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Assessing Transmission of Lymphatic Filariasis Using Parasitologic, Serologic, and Entomologic Tools after Mass Drug Administration in American Samoa

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Abstract. Assessing the interruption of lymphatic filariasis transmission after annual mass drug administration (MDA) requires a better understanding of how to interpret results obtained with the available diagnostic tools. We conducted parasitologic, serologic, and entomologic surveys in three villages in American Samoa after sentinel site surveys suggested filarial antigen prevalence was < 1% after five annual MDAs with diethylcarbamazine and albendazole. Antigen and antifilarial antibody prevalence ranged from 3.7% to 4.6% and from 12.5% to 14.9%, respectively, by village. Only one person was microfilaria positive. Although no children less than 10 years of age were antigen positive, antifilarial antibody prevalence in this age group was 5.1% and antibody-positive children were detected in all three villages. *Wuchereria bancrofti*–infected mosquitoes were also detected in all three villages. Thus, monitoring of infections in mosquitoes and antifilarial antibody levels in children may serve as indicators of local transmission and be useful for making decisions about program endpoints.

INTRODUCTION

Lymphatic filariasis (LF) is a helminth infection caused by a parasitic worm that is transmitted between humans by a mosquito vector. Both debilitating and socially stigmatizing, LF is a leading cause of global disability. More than 120 million persons in 80 countries are already infected and more than 40 million persons are incapacitated or disfigured by chronic manifestations of filarial disease.[1] Fortunately, there are tools available for identifying infected persons who represent the reservoir for infection and for treating affected persons and communities with drugs that target circulating stages of the parasite.[2] The availability of these tools has led to the development of filariasis elimination programs based on mass drug administration (MDA).[3–5] The success of MDA depends on interrupting parasite transmission by reducing the level of microfilariae circulating in the blood.[5]

The Pacific Program to Eliminate Lymphatic Filariasis (PacELF) was created in 1999 with the goal of eliminating LF by 2010 in 22 countries and territories in the Pacific region where an estimated 1.8 million persons were infected.[6,7] American Samoa, one of the LF-endemic settings in the Pacific, has a long history of LF surveys and interventions. In 1930, 44–64% of adult residents of Tutuila were microfilaria positive with 6.7% exhibiting elephantiasis.[8] Prevalence in subsequent surveys was lower, perhaps as a consequence of vector control operations.[9] MDA campaigns using diethylcarbamazine (DEC) in 1963 and 1965 reduced microfilaremia to levels of 1–2%.[10] In the absence of further interventions in the 1980s and 1990s, antigen prevalence was 16.5% in a 1999 survey of residents of 18 villages.[6] In 2000, the American Samoa Department of Health (DOH), with support from the World Health Organization, commenced annual MDA with DEC and albendazole. Initial coverage was low ($\leq$ 50%), but communication and distribution strategies were modified, and in 2003, 2004, and 2005, coverage was increased to 70%, 65%, and 66% respectively. Surveys of the villages that served as sentinel sites demonstrated a decrease in antigen prevalence from 13% in 2003 to 0.95% in 2006.[11] Based on these results, additional surveys were conducted to determine whether transmission of LF had been interrupted.

Understanding how different measures of infection are related to residual transmission is important for making programmatic decisions about stopping MDA. Ideally, program managers should be able to use simple tools and survey methods to determine whether infection rates are below the thresholds required to maintain transmission for different parasite-vector combinations.[2] Microfilaremia is the gold standard for monitoring filarial infection. However, the sensitivity of blood tests for microfilaremia declines after MDA as microfilaria prevalence and intensity decrease. Rapid antigen tests are more sensitive than microfilaria detection and provide the additional advantage of daytime blood sampling in areas of the world where the parasite is nocturnally periodic.[12] PacELF guidelines for stopping MDA are based on the use of the antigen test in community-wide surveys.[13] Questions about the sensitivity of antigen tests for monitoring filarial exposure have prompted consideration of the use of antibody tests. In principle, antibody tests provide a cumulative measure of filarial exposure and the detection of antifilarial antibody in children born after MDA reflects the potential for transmission.[2] American Samoa provides excellent opportunities for comparing microfilaremia, antigenemia, and antifilarial antibody assays with mosquito infection rates in a low endemicity setting where *Aedes polynesiensis* is the major vector. We present the results of parallel parasitologic and serologic surveys in three villages. Entomologic findings are published in the accompanying paper.[14]

