Molecular xenomonitoring as a complement to human antigen seroprevalence for lymphatic filariasis surveillance in Samoa

Brady McPherson  
Australian Defence Force Malaria and Infectious Disease Institute

Helen J. Mayfield  
University of Queensland

Angus McLure  
Australian National University

Katherine Gass  
The Task Force for Global Health

Take Naseri  
Samoa Ministry of Health

Robert Thomsen  
Samoa Ministry of Health

Steven A. Williams  
Smith College

Nils Pilotte  
Quinnipiac University

Therese Keams  
Menzies School of Research Health

Patricia M. Graves  
James Cook University

Colleen L. Lau  
University of Queensland

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Abstract

Molecular xenomonitoring (MX), the detection of filarial DNA in mosquitoes using molecular methods (PCR), is a potentially useful surveillance strategy for lymphatic filariasis (LF) elimination programs. Delay in filarial antigen (Ag) clearance post-treatment limits the usefulness of human surveys as an early indicator of the effectiveness of mass drug administration (MDA), and MX may be more sensitive in this setting. We compared prevalence of infected mosquitoes pre- and post-MDA (2018 and 2019) in Samoa, and investigated associations between presence of PCR-positive mosquitoes and Ag-positive humans. We observed a statistically significant decline in mosquito infection prevalence post-MDA, but no change in human Ag prevalence during this time. Presence of PCR-positive pools of ‘all species’ was most sensitive (78.6%) for detecting villages with Ag-positive humans, while *Ae. polynesiensis* provided the highest positive predictive value (81.8%). Our study provides promising evidence for MX as a complement to human surveys in post-MDA surveillance.

Introduction

Lymphatic filariasis (LF) is a globally significant parasitic disease which can result in severe morbidity and disability, such as lymphedema, elephantiasis or scrotal hydroceles. Elimination programs for vector-borne diseases such as LF, malaria and onchocerciasis represent significant global heath investments. Since 2000, the World Health Organization’s (WHO) Global Programme to Eliminate LF has facilitated mass drug administration (MDA) of anti-filarial drugs to >910 million people in 68 countries. Despite huge successes over >20 years, LF remains endemic in many countries. Most infections are asymptomatic but may still contribute to transmission. Identifying residual infections is therefore critical for programmatic decision-making such as stopping or resuming MDA.

The standard diagnostic test used in LF programmatic surveys detects circulating filarial antigen (Ag), produced by adult worms\(^1\). Although Ag can be detected quickly and inexpensively in the field using rapid diagnostic tests such as the Alere Filariasis Test Strip\(^1\), they cannot distinguish between current and recently cleared infections. After treatment with anti-filarial medications, Ag persist for months or years, and therefore may not be sufficiently sensitive for determining whether transmission has been interrupted\(^2\)\(^-\)\(^3\). As countries work towards elimination and LF Ag prevalence drops to very low levels, highly sensitive surveillance methods will be required to detect changes in prevalence and monitor progress. An alternative indicator of transmission is the presence of microfilaria (Mf, immature parasites) in the blood, which confirms active infection. However, Mf may not be detected even if adult worms are present, e.g., if the worms are too young, too old, or have not mated.

Molecular Xenomonitoring (MX) involves testing mosquitoes for filarial DNA using the polymerase chain reaction (PCR). Unlike Ag tests, the mosquitoes’ short life span means that a positive PCR indicates infectious people were recently nearby\(^4\). MX may therefore provide a more sensitive signal of transmission than Ag\(^5\). MX has been used in research settings in American Samoa\(^6\), Sri Lanka\(^3\) and India\(^7\), although significant knowledge gaps still exist regarding survey design and interpretation of results. Recent studies have highlighted the need for more efficient mosquito collection methods, better understanding of the relationship between results from MX and human surveys\(^8\), and whether speciation of mosquitoes improves the usefulness of MX results (especially in countries where entomology expertise is limited)\(^6\)\(^9\).
LF is endemic in Samoa, transmitted by *Aedes* mosquitoes including the highly efficient *Aedes polynesiensis*, with a flight range of ~150m\(^{10,11}\). Samoa has conducted LF elimination programs under GPELF since 1998\(^{12}\), but programmatic surveys in 2013 and 2017\(^{12}\) and research projects in 2018\(^{13}\) showed ongoing transmission. In 2018, Samoa was the first country to distribute nation-wide triple-drug MDA (ivermectin, albendazole and diethylcarbamazine)\(^{14}\). Evaluating the effectiveness of this intervention is therefore of interest globally, but a key challenge is the ability to detect reductions in infection prevalence post-MDA. Considering that Ag could persist for months or years after treatment\(^{15}\), MX might provide a more sensitive surveillance strategy in the post-MDA setting.

