

Article

Triple-drug mass drug administration is effective for lymphatic filariasis microfilaria clearance in Samoa

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Abstract: Following the first triple-drug MDA for lymphatic filariasis in Samoa in 2018, unexpected persistence of microfilaria (Mf) positivity in 18 (15%) of 121 antigen-positive persons was observed in a nationwide household survey 1-2 months later, raising concerns about MDA efficacy. In 2019, a monitored treatment study was done in 2019 before and 7 days after directly observed weight-based treatment. Mf presence and density were evaluated using 1 mL membrane filtered venous blood, and 60uL thick blood films on slides prepared from venous or fingerprick blood. All 14 participants were still Mf positive on filters from venous blood pre-treatment samples, but two were negative by slide made from the same samples. Mf were cleared completely by day 7 in 12 of 13 participants followed up, and by day 30 in the remaining participant. Filtered blood using EDTA samples (to reduce clumping of Mf) is preferred over slides alone for improving the likelihood of detecting Mf and estimating their density. The triple-drug MDA strategy was effective at clearing Mf by day 30 when given and taken at the correct dose.

Keywords: Lymphatic filariasis, Samoa, microfilaria, DEC, albendazole, ivermectin

1. Introduction

Lymphatic filariasis (LF) is a helminth worm transmitted by mosquitoes. The adult worms live in the lymphatic system, potentially causing chronic disability due to limb and scrotal swelling. A mated pair of adult worms lives for 5 to 7 years and can produce millions of microfilariae (Mf) that circulate in peripheral blood. In 2000, it was estimated that 199 million people worldwide were infected with LF (1). The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has conducted an intensive mass drug administration (MDA) programme since 2000 to interrupt transmission of LF (2), and the burden of infection is estimated to have been reduced by almost 75% to 51 million infections in 2018 (1). By 2020, 7.7 billion MDA treatments had been delivered and 17 countries or territories (eight of which are in the Pacific region (3)) have been validated as having eliminated LF as a public health problem (2, 4).

However, some countries, especially those with efficient *Aedes* vectors such as Samoa and American Samoa, are experiencing challenges including stagnation of progress, delay in MDA initiation, persistence of transmission hotspots, failing Transmission Assessment Surveys (TAS) during post-MDA surveillance, and resurgence (increasing LF antigen prevalence) after MDA had stopped (5-8).

For those reasons, in 2017 the GPELF recommended introduction of triple-drug MDA using ivermectin, diethylcarbamazine (DEC), and albendazole (IDA) to address some of these obstacles (9). The triple-drug regimen is expected to rapidly clear all Mf within one week of treatment (10) and partially sterilize the adult worms, although Mf may reappear in a small proportion of treated people one or two years later (11, 12).

The Pacific region has a long history of high LF prevalence and MDA interventions. Before GPELF, Samoa had conducted eight rounds of MDA with DEC since 1965, followed by two rounds of DEC plus ivermectin in the 1990s (13). Samoa was also the first country to initiate MDA with DEC and albendazole in 1999 at the beginning of the Pacific Programme to Eliminate LF (PacELF) before the official start of GPELF in 2000 (14). Under PacELF, Samoa conducted five nationwide MDA rounds between 1999 and 2003, but did not achieve the recommended threshold in a community cluster survey of all ages in 2004 (15), so a further three nationwide rounds of two-drug MDA were distributed in 2006, 2008 and 2011. After failing a TAS in one evaluation unit in 2013, two further targeted rounds of MDA were distributed in that area only in 2015 and 2017, but all three evaluation units in Samoa failed TAS-2 later in 2017. Due to apparently resurging antigen prevalence back to 1999 levels, the country initiated triple-drug MDA with the first nationwide distribution successfully completed in August 2018 (16).

A large population representative LF community survey as part of the Surveillance and Monitoring for Elimination of LF and Scabies in Samoa (SaMELFS) project was conducted in Oct-Nov 2018, 8-11 weeks after the August 2018 MDA, and showed antigen prevalence of 4.0% (95% CI 2.8–5.6%) in 3940 participants aged 5 years and over (8). The MDA coverage was reported to be very good with 80.2% of the total population reported taking MDA (16). Amongst the 121 participants who were antigen-positive and had slides available (8), 18 were Mf-positive. Surprisingly, 14 of the 18 Mf-positive persons reported taking the IDA pills in 2018, raising concerns about the effectiveness of IDA in Samoa. The estimated post-MDA Mf prevalence of 0.6% (0.3–1.0%) nationwide and 1.7% (0.7–4.1%) in known hotspots (8) also caused concern that the IDA might not be as effective as expected in Samoa.

This “monitored treatment” study is a preliminary investigation into the reason(s) for persistent Mf in Ag-positive people, the majority of whom reported taking IDA in Samoa in 2018. The primary objective of the study was to assess the effectiveness of appropriately dosed IDA in clearing Mf from Mf-positive people identified in the Oct-Nov 2018 survey. We revisited these Mf-positive persons in March-April 2019, i.e. 5-6 months after the previous survey. This study aims to exclude three hypotheses as the potential reasons for Mf persistence: (i) inadequate dosage due to incorrect weighing or administration of pills, (ii) incorrect reporting about participation, i.e. non-compliance or partial compliance in taking pills, and (iii) drug resistance in the local filarial worm population.

