376.30–1,553.49)	928.69 (861.89–995.48)	1,426.62 (1,295.41–1,557.82)	712.64 (612.72–812.57)
Geometric mean of positive counts	2,825.0 (2,660.84–2,999.22)	1,484.72 (1,394.95–1,580.27)	2,550.30 (2,336.56–2,783.59)	1,123.82 (1,017.06–1,241.78)
Contraindication				
None	15,458 (95.5%)	18,098 (96.8%)	6,716 (96.2%)	6,814 (97.6%)
<i>L. loa</i> mf > 20 000 per mL	344 (2.1%)	283 (1.5%)	134 (1.9%)	87 (1.2%)
Pregnancy	165 (1.0%)	250 (1.3%)	61 (0.9%)	57 (0.8%)
Bad state of health	215 (1.4%)	66 (0.4%)	72 (1%)	25 (0.4%)
Treated with ivermectin	15,369	17,994	6,692	6,798
Refusals	89	104	24	16

**Table 1. Participants' characteristics and frequencies of contraindications to ivermectin treatment in 2015 and 2017**

**Table 2: Transition matrix of *L. loa* microfilarial density in individuals treated with ivermectin in 2015**

	<i>L. loa</i> microfilarial density in 2017							Total
	0	1–100	101–500	501– 2,000	2,001– 10,000	10,000– 20,000	>20,000	
0	5,274	11	201	38	7	0	0	5,531
1–100	3	0	0	0	0	0	0	3
101–500	204	0	31	20	6	0	0	261
501–2,000	179	1	54	40	14	0	0	288
2,001–10,000	144	0	74	130	82	2	2	434
10,001–20,000	14	0	9	50	73	7	0	153
20,000–26,000	2	0	1	7	12	0	0	22
Total	5,820	12	370	285	194	9	2	6,692

**Table 3: Transition matrix of *L. loa* microfilarial density in individuals not treated with ivermectin in 2015**

		<i>L. loa</i> microfilarial density in 2017							Total
		0	1–100	101–500	501– 2,000	2,001– 10,000	10,000– 20,000	>20,000	
<i>L. loa</i> microfilarial density in 2015	0	109	0	6	2	1	0	0	118
	1–100	0	0	0	0	0	0	0	0
	101–500	6	0	1	2	3	1	0	13
	501–2,000	1	0	1	2	3	1	0	8
	2,001–10,000	0	0	0	3	8	0	0	11
	10,001–20,000	0	0	0	0	3	2	2	6
	>20,000	2	0	0	3	19	29	81	134
	Total	118	0	8	12	37	33	83	291

**Table 4. Transition matrix of contraindications to ivermectin in the cohort of participants**

		2017				
	Contraindication	None	<i>L. loa</i> mf > 20,000 per mL	Pregnancy	Bad state of health	Total
2015	None	6,645	2	52	17	6,716
	<i>L. loa</i> mf > 20,000 per mL	51	83	0	0	134
	Pregnancy	55	1	5	0	61
	Bad state of health	63	1	0	8	72
	Total	6,814	87	57	25	6,983

