Am. J. Trop. Med. Hyg., 80(5), 2009, pp. 774–781

Xenomonitoring of *Wuchereria bancrofti* and *Dirofilaria immitis* Infections in Mosquitoes from American Samoa: Trapping Considerations and a Comparison of Polymerase Chain Reaction Assays with Dissection

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Abstract. Entomologic monitoring of filarial infections, xenomonitoring, may have advantages in certain epidemiologic situations to assess the presence of infections in humans. Hemalum staining and dissection and polymerase chain reaction (PCR) were compared to determine the filarial infection status of *Aedes* (*Stegomyia*) mosquitoes in American Samoa. The overall prevalences of *Wuchereria bancrofti* and *Dirofilaria immitis* infections in *Ae. polynesiensis* were, respectively, 0.16% and 1.06% by dissection and 0.69% and 1.77% by PCR. Human filarial worm DNA rates in *Aedes aegypti* and *Aedes upolensis* were 1.16% and 0.38%, respectively. The results suggest that *W. bancrofti* transmission to humans may be continuing at low levels in some villages despite recent completion of 5 years of mass drug administration. PCR testing of mosquitoes collected using the BG-Sentinel traps represents a promising alternative to landing catches for assessing the transmission of filariasis in areas where *Ae. polynesiensis* and related species are the primary vectors.

INTRODUCTION

The parasitic nematodes *Wuchereria bancrofti* and *Dirofilaria immitis* are endemic in the islands that constitute the US territory of American Samoa. *W. bancrofti* is the major causative agent of lymphatic filariasis (LF), a debilitating disease that affects > 120 million individuals worldwide in 83 different countries,[1] whereas *D. immitis*, the etiologic agent for dog heartworm, is a major veterinary problem throughout many countries in temperate and tropical regions. In American Samoa, *W. bancrofti* is transmitted principally by the mosquito *Aedes* (*Stegomyia*) *polynesiensis* Marks, a semi-domesticated, primarily diurnal, mosquito distributed throughout much of the South Pacific.[2,3] *Ae. polynesiensis* is also an important vector of *D. immitis* in the Samoan islands.[4,5]

The passage of World Health Assembly resolution 50.29 in 1997 called for the elimination of LF. In 1999, 22 island nations and territories of the Pacific established the Pacific Program to Eliminate Lymphatic Filariasis (PacELF). The aim of this program is the elimination of LF as a public health problem in the Pacific by 2020, as well as the alleviation of the debilitating effects of the disease for those already infected. Control efforts are based on the annual co-administration of diethylcarbamazine (DEC) with albendazole.

Previous mass drug administration (MDA) programs in the Pacific countries were based on the use of DEC alone. In Samoa, the administration of DEC in two separate campaigns in 1966 and 1971 led to reductions in the microfilarial (Mf) rate from 21% in 1964 to 0.14% in 1973, but after cessation of these programs, the Mf rate rebounded to 2.1% by 1975.[6] Similarly, in French Polynesia, DEC administration at 6-month intervals during a 34-year period to residents of Maupiti failed to eliminate the parasite; in 2000, an Mf rate of 0.4% and antigenemia of 4.6% were found.[7] Much of the difficulty associated with the control of LF in the Polynesian islands can be attributed to the efficiency that *Ae. polynesiensis* exhibits as a vector. This species paradoxically serves as a more efficient vector in communities with low-density microfilaremia,[8] a situation that arises after multiple rounds of MDA.

The current PacELF program, based on the two-drug strategy, has yielded promising results. In American Samoa in 1999, before the PacELF MDA campaigns, convenience sampling of 18 villages showed an antigen positive rate of 16.5%.[9] In 2006, random testing of residents from four sentinel villages after five PacELF MDAs showed a reduction in the antigen positive rate to 0.95%.[10] Although Mf-testing and antigen testing in human populations are powerful tools, the persistence of filarial antigens in the human host makes real-time assessments of ongoing transmission in a community difficult.[11] Furthermore, there are reports of decreased sensitivity of antigen testing using ICT cards after multiple rounds of MDA.[12] This potential lack of sensitivity could call into question the reliability of using antigen testing for long-term monitoring or for establishing treatment endpoints.[12]

There is an increased need for the development of additional diagnostic tools that can evaluate the effectiveness of current control measures and provide evidence that MDA in a given community can be terminated.[11] One potential tool for assessing the progress of LF control programs in endemic communities is vector monitoring or xenomonitoring. Molecular xenomonitoring (MX), the use of polymerase chain reaction (PCR) to detect parasite DNA, in particular, serves as a real time measure of the rate at which humans expose mosquitoes to Mf.

The practicalities of MX depend on the ability to adequately sample the vector population, the ability to accurately determine the infection status in the vector, and the ability to relate infections in the vector population to infections in the human population. In the South Pacific from Fiji to French Polynesia as well as in parts of Southeast Asia, where *Aedes* are important vectors of LF, human landing catches have been the only method capable of collecting large numbers of aedine mosquitoes. However, this technique increases the risk to workers

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of exposure to vector-borne diseases and is unsuitable during periods of arboviral transmission. Therefore, the development and validation of a trap or collection method that collects adequate numbers of host-seeking *Aedes* females is essential.

Dissection and microscopic examination of mosquitoes have traditionally been the gold standard for determining filarial infection rates in mosquitoes. Although this method has the advantage of allowing investigators to identify the filarial worm life stage the mosquito is harboring (including the infective L3 stage), the method is labor intensive and time consuming and thus may not be practical for large-scale surveillance programs (i.e., as infection prevalence in mosquitoes declines, an increasing number of mosquitoes must be analyzed to determine the infection prevalence). PCR-based assays have been developed to detect the DNA of the human filarial worms *Brugia malayi*[13] and *W. bancrofti*[14] in blood and mosquitoes. These methods were later adapted for pools of mosquitoes.[15] These assays are highly sensitive, detecting 1 Mf in pools of 50–100 mosquitoes.[16] Variations of this technique have been used in field studies involving a variety of mosquito species including *Anopheles punctulatus*,[17] *Culex quinquefasciatus*,[18–20] *Culex pipiens*,[21,22] *Ae. polynesiensis,*[23] and *Mansonia annulifera*, *Mansonia uniformis*, and *Mansonia indiana*.[24] Studies suggest that xenomonitoring with PCR to detect parasite DNA is both sensitive and compatible with analyses of large sample sizes. In Egypt, PCR-based monitoring of mosquitoes for the presence of filarial DNA is a component in evaluating the progress of the filariasis control program.[25]

In this paper, the use of xenomonitoring was studied in an area after five annual MDAs with DEC and albendazole. Host-seeking female *Ae. polynesiensis* mosquitoes and female *Ae. aegypti* and *Ae. upolensis* mosquitoes from three villages on the island of Tutuila, American Samoa, were collected by BG-Sentinel mosquito traps (BioGents, Regensburg, Germany), and variations in trap efficacy among villages and within a village over time were studied. *Ae. polynesiensis* mosquitoes were screened for infection with *W. bancrofti*, the causative agent of human LF, and were also screened for infection with *D. immitis*, the causative agent of dog heartworm, by both PCR assays and hemalum staining with dissection to validate the PCR assay. *Ae. aegypti* and *Ae. upolensis* mosquitoes were also screened for infection with *W. bancrofti* by PCR. Finally, the potential role that these methods could play in assessing filarial infection and transmission in endemic communities that are nearing LF program endpoints is discussed.