2. Materials and Methods

Study setting

Samoa is an independent country in the South Pacific (population ~200,000 in 2018), consisting of two main tropical islands, Upolu and Savai'i, and a number of sparsely populated smaller adjacent islands. Samoa is divided into four administrative regions, with ~19% of the population living in Apia Urban Area (AUA), 35% in Northwest Upolu (NWU), 23% in Rest of Upolu (ROU), and 22% in Savai'i (SAV) (17). The larger islands are made up of tropical forests, mountains, valleys, wetlands, and fringing reefs, and the majority of the population live in small coastal villages in a rural setting.

Study design

We revisited Mf-positive persons in March-April 2019, i.e. 5-6 months after the 2018 survey, to provide treatment with one dose of IDA. We assessed the effectiveness of IDA in clearing Mf by comparing Mf presence and density at baseline (pre-treatment) and after directly-observed treatment using recommended weight-based dosages of all three medications. We collected blood samples at baseline, and at 3 hours and 7 days after treatment. The plasma sample at 3 hours post-treatment was collected to allow for pharmacokinetic studies (plasma concentrations of ivermectin, DEC and albendazole) to determine whether the recommended dosages were sufficient for achieving effective plasma concentrations. If Mf were not cleared by 7 days post-treatment, repeat samples were collected until Mf clearance was achieved.

Ethics approval and consent

Ethics approvals were granted by the Samoan Ministry of Health and The Australian National University Human Research Ethics Committee (protocol 2018/341). The study was conducted in close collaboration with the Samoa Ministry of Health, the WHO country office in Samoa, and the Samoa Red Cross.

Treatment of Mf-positive persons

Each person was weighed by a doctor or nurse using medical-grade scales. Treatment with ivermectin (150-200 ug/kg, Merck), DEC (6 mg/kg, Eisai), and albendazole (one 400mg tablet) was provided according to weight as per the Samoa Ministry of Health 2018 MDA schedule (16) shown in **Table 1**. Medications were donated for the triple drug MDA in 2018. Treatment was directly observed by study team members.

Table 1: Weight-based dosing schedule for triple-drug MDA in Samoa, 2018.

Weight range (kg)	Number of ivermectin tablets (3mg)	Number of DEC tablets (100mg)	Number of albendazole tablets (400mg)	Total number of tablets
<15 kg (or 2-4 years old)	0	1	1	2
15-23 kg	1	1	1	3
24-38 kg	2	2	1	5
39-53 kg	3	3	1	7
54-68 kg	4	4	1	9
69-83 kg	5	5	1	11
84-98 kg	6	6	1	13
99-124 kg	7	7	1	15
>124kg	8	8	1	17

Source: Samoa Ministry of Health

Blood samples and laboratory methods

Detection of filarial Mf on filtered blood was first described by Wylie in 1970 (18) for veterinary parasites, with modified methods proposed by Chularerk and Desowitz in 1970 (19) and by Dickerson et al for polycarbonate filters in 1990 (20). The method has been used extensively in the Pacific region including in Tonga (21), Fiji (22), Samoa (22) and PNG (23), including most recently in clinical trials of safety and efficacy of the triple-drug MDA regime (10). Previous studies have usually used blood with EDTA or citrate as anticoagulant, although heparin with Teepol detergent was used in one study in Tonga (19).

A recent systematic review attempted to standardize the diagnostic methods used for assessing Mf prevalence in blood samples collected from participants (24). The methods evaluated were counting chamber (>50 uL blood); membrane filtration (1 mL blood); Knotts technique (1 mL); and slides made

with ≥ 40 uL of blood. Estimated Mf prevalence in the persons concurrently sampled was 2.39 (95% CI 1.62-3.53) times higher when using membrane filtration and 1.37 (95%CI 0.81-2.30) times higher when using slides with ≥ 40 uL blood (24), compared to 20 uL blood slides. Previous studies in Haiti with *W. bancrofti* have observed higher Mf densities on slides (prepared using 20 uL fingerprick capillary whole blood) than in concurrent filtered venous samples (25, 26). Therefore, we decided also to evaluate slides made from venous and fingerprick blood in order to investigate the validity of this finding and maximize the sensitivity of the study for detecting Mf clearance.

Based on the above findings from previous studies, we used both membrane filters and 60 uL three-line slides to evaluate Mf presence and density. Plasma samples were prepared from the venous blood and stored for future pharmacokinetic studies if required.

After consulting recent users of the membrane filtration technique, we chose initially to use heparinized blood samples. Although heparin is known to cause clumping of leucocytes and Mf in blood samples (27), its advantage is that the same blood sample can be used for the Alere Filariasis Test Strip (FTS) antigen test, for which EDTA or citrate blood is not recommended. We decided to also test venous samples in EDTA for practical reasons that evolved as the study progressed.