In this study, we aimed to evaluate the effectiveness of MX for LF surveillance by comparing the results of spatially-representative MX surveys with human Ag prevalence surveys conducted pre- and post-MDA (2018 and 2019) in Samoa. The main objectives were to: a) estimate the prevalence of LF infection in mosquitoes pre- and post-MDA; b) assess the usefulness of MX as an indicator of the presence of Ag-positive humans at primary sampling unit (PSU); and c) compare the sensitivity of MX and human Ag for detecting changes in infection prevalence for post-MDA surveillance. Results were considered in a practical context based on what can realistically be achieved on the ground and how findings can be used to assist programmatic decision-making.

**Results**

**Mosquito abundance and distribution**

In 2018, one to three months before the 2018 MDA, an MX survey was conducted in 28 randomly selected PSUs on the two main islands of Samoa (Upolu and Savai‘i). A total of 8,506 female mosquitoes were trapped, with a mean of 320 per PSU (range 147-781, median 278). *Culex* were the predominant category (n=4,333, 48%), followed by *Aedes polynesiensis* (n=2,498, 28%), other *Aedes* spp. (n=1,443, 16%) and *Ae. (Finlaya)* spp. (n=698, 8%) (Supplementary Table S1). The predominant species in the *Culex* and *Aedes* (other) categories were *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Numbers of *Ae. polynesiensis* by PSU ranged from 9 to 248 (mean 89, median 69) (Fig. 1a). Summaries for other species are available in Supplementary Table S1.

In 2019, six to nine months after the 2018 MDA, a repeat MX survey was conducted in Upolu, Savai‘i, and Manono islands across 35 PSUs (30 randomly selected and five purposively selected because of high Ag prevalence in previous human surveys). A total of 34,299 female mosquitoes were trapped, with a mean of 980 per PSU (range 290-3,719, median 855). *Cx. quinquefasciatus* was the predominant species (n=17,807, 52%), followed by *Ae. polynesiensis* (n=10,540, 31%), *Ae. aegypti*. (n=3396, 10%) and *Ae. (Finlaya)* spp. (n=1377, 4%) (Supplementary Table S2). Numbers of *Ae. polynesiensis* varied by PSU with a range of 22 to 1,046 (mean 89, median 69) (Fig. 1a). Summaries for other species are available in Supplementary Table S2.

**Estimated prevalence of LF infection in mosquitoes**

In 2018, mosquitoes were sorted into 475 pools (mean 18 mosquitoes/pool, range 1-25); 18% of pools were positive for *W. bancrofti* DNA by PCR. The highest positivity rate of pools was in *Aedes* spp. (other) (30%), followed by *Ae. polynesiensis* (24%) (Table 1). Overall estimated infection prevalence in all species combined was 0.9% (95% credible interval [Crl] 0.2-2.3), with the highest prevalence in *Aedes* spp. (other) (1.6%) followed by
*Ae. polynesiensis* (1.2%). Highest prevalence was found in Northwest Upolu (NWU), north-east coast of Upolu (Musumusu and Faleapuna) and southeast Savai’i (Papa & Tafua) (Fig. 2a, Supplementary Table S3).

In 2019, mosquitoes were sorted into 2,638 pools (mean 13 mosquitoes/pool, range 1-25). Most pools consisted of *Cx. quinquefasciatus* (39%) or *Ae. polynesiensis* (27%). In total, 7% of pools were PCR-positive. The highest positivity rate of pools was in *Ae. polynesiensis* (14%), followed by *Ae. aegypti* (7%) (Table 1). Estimated infection prevalence in all species combined was 0.3% (95% CrI 0.05-1.0%), with the highest prevalence in *Ae. polynesiensis* (0.6%). Highest prevalence was observed in NWU within three purposively chosen PSUs not sampled in 2018: Lauli’i (7.5%), Fasito’o Tai (7.1%) and Faleasiu (6.4%) (Fig. 2b, Supplementary Table S4).
Table 1

Detection of *W. bancrofti* DNA in female mosquito pools in 2018 (28 PSUs) and 2019 (35 PSUs) in Samoa. Results for 2019 are split by randomly (R) or purposively (P) selected PSUs. Purposive PSUs were not surveyed in 2018.