MATERIALS AND METHODS

Study area. The study was conducted in the US territory of American Samoa (Figure 1) on the island of Tutuila (14°20′ S, 170°48′ W). Bancroftian filariasis in American Samoa is transmitted primarily by the mosquito *Ae. polynesiensis,* with *Aedes samoanus, Aedes tutuilae*, and *Ae. upolensis* playing lesser roles in transmission.[2–4,26] Mosquitoes were collected from three villages located in the Western health district of Tutuila: Afao, Asili, and Seetaga, between June 26 and July 19, 2006 (Figure 1). Samples from the human population were collected in parallel, and the results of those studies are reported in the accompanying paper.

Mosquito collections. Mosquitoes were collected using BG-Sentinel mosquito traps baited with BG-Lure.[27] Ten traps were deployed in each of the three villages. Trap sites were established throughout the villages in areas sheltered from direct sunlight and rain. The sites were at least 75–100 m from the next nearest trap site. Mosquitoes were collected twice daily (10:00 am and 6:30 pm), and trapping was conducted over two periods of 4 consecutive days each for a total of 8 trap days per village. The two 4-day collection periods were separated by 10 days. The mosquitoes collected at each trap location were held separately and transported to the laboratory, where they were anesthetized with CO_2 and identified using morphologic keys.[28,29] Female mosquitoes were pooled by trap location and time of collection and placed into 1.5-mL Eppendorf tubes containing 98% ethanol. Preserved female mosquitoes from each tube were randomly assigned to one of two experimental groups for analysis by either dissection or PCR.

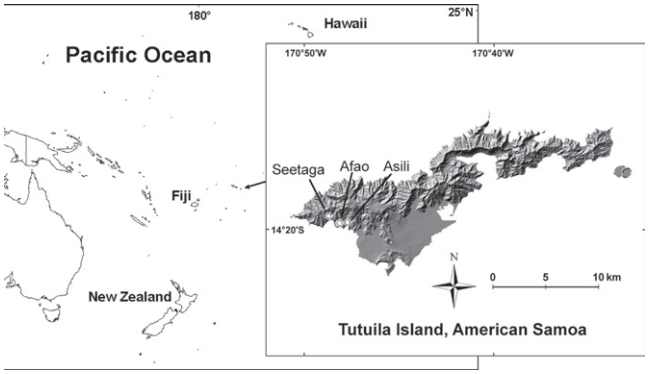

FIGURE 1. Map of Tutuila, American Samoa, showing geographic location of village study sites.

Mosquito staining. *Ae. polynesiensis* mosquitoes were stained using hemalum (Mayer's) stain (VWR, West Chester, PA) following a modification of Nelson.[30] Briefly, specimens were washed for 30 minutes in descending dilutions of ethanol (70%, 55%, and 25%) and were stained for 7 days in hemalum stain. After removal of the stain, the specimens were washed for 3 days in distilled water and stored in glycerol until dissected.

Dissection and microscopy. Mosquitoes were dissected individually in glycerol. The head and thorax were dissected separately from the abdomen on a glass slide using a Nikon SMZ-U Stereoscopic Zoom Dissecting microscope (Nikon Instruments, Melville, NY) at ×8 magnification. The number, location, and developmental stage of filarial worms were noted. Positive slides were re-screened at ×20–40 magnification to determine the species of filarial worm present in the mosquito specimen. Identifications were verified by a second parasitologist.

Extraction of DNA from mosquitoes. DNA from pools of *Ae. polynesiensis* (average pool size = 4.9 mosquitoes), *Ae. aegypti* (average pool size = 1.92 mosquitoes), and *Ae. upolensis* (average pool size = 2.10 mosquitoes) was extracted using a modification of the Qiagen DNeasy kit protocol (Qiagen, Hilden, Germany). Briefly, mosquitoes were dried overnight and placed in 2-mL grinding tubes containing 180 µL 1× phosphate-buffered saline (PBS) and a 0.177″ zinc-plated ball bearing. Mosquitoes were vortexed on a Fisher Vortex Genie 2 vortex mixer (Fisher Scientific, Waltham, MA) using a Mo Bio Horizontal vortex adapter (Mo Bio, Carlsbad, CA) for 15 minutes to macerate the specimens. The tubes were spun briefly, and 200 µL of lysis buffer (Buffer AL) and 20 µL of proteinase K were added to the samples. The samples were vortexed briefly and incubated at 70°C for 10 minutes.

An additional 20 μL of proteinase K was added to each tube, and the samples were incubated at 56°C for 60 minutes. The incubated material was then spun at 13,000*g* for 5 minutes, and the supernatant was added to 200 μL of 98% ethanol. This mixture of supernatant and ethanol was applied to the Qiagen DNeasy spin column. The column was washed twice with buffer AW1 and once with buffer AW2. DNA was eluted from the column into a labeled tube by adding 125 μL of AE elution buffer (performed twice). The purified DNA was used for the PCR assay.

Extraction controls. A negative DNA extraction control from a pool of non-infected laboratory reared *Ae. polynesiensis*, *Ae. albopictus,* or *Ae. aegypti* mosquitoes was run with each set of PCR reactions. In addition, a positive DNA extraction control and positive PCR control were also included with each set of PCR reactions. The positive DNA extraction control consisted of a pool of laboratory-reared mosquitoes spiked with blood containing *W. bancrofti* Mf or spiked with three to six *D. immitis* L3 stage larvae. The positive PCR control consisted of *W. bancrofti*–positive human blood without mosquitoes or three to six *D. immitis* L3 stage larvae without mosquitoes.

PCR amplification with *W. bancrofti* primers. PCR assays were performed using the NV-1 and NV-2 primers.[14,15] The target sequence for these primers is the SspI repeat, a gene present at ~500 copies per haploid genome. Amplification with these primers yields a 188-bp fragment. Each 50-μL PCR reaction contained 1× Qiagen Taq buffer, 3 mmol/L $MgCl_2$, 0.20 mmol/L each of dATP, dCTP, dGTP, and dTTP, 10 pmol of NV-1 and NV-2 primer, 1.25 U HotStarTaq DNA polymerase, and 1 μL genomic DNA. PCR reactions were run on a BioRad I-Cycler (BioRad, Hercules, CA), and reaction conditions consisted of a single step of 95°C for 15 minutes, followed by 54°C for 5 minutes. After these initial two steps, the reactions were subjected to 35 cycles of 72°C for 30 seconds, 94°C for 20 seconds, and 54°C for 30 seconds. The final step was a 5-minute extension at 72°C. PCR products were size fractionated on 2% agarose gels stained with GelRed (Biotium, Hayward, CA). Agarose gels were run at 70 V for 1 hour and visualized under UV light. Samples that were positive for the *W. bancrofti* SspI repeat were verified by repeating the PCR reaction in duplicate. A pool was confirmed positive if at least one of the two repeat samples were also positive.

