

The impact of *Loa loa* microfilaraemia on research subject retention during a whole sporozoite malaria vaccine trial in Equatorial Guinea

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Loa loa microfilariae were found on thick blood smears (TBSs) from 8 of 300 (2.7%) residents of Bioko Island, Equatorial Guinea, during a *Plasmodium falciparum* sporozoite malaria vaccine clinical trial. Only one subject was found to have microfilaraemia on his first exam; parasites were not discovered in the other seven until subsequent TBSs were performed, at times many weeks into the study. All infected individuals were asymptomatic, and were offered treatment with diethylcarbamazine, per national guidelines. *L. loa* microfilaraemia complicated the enrolment or continued participation of these eight trial subjects, and only one was able to complete all study procedures. If ruling out loiasis is deemed to be important during clinical trials, tests that are more sensitive than TBSs should be performed.

Keywords: *Loa loa*, loiasis, malaria vaccine, microfilaraemia, microfilaria

Introduction

The filarial nematode *Loa loa* is common in West and Central Africa, with a reported prevalence of >20% on Bioko Island, Equatorial Guinea.¹ Other than causing Calabar swellings² and eye worm,³ loiasis has generally been regarded as a chronic, largely asymptomatic infection. However, serious cardiac, renal, pulmonary and neurologic complications have been reported⁴ and evidence suggests that high levels of *L. loa* microfilaraemia may be associated with an increase in all-cause mortality.⁵ Loiasis is of particular concern in areas endemic for onchocerciasis and lymphatic filariasis, since ivermectin used in mass treatment programs can cause potentially fatal encephalopathy in people with high levels of *L. loa* microfilaraemia.⁶ The World Health Organization recommends diethylcarbamazine (DEC) as the first-line treatment for loiasis,⁷ but it too can cause severe encephalopathy in patients with hypermicrofilaraemia.^{8,9} We report eight cases of asymptomatic *L. loa* microfilaraemia occurring in adult subjects in a clinical trial of two *Plasmodium falciparum* sporozoite

(PfSPZ) malaria vaccines on Bioko Island, Equatorial Guinea, and the impact this had on the study.

Patients

Giemsa-stained thick blood smears (TBSs) were examined from 300 Equatoguinean residents of Bioko Island ages 6 months–65 y during a clinical trial of two malaria vaccines,¹⁰ the radiation-attenuated PfSPZ vaccine^{10–14} and the chemo-attenuated PfSPZ-CVac (PfSPZ chemoprophylaxis vaccine).¹⁵ Written informed consent was obtained from all study participants. Venous blood for TBSs was drawn at screening visits prior to enrolment in the trial and 5–20 additional times (depending on study group) during the subsequent vaccination and follow-up period in order to assess for malaria parasitaemia. Blood samples were generally drawn between 10:00 h and 14:00 h, which corresponds to the period of highest sensitivity for *L. loa* microfilaria detection.⁹ *L. loa* microfilariae were found in 8 of 300 (2.7%) subjects tested (0 of 152

Table 1. Characteristics and outcomes of eight cases of asymptomatic *L. loa* microfilaraemia in a malaria vaccine trial in Equatorial Guinea.

Patient number	Gender	Age (years)	Number of smears before microfilariae detected	Microfilariae/mL at time of initial detection	qPCR at time of initial microscopic detection	Eosinophil count at time of initial detection ($\times 10^3/\text{mm}^3$)	Treatment	Eosinophil count post-treatment ($\times 10^3/\text{mm}^3$)	Outcome
1	M	21	2	2500	Positive	0.81	DEC $\times 2$	–	Cleared microfilariae
2	M	61	3	3000	Positive	1.93	DEC	0.46	Cleared microfilariae
3	M	30	5	3000	Positive	1.21	DEC	0.42	Cleared microfilariae
4	M	25	1	15000	ND	0.83	DEC	–	Cleared microfilariae
5	M	23	2	1500	ND	1.36	None	–	Lost to follow-up
6	M	21	14	1500	ND	1.15	None (DEC not taken)	–	Cleared microfilariae without treatment
7	F	50	3	6000	Negative	0.48	DEC $\times 2$	0.16	Cleared microfilariae, then recurred and was re-treated
8	M	24	6	500	ND	1.1	DEC	0.64	Cleared microfilariae, continued in trial

ND: not done.

Eosinophilia was defined as an eosinophil count $>0.5 \times 10^3/\text{mm}^3$ in peripheral blood.