| Year | Species category | Females (n) | Pools (n) | Positive Pools (n) | Positive pool (%) | Estimated infection prevalence* (%) (95% Crl) |
|------|------------------|-------------|-----------|--------------------|-------------------|---------------------------------------------|
|      |                  | R           | P         | R                  | P                 | R                                          | |
| 2018 | *Ae. polynesiensis* | 2451        | NA        | 136                | NA                | 33                          | 24.3 | NA  |
|      |                  |             |           |                    |                   | 1.2 (0.3-2.9)                | |
|      | *Ae. (Finlaya) spp.* | 219         | NA        | 33                 | NA                | 1                           | 3.0  | NA  |
|      |                  |             |           |                    |                   | 0.4 (0-1.6)                 | |
|      | *Aedes spp. (other, including aegypti)* | 1866 | NA | 105                | NA                | 31                          | 29.5 | NA  |
|      |                  |             |           |                    |                   | 1.6 (0.4-4.1)               | |
|      | *Culex spp. (all)* | 3970        | NA        | 201                | NA                | 21                          | 10   | NA  |
|      |                  |             |           |                    |                   | 0.4 (0.1-1.0)               | |
|      | All species      | 8506        | NA        | 475                | NA                | 86                          | 18.1 | NA  |
|      |                  |             |           |                    |                   | 0.9 (0.2-2.3)               | |
| 2019 | *Ae. polynesiensis* | 8,833       | 1,703     | 612                | 111               | 56                          | 9.2  | 41.4 |
|      |                  |             |           |                    |                   | 0.6 (0.1-2.4)               | 1.8  | (0.2-6.9) |
|      | *Ae. (Finlaya) spp.* | 1,232       | 145       | 170                | 28                | 1                           | 0.6  | 3.6  |
|      |                  |             |           |                    |                   | 0.2 (0-0.8)                 | 0.5  | (0-2.6) |
|      | *Ae. aegypti*    | 2,861       | 535       | 399                | 70                | 26                          | 6.5  | 10   |
|      |                  |             |           |                    |                   | 0.5 (0.1-2)                 | 1.5  | (0.1-5.6) |
|      | *Aedes spp. (all other)* | 1,002 | 134 | 169                | 36                | 4                           | 2.4  | 8.3  |
|      |                  |             |           |                    |                   | 0.5 (0-1.7)                 | 1.8  | (0.1-4.9) |
|      | *Cx.*            | 16,448      | 1,359     | 917                | 100               | 25                          | 2.7  | 3    |
|      |                  |             |           |                    |                   | 0.1                         | 0.3  |     |
Comparison between mosquito infection prevalence in randomly vs purposively selected PSUs in 2019.

In 2019, crude infection prevalence in all mosquito species in randomly selected PSUs was 0.30% (95% CrI 0.05-1.05%), lower than the 1.1% (95% CrI 0.1-3.9%) in purposively selected PSUs. This difference remained statistically significant after adjusting for mosquito species, region, and clustering within PSUs (OR: 0.24, 95% CrI 0.06-0.86). For both randomly and purposively selected PSUs, the highest prevalence was observed in *Ae. polynesiensis* (0.6% and 1.8% respectively), followed by *Ae. aegypti* (0.5% and 1.5% respectively).

For the 28 PSUs that were sampled in both years, estimated mosquito infection prevalence for the country was higher in 2018 than 2019 for both the entire sample (‘any species’) (0.9% vs 0.3%) and *Ae. polynesiensis* (1.2% vs 0.5%). This decreasing trend was observed across all species categories nationally and in all regions, although not all differences were statistically significant (Fig. 3, Supplementary Table S5).

After adjusting for mosquito species and clustering within PSUs, infection prevalence at the national level was lower in 2019 than 2018 (odds ratio [OR] 0.4, 95% CrI 0.2-0.6) and in three regions (NWU: OR 0.6 [95% CrI 0.4-0.9], ROU: OR 0.1 [95% CrI 0.0-0.2], SAV: OR 0.3 [95% CrI 0.1-0.8]). Estimated prevalence declined in AUA, but the difference was not statistically significant (OR 0.6, 95% CrI 0.2-1.5). Given the low prevalence, these ORs closely approximate prevalence ratios. For example, infection prevalence in mosquitoes in ROU was ~90% (i.e. 1 – 0.1) lower in 2019 than in 2018. This trend was seen across all PSUs, but only statistically significant in 10 PSUs (Supplementary Table S6).

In 2018, 50% (14/28) of PSUs contained at least one PCR-positive pool of *Ae. polynesiensis* and 82% (23/28) contained at least one PCR-positive pool of ‘any species’. Results were similar in 2019, with 63% (22/35) of PSUs returning at least one PCR-positive pool of *Ae. polynesiensis* in 2018 and 80% (28/35) returning at least one PCR-positive pool of ‘any species’ (Fig. 4).

**Human Ag and Mf infection**

In 2018, we recruited 1542 participants aged 5-9 years and 1551 participants aged ≥10 years from 30 randomly selected PSUs. In 2019, we recruited 1811 participants aged 5-9 years and 1815 participants aged ≥10 years from the same 30 PSUs. The numbers of participants in 2018 and 2019 in each of the four regions are given in Supplementary Table S7 and Supplementary Table S8 respectively. Nationally, age-adjusted Ag prevalence in the
6-9 months post-MDA was similar in 2018 and 2019 (Fig. 5). Results at the regional level were mixed, with two regions (NWU and SAV) showing an increase from 2018 to 2019 in both age groups. Only one region (AUA) showed a decrease in Ag prevalence in 2019 in both age groups.