PCR amplification with *D. immitis* primers. PCR assays were performed using primers based on a tandemly repeated *D. immitis* surface antigen present at 20–50 copies per haploid genome.[31,32] Amplification with these primers yielded a 378-bp fragment. Each 50-μL PCR reaction contained 1× Qiagen Taq buffer, 3 mmol/L $MgCl_2$, 0.20 mmol/L each of dATP, dCTP, dGTP, and dTTP, 10 pmol of *D. immitis* surface antigen forward and reverse primers, 1.25 U HotStarTaq DNA polymerase, and 1 μL genomic DNA. PCR reactions were run on a BioRad I-Cycler (BioRad). The PCR conditions consisted of a single step of 95°C for 15 minutes, followed by 50°C for 5 minutes; after these initial two steps, the reactions were subjected to 35 cycles of 72°C for 1 minute, 90°C for 1 minute, and 50°C for 1 minute. The final step was a 5-minute extension at 72°C. PCR products were size fractionated on 2% agarose gels stained with GelRed (Biotium, Hayward, CA). Agarose gels were run at 70 V for 1 hour and visualized under UV light. Samples positive for the DNA of the *D. immitis* surface antigen repeat were verified by repeating the PCR reaction in duplicate. A pool was confirmed positive if at least one of the two repeat samples was also positive.

Statistical analyses. Analyses of mosquito abundance were performed on the full collection data set (N = 4,367). Comparisons between the numbers of mosquitoes captured in each of the three villages and comparisons between the numbers of mosquitoes captured at the different trap locations within the villages were carried out using the Kruskal-Wallis test. Comparisons between the number of mosquitoes captured in the 10:00 am and the 6:30 pm collections and comparisons between the numbers of mosquitoes collected during the first 4-day sampling period versus those collected during the second 4-day sampling period were carried out using the Wilcoxon rank sum test. Analyses involving infection status were performed on the subset of the collection data that underwent PCR or dissection (N = 3,816). Mosquito infection rates by PCR as well as by dissection were calculated using PoolScreen2 software,[33] which provided maximum likelihood estimates (MLE) with 95% confidence intervals (CIs) based on the likelihood ratio method. The Fisher exact test was used to assess the association between infection and method of identification (PCR or dissection) at the village level and overall study area level. The Fisher exact test was used to test for associations between the two 4-day sampling periods and the presence of *W. bancrofti*–infected mosquitoes and to test for associations between morning and evening collections and the presence of *W. bancrofti*–positive mosquitoes. $P < 0.05$ was considered statistically significant, except in cases where multiple pairwise comparisons between the three villages were considered or a test was stratified by village. In these situations, the Bonferroni-corrected level of significance of 0.017 was used to ascertain significance. Statistical analyses were carried out with SAS Version 9.1 (SAS Institute, Cary, NC).

RESULTS

Mosquito abundance. Female mosquitoes were collected in three villages in the Western Health District of the island of Tutuila, American Samoa, over a 4-week period in June and July 2006. A total of 4,367 female *Ae. polynesiensis*, 267 *Ae. aegypti*, and 267 *Ae. upolensis* were collected in the three villages (Table 1). There were significant differences in the number of female *Ae. polynesiensis* mosquitoes collected in each of the three villages ($P < 0.001$). Pairwise comparisons showed that Asili and Seetaga, as well as Asili and Afao, collected significantly different numbers of *Ae. polynesiensis* ($P < 0.001$ for both comparisons), whereas Afao and Seetaga did not ($P = 0.14$). There was also a significant difference when comparisons were made among traps within each of the three villages ($P < 0.001$ for all three villages). Overall, there were significantly fewer *Ae. polynesiensis* mosquitoes captured in the morning (10:00 am) than in the evening (6:30 pm) collections ($P < 0.001$; Figure 2). When stratifying the analysis by village, the same result holds for Afao ($P < 0.001$) and Asili ($P < 0.001$) but not Seetaga ($P = 0.041$). There was not a significant difference within the three-village study area in the numbers of *Ae. polynesiensis* mosquitoes collected during the first 4-day sampling period versus the second 4-day sampling period ($P = 0.18$; Figure 3). When stratifying the analysis by village, the same result holds in Afao ($P = 0.41$) and Asili ($P = 0.33$), but not in Seetaga, where there were significantly more *Ae. polynesiensis* mosquitoes collected during the first sampling period than the second ($P = 0.014$).

TABLE 1
Number of female *Ae. polynesiensis*, *Ae. upolensis,* and *Ae. aegypti* mosquitoes collected from 10 trap locations in three villages on the island of Tutuila, American Samoa, 2006

Village	Trap	*Ae. polynesiensis**	*Ae. aegypti*†	*Ae. upolensis*‡
Afao§				
	1	83	9	2
	2	82	12	0
	3	82	4	0
	4	87	2	3
	5	311	17	3
	6	41	13	0
	7	22	10	0
	8	162	11	5
	9	86	2	1
	10	191	9	9
	Total	1,147[a]	89[a]	23[a]
Asili§				
	1	206	14	45
	2	261	28	57
	3	250	9	9
	4	135	11	7
	5	232	18	2
	6	227	8	2
	7	95	12	0
	8	269	9	33
	9	70	5	10
	10	544	14	37
	Total	2,289[b]	128[a]	202[b]
Seetaga¶				
	1	227	11	11
	2	96	3	2
	3	95	2	3
	4	44	8	2
	5	129	2	1
	6	160	2	16
	7	29	11	1
	8	66	3	0
	9	43	6	1
	10	42	2	5
	Total	931[a]	50[b]	42[a]
Cumulative total		4,367	267	267

*Total number of *Ae. polynesiensis* collected within a village. Values with different superscript letters within a column are significantly different at $P < 0.0001$.
†Total number of *Ae. aegypti* collected within a village. Values with different superscript letters within a column are significantly different at $P < 0.0001$.
‡Total number of *Ae. upolensis* collected within a village. Values with different superscript letters within a column are significantly different at $P < 0.0001$.
§Denotes villages in which among the 10 traps sampled at least 1 trap was significantly different from the others in the number of *Ae. polynesiensis* mosquitoes collected ($P < 0.0001$), *Ae. aegypti* mosquitoes collected ($P < 0.0001$), and *Ae. upolensis* mosquitoes collected ($P < 0.002$).
¶Denotes villages in which among the 10 traps sampled at least 1 trap was significantly different from the others in the number of *Ae. polynesiensis* mosquitoes collected ($P < 0.0001$) and *Ae. upolensis* mosquitoes collected ($P < 0.002$).