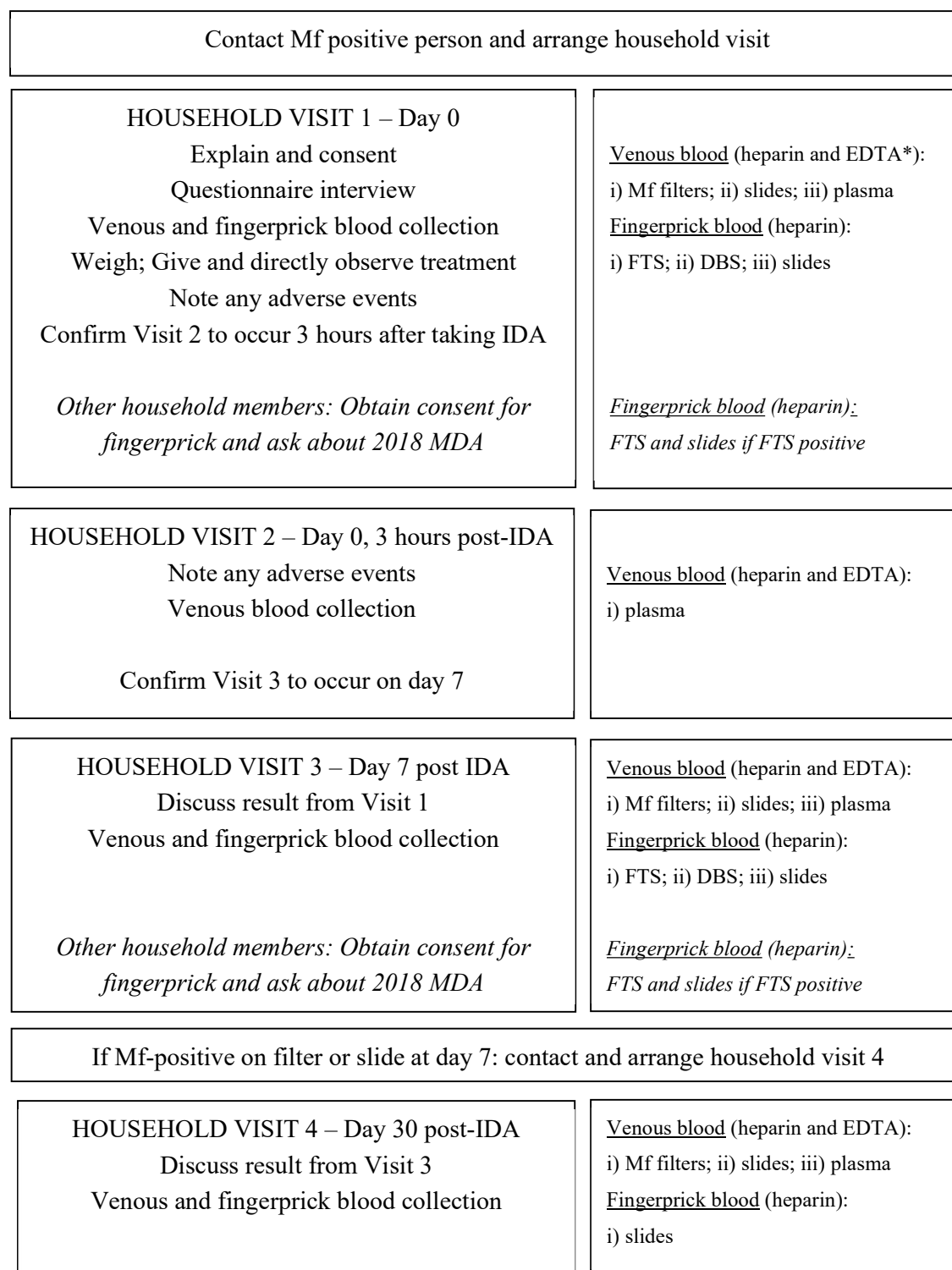
Samples taken from study participants included venous blood in 10 ml lithium heparin and EDTA Vacutainers (BD, Macquarie Park, NSW, Australia) and finger-prick blood (using contact-activated spring-loaded high blood flow 1.5 x 2mm lancets BD cat 366594) in lithium heparin Microtainers (BD cat 365965). Blood samples were collected in both heparin and EDTA where possible, as well as fingerprick samples in heparin Microtainers for the FTS tests and to compare Mf counts from venous and capillary blood.

With heparin, clumping was very marked and occurred at both macro and micro levels (**Supplementary Fig 1**), making counting on high density Mf samples very difficult (**Supplementary Fig 2**). The clumping was minimized with EDTA, making the filters much clearer and Mf easier to count. Thereafter, we collected blood in both heparin and EDTA tubes for the filters where possible, and took both (plus slides) into account in determining Mf presence. To enable direct comparisons of Mf counts over time for each individual, these were assessed on samples taken with the same anticoagulant (with one exception due to sample availability).

Household members of the study participants were also interviewed about MDA participation in 2018, and with their consent, were tested by FTS on fingerprick blood samples. If Ag-positive, slides were prepared, stained and examined for Mf in Samoa.