In 2018, 13 Mf-positive participants were identified in the 30 randomly selected PSUs, and 10 out of these 30 PSUs (33.3%) had at least one Mf-positive participant. Prevalence of Mf infection was likely underestimated in 2018, because the survey was conducted after MDA. In 2019, 17 Mf-positive participants were identified in the same 30 random PSUs and at least one Mf-positive participant was found in 10 of the 30 PSUs (33.3%), but not in the same 10 PSUs as 2018. Six PSUs had Mf-positive people both years, four PSUs only in 2018, and four PSUs only in 2019. Fourteen PSUs (46.7%) had an Mf-positive person one or both years.

**Presence of PCR-Positive pools as indicator of Ag-positive humans in a PSU**

At the PSU level, there was no significant association between the presence of Ag-positive humans and PCR-positive mosquito pools in 2018 or 2019, whether considering all mosquito species (Fisher exact p-value 0.73 in 2018; 0.83 in 2019), all *Aedes* (Fisher exact p-value 0.89 in 2018; 0.73 in 2019), or *Ae. polynesiensis* only (Fisher exact p-value 0.68 in 2018; 0.52 in 2019). Consequently, the sensitivity, specificity, positive predictive value, and negative predictive value of PCR-positive pools to identify Ag-positive PSUs were no better than random classification (Supplementary Table S9). However, combining MX (all species) and Ag surveillance of humans identified more positive PSUs (those with positive humans and/or mosquitoes detected) than each method alone, with 96% (27/28) of PSUs being positive for MX (all species) and/or Ag in 2018 and 97% (34/35) in 2019. Ag surveillance used alone identified 79% (22/28) in 2018 and 80% (28/35) in 2019. For MX, using ‘all species’ identified the most positive PSUs with 82% (23/35) in 2018 and 80% (28/35) in 2019, followed by ‘all Aedes’ with 75% (21/28) in 2018 and 69% (24/35) in 2019, and *Ae. polynesiensis* with 50% (14/28) and 63% (22/35) in 2019 (Fig. 6).

**Detecting a change in human or mosquito infection prevalence post-MDA**

Mapping the results from Fig. 6 by PSU shows the spatial distribution of MX and human Ag (and Mf) results (Fig. 7). PCR-positive mosquitoes and Ag-positive humans were found in all regions in both years. In 2018, of the 23 PSUs with PCR-positive pools, nine (39%) did not return any positive *Ae. polynesiensis* pools; in 2019, this proportion reduced to 21% (six out of 28 PSUs).

Figure 8 represents the change in mosquito infection or human Ag prevalence between 2018 and 2019, expressed as ORs where an OR of one indicates no change, OR <1 a reduction, and OR of >1 an increase in prevalence. MX showed a significant reduction in mosquito infection prevalence from 2018 to 2019 nationally and in all regions for ‘all species’ and *Ae. polynesiensis*. There was no significant change in Ag prevalence in participants aged ≥10 years or 5-9 years at the national level. Variable patterns were observed at the region level, with a significant increase in Ag prevalence between 2018 and 2019 in 5-9 year-olds in NWU and in ≥10 year-olds in SAV, but a significant decrease in ≥10 year-olds in AUA.

**Discussion**
This study provided evidence that MX can be useful for LF surveillance, and potentially more sensitive than human Ag prevalence for detecting changes in infection levels immediately following triple-drug MDA. After one round of triple-drug MDA in Samoa, MX detected a decline in LF prevalence in mosquitoes, while there was no significant change in human Ag prevalence. We compared MX results for the main vector *Ae. polynesiensis* and ‘all species’ combined and found that spatial and temporal trends in mosquito infection prevalence were similar.

Higher numbers of female mosquitoes were caught in 2019 than 2018, partly due to drier conditions in 2018 and additional PSUs and households surveyed in 2019. In 2019, survey teams were more proactive with trap placements to increase catches (i.e. if few mosquitoes were trapped in the first 24 hours, the trap was moved to another part of the house for the next 24 hours). The increased catch numbers in 2019 resulted in more pools, leading to higher workload and laboratory costs but provided more precise infection prevalence estimates. Mosquito species trapped in Samoa were as expected, with *Cx. quinquefasciatus* predominating, followed by *Ae. polynesiensis* and *Ae. aegypti* in both years.