For both *Ae. aegypti* and *Ae. upolensis,* there were significant differences in the number of mosquitoes collected in each of the three villages ($P < 0.001$ for both species; Table 1). Pairwise village comparisons for *Ae. aegypti* were significant at the Bonferoni-corrected level for Asili and Seetaga, as well as Afao and Seetaga, but not for Afao and Asili ($P = 0.033$). Pairwise village comparisons for *A. upolensis* were significant at the Bonferoni-corrected level for Asili and Seetaga, as well as Afao and Asili, but not for Afao and Seetaga ($P = 0.13$). There were also significant differences when comparisons were made among traps within each of the three villages for both species except for *Ae. aegypti* in Seetaga ($P = 0.059$). At the three-village study area level, there were significantly more *Ae. aegypti* collected in the morning than the evening ($P < 0.001$). At the village level, this remained true for Afao ($P = 0.008$) and Asili ($P < 0.001$) but not Seetaga ($P = 0.17$). Overall, there was no significant difference in the number of

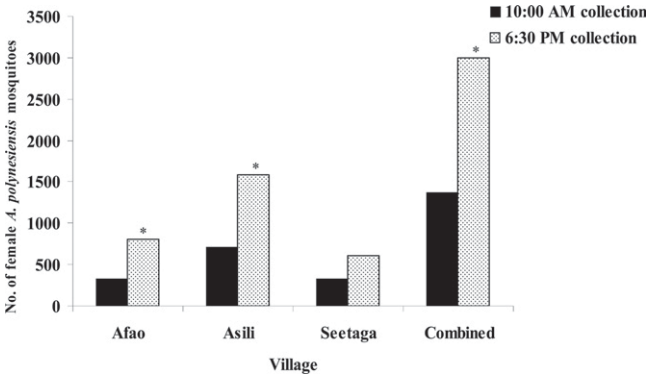

FIGURE 2. *Aedes polynesiensis* collections as a function of sampling time. The difference in the number of mosquitoes collected at 10:00 am (solid bars) and 6:30 pm (shaded bars) in the villages of Afao, Asili, and Seetaga. * Significant difference ($P < 0.001$) from 10:00 am sampling time.

Ae. upolensis mosquitoes collected in the morning relative to the evening ($P = 0.97$); this is also true at the village level for all three villages. At the overall study area level, there was no significant difference in the numbers of *Ae. aegypti* or *Ae. upolensis* collected in the first sampling period compared with the second sampling period ($P = 0.36$ and $P = 0.33$, respectively). This is true at the village level as well.

***W. bancrofti* infection rates in *Ae. polynesiensis*.** A total of 1,922 female *Ae. polynesiensis* were screened for infection with *W. bancrofti* and *D. immitis* by PCR and 1,894 *Ae. polynesiensis* were screened for infection by the two parasites by hemalum staining and dissection. PCR assays were performed on 390 pools, with each pool containing an average of 4.9 *Ae. polynesiensis* mosquitoes. The prevalence of *W. bancrofti* infection in *Ae. polynesiensis* by the staining and dissection technique ranged from 0% to 0.23% in all three villages, with a mean value of 0.16% (95% CI: 0.03–0.46%) when all three villages were pooled (Figure 4). Maximum likelihood estimates (MLEs) of infection by PCR analysis ranged from 0.52% to 0.90%, with a mean value of 0.69% (95% CI: 0.34–1.2%) when all three villages were pooled (Figure 4). There is a significant association between the method of identification and *W. bancrofti* infection prevalence ($P = 0.0201$), with PCR identifying more infections than dissection.

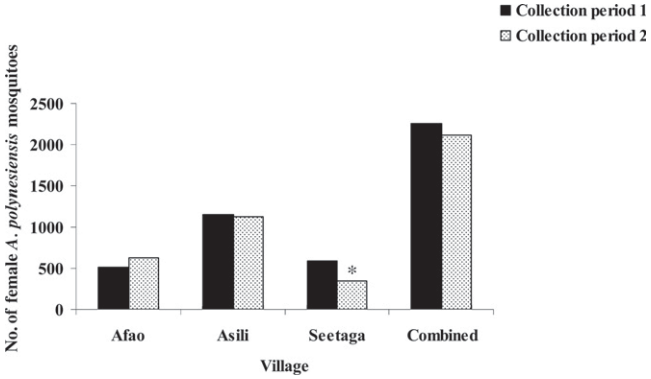

FIGURE 3. *Aedes polynesiensis* collections as a function of sampling period. The difference in the numbers of mosquitoes collected from Sampling Period 1 (solid bars) and Sampling Period 2 (shaded bars) in the villages of Afao, Asili, and Seetaga (4-day sampling periods). * Significant difference ($P < 0.05$) from first collection period.

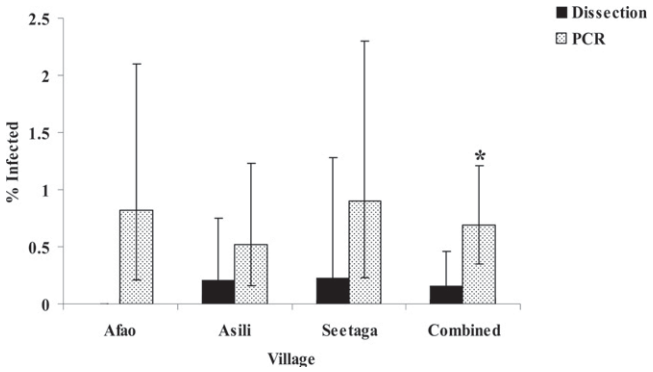

FIGURE 4. *Wuchereria bancrofti* prevalence of infection by staining and dissection (solid bars) and PCR (shaded bars) in *Ae. polynesiensis* mosquitoes. The error bars represent the 95% CI of the mean. * Significant difference ($P < 0.05$) from dissection.

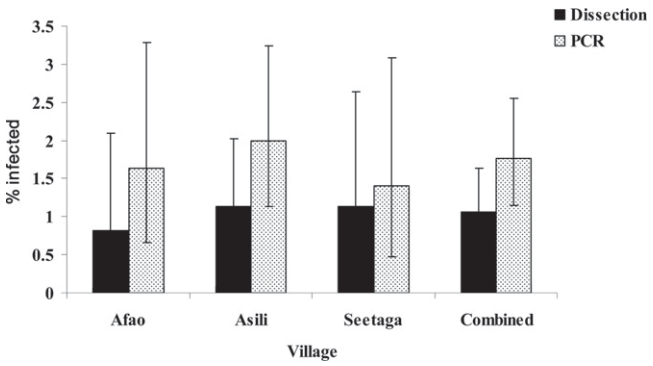

FIGURE 5. *Dirofilaria immitis* prevalence of infection by staining and dissection (solid bars) and PCR (shaded bars) in *Ae. polynesiensis* mosquitoes. The error bars represent the 95% CI of the mean.