# Figures

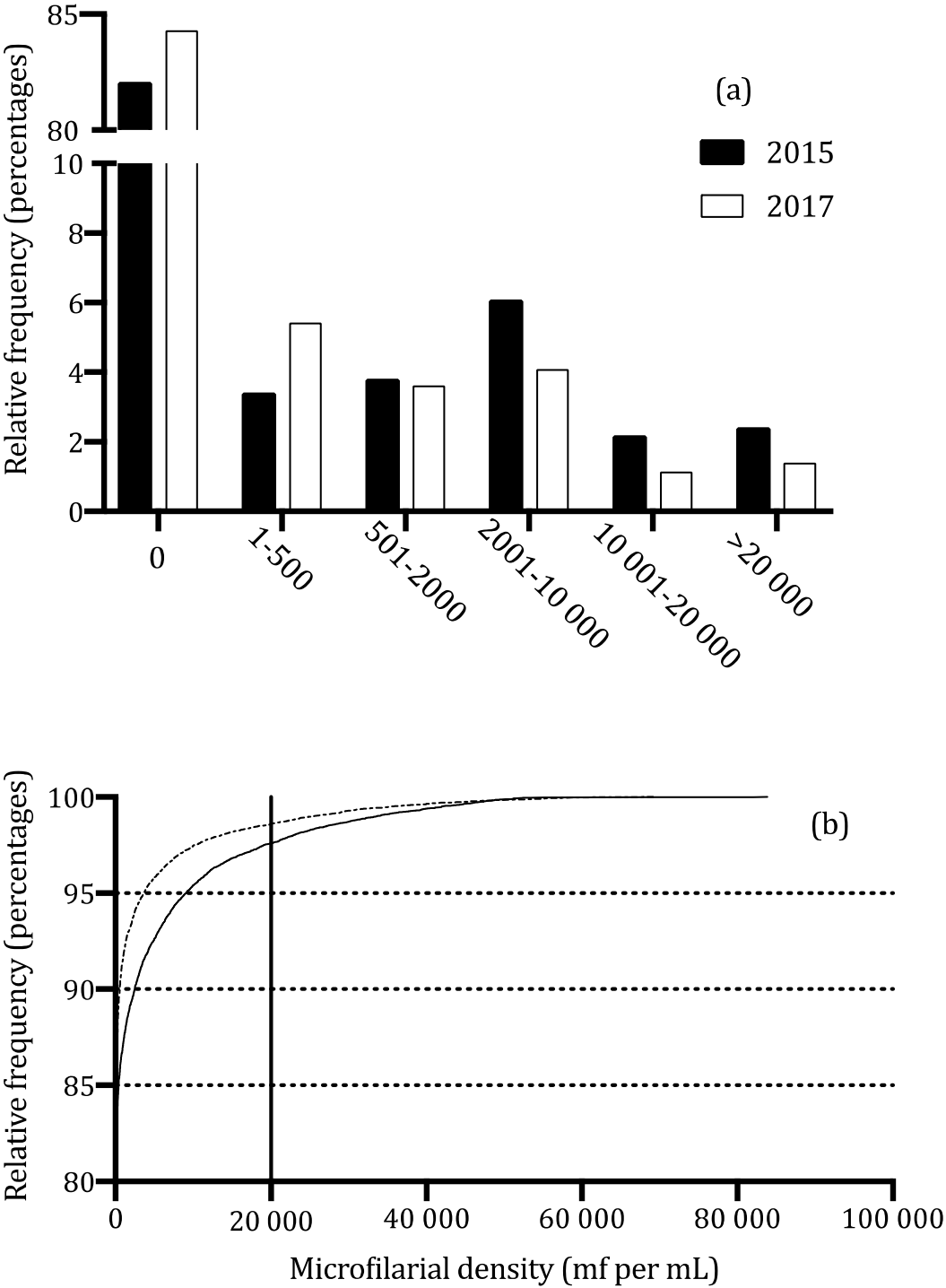
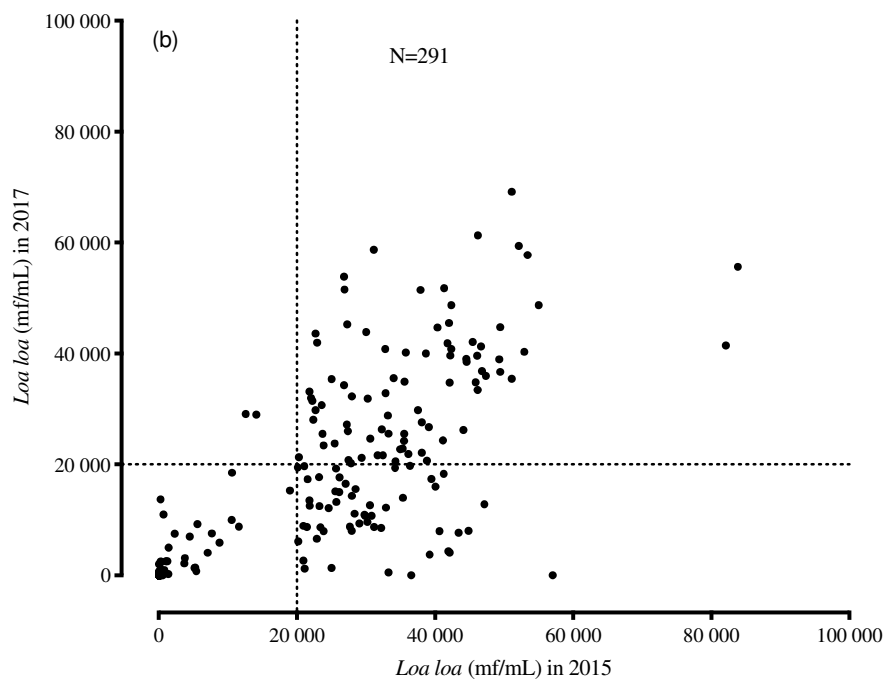
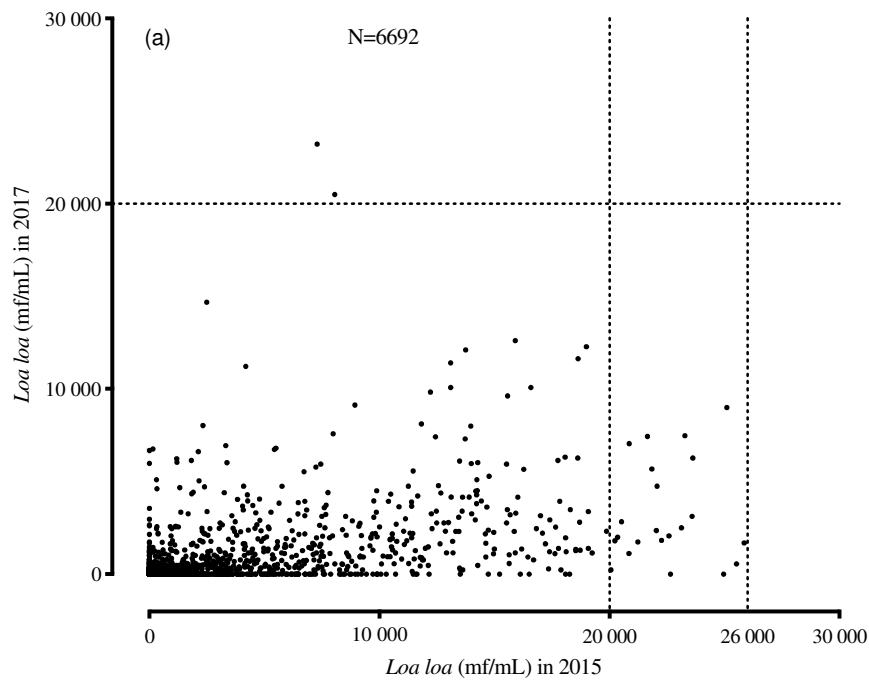