All blood was kept cool in insulated containers during transport to the field laboratory. A flow chart illustrating the schedule of collections is given in **Figure 1** and summarized below:

Figure 1: Flowchart of follow up of Mf positive people and their household members in the Monitored Treatment study, Samoa 2019



* EDTA filters were done starting with participant ID 10; obtained for 2 of 14 participants at visit 1

- **Venous blood** samples in heparin or EDTA were used on the same day of collection or (rarely) stored at 4 deg C until the next morning. They were used for:
 - i) membrane filters
 - ii) three-line thick blood films on slides (60uL)
 - iii) collection of plasma (for potential pharmacokinetic assays) by centrifuging the remaining sample at >1000g for 10 mins. Plasma samples were frozen in a domestic freezer initially and transferred weekly for storage at minus 70 deg C.
- **Fingerprick blood** samples were used on the day of collection or the next day after storage at 4 deg C to:
 - i) test for circulating filarial antigen (Ag) using Alere Filariasis Test Strips (FTS),
 - ii) prepare dried blood spots on TropBio filter papers (Cellabs) for future testing for anti-filarial antibodies (Ab), and
 - iii) prepare three-line thick blood films on slides (60 uL per slide)

Procedure for membrane filters

The venous blood samples were handled and prepared for Mf detection using procedures derived from previous studies (10, 20). Briefly, we used Millipore Isopore 25 mm polycarbonate filter discs and Swinnex EMD 25mm filter holders (Merck, Brisbane, QLD, Australia). After assembling the filter, 10 mL of distilled or bottled water was passed through the filter assembly using a 10mL syringe. Venous blood samples in Vacutainers were mixed thoroughly by inverting four to five times, then 1 mL of blood was drawn up into a 1mL syringe using a 20G needle and gently passed through the filter. Immediately afterwards, two lots of 10mL of distilled water were passed through the filter to lyse the red blood cells, followed by 10mL of air to dry the filter. The filter was removed from the assembly using tweezers, and placed face up on a microscope slide to dry before staining with Giemsa.

Second filters were collected for potential future sequencing of Mf from each venous sample where possible (heparin and EDTA). The procedure was the same as preparing filters for staining except that after lysing the blood with water, 1 x 1mL of RNALater solution (ThermoFisher, Townsville, Qld) was passed through the filter, followed by 10 mL of air. Filters were placed using tweezers into Eppendorf tubes which were filled to the brim with RNALater and stored at -70 deg C.

Filariasis Test Strips

The Alere Filariasis Test Strip (FTS) (Scarborough, ME, USA) was used to detect circulating filarial Ag. Using a micropipette, 75µl of heparinized blood was placed onto the FTS, and assessed after 10 minutes per manufacturer's instructions. If sufficient blood was available, positive tests were repeated to confirm the result.

Blood slides

For all blood samples with a positive FTS result, thick blood slides were prepared according to WHO guidelines as previously described (8). Three 20µL lines of blood were placed on to a single slide using a pipettor, and up to three slides were prepared if sufficient blood was available. After 72 hours of drying time, slides were dehaemoglobinised in water for 10-15 minutes, carefully removed and dried. One slide from each family member was stained and examined immediately; the remainder of the slides were stored and shipped to Australia.

Staining of filters and slides

Filters were allowed to dry overnight and slides were dried for 3 days. All were protected from ants while drying. Slides were dehaemoglobinised in bottled water for 15 minutes, removed gently then allowed to air dry thoroughly. Filters and slides were stained with 2% Giemsa (VWR Giemsa Stain

Improved R66 Gurr; Bio-Strategy Pty Limited Campbellfield, VIC Australia) in distilled water for 50 minutes, allowed to dry and examined x100 and x400 magnification.

All slides and filters were read independently and blindly by two readers (PG and JS) and the results reported are the average counts. The numbers of Mf on the whole filter or slide were counted.

Statistical analysis

Mf counts were highly skewed and were transformed to logs for statistical comparisons; 1 was added to all counts to allow for 0 counts in log transformations and plots. Log (Mf+1)/mL counts in heparinised blood for different sample types (venous and fingerprick) and specimens (filter or slide) at day 0 were compared using one-way ANOVA with non-independent samples. Median Mf and median log (Mf+1) counts at day 0 were compared using non-parametric K-sample tests and Wilcoxon matched pairs signed rank test. Pearson’s correlation coefficient was estimated for counts from individuals using different sample and specimen types. Analysis was done using STATA 16.

3. Results

Study participants

Of the 18 Mf-positive people identified in 2018 SaMELFS survey, 17 gave information in 2019 (in one case by telephone) about MDA participation, and 14 participants were enrolled in the monitored treatment study involving blood sampling. Four people did not enrol in the monitored treatment and follow-up due to declining to participate (n=1), being overseas (n=1), ineligible due to breastfeeding (n=1) and being unable to schedule a visit (n=1). The 14 full participants lived in 12 different villages (10 in Northwest Upolu (NWU), one in Rest of Upolu, and one in Savai’i). No Mf positive persons were identified in 2018 in Apia Urban Area. The villages of Vaiusu and Leauva’a (both NWU) had two Mf positive participants, on in each of two separate households; the other villages each had one Mf positive participant in one household. Their ages ranged from 5 to 74 years, and there were 8 females and 6 males (Table 2). The range of weights of adult participants (aged 20 to 74) was 67 to 110 kg, with total numbers of tablets given ranging from 9 to 15 according to Table 1. The one 5 year old child weighed 18 kg and was given one tablet each of ivermectin, DEC and albendazole.

At the time of the 2018 survey, each person or their parent/guardian was asked if they were aware of the MDA, eligible for MDA, and whether they were offered it. Then they were asked if they took all the pills, or if not why not. Results for the whole survey were presented by Willis et al (16), and for the current study participants in Table 2. When 17 of the 18 Mf positive persons from 2018 were interviewed and probed in more depth in the current study in 2019 about participation in the 2018 MDA, some of their responses in 2019 differed from their previous reports made at the time of the 2018 survey (Table 2).