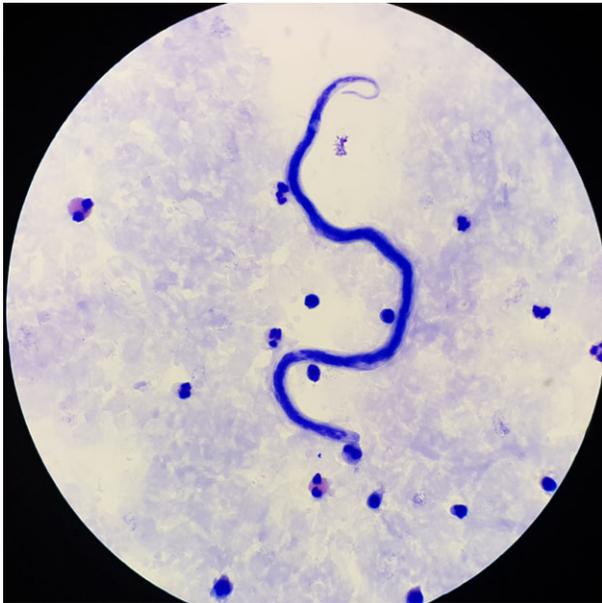


Figure 1. *L. loa* microfilaria seen on light microscopy of a TBS of one of the study subjects.

subjects ages 6 months–17 y and 8 of 148 subjects ages 18–65 y). Details of the eight cases are displayed in Table 1. All individuals with *L. loa* microfilaraemia were adults; there were seven males and one female, ranging in age from 21 to 61 y (median 24.5). All were asymptomatic and had unremarkable physical exams. Microfilariae (see Figure 1) were detected in one of the affected individuals (patient 4) on his first TBS, but were not

seen in the other seven until their second (n=2), third (n=2), fifth (n=1), sixth (n=1) or fourteenth (n=1) (median 3) blood smears, 2–35 weeks (median 11.5) after the initial negative smear.

Eosinophil counts were measured with a Pentra C+ haematology analyser at the point of initial detection of microfilariae. Eosinophilia (defined as a peripheral blood eosinophil count $>0.5 \times 10^3/\text{mm}^3$) was seen in seven of eight of the affected subjects at the time of diagnosis, with counts ranging from 0.48 to $1.93 \times 10^3/\text{mm}^3$ (median $1.13 \times 10^3/\text{mm}^3$). By comparison, the eosinophil count for the 124 subjects enrolled in the study but not diagnosed with loiasis ranged from 0.09 to $4.54 \times 10^3/\text{mm}^3$ (median $0.49 \times 10^3/\text{mm}^3$) ($p=0.011$ by Wilcoxon rank sum test). Creatinine levels were determined with a COBAS Integra 400 plus analyser at the time of the first positive TBS. All creatinine levels were normal (mean = 0.81 mg/dL) and electrocardiograms were unremarkable.

The eight individuals were referred to the National Program for Onchocerciasis and Other Filariasis Control, where the diagnosis of loiasis was confirmed by repeat blood smear. The program prescribed a 21-d course of self-administered oral DEC, as per national guidelines; 12.5 mg on day 1, 25 mg on day 2, 50 mg on day 3, 50 mg twice daily on day 4 and 50 mg three times daily on days 5–21. Six of eight subjects reportedly completed treatment. Follow-up TBSs were free of microfilariae in five of these subjects (patients 2, 3, 4, 7 and 8) and the sixth cleared microfilariae after a second 21-d course of DEC (patient 1). One of these subjects (patient 7) completed treatment, was cleared of microfilariae and subsequently had a microfilaria-positive TBS. She was prescribed a second course of DEC. No known adverse effects of DEC were seen or reported. Eosinophil counts performed 4–10 weeks (median 7.5) after treatment were reduced to normal in three of the four subjects who were tested.

A pair of experienced microscopists on the research team retrospectively performed microfilaria counts on all eight of the TBS slides in which *L. loa* was first detected. In brief, 10 μ L of blood collected in ethylenediaminetetraacetic acid was placed on a 10-mm \times 20-mm rectangle on a glass slide, dried and Giemsa stained and 2 μ L of blood were examined (five to six passes across the width of the rectangle depending on the objective field number). Microfilaria counts ranged from 500 to 15 000 microfilaria/mL (Table 1). After conclusion of the trial, blood samples from the time of initial microscopic detection of microfilariae in four of the eight patients were also analysed for *L. loa* DNA by a published quantitative polymerase chain reaction (qPCR) assay.¹⁶ *L. loa* DNA was found in blood samples from patients 1, 2 and 3, but not in patient 7 (Table 1).