Although there were 13 positive PCR pools, only three mosquitoes were found to be infected with *W. bancrofti* by staining and dissection. Two of these mosquitoes (both from the village of Asili) contained infective L3 stage larvae. The third mosquito, from the village of Seetaga, contained L1 and L2 stage larvae. The overall *W. bancrofti* L3-infective rate by dissection was 0.11% (95% CI: 0.01–0.37%).

There was a significant difference in the number of *W. bancrofti* PCR-positive mosquito pools from mosquitoes collected in the first 4 days of sampling compared with the number of *W. bancrofti* PCR-positive mosquito pools from mosquitoes collected in the second 4-day sampling period ($P = 0.004$), with the second sampling period identifying more positive pools than the first (11 and 2, respectively). There was no difference in the number of *Ae. polynesiensis* mosquitoes, collected in the first four days of sampling, that were positive by dissection compared with the number of positives from the second 4-day collection period ($P = 0.59$). There was no association between *W. bancrofti* infection and the time (morning or evening) of mosquito collection, regardless of the method used ($P = 1.00$ for both dissection and PCR).

***D. immitis* infection rates in *Ae. polynesiensis*.** The prevalence of *D. immitis* infection in *Ae. polynesiensis* mosquitoes by staining and dissection in the three surveyed villages ranged from 0.82% to 1.14%, with a mean value of 1.06% (95% CI: 0.68–1.63%) when the three villages were pooled (Figure 5). Point estimates of infection by PCR in the three surveyed villages yielded MLEs that ranged from 1.4% to 2.0% (Figure 5). The MLE by PCR for mosquitoes from the three villages when pooled together was 1.77% (95% CI: 1.6–2.55%). The overall infection rates as estimated by PCR (1.77%) and dissection (1.06%) were not significantly different ($P = 0.07$). The overall *D. immitis* L3-infective rate by dissection was 0.11% (95% CI: 0.01–0.37%).

***W. bancrofti* infection rates in *Ae. aegypti* and *Ae. upolensis*.** A total of 262 female *Ae. aegypti* mosquitoes and 264 female *Ae. upolensis* mosquitoes were screened for infection with *W. bancrofti* by PCR. Maximum likelihood estimates of infection in *Ae. aegypti* ranged from 0% to 1.58%, with a mean value of 1.16%, when all three villages were pooled (95% CI: 0.02–3.33%; Figure 6). There was no difference in the *W. bancrofti* infection rate between *Ae. polynesiensis* and *Ae. aegypti* ($P = 0.77$). The maximum likelihood estimate of infection in *Ae. upolensis* ranged from 0% to 0.51% (with only a single positive pool observed in the village of Asili). The maximum likelihood estimate of infection for all three villages when pooled together was 0.38% (95% CI: 0.02–1.95%; Figure 6). There was no difference in the *W. bancrofti* infection rate between *Ae. polynesiensis* and *Ae. upolensis* ($P = 0.21$).

DISCUSSION

As of 2005, MDA programs involving the administration of microfilaricidal drugs had been initiated in 42 of the 83 LF-endemic countries.[34] Monitoring of filarial transmission in communities that are participating in MDA programs is essential to determine the progress a community is making in its control efforts and determining MDA endpoints and certifying when transmission has been halted. The monitoring of mosquito populations for filarial worm infection has the potential to provide insight into the current transmission of LF in an endemic community. The detection of *W. bancrofti* DNA in *Ae. polynesiensis* confirms that infected individuals are still present in American Samoan villages despite the administration of multiple rounds of MDA, indicating that transmission from humans to mosquitoes may be occurring. Furthermore, the study confirms that PCR is a more sensitive technique than dissection for detecting infections in mosquito vectors.[11,16,22,23,35] Finally, this study shows that BG-Sentinel traps are an effective means of sampling *Ae. polynesiensis* mosquitoes and other mosquitoes within the *Stegomyia* subgenus.

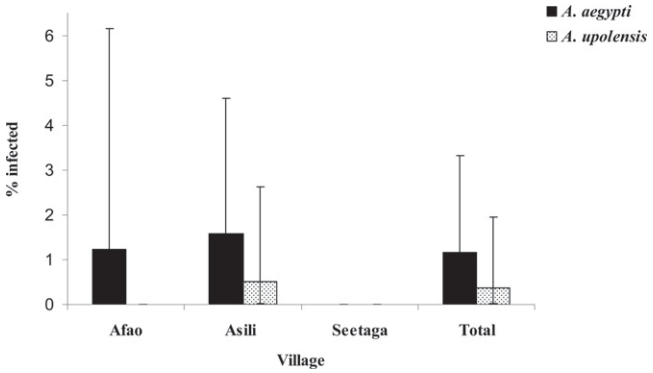

FIGURE 6. *Wuchereria bancrofti* prevalence of infection by PCR in *Ae. aegypti* (solid bars) and *Ae. upolensis* (shaded bars) mosquitoes. The error bars represent the 95% CI of the mean.

This study assessed two analytical techniques for determining filarial worm infections in mosquitoes: 1) hemalum staining coupled with dissection, long considered the "gold standard," and 2) the PCR assay. This is the first field study to evaluate human filarial worm infection rates in mosquitoes by both dissection and PCR from villages in the South Pacific that have participated in multiple rounds of the two-drug regimen MDA. Transmission of LF in the Polynesian region is unique relative to other endemic regions because of the diurnal nature of the mosquito vector and subperiodic circulation patterns of the microfilariae. Because of this, it is critical that the potential techniques for the detection of filarial worms in mosquitoes be tested within this vector/parasite system in multiple geographic locales. A comparison of these two methods was made by testing the mosquitoes for the presence of both *W. bancrofti* and *D. immitis*. Testing for the presence of *D. immitis* was included as a means of validating the work with *W. bancrofti*. Because there has been little to no treatment of canines for dog heartworm in American Samoa and because *Ae. polynesiensis* has been well established as a vector of *D. immitis*,[4,36] both analytical methods were simultaneously tested in vector–parasite systems that were low prevalence (*W. bancrofti*) and high prevalence (*D. immitis*). As expected, *D. immitis* infection rates were relatively high. The 1.06% infection rate determined by dissection was substantially higher than the 0.6% rate found in dissections from a wider survey conducted in 1963.[4]

Both methods were effective at detecting both *W. bancrofti* and *D. immitis* in mosquitoes. PCR was the more sensitive technique for the detection of both species of filarial worms, although the difference between dissection and PCR was statistically significant only for *W. bancrofti*–infected mosquitoes. The use of PCR should lead to rapid, high-throughput screening of mosquito vectors—a critical element in any program involving entomologic monitoring in a post-MDA environment. Although this study showed that the prevalence of *W. bancrofti* infection in *Ae. polynesiensis* in these three villages was < 1%, it should be noted that infected mosquitoes were found at approximately the same rate within each of the three villages, and serologic results from a parallel study involving residents of these three villages indicated that several children younger than 10 years of age tested positive for anti-filarial IgG4 antibody, indicating potentially recent exposure to or prepatent infection with *W. bancrofti*.[37] Thus, the evidence would suggest that residual transmission of LF, albeit at low levels, may be occurring within these villages. However, additional studies involving the screening of a substantially greater number of mosquitoes are necessary to fully understand the dynamics of LF transmission in American Samoa. The use of PCR should allow for the rapid screening of large numbers of mosquitoes. In addition, the study also showed that these infected mosquitoes can be easily preserved in ethanol for shipping to facilities where either PCR or dissection can be performed.