Table 2: Participant characteristics and MDA (2018) participation reported in 2018 and 2019.

Participant ID number	Age (years)	Gender	Reported (in 2018) taking 2018 IDA	Reported (in 2019) taking 2018 IDA
1	59	M	Yes	No
2	46	F	No	No
3	50	F	Yes	Yes
4	44	F	No	No
5	60	M	Yes	Yes
6	52	F	Yes	Yes
7	20	F	Yes	No
8	5	F	Yes	Yes
9	44	M	Yes	Incomplete ¹

10	64	F	No	No
11	34	F	Yes	No
12	74	M	Yes	Yes
13	58	M	Yes	Yes
14	50	M	Yes	Yes
No blood samples: interview only or missing in 2019				
15	27	M	Yes	Unknown
16	73	M	Yes	No
17	41	F	No	No
18	31	F	Yes	No

¹Reported that they were given only two types of pills in 2018, and not weighed using scales

In 2018, 14 of the 18 participants answered Yes about MDA participation. In 2019, five of these (3 in the full follow-up group) changed their answers to No, and one other reported that they had not taken all three drugs (**Table 3**). Four of 17 people answered “No” consistently and 7 gave ‘Yes’ answers on both occasions. i.e. 11 of 17 (10 of 14 followed-up) were consistent in their answers between the two surveys.

Table 3: MDA participation reported in 2018 and 2019.

	Participant			Family member			Total
MDA participation 2018, reported in 2018 and 2019	All Mf pos 2018	Ag pos, Mf pos 2018; Interview only 2019	Ag pos, Mf pos 2018; Interview, treatment and follow up 2019	All interviewed and tested 2019	Ag neg, Mf neg 2019	Ag pos, Mf neg 2019	
Yes to Yes	7	0	7	23	19	4	30
No to No	4	1	3	2	1	1	6
Yes to Incomplete	1	0	1	0	0	0	1
Yes to No	5	2	3	1	1	0	6
No to Yes	0	0	0	1	1	0	1
Yes to Unknown	1	1	0				1
TOTAL	18	4	14	27	22	5	45 (44 [1 missing] for 2019)

There were 53 household members of Mf-positive persons who participated in the current study, of whom 27 participated in both the 2018 and 2019 surveys and had MDA participation answers both times. Among these 27 family members, 23 gave consistent Yes answers and two consistent No answers i.e. 25 out of 27 were consistent between the 2018 and 2019 interviews (**Table 3**). One family member changed their answer from Yes to No, while another changed in the opposite direction. In family members who were interviewed both years, 24 of 27 (88.9%) answered Yes in 2018 as did the same proportion in 2019.

Overall, combining participants and family members, MDA participation at the 2018 MDA (Yes) was reported by 38/45 persons in 2018 (84.4%) and 31/44 after second interview in 2019 (70.5%). Taking the 44 people together (17 Mf positives including 14 full study participants and 27 household

members), 33 gave consistent answers (30 Yes-Yes, 6 No-No); 6 changed from Yes to No, one changed from Yes to Incomplete, and one changed from No to Yes (**Table 3**).

Antigen and Mf results at baseline in 2019

All 14 participants were Ag-positive by FTS at visit 1 (pre-treatment) and had Mf detected on at least one of their pre-treatment blood samples (**Table 4**).

Among the 52 family members in 13 households tested by FTS in 2019, 11 (21.2%) were Ag-positive. For the 27 family members in 11 of these households who were tested by FTS in 2019 and who had MDA responses recorded at both 2018 and 2019 surveys, 5 (18.5%) were Ag positive (**Table 3**). Of the 27 family members reporting on MDA at both years, 25 were tested by FTS in both 2018 and 2019 (two declined blood test in 2018). Four (all of whom consistently reported taking MDA) of the 25 were antigen positive both times; one converted from antigen negative to positive (did not take MDA); one converted from Ag positive to negative (reporting taking MDA); and the remaining 19 were Ag negative in both surveys.

None of the Ag positive family members was Mf-positive on either of two 60 uL slides in 2019. All Ag positive family members were treated.

Mf counts in study participants before and after treatment

For the 14 current study participants, the Mf counts are shown in **Table 3** for the 2018 survey and the current 2019 study at days 0, 7 and (for one participant) 30. At the 2019 visit 1, Mf were identified on filters from all 14 participants, with counts ranging from 2 to 632 per mL. The different sample types (filters or slides) were concordant (positive/negative) in 12 out of 14 (86%) persons (**Table 2**). The exceptions were:

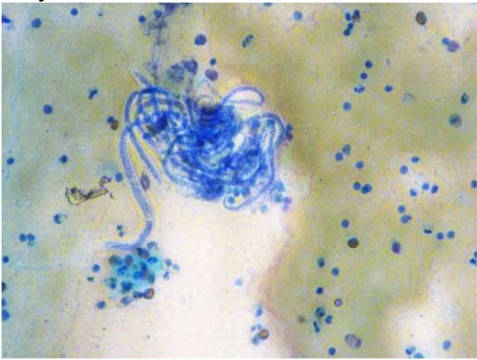
- ID 6 who was Mf-negative on the venous heparin slide but positive on all other sample and specimen types;
- ID 8 who was Mf-positive with 2 Mf/mL on the venous filter but negative on all other sample and specimen types.