In PSUs where a direct comparison was possible (sampled both years), LF prevalence in mosquitoes declined between 2018 and 2019 across all species and genera. It is highly plausible that most mosquitoes categorised as ‘*Aedes* other’ in 2018 were *Ae. aegypti*. Under this assumption, *Ae. aegypti* appeared to have a higher pool PCR-positivity rate and infection prevalence than *Ae. polynesiensis* in 2018, but the observation was reversed in 2019. *Aedes* spp. had a dramatically higher pool positivity rate and infection prevalence than that of *Culex* spp. The large numbers of *Culex* spp. pools therefore diluted the overall infection prevalence of ‘all species’ combined. Where prior knowledge of endemic mosquito species distribution and infection prevalence are available, this can be used to inform the pooling strategy for MX.

The pool PCR-positivity rate and mosquito infection prevalence were three times greater in the purposively selected than the randomly selected PSUs. This was expected since the purposively selected PSUs had historically high LF prevalence but highlights the importance of local knowledge for identifying areas of high transmission. Mosquito infection prevalence in randomly selected PSUs was lower in 2019 than 2018 nationally and in each region (Fig. 3 and Fig. 8), but this difference was not statistically significant for AUA. Even though our study was designed to detect differences at national and regional levels, we also observed statistically significant reductions in mosquito infection prevalence between 2018 and 2019 in 10 PSUs, and prevalence did not increase in any PSUs (Supplementary Table S6).

Unsurprisingly, using ‘all species’ identified more positive PSUs than using only *Aedes* spp. or *Ae. polynesiensis*. Comparison between mosquito categories showed higher infection prevalence in *Aedes* spp. than *Culex* spp. Nevertheless, analyses using ‘all species’ or only *Ae. polynesiensis* had concordant spatial and temporal trends, though the larger sample sizes with ‘all species’ resulted in tighter confidence intervals, illustrating one advantage of using all mosquito samples available. However, sorting mosquitoes by species requires substantial expertise. MX surveys could be greatly simplified if sorting was restricted to the genus level. Promisingly, despite the differences in infection prevalence between *Culex* and *Aedes* mosquitoes and between *Ae. polynesiensis* and other *Aedes* mosquitoes, adjustment for genus or species had little effect on the point estimate or confidence interval for the difference in prevalence between 2018 and 2019 at national, regional or PSU levels (Supplementary Figure S1 and Supplementary Figure S2 ). This finding suggests that, for the purposes of detecting temporal trends, sorting by genus or species in our study made little difference to the results or their interpretation. Whether this observation can be generalised to future surveys in Samoa or elsewhere is uncertain.
If the relative abundance of species captured were substantially different between two time-points or two survey locations to be compared, failing to sort by species may bias comparisons.

The imperfect sensitivity of each surveillance approach suggests that using MX and Ag surveillance together may be beneficial if the aim is to identify (and respond to) as many focal areas of transmission as possible. Combining these surveillance methods may also assist in understanding transmission as MX may be a better indicator of recent transmission than Ag (assuming PCR testing can be conducted in a reasonable timeframe). Because evidence of infection (human and/or mosquito) was observed in nearly all PSUs, the small number of negative PSUs (for both human and mosquito infection) made it difficult to assess the predictive accuracy of PCR-positive mosquitoes for the presence of Ag-positive people.

When comparing MX and Ag for post-MDA surveillance, we found a statistically significant decrease in infection prevalence in mosquitoes at the country level for ‘all species’ and *Ae. polynesiensis*, but no significant change overall in Ag prevalence in 5-9 year-olds or in those aged ≥10 years. This could be due to a slower change in Ag compared to mosquito infection. These results demonstrate each surveillance method’s sensitivity to changing prevalence, and when used together may provide a more complete picture. MX may provide a more accurate picture of the transmission at specific time points, whereas Ag, due to its more gradual decline, provides a smoother trend over time. While the significant reduction in mosquito infection prevalence suggests encouraging impact on LF transmission by the MDA, transmission by mosquitoes was not completely interrupted, and we do not know the target threshold of mosquito infection prevalence required for demonstrating elimination. Mf prevalence did not decline between years, and Mf were detected in residents of about one third of villages each year. However, the 2018 human survey was conducted immediately post-MDA; although this was not expected to influence Ag results, it prevented comparison of Mf results between years. Further studies of Mf persistence and reappearance after MDA are required to fully understand the expected relationship between Mf and MX results.

Our results should be considered in light of the study’s limitations. In 2018, the sample size of households required to trap sufficient mosquitoes was calculated using the best available evidence, but dry weather conditions led to lower-than-expected catches. Operational challenges in 2018 led to simplified pooling by reducing the number of mosquito categories. In 2019, protocol changes and wetter conditions led to increased catches. Mosquitoes were pooled between households in 2018, so prevalence estimates could not be adjusted for household-level clustering. Despite these limitations, we were able to identify a statistically significant decline in infection prevalence in mosquitoes, which was not observed using human Ag prevalence.