It should also be noted that the presence of *D. immitis* in *Ae. polynesiensis* mosquitoes may constitute an additional public health problem in American Samoa. Although rare, pulmonary human dirofilariasis has been observed throughout the United States as well as in Australia and Japan.[38,39] Humans are a dead-end host for *D. immitis*, but infection can lead to the development of pulmonary lesions. These lesions can be mistaken for malignancies, and patients are often subjected to costly and unnecessary surgical procedures.[39] In American Samoa, human exposure to *D. immitis* is a strong possibility because the principal vector, *Ae. polynesiensis,* is an opportunistic feeder that blood feeds on humans and domesticated mammals. The potential risk of human infection will remain without programs to control heartworm infection in canines or to reduce the mosquito vector population.

Previous studies have shown that BG-Sentinel traps are effective for capturing host-seeking *Ae. aegypti*.[40–43] In this study, these traps were effective in capturing female *Ae. polynesiensis* mosquitoes and female *Ae. aegypti* and *Ae. upolensis* mosquitoes. Preliminary studies conducted in American Samoa showed that the BG-Sentinel trap without carbon dioxide was comparable to a Fay-Prince trap with carbon dioxide.[27] This study also showed that BG-Sentinel traps collect greater numbers of *Ae. polynesiensis* mosquitoes in the late afternoon hours compared with morning collections, thus corroborating earlier studies that indicated that the peak biting time for *Ae. polynesiensis* was the late afternoon.[36,44] This observation supports the argument that mosquito collections in BG-Sentinel traps accurately reflect the number of active host-seeking female mosquitoes at each trap location. The use of BG-Sentinel traps could eliminate the reliance on human landing catches, thereby making xenomonitoring a possibility in *Aedes* transmission areas by eliminating unnecessary exposure of field workers to the bites of mosquitoes possibly infected with dengue or other arboviruses.

In addition to detecting *W. bancrofti* and *D. immitis* in *Ae. polynesiensis,* the study also detected *W. bancrofti* infections in *Ae. upolensis*, a secondary vector of LF in American Samoa,[3] and in *Ae. aegypti*, a species that does not allow *W. bancrofti* to develop to the second or third larval stages.[45] It is not surprising that pools of *Ae. aegypti* were PCR positive for *W. bancrofti,* because Fisher and others[46] showed that DNA from *B. malayi* could be detected by PCR for up to 3 weeks in pools of *Ae. aegypti* Liverpool strain mosquitoes (a competent laboratory vector) as well as in *Ae. aegypti* Rockefeller strain mosquitoes (a non-transmitting strain) after ingestion of an Mf-infected blood meal. Additionally, the persistence of *B. malayi* DNA for up to 3 days was observed in 80% of pools from *Cx. pipiens* Iowa, a non-transmitting species that does not allow the Mf to penetrate the midgut.[46] Because of the potential for DNA persistence in field-collected mosquitoes, it is important to remember that molecular xenomonitoring (MX) by conventional PCR is a tool to make indirect estimations of LF in human populations. At best, it allows for an immediate real-time assessment of the presence of Mf in an endemic community. Under this definition of MX, both vector and non-vector species from endemic communities could be screened by PCR. The recent report of a qRT-PCR assay that detects a *B. malayi* L3 stage-specific transcript in infected Liverpool strain of *Ae. aegypti* mosquitoes[47] is the first step in the development of advanced molecular tools that will allow for more precise estimates of one parameter of the mosquito-to-human transmission potential.

The detection of *W. bancrofti* DNA in *Ae. aegypti* mosquitoes has interesting implications for some mosquito monitoring programs. *Ae. aegypti* is a highly anthropophilic and endophagic species and, although it is not a natural vector of LF, the results from this study showed that this species could be used to document the presence of Mf within an endemic community. Furthermore, it is possible that far fewer numbers of *Ae. aegypti* would need to be screened to determine whether Mf-positive individuals are in a community because of its

propensity to exclusively take human blood meals. The ability of BG-Sentinel mosquito traps to collect sufficient numbers of host-seeking females could allow for the screening of this species to serve as a sentinel of *W. bancrofti* presence within a community. Nonetheless, within this study, there are clear advantages to using the primary vector, *Ae. polynesiensis,* as opposed to a "sentinel" species such as *Ae. aegypti*. There was no statistical difference in infection rates between the two species and the catch rate for *Ae. polynesiensis* was 16 times greater than that of *Ae. aegypti*. Furthermore, in American Samoa. *Ae. polynesiensis* is generally more abundant in pupal density and landing catch rates than *Ae. aegypti*.[48]

Although PCR should greatly increase our ability for high-throughput screening of potential LF vectors at a reduced cost-per insect, the costs, especially those of time and manpower, associated with a comprehensive mosquito-trapping strategy must be considered. This study showed that there were significant differences in the number of *Ae. polynesiensis* mosquitoes collected in the first 4-day trapping period that were *W. bancrofti* positive compared with the number of *Ae. polynesiensis* collected during the second 4-day trapping period that were *W. bancrofti* positive. If only the first of the two trapping periods were included in this study, there would have been an underestimation of the overall infection rate in the mosquito populations of the three villages. In addition, based on differences in the number of mosquitoes collected per trap, the study shows that there is high spatial variability in the density of *Ae. polynesiensis* among the trap sites within each of the three study villages. Given the short flight range of this species along with widely varying spatial densities, any xenomonitoring strategy used within the islands of the Pacific that involves the collection and screening of *Ae. polynesiensis* mosquitoes will need to include multiple traps within a study site as well as a well-planned and executed longitudinal sampling strategy. Nonetheless the BG-Sentinel trap should prove to be a powerful tool for collecting the numbers of *Ae. polynesiensis* mosquitoes needed to make accurate estimates of the prevalence of filarial worm infection within the mosquito population. Farid and others[25] have proposed an upper confidence level of 0.25% for filarial worm infection rates in mosquitoes in regions where *Culex* mosquitoes are the primary vector. Additional parallel studies of infections in humans and *Ae. polynesiensis* mosquitoes in regions of the Pacific that are engaged in MDA programs are still needed to determine the parasite DNA rate in *Ae. polynesiensis* mosquitoes that might signify an endpoint for MDA.