Examples of Mf observed in Participant 1 are shown in **Figure 2**.

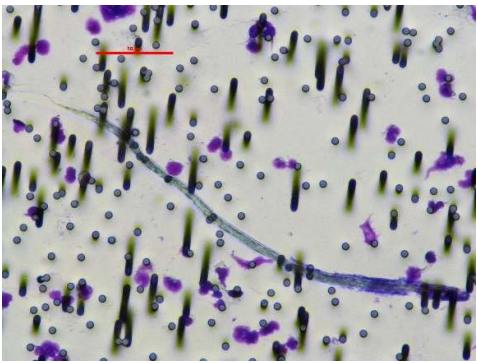
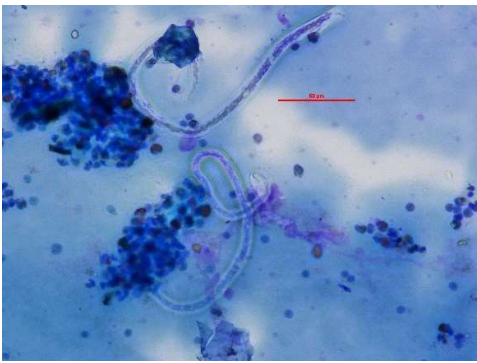
One person (ID 11) experienced adverse reactions to the MDA medications, withdrew from the study at visit 2 (3 hours post-treatment) and declined to provide further blood samples.

Of the 13 participants who agreed to provide a post-treatment blood sample at visit 3, 12 were cleared of detectable Mf on all their 7-day post-treatment blood samples and specimen types examined (**Table 4**). One person (ID 1) had persistent Mf at day 7 (at a reduced density) in one filter (EDTA) and one slide (venous heparin), but was negative on heparin filter and heparin fingerprick slide. They had no detectable Mf in any sample at day 30 (no additional IDA doses were given) (**Table 4**).

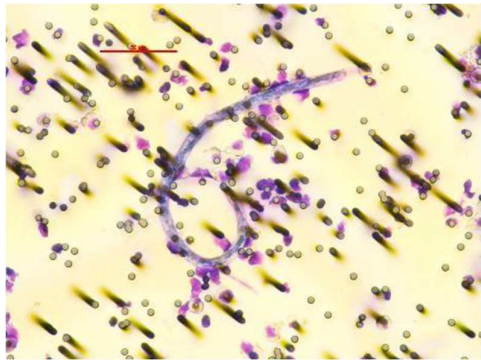
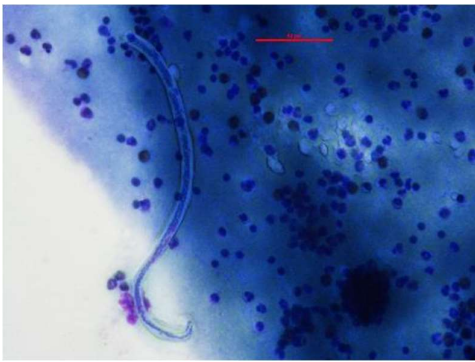
Figure 2: Participant ID 1: Microfilariae in slides and filter of 59 year old male in 2018 and 2019 (day 0 and 7).



A: 2018 slide from fingerprick heparin blood, x400



B: 2019 day 0 slide from heparin fingerprick blood and filter from venous EDTA blood, x400



C: 2019 day 7 slide from heparin venous blood and filter from venous EDTA blood, x400

Table 4: Mf counts per mL by sample type, anticoagulant and specimen type.

	Mf/mL												
	2018	2019 day 0, pre-treatment				2019 day 7 post-treatment				2019 day 30 post-treatment			
Sample type	Finger-prick	Venous			Finger-prick	Venous			Finger-prick	Venous			Finger-prick
Anti-coagulant	Hep	Hep	EDTA	Hep	Hep	Hep	EDTA	Hep	Hep	Hep	EDTA	Hep	Hep
Specimen type	Slide*	Filter	Filter	Slide*	Slide*	Filter	Filter	Slide*	Slide*	Filter	Filter	Slide*	Slide*
ID number													
1	1142	11		875	3467	0	5	17	0	0	0	0	0
2	17	26		33	17	0	0	0	0				
3	342	331		100	275		0		0				
4	33	634		408	242	0	0	0	0				
5	350	336		875	792	0	0	0	0				
6	33	37		0	67	0	0	0	0				
7	150	40		17	25	0	0	0	0				
8	67	2		0	0	0		0	0				
9	75	229		242	67	0	0	0	0				
10	333	489		350	467	0	0	0	0				
11	100	247	373	83	292	withdrew							
12	117	7 ^{fp}		17	50	0	0	0	0				
13	367	538		817	608	0	0	0	0				
14	108	265	308	200	33	0	0	0	0				

* : 60 uL of blood examined on each of two slides; average count extrapolated to 1 mL

^{fp}: venous draw failed; 0.7 mL of fingerprick blood used for heparin filter, adjusted to 1 mL equivalent count