In conclusion, our study suggests that in the immediate post-MDA period, MX might be better for detecting declining prevalence than Ag surveillance in adults or children. However, if the goal is to detect the presence of residual transmission, Ag and MX surveys may provide complementary information. In MX, using both the primary mosquito species and other species increased the sample size and improved the ability to detect residual transmission and changes in prevalence. In our study, adjusting for species made very little difference to estimates of temporal trends, suggesting that the labour-intensive process of sorting mosquitoes into species categories did not influence the overall results or their interpretation. In countries where the expertise required to trap and identify mosquitoes is limited, omitting speciation could make MX more feasible. Further research is required to determine whether our findings are generalisable to future surveys in Samoa and to other settings.

### Methods
Study region

Samoa is a tropical island nation in the South Pacific with approximately 201,316 residents in 2018,\textsuperscript{16} the majority of whom live on the islands of Upolu and Savai’i (Fig. 9). Villages are predominantly rural, with urbanised areas around the capital of Apia and the Savai’i ferry port of Salelologa. Average rainfall is 3000-6000 mm/year\textsuperscript{17} and inland areas remain largely forested. The predominant LF vector species is the day-biting \textit{Ae. polynesiensis}, with evidence that night-biting \textit{Ae. samoanus} (included in \textit{Ae. (Finlaya) subgenus}) also able to transmit \textit{W. bancrofti}\textsuperscript{18}.

Site selection

In 2018, 30 villages were randomly selected from a line list of 338 villages in the 2016 national census. For eight selected villages with population of <600, the next village on the list was added to the PSU. Five additional villages were purposively selected by the Samoa Ministry of Health due to high Ag prevalence in previous surveys\textsuperscript{12,19}. Within each PSU, 15 households were selected using a ‘virtual walk’ method as described previously\textsuperscript{13}. If a selected location was not a household, or was uninhabited (destroyed, abandoned, unoccupied), it was replaced by the closest house. If a PSU consisted of two villages, the number of house locations per village was proportionate to each village's population. Spatial data on country, island, region and village boundaries in Samoa were obtained from the Pacific Data Hub (pacificdata.org) and DIVA-GIS (diva-gis.org). Geographic information systems software ArcMap (v10.6, Environmental Systems Research Institute, Redlands, CA) was used to manage spatial data and produce maps.

Ethical approval

was obtained from human research ethics committees at the Samoa Ministry of Health and The Australian National University (protocol 2018/341). The study was conducted in collaboration with the Samoa Department of Health, WHO Samoa country office, Samoa Red Cross, The Task Force for Global Health, and the United States Centers for Disease Control and Prevention. permission was sought from village chiefs before entering a village and verbal and written consent was obtained from all participants, or from the parents or guardians of participants under the age of 18. All methods were performed in accordance with the relevant guidelines and regulations.

Data collection

Mosquito survey

Mosquito surveys were conducted on Upolu and Savai’i in June-July of 2018 (3-6 months pre-MDA), and on Upolu, Savai’i and Manono Island in May-June of 2019, six to nine months post-MDA (survey timeline in Supplementary Figure S4). In 2018, mosquito surveys were conducted in only 28 of the 30 randomly selected PSUs due to logistic reasons and insufficient time before MDA, and the purposively selected PSUs were not surveyed. Mosquito surveys were conducted in all 35 PSUs in 2019.

In 2018, mosquito surveys were conducted at 10 households per PSU. The target sample size for mosquitoes was based on a positivity threshold of an upper 1-sided confidence interval <0.25%, using results from Schmaedick et. al. (2014)\textsuperscript{8} in American Samoa, which found infection prevalence in \textit{Ae. polynesiensis} of >0.25%
(0.28%, 95% CI: 0.20%, 0.39%). Using a design effect of two, the estimated target sample size was 13,700 mosquitoes. Based on the work of Chambers et al (2009) in American Samoa, the expected catch per 10 traps per 24 hours was 180 mosquitoes. Thus, for 30 PSUs with 10 trap sites/PSU, the expected catch was 10,800/48 hours, the maximum possible time per PSU to enable most PSUs to be surveyed before the start of MDA. The number of mosquitoes caught in 2018 was lower than expected and therefore, in 2019 the number of households per PSU was increased from 10 to 15 for a total of 525 ‘48-hour’ household trapping periods compared to 280 in 2018.

Traps were placed at each household for 48 hours. We used BioGents Sentinel Mosquito Traps (Models 1 and 2 with attractant lures), based on previous findings of their effectiveness when targeting Aedes mosquitoes in Samoa. Traps were serviced twice daily in 2018 and once daily in 2019, to collect mosquito bags and replace batteries. Mosquitoes at ambient temperatures can be damaged during transit as they fly around inside bags, and hamper speciation. To minimise this, trap-bags of mosquitoes were transported in chilled containers. Mosquitoes were euthanized by placing the trap-bag in a freezer, or in a resealable bag containing an acetone-soaked cotton-ball. Male mosquitoes were discarded.