Received August 29, 2008. Accepted for publication December 4, 2008.

Acknowledgments: The authors thank Jeanette Tuileto'a, Sanerive Fuiava, and Olaiaiga Tupa'i for technical assistance in the field, Onosa'i Aulava for helping arrange mosquito trapping sites, and the American Samoa Department of Health, the American Samoa Community College Nursing Program, the American Samoa Community College Division of Community and Natural Resources, and the residents of Afao, Asili, and Seetaga for participation. The authors also thank the Filariasis Research Reagent Repository Center (FR3) for providing L3 stage *Dirofilaria immitis* and Mark Eberhard for assistance in microscopic identification of filarial worm species.

Financial support: Funding for this project was provided by GlaxoSmithKline. EWC was supported by an appointment to the Emerging Infectious Diseases (EID) Fellowship Program administered by the Association of Public Health laboratories (APHL) and funded by the Centers for Disease Control and Prevention as well as by support from the Atlanta Research and Education Foundation, Decatur GA. SM's research is supported by an appointment at the Division of Parasitic Diseases, National Center for Zoonotic Vector-Borne and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA, and the support of Atlanta Research and Education Foundation, Decatur, GA.

Disclaimer: The views of the authors are their own and do not necessarily represent those of the Centers for Disease Control and Prevention.

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REFERENCES

1. Michael E, Bundy DA, Grenfell BT, 1996. Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology 112:* 409–428.
2. Belkin JH, 1962. *The Mosquitoes of the South Pacific.* Berkeley and Los Angeles: University of California Press.
3. Ramalingam S, Belkin JN, 1964. Vectors of sub-periodic Bancroftian filariasis in the Samoa-Tonga area. *Nature 201:* 105–106.
4. Ramalingam S, 1968. The epidemiology of filarial transmission in Samoa and Tonga. *Ann Trop Med Parasitol 62:* 305–324.
5. Samarawickrema WA, Kimura E, Sones F, Paulson GS, Cummings RF, 1992. Natural infections of *Dirofilaria immitis* in *Aedes* (*Stegomyia*) *polynesiensis* and *Aedes* (*Finlaya*) *samoanus* and their implication in human health in Samoa. *Trans R Soc Trop Med Hyg 86:* 187–188.
6. Ichimori K, 2001. Entomology of the filariasis control programme in Samoa, *Aedes polynesiensis* and *Ae. samoanus. Med Entomol Zool 52:* 11–21.
7. Esterre P, Plichart C, Sechan Y, Nguyen NL, 2001. The impact of 34 years of massive DEC chemotherapy on *Wuchereria bancrofti* infection and transmission: the Maupiti cohort. *Trop Med Int Health 6:* 190–195.
8. Pichon G, 2002. Limitation and facilitation in the vectors and other aspects of the dynamics of filarial transmission: the need for vector control against *Anopheles*-transmitted filariasis. *Ann Trop Med Parasitol 96 (Suppl 2):* S143–S152.
9. Burkot TR, Taleo G, Toeaso V, Ichimori K, 2002. Progress towards, and challenges for, the elimination of filariasis from Pacific-island communities. *Ann Trop Med Parasitol 96 (Suppl 2):* S61–S69.
10. Liang JL, King JD, Ichimori K, Handzel T, Pa'au M, Lammie PJ, 2008. Impact of five annual rounds of mass drug administration with diethylcarbamazine and albendazole on *Wuchereria bancrofti* infection in American Samoa. *Am J Trop Med Hyg 78:* 924–928.
11. Weil GJ, Ramzy RMR, 2007. Diagnostic tools for filariasis elimination programs. *Trends Parasitol 23:* 78–82.