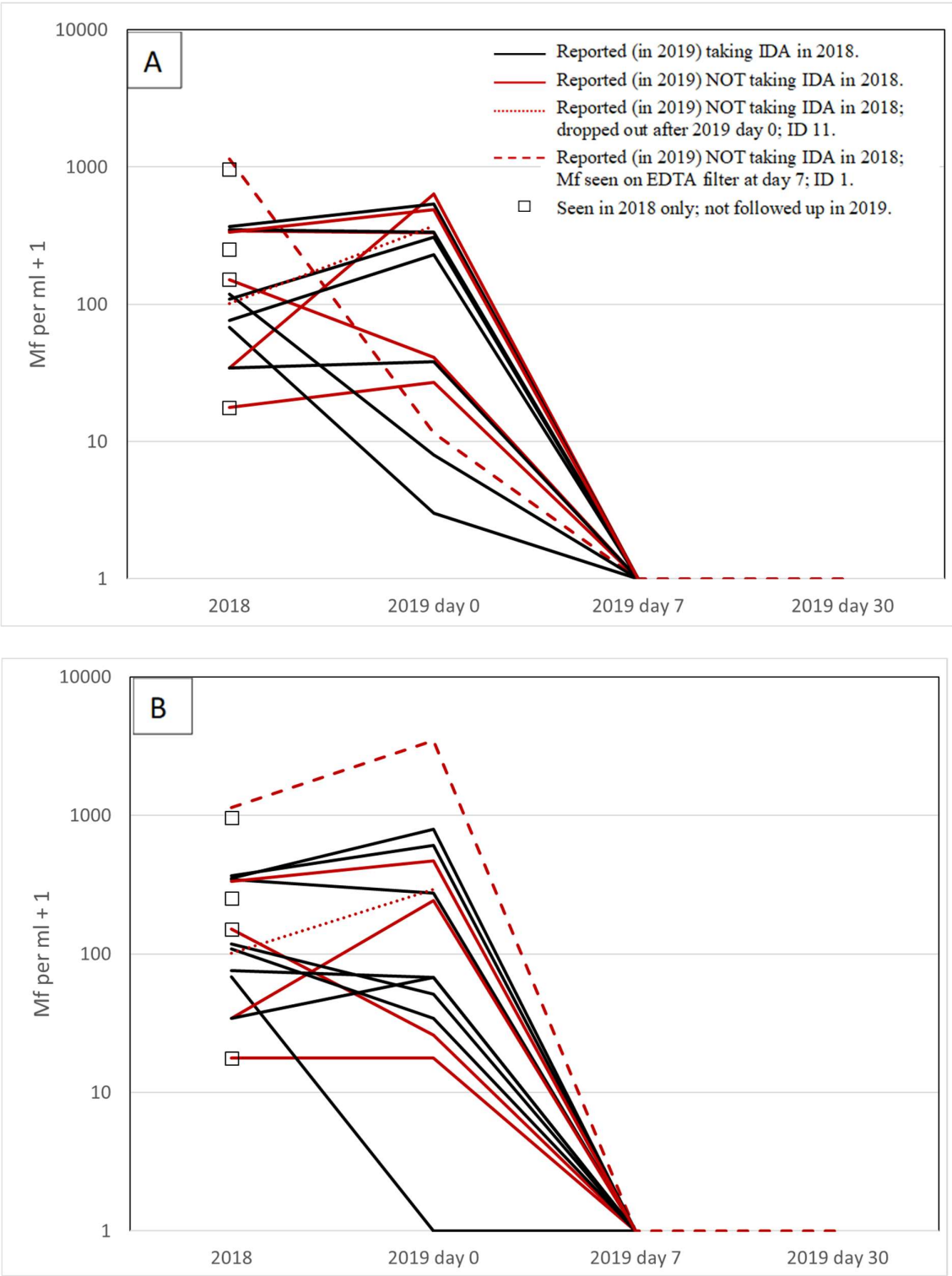
To compare Mf counts over time for each person, we used counts from filters using the same anticoagulant (heparin in most cases) with one exception (ID 3) where we had only heparin blood filter at day 0 and only EDTA blood at day 7. Mf counts over time for each person are shown in **Figure 3**. Note that in 2018, only fingerprick blood slides were done. **Figure 3A** shows the results for 2018 (fingerprick) and 2019 for venous filter samples, while **Figure 3B** shows the results for fingerprick slide samples at all time points.

Mf counts on fingerprick slides in 2018 for the 4 Mf positive persons who were not followed up in 2019 are shown as squares in **Figure 3A** and **3B**. Their counts in 2018 were within the range of the followed-up participants.

Pharmacokinetics

Considering that we observed Mf clearance in 12 of 13 (92.3%) participants by day 7 and 100% Mf clearance in all 13 participants by day 30, pharmacokinetic studies were deemed to be not necessary and not conducted.

Figure 3: Mf counts per mL in heparinized* blood samples A: Fingerprick slides in 2018 and venous filters in 2019. B: Fingerprick slides in 2018 and 2019. *EDTA for ID3 at day 7



Comparisons of Mf density in different sample types.

The differences between counts observed in Table 4 were compared and the results given in the supplementary material (**Table S1** and **Figure S3**). Mean and median counts were significantly higher in venous filter samples than in venous or fingerprick slide samples (**Table S2**) but not different between the two types of fingerprick specimens. Correlations between counts ranged from 0.529 to 0.729, and were significant between venous filters and both slide types (**Table S2**).