The discovery of *L. loa* microfilaraemia in these otherwise healthy individuals had a significant impact on the conduct of the clinical trial and the retention of participants. The study protocol specified that all subjects be microscopically free from helminth infections at the time of enrolment. The protocol also mandated treatment of parasitic diseases in accordance with national guidelines, after which subjects could be allowed to continue in the study at the discretion of the investigators. Two subjects (patients 4 and 5) were found to have microfilaraemia before they were enrolled in the trial. They were prescribed DEC and given follow-up. Patient 4 completed treatment and cleared his microfilariae, but patient 5 never began treatment and was lost to follow-up. The remaining subjects had their first positive TBS for *L. loa* weeks into the trial, in the midst of a fixed study schedule. Three subjects (patients 1–3) who had received one or two doses of malaria vaccine prior to their diagnosis of loiasis were excluded from further vaccination since they were taking DEC at the time they were scheduled to be vaccinated. One subject (patient 6) was prescribed DEC, had a total of nine subsequent TBSs that were negative, received his final vaccine dose and later reported that he had never taken his treatment. Patient 7, whose vaccination was delayed due to treatment of microfilaraemia, eventually developed a separate medical condition that caused her to be withdrawn from the trial. In all, only one of the six enrolled adults diagnosed with *L. loa* was treated, cleared of microfilariae and able to participate in all remaining trial procedures (patient 8). Losing five subjects amounted to a significant reduction in sample size, as a total of 65 adults were enrolled in the trial.

Discussion

Eight (2.7%) of the 300 individuals who had TBSs performed during this vaccine trial¹⁰ were found to have *L. loa* microfilaraemia. Results were confirmed by a *L. loa*-specific qPCR in three of the four patients whose samples were retrospectively tested. The prevalence of loiasis on Bioko Island has previously been reported to be >20%,¹ based on self-reported histories of ever having had eyeworm. This method may have overestimated the current prevalence of *L. loa* infection,¹⁷ particularly since mass administration of ivermectin for onchocerciasis control has occurred on Bioko Island since 1989.¹⁸ The microfilaricidal effects of ivermectin on *L. loa* are well established¹⁹ and the prevalence of *L. loa* microfilaraemia has been shown to decrease markedly in regions where ivermectin mass administration has been employed.^{19,20}

Assessing the prevalence of loiasis is complicated by the fact that many individuals who harbour adult worms are amicrofilaraemic²¹ and that some care is required to accurately distinguish *L. loa* from *Mansonella perstans* microfilariae by microscopy.²² Of interest, a recent cross-sectional study of 243 residents of Bioko Island found the prevalence of *L. loa* microfilaraemia to be 0.7% and *M. perstans* to be 8.8% by qPCR of whole blood.²³

From a clinical standpoint, there was nothing extraordinary about the eight cases of *L. loa* microfilaraemia reported here. All were asymptomatic at the time of diagnosis, which is common among residents of endemic areas.²⁴ A single course of DEC led to sustained clearance of microfilariae in four of six patients who were treated; one patient required a second course of DEC for persistent parasitaemia and another was re-treated when microfilaraemia recurred several weeks after demonstrated clearance. This is consistent with other reports.^{25,26} Typically, all patients had eosinophilia, which resolved in most cases with anti-filarial treatment.^{26,27} Although treatment with DEC has been associated with adverse reactions such as pruritus, Calabar swelling, dizziness, headache, arthralgias, fever and even encephalopathy,^{8,9,25,27,28} it was well tolerated in our patients.

What makes these cases relevant is their impact on the malaria vaccine trial. With respect to recruitment of participants, the protocol prescribed that all subjects be free from helminth infections at enrolment, as determined by microscopic examination of TBSs. This approach was taken due to concerns about a potential negative effect of these infections on vaccine-induced protective immunity.²⁹ Because only one of the eight affected individuals was able to complete this trial, we could not assess the impact of *L. loa* microfilaraemia on the immune response to the vaccines studied. Still, it is clear that performing TBSs during screening visits was not adequate to rule out loiasis in this population. Only 1 of the 300 (0.3%) Equatoguinean potential participants had microfilariae on their initial TBS during screening. Another 7 of 300 (2.3%) were not found to have microfilaraemia until weeks later. In fact, a median of three negative TBSs were recorded among subjects who eventually displayed microfilaraemia.

It is known that the sensitivity of standard Giemsa-stained TBSs to detect loiasis is low; less than half of individuals who harbour adult worms have detectable microfilaraemia.²¹ Antibody tests could be used, but their inability to distinguish past from current infections³⁰ would be likely to inflate the number of suspected active *L. loa* cases. Quantitative PCR has been shown to be more sensitive than microscopy for the detection of *L. loa* microfilariae^{16,23} and is a technology that is increasingly available in the field. In fact, in the current study, qPCR was used in tandem with microscopy to diagnose *P. falciparum* infection. In response to the detection of microfilariae in study subjects, after the trial, qPCR assays for *L. loa* and *M. perstans* were also established at the research site.