Female mosquitoes were sorted under a stereomicroscope (Make: Olympus, Model: SZ61) using established taxonomic keys. Four categories were used in 2018: Ae. polynesiensis, Ae. (Finlaya) spp. (including Ae. samoanus), Culex spp., and all other Aedes spp. Additional entomological capacity in 2019 enabled sorting into nine categories: Ae. polynesiensis, Ae. aegypti, Ae. albopictus, Ae. upolensis, Ae. (Finlaya) spp., Aedes spp (other), Cx quinquefasciatus and Culex spp (other). Mosquitoes that could not be speciated were identified to genus level as Aedes spp (other) or Culex spp (other). All classification at the species level was carried out by qualified entomologists or trained technicians. To reduce workload, initial sorting by sex and genus (Aedes vs. Culex) was done by non-entomologist who had been trained and were competent in making this initial distinction.

Each species/category of mosquito was placed into pools of 1-25 mosquitoes in 1.5mL Eppendorf tubes. In 2018, mosquitoes for each species and PSU were pooled separately, but mosquitoes from different households were sometimes combined. Increased catch numbers in 2019 enabled a more precise pooling strategy, where each pool contained a single species from one household. Pooled mosquitoes in open tubes were oven-dried at 60°C for three hours, and tubes placed in boxes with silica gel desiccant and shipped to Smith College. Samples underwent DNA extraction utilizing the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Extractions occurred using a modified version of the manufacturer’s suggested protocol which has been previously described. Following extraction, all samples were tested for the presence of W. bancrofti using the real-time PCR assay utilizing all recommended recipes and protocols.

**Human seroprevalence survey**

Human surveys were conducted in all 35 PSUs in both years, with a target of 15 households per PSU. The baseline survey was conducted in Sept-Nov 2018, within three months of the start of MDA. The post-MDA survey was conducted from March-May 2019, six to nine months post-MDA and immediately prior to the 2019 MX survey (see Supplementary Figure S4 for survey timeline). Survey design has been previously described.

**Data analysis**

**Mosquito abundance, distribution, and prevalence**
We calculated mosquito abundance for each species and PSU as the number of female mosquitoes tested from all traps over the two-day trapping period in each year. We reported abundance by region, PSU and species. Mosquitoes not classified as *Culex* or *Aedes* were excluded.

Prevalence of mosquitoes infected with *W. bancrofti* was estimated from pool tested results using the R package PoolTestR\textsuperscript{26}. When estimating prevalence for a single PSU for a single species, genus, or without any adjustment for mosquito species, the function PoolPrev was used to calculate the maximum likelihood prevalence. For other estimates, the function PoolPrevBayes was used with default uninformative priors to fit Bayesian, mixed effect, multivariable logistic regression models modified for pooled data with variable pool sizes.

To compare prevalence between randomly and purposively selected PSUs in 2019, we fitted a multivariable model with fixed/population effects for region, species and selection method (random/purposive) and random/group effects for PSU. The OR and 95% CrI intervals for purposively vs randomly selected villages was used to determine differences and statistical significance. To examine the sensitivity of MX for detecting changes in infection rates from MDA, we compared the estimated prevalence in 2018 and 2019. For models comparing prevalence between years, we only included data from the 28 PSUs sampled in both years and collapsed species categories to those used in 2018.

To examine changes over time at the national or PSU level, we fitted multivariable models with fixed/population effects for vector species and, random/group effects for each PSU at baseline (2018), and random/group effects for the temporal change in each PSU. Region was not included as a covariate as approximate leave-one-out cross validation\textsuperscript{27} indicated including it did not improve the model. To examine changes over time by region, we modified the model to include fixed/population effects for each region in 2018 and fixed/population effects for the temporal change. From these models we calculated ORs (which approximate prevalence ratios as prevalence was low) and 95% CrI to quantify differences between years at national, regional and PSU levels. As a sensitivity analysis, we fitted alternative models adjusting for genus rather than species, or without adjustment for either. The presence/absence results for PSUs for *Ae. polynesiensis* and ‘all species’ were mapped.

**Human Ag and Mf prevalence**

Human Ag prevalence for each year was estimated at national and regional levels, and in the 30 randomly selected PSUs. Statistical analyses were performed using Stata (version 16, Stata Corp, College Station). Estimates were calculated using the *proportion* command for each age category (5-9 and $\geq 10$ years), adjusting for selection probability at PSU and individual levels, and for clustering at PSU level. Results were standardized by gender and (for those aged $\geq 10$ years) by age group using 5-year categories in the 2016 census. Finite population correction (FPC) was applied since sampling was without replacement. For human Ag, ORs were estimated by region and overall for each age group (5-9 and $\geq 10$ years) using *melogit*, adjusting for selection probability at PSU and individual levels, and for clustering at PSU. Age, gender and region were included as fixed effects. After stratification by region, OR for the 5-9 year-old group could not be estimated in two regions (AUA and ROU) because there were no Ag-positives in 2019.