MATERIALS AND METHODS

Study area. The study took place in the American Samoan villages of Afao, Asili, and Seetaga in July 2006, approximately 10 months after the most recent round of MDA. Although these villages participated in past MDAs, they had not served as sentinel or spot check villages and had not been surveyed after the implementation of MDA. Village households were

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mapped using a global positioning system and each house was assigned a number.

Study population. Persons ≥ 5 years of age were enrolled, registered, and assigned an identification number by staff of the Department of Health. The number of persons registered for the study was approximately the same as the number of persons ≥ 5 years of age who were registered in the 2000 census. In Afao, Asili, and Seetaga, the number of persons tested for filarial infection was 97%, 93%, and 96%, respectively, of the number of persons registered. Participation was voluntary and verbal consent was given. Both registration and verbal consent forms were translated into Samoan. Personal information, including age and sex, along with the recall of MDA participation in the previous years were recorded, including how many years the drugs were taken, and if the drug was taken every year. Study protocols were reviewed and approved by Institutional Review Boards for both the Centers for Disease Control and Prevention (CDC, Atlanta, GA) and the American Samoa Department of Health.

Blood collection. A total of 120 μL of blood was collected by finger prick into capillary tubes. One hundred microliters was used for antigen testing in the field and the remaining 20 μL was used to prepare blood spots for later antibody screening. Blood spots were placed on Whatman (Maidstone, United Kingdom) no. 2 filter paper, allowed to dry, placed in plastic bags, taken to CDC, and stored at –20°C prior to antibody testing.

Antigen testing. Filarial antigen testing was done in the field with the immunochromatographic test (ICT) cards (Binax, Portland, ME) using 100 μL of blood.[12] Tests were read after 10 minutes according to the manufacturer's instructions and recorded as positive, negative, or unreadable. If the ICT result was positive, an additional 20 μL of blood was taken for microfilaria testing. All testing was done in daylight hours because *Wuchereria bancrofti* is diurnally sub-periodic in American Samoa.

Microfilaremia testing. Persons who tested positive for antigen by ICT were further tested for circulating microfilariae. Twenty microliters of blood was collected and smeared onto glass slides in the field, stained with Giemsa, and examined microscopically.

Antibody testing. A hole punch was used to punch two 6-mm samples from each filter paper blood spot. Samples were eluted overnight from blood spots at 4°C in 200 μL of phosphate-buffered saline–0.05% Tween 20. An enzyme-linked immunosorbent assay was used to detect antifilarial IgG4 to recombinant antigen Bm14 as previously described.[15] Antibody responses were standardized using a positive control serum that was included on each plate as a standard curve. A predetermined cutoff, based on the mean plus three standard deviations of the response of samples from nonendemic controls, was used to distinguish positive and negative results.

Treatment. Antigen-positive persons were treated with DEC and albendazole by Department of Health staff upon the conclusion of testing. All community residents were advised to continue to participate in MDA, regardless of ICT results.

Statistical analysis. Data were entered and prevalence rates were calculated using Microsoft Excel® (Redmond, WA). Prevalence rates were compared using a chi-square test.

RESULTS

Study population. A total of 579 persons ≥ 5 years of age were registered in Afao, Asili, and Seetaga (Table 1). Of persons registered, 88.1% reported being treated during the MDA in 2005. Pregnancy, refusal, and being off the island were the principle reasons given for noncompliance among persons who were not treated.