Figure 1. (a) Frequency distribution and (b) cumulative frequency distribution of *L. loa* microfilarial density in 16,182 individuals examined in 2015 (solid line) and in 18,697 individuals examined in 2017 (dotted line)



**Figure 2.** *L. loa* microfilarial density in a cohort of 6,983 individuals examined in 2015 and 2017. (a) Individuals treated with ivermectin in 2015 and (b) Individuals not treated with ivermectin in 2015



## Appendix

Pre-treatment <i>L. loa</i> microfilarial density (mf per mL)	Number of individuals	TaNT			Number of individuals	Gardon et al 1997		
		Arithmetic mean of <i>L. loa</i> microfilarial density (mf per mL)		% Reduction		Arithmetic mean of <i>L. loa</i> microfilarial density (mf per mL)		% Reduction
		Baseline	18 months			Baseline	12 months	
0	5,649	0	18.8	-	39	0	0.5	-
1–100	3	39.0	0	100.0	41	37.6	9.8	74.0
101–500	274	268.1	255.3	30.2	40	267.0	152.5	42.9
501–2,000	296	1,150.0	448.6	66.9	19	1,029.5	91.6	91.1
2,001–10,000	445	4,943.3	1,295.8	75.4	45	4,459.1	916.0	79.5
10,001–20,000	160	13,946.9	3,464.3	79.4	27	14,277.8	2,906.7	79.6

**Table 1. Arithmetic mean of *L. loa* microfilarial density before and after 18 months (present study) and 12 months (Gardon et al 1997) after a first single dose of ivermectin**