**Comparing MX and human Ag as surveillance methods**

We compared MX and human Ag as surveillance methods in terms of presence/absence of positives, and prevalence difference between years. We measured correlation between Ag and MX results using a one-sided
Fisher’s exact test implemented with fisher.test in R\textsuperscript{28}. We assessed sensitivity and usefulness of MX for predicting the presence of Ag-positive humans in a PSU by using Ag presence/absence as the ‘reference’ state. Analyses were conducted for different mosquito categories to determine if the time, effort and expertise required to speciate mosquitoes improved predictive accuracy. We used the same presence/absence data to illustrate the concordance between the two surveillance methods (Supplementary 6), and reported concordance as percentage of PSUs in which the two methods provided the same result (both positive/both negative). Results were mapped to show the findings for each surveillance method by PSU and year.

To compare each surveillance method pre- and post-MDA, we used prevalence estimates by year in 28 PSUs that were surveyed in both years. We calculated ORs (2019 vs 2018) to determine significant changes in prevalence post-MDA. We did not conduct analyses for associations between the presence of PCR-positive pools and Mf-positive persons because Ag is the standard indicator currently used for LF surveillance. Also, the 2018 human survey was conducted immediately post-MDA, which would have affected Mf results.

**Data Availability**

All relevant data are within the paper. We are unable to provide individual-level antigen prevalence data and demographic data because of the potential for breaching participant confidentiality. The communities in Samoa are very small, and individual-level data such as age, sex, and village of residence could potentially be used to identify specific persons. For researchers who meet the criteria for access to confidential data, the data are available on request from the Human Ethics Officer at the Australian National University Human Research Ethics Committee, email: human.ethics.officer@anu.edu.au.

**Declarations**

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Figures
Figure 1

Number of female mosquitoes caught by species category and primary sampling unit (PSU) in a) 2018 and b) 2019 in Samoa. Values provided in Supplementary Table S1 and Supplementary Table S2.
Figure 2

Estimated prevalence of female mosquitoes infected with *W. bancrofti* by PSU and species category in a) 2018 and b) 2019 in Samoa. Values and confidence intervals provided in Supplementary Table S3 and Supplementary Table S4.
Figure 3

Estimated infection prevalence (%) by mosquito species, region and year (using data from the 28 randomly selected PSUs surveyed in both 2018 and 2019), Samoa. AUA=Apia Urban Area; NWU=North West Upolu; ROU=Rest of Upolu; SAV=Savai‘i. Values provided in Supplementary Table S5.
Figure 4

Presence of female mosquitoes (Ae. polynesiensis and 'any species') PCR-positive for *W. bancrofti* by PSU. Data from 2018 shown in the left hemisphere, and 2019 in the right hemisphere.
a) 5 - 9 year-olds

Figure 5

Adjusted LF prevalence from human survey for Ag in 2018 and 2019 for 30 randomly selected PSUs. Adjusted for selection probability at PSU and household (>10 years) or individual (5-10 years) levels, clustering at the PSU level, with finite population correction, and standardized for age and gender.

b) 10+ year-olds
**Figure 6**

PSU positivity by surveillance method for 2018 and 2019 showing concordance between human Ag positivity and MX positivity for *Ae. polynesiensis*, all *Aedes* spp., and all species.
Figure 7

Presence or absence of Ag-positive and Mf-positive humans and PCR-positive mosquitoes by PSU in a) 2018 and b) 2019. PCR results for ‘any species’ and Ae. polynesiensis shown in the left hemisphere of each circle. Human Ag and Mf results shown in the right hemisphere.
Figure 8

Change in prevalence between 2018 and 2019, expressed as an odds ratio (OR), for mosquito infection prevalence (MX for all species and Ae. polynesiensis), and human Ag prevalence (in those aged ≥10 years, and 5-9 years). Given the low prevalence, the ORs are approximately equal to prevalence ratios. ORs <1 indicate decrease in infection prevalence in 2019 compared to 2018, ORs >1 indicate an increase, and OR of 1 indicate no change. OR could not be calculated for 5-9 year-olds in AUA and ROU because no antigen-positive cases were detected in these groups in 2019.
Figure 9

Map of Samoa showing approximate locations of the 35 primary sampling units (PSU). Villages included in each PSU are given in Supplementary Figure S3. Insets show a) location of Samoa, and b) closeup of Apia Urban Area (AUA) and North West Upolu (NWU) regions.

Supplementary Files

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- McPhersonetalSupplementary3101.docx