12. Njenga SM, Wamae CN, Njomo DW, Mwandawiro CS, Molyneux DH, 2008. Impact of two rounds of mass treatment with diethylcarbamazine plus albendazole on *Wuchereria bancrofti* infection and the sensitivity of immunochromatographic test in Malindi, Kenya. *Trans R Soc Trop Med Hyg 102:* 1017–1024.
13. Lizotte MR, Supali T, Partono F, Williams SA, 1994. A polymerase chain reaction assay for the detection of *Brugia malayi* in blood. *Am J Trop Med Hyg 51:* 314–321.
14. Zhong M, McCarthy J, Bierwert L, Lizotte Waniewski M, Chanteau S, Nutman TB, Ottesen EA, Williams SA, 1996. A polymerase chain reaction assay for detection of the parasite *Wuchereria bancrofti* in human blood samples. *Am J Trop Med Hyg 54:* 357–363.
15. Chanteau S, Luquiaud P, Failloux AB, Williams SA, 1994. Detection of *Wuchereria bancrofti* larvae in pools of mosquitoes by the polymerase chain reaction. *Trans R Soc Trop Med Hyg 88:* 665–666.
16. Nicolas L, Luquiaud P, Lardeux F, Mercer DR, 1996. Polymerase chain reaction assay to determine infection of *Aedes polynesiensis* by *Wuchereria bancrofti. Trans R Soc Trop Med Hyg 90:* 136–139.
17. Bockarie MJ, Fischer P, Williams SA, Zimmerman PA, Griffin L, Alpers MP, Kazura JW, 2000. Application of a polymerase chain reaction-ELISA to detect *Wuchereria bancrofti* in pools of wild-caught *Anopheles punctulatus* in a filariasis control area in Papua New Guinea. *Am J Trop Med Hyg 62:* 363–367.
18. Goodman DS, Orelus JN, Roberts JM, Lammie PJ, Streit TG, 2003. PCR and mosquito dissection as tools to monitor filarial infection levels following mass treatment. *Filaria J 2:* 11.
19. Hoti SLPK, Vasuki V, Lizotte MW, Hariths VR, Sushma N, Gunasekaran K, Ramaiah KD, Vanamail P, Mariappan T, Williams SA, 2002. Evaluation of Ssp I polymerase chain reaction assay in the detection of *Wuchereria bancrofti* infection in field-collected *Culex quinquefasciatus* and its application in the transmission studies of lymphatic filariasis. *J Appl Ent 126:* 417–421.
20. Vasuki V, Hoti SL, Sadanandane C, Jambulingam P, 2003. A simple and rapid DNA extraction method for the detection of *Wuchereria bancrofti* infection in the vector mosquito, *Culex quinquefasciatus* by SspI PCR assay. *Acta Trop 86:* 109–114.
21. Ramzy RM, El Setouhy M, Helmy H, Ahmed ES, Abd Elaziz KM, Farid HA, Shannon WD, Weil GJ, 2006. Effect of yearly mass drug administration with diethylcarbamazine and albendazole on bancroftian filariasis in Egypt: a comprehensive assessment. *Lancet 367:* 992–999.
22. Ramzy RMR, Farid HA, Kamal IH, Ibrahim GH, Morsy ZS, Faris R, Weil GJ, Williams SA, Gad AM, 1997. A polymerase chain reaction-based assay for detection of *Wuchereria bancrofti* in human blood and *Culex pipiens. Trans R Soc Trop Med Hyg 91:* 156–160.
23. Plichart C, Sechan Y, Davies N, Legrand A-M, 2006. PCR and dissection as tools to monitor filarial infection of *Aedes polynesiensis* mosquitoes in French Polynesia. *Filaria J 5:* 2.
24. Hoti SL, Vasuki V, Lizotte MW, Patra KP, Ravi G, Vanamail P, Manonmani A, Sabesan S, Krishnamoorthy K, Williams SA, 2001. Detection of *Brugia malayi* in laboratory and wild-caught Mansonioides mosquitoes (Diptera: Culicidae) using Hha IPCR assay. *Bull Entomol Res 91:* 87–92.
25. Farid HA, Morsy ZS, Helmy H, Ramzy RMR, El Setouhy M, Weil GJ, 2007. A critical appraisal of molecular xenomonitoring as a tool for assessing progress toward elimination of lymphatic filariasis. *Am J Trop Med Hyg 77:* 593–600.
26. Samarawickrema WA, Kimura E, Spears GF, Penaia L, Sone F, Paulson GS, Cummings RF, 1987. Distribution of vectors, transmission indices and microfilaria rates of subperiodic *Wuchereria bancrofti* in relation to village ecotypes in Samoa. *Trans R Soc Trop Med Hyg 81:* 129–135.
27. Schmaedick MA, Ball TS, Burkot TR, Gurr NE, 2008. Evaluation of three traps for sampling *Aedes polynesiensis* and other mosquito species in American Samoa. *J Am Mosq Control Assoc 24:* 319–322.
28. Huang Y-M, 1977. The mosquitoes of Polynesia with a pictorial key to some species associated with filariasis and/or dengue fever. *Mosquito Systematics 9:* 298–322.
29. Ramalingam S, 1976. An annotated checklist and keys to the mosquitoes of Samoa and Tonga. *Mosquito Systematics 8:* 298–318.
30. Nelson GS, 1958. Staining of filarial larvae in insects before dissection. *Bull World Health Organ 19:* 204.
31. Culpepper J, Grieve RB, Friedman L, Mika-Grieve M, Frank GR, Dale B, 1992. Molecular characterization of a *Dirofilaria immitis* cDNA encoding a highly immunoreactive antigen. *Mol Biochem Parasitol 54:* 51–62.
32. Poole CB, Grandea AG 3rd, Maina CV, Jenkins RE, Selkirk ME, McReynolds LA, 1992. Cloning of a cuticular antigen that contains multiple tandem repeats from the filarial parasite *Dirofilaria immitis. Proc Natl Acad Sci USA 89:* 5986–5990.
33. Katholi CR, Toe L, Merriweather A, Unnasch TR, 1995. Determining the prevalence of *Onchocerca volvulus* infection in vector populations by polymerase chain reaction screening of pools of black flies. *J Infect Dis 172:* 1414–1417.
34. WHO, 2006. Global programme to eliminate lymphatic filariasis. *Wkly Epidemiol Rec 81:* 221–232.
35. Williams SA, Laney SJ, Bierwert LA, Saunders LJ, Boakye DA, Fischer P, Goodman D, Helmy H, Hoti SL, Vasuki V, Lammie PJ, Plichart C, Ramzy RMR, Ottesen EA, 2002. Development and standardization of a rapid, PCR-based method for the detection of *Wuchereria bancrofti* in mosquitoes, for xenomonitoring the human prevalence of bancroftian filariasis. *Ann Trop Med Parasitol 96:* S41–S46.
36. Samarawickerma WA, Sone F, Cummings RF, 1987. Pa'au M, Seasonal abundance, diel biting activity and paraity of *Aedes polynesiensis* marks and *A. samoanus* (Grunberg) (Diptera: Culicidae) in Samoa. *Bull Entomol Res 77:* 191–200.
37. Mladonicky J, King J, Liang JL, Chambers EW, Pa'au M, Schmaedick MA, Burkot TR, Bradley M, Lammie PJ, 2009. Assessing transmission of lymphatic filariasis using parasitologic, serologic and entomologic tools following mass drug administration in American Samoa. *Am J Trop Med and Hyg 80:* 769–773.
38. Dissanaike AS, 1979. Zoonotic aspects of filarial infections in man. *Bull World Health Organ 57:* 349–357.
39. Theis JH, 2005. Public health aspects of dirofilariasis in the United States. *Vet Parasitol 133:* 157–180.
40. Krockel U, Rose A, Eiras AE, Geier M, 2006. New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. *J Am Mosq Control Assoc 22:* 229–238.
41. Maciel-de-Freitas R, Eiras AE, Lourenco-de-Oliveira R, 2006. Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes aegypti* (Diptera: Culicidae). *Mem Inst Oswaldo Cruz 101:* 321–325.
42. Williams CR, Long SA, Russell RC, Ritchie SA, 2006. Field efficacy of the BG-Sentinel compared with CDC Backpack Aspirators and CO2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. *J Am Mosq Control Assoc 22:* 296–300.
43. Williams CR, Long SA, Webb CE, Bitzhenner M, Geier M, Russell RC, Ritchie SA, 2007. *Aedes aegypti* population sampling using BG-Sentinel traps in north Queensland Australia: statistical considerations for trap deployment and sampling strategy. *J Med Entomol 44:* 345–350.
44. Jachowski LA, 1954. Filariasis in American Samoa. *Am J Hyg 60:* 186–203.
45. Lee DJ, Hicks MM, Griffiths M, Debenham ML, Bryan JH, Russell RC, Geary M, Marks EN, 1987. *The Culicidae of the Australasian Region.* Canberra, Australia: Commonwealth Department of Health.
46. Fischer P, Erickson SM, Fischer K, Fuchs JF, Rao RU, Christensen BM, Weil GJ, 2007. Persistence of *Brugia malay*i DNA in vector and non-vector mosquitoes: implications for xenomonitoring and transmission monitoring of lymphatic filariasis. *Am J Trop Med Hyg 76:* 502–507.
47. Laney SJ, Buttaro CJ, Visconti S, Pilotte N, Ramzy RM, Weil GJ, Williams SA, 2008. A Reverse transcriptase-PCR assay for detecting filarial infective larvae in mosquitoes. *PLoS Negl Trop Dis 2:* e251.
48. Burkot TR, Handzel T, Schmaedick MA, Tufa J, Roberts JM, Graves PM, 2007. Productivity of natural and artificial containers for *Aedes polynesiensis* and *Aedes aegypti* in four American Samoan villages. *Med Vet Entomol 21:* 22–29.