In summary, screening TBSs were likely inadequate to assess the true number of participants with loiasis in this study. If ruling out infection with *L. loa* is desired in early phase studies of vaccines against malaria and other pathogens, a more sensitive test such as qPCR could be done during initial screening to decrease the risk of enrolling individuals with occult loiasis that might later become patent by TBS.

An argument can be made against the need to detect and treat helminths prior to clinical trial enrolment in areas where they are endemic; the logic being that the drug or vaccine under investigation should be able to pass muster in a population where these parasitic infections are common. This may be a reasonable approach in larger later-phase trials, where randomization should balance the number of infected subjects in the comparison groups. In fact, TBS positivity for *L. loa* was removed as an exclusion criterion in a subsequent malaria vaccine trial conducted in the same population. In the current study, the incidental appearance of *L. loa* microfilariae on enrolled participants' TBSs caused considerable disruption of the trial schedule. These subjects' participation was effectively suspended for a minimum of 3 weeks while their diagnosis was confirmed, treatment provided and clearance of microfilariae verified. Four of the participants with loiasis were taking DEC at the time their vaccinations were scheduled; three were withdrawn from the trial and vaccination was delayed in the fourth. Some consideration was given to delaying treatment for asymptomatic loiasis until trial procedures were performed, but given this parasite's potential for significant morbidity and increased mortality,^{4,5,9} this approach was rejected. An alternative would have been to provide treatment without suspending trial procedures, although it would be important to ascertain that ongoing trial participation did not interfere with successful cure.

One issue that bears mentioning pertains to the safe treatment of loiasis. While this parasite has the potential to cause serious disease and should be treated, treatment is not without risk. Not all of the medical and laboratory personnel involved with the trial were initially familiar with this pathogen. As such, all microscopic diagnoses of *L. loa* microfilaraemia were confirmed by qualified personnel in the Equatoguinean National Program for Onchocerciasis and Other Filariasis Control. Treatment with DEC in accordance with national guidelines was offered to each patient and prescribed by National Program staff. Still, a later re-examination of the TBS slides in which *L. loa* was first detected showed microfilaria counts >2500/mL in four of the eight subjects with loiasis. Pretreatment with albendazole to decrease microfilarial load should have been considered in these patients prior to administration of DEC, given the risk of encephalopathy.^{8,9,31} Fortunately, none of the subjects treated for *L. loa* microfilaraemia experienced any adverse effects from DEC. Of note, although individuals with demonstrated *L. loa* microfilaraemia could not be enrolled and/or continue in the trial without evidence of parasitological cure (as was the case with other helminth infections), they were not compelled to accept treatment. In fact, two of the eight individuals diagnosed with loiasis opted to forgo treatment, although one of them initially claimed to have taken his DEC, then later confessed that he had not.

Conclusions

Although these eight cases of *L. loa* microfilaraemia were asymptomatic and generally responded well to treatment with DEC when it was accepted, what is relevant is the impact that this parasite had on the malaria vaccine trial. Based on published literature, loiasis could not reliably be ruled out by TBS during screening of participants. The incidental appearance of microfilariae on

subsequent blood smears, weeks into the trial, led to the eventual exclusion of most affected participants, reducing the overall sample size. If *L. loa* microfilaraemia is deemed to be incompatible with clinical trial participation, a more sensitive method of diagnosis, such as qPCR, should be utilized.

Authors' contributions: SLH, SA, TLR and CD conceived and designed the malaria vaccine trial. SLH, SA, TLR, CD, AO, VUN, AM, MM, EN, TS, JR, MEOM, BENP, SRM, PFB and LWPC were responsible for study implementation. RN provided confirmation of microfilaraemia and prescribed treatment for participants with loiasis. PR was responsible for statistical analysis. SRM was responsible for writing the original draft. SLH, SA, TLR, CD, TS, LWPC and SRM were responsible for review and editing the article. All authors have read and agreed to the published version of the article. SRM and SLH are guarantors of the paper.

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Competing interests: SLH, TLR, PFB, PR and LWPC are employed by Sanaria Inc., which developed the PfSPZ vaccine and PfSPZ-CVac. SRM was employed by Sanaria Inc. at the time of the clinical trial but was not at the time the manuscript was prepared and submitted. AO was the recipient of a UK Medical Research Council African Research Leader award for work unrelated to this manuscript. All other authors declare no competing interests.

Ethical approval: The study was approved by the Comité Ético Nacional de Guinea Equatorial, additionally reviewed by the MaGil Institutional Review Board in Rockville, MD, the Ifakara Health Institute Institutional Review Board in Tanzania and the Ethics Committee of Northwestern and Central Switzerland.

Data availability: The data underlying this article are available in this published article.

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