4. Discussion

IDA was highly effective for clearing Mf in Samoa when medications were taken while directly observed, and using the recommended weight-based dosages. Mf were cleared completely by day 7 after treatment in 12 of 13 participants followed-up, and by day 30 in the remaining participant. Overall, this study found that persistence of Mf in a proportion of individuals after the triple-drug MDA programme in 2018 was unlikely to have been due to drug resistance in the local filarial worm population. More likely explanations include inadequate dosage due to incorrect weighing, administration of insufficient numbers of pills, and/or flawed reporting about participation, i.e. non-compliance or partial compliance in taking pills. Thus future MDAs must provide accurate weighing scales, as well as thorough training for MDA drug distributors in correct dosage by weight. The practice of directly observed treatment must be emphasized in future MDAs.

Our results showed that reported MDA taking may not be accurate. Based on the findings of full clearance of Mf after observed correct treatment doses in the current study, it seems unlikely that the seven participants who consistently reported Yes to the 2018 MDA when asked in both 2018 and 2019 actually took the full correct dose. This may be an error in the dose provided, or lack of swallowing all the pills. It may also be due to social desirability bias (desire of participants to respond with the 'correct' answer that they think surveyors want to hear, especially in front of family members), or incorrect proxy reporting for children or absent family members. In Samoa, surveys tend to be conducted in family groups, with no privacy. Persistence of Mf positivity after MDA is more understandable in those who consistently reported No in both surveys (3 of 14) or changed answers from Yes to No or Incomplete (4 of 14 participants).

Incorrect reporting of MDA participation as Yes in up to 11 of the Mf positive participants may not be as grave a problem as it initially appears however. The participants represent a biased sample of the survey population since they were identified and selected due to being Mf-positive (presumably because they were more likely not to have taken MDA). Reported MDA compliance in family members did not change between surveys to a similar extent, and their compliance in both years (89.9%) was no different than that reported in the full survey (16). Nevertheless the findings show that it is important to ask survey questions in a manner most likely to elicit an honest response. For example, interviewing people individually rather than in front of their families, avoiding proxy responses from one person for a whole family, or stressing that there is no 'right' answer, and probing further if answers seem rushed. When MDA is given to some children at school, as it was in Samoa, parents or guardians may be unaware of their children's participation or not, and 'don't know' answers should be acceptable.

Regarding the laboratory methods, filters sample a much larger volume of blood than slides and were better for assessing Mf presence and density. However the method is time consuming, provides greater exposure of lab workers to blood and potential aerosols, and is difficult to apply on a large scale. Slides made from 60 uL of either venous or fingerprick blood were acceptable for determining Mf clearance, but using slides exclusively would have missed some Mf positive participants. All participants (N=14) were still Mf-positive by venous blood on filters before treatment, but two participants were negative by slide from the same venous sample, and one of these two was also

negative on the fingerprick slide. This finding of greater sensitivity of filters for determining Mf positivity corresponds with the review of Vinkeles Melchers et al (24).

A limitation of the study is the relatively small sample size and the inability to follow up all those who were identified as Mf positive in 2018. Also at the beginning of the study we did not know the best anticoagulant, sample or specimen type to use and therefore the methods evolved during the study. We made slides from anticoagulated blood which limited our ability to compare with previous studies of Mf density which used fingerprick slides made from whole blood, either directly from the finger or collected in capillary tubes (25, 26). We also used high flow spring loaded lancets compared to the manual type used in earlier studies.

Mf counts had a greatly skewed distribution; counts in different sample and specimen types were compared after log transformation by both parametric and non-parametric tests, which gave consistent results. Estimated mean log and median Mf densities were significantly higher in the filtered venous blood than on slides (either from the same venous sample or from fingerpricks). Comparisons of counts detected no significant difference in densities on slides made concurrently from venous or fingerprick blood.

Our finding of no difference in estimated density between venous and fingerprick slide samples contrasts with previous findings implying that density was higher in fingerprick than venous blood (25, 26). However, previous studies compared Mf density from venous filters with fingerprick slides made with whole blood (20uL applied in one circular sample rather than 60uL in 3 lines), not heparinized blood as used in this study for both venous and fingerprick slides. It is not clear why slides from venous blood samples in our study would yield lower counts than filters from the same blood samples. The explanation for both findings in our study may be that Mf in heparinised blood do not stick as well to slides as to filters, or to slides made with whole blood.

We found that EDTA is preferable to heparin as anticoagulant for filtered blood (to reduce clumping of Mf and make counting easier). Thus filtered blood using EDTA is preferred over slides alone for greater sensitivity of detecting Mf and counting their density. This means that separate samples need to be taken for FTS antigen determination if needed, since the manufacturer specifies heparinized blood. For studies with large numbers of samples where filters from venous blood are not feasible, slides made with whole blood may be more sensitive than those made with heparinized or EDTA blood, and this should be tested given the greater emphasis being placed on Mf prevalence as elimination nears.

5. Conclusions

The triple-drug MDA strategy in Samoa was effective at clearing Mf by 30 days post-treatment when given and taken at the recommended weight-based dosage. Our study did not identify any evidence of drug resistance to IDA in Samoa.

Supplementary Materials: The Supplementary file includes Figures S1, S1, S3, S4 and Tables S1 and S2 with explanatory text.

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Conflicts of Interest:

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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