Prevalence. The overall prevalence of microfilaremia, antigenemia, and antifilarial antibody are presented in Table 2. Antigen prevalence was 4.4% in Afao, 3.7% in Asili, and 4.6% in Seetaga. Of the 24 antigen-positive persons, only one person in Afao was microfilaria positive on the basis of detection of microfilariae on a 20-μL blood film. The microfilaria prevalence for each village represents a minimum prevalence because only ICT-positive persons had a blood film prepared and examined. The Bm14 antibody prevalence was significantly higher than antigen prevalence in all three villages ($P < 0.01$ for all three comparisons) and ranged from 12.5% to 14.9%. Overall, antigen or antibody prevalence did not differ significantly by village ($P > 0.05$). The single microfilaria-positive person was also ICT positive and Bm14 positive. Of 23 ICT-positive persons tested for antifilarial antibody, 12 (52%) were Bm 14 positive. Antifilarial antibody levels were greater among antigen-positive than antigen-negative persons ($P < 0.001$).

The age-specific prevalences of antigenemia and antibody responsiveness are shown in Figure 1. The one person who was microfilaria positive was more than 40 years of age. Antigen prevalence increased significantly with age in Asili and Seetaga ($P = 0.011$ and 0.007, respectively, by chi-square for trend). In all three villages, antibody prevalence significantly increased with age ($P < 0.01$, by chi-square for trend). There were no antigen-positive children less than 10 years of age; however, all three villages had antibody-positive children less than 10 years old and overall antibody prevalence for this group was 5.1%.

DISCUSSION

The first years of the global filariasis elimination program have provided abundant evidence that annual MDA is an effective strategy for reducing filarial infection prevalence.[16–19] In principle, infection prevalence can be reduced to a point below which transmission can not be maintained. However, defining this threshold has been problematic. Despite the availability of new tools to monitor filarial infection and exposure in humans, we still do not have a good understanding of how these measurements relate to transmission dynamics. We investigated the relationships of microfilaremia, antigenemia, and antifilarial antibody to entomologic indices of infection in villages in American Samoa after MDA reduced antigenemia prevalence in sentinel sites to less than 1%.[11,14] Our results argue that a potential for filarial transmission still exists and

TABLE 1
Study population demographics of three villages, American Samoa

Characteristic	Afao	Asili	Seetaga
Male	86	102	111
Female	74	93	113
Total tested*	160	195	224
Median age, years	23	24	26
Age range, years	5–81	5–81	5–78

*Of persons registered: 165 in Afao, 205 in Asili, and 227 in Seetaga.

TABLE 2

Village-specific infection prevalence for lymphatic filariasis, American Samoa*

Village	ICT positive (%)	Mf positive (%)†	Bm14 positive (%)	*P*‡
Afao	7/160 (4.4)	1/7 (0.6)	19/152 (12.5)	0.009
Asili	7/191 (3.7)	0/7 (0)	25/172 (14.5)	0.002
Seetaga	10/218 (4.6)	0/10 (0)	32/214 (14.9)	0.003

* ICT = immunochromatographic test; Mf = microfilaria.
† Minimum Mf prevalence for the village assuming that all ICT-negative persons are microfilaria negative.
‡ *P* for comparison of antigen and antibody prevalence by chi-square test.

that antifilarial antibody responses in children may serve as a useful programmatic indicator of transmission.

Establishing optimal tools for program monitoring and definition of program endpoints has been a challenge for LF programs.[2] In principle, children born after the introduction of MDA represent the best population for detecting incident infections. However, patent infections may not develop for one or more years after infective mosquito bites and monitoring microfilaremia requires night blood collections in much of the world.[20] Monitoring antigenemia is now simpler with the availability of a rapid, sensitive, and specific diagnostic test, but is similarly, a lagging indicator with respect to monitoring active transmission.[12] Based on our results, monitoring antifilarial antibody responses may provide an important alternative. The Bm14 assay is sensitive for the detection of *W. bancrofti* and *Brugia* infections and is highly specific for filarial parasites.[21,22] In addition, antibody responses in primates develop before patent infection.[23] Thus, positive responses in young children are suggestive of either recent filarial exposure or infection. We detected no antigen-positive children less than 10 years of age in any of the three villages we tested, but, Bm14-positive children were found in all three villages (Figure 1). It is possible that these responses reflect exposures that took place prior to MDA. However, the possibility of more recent exposure can not be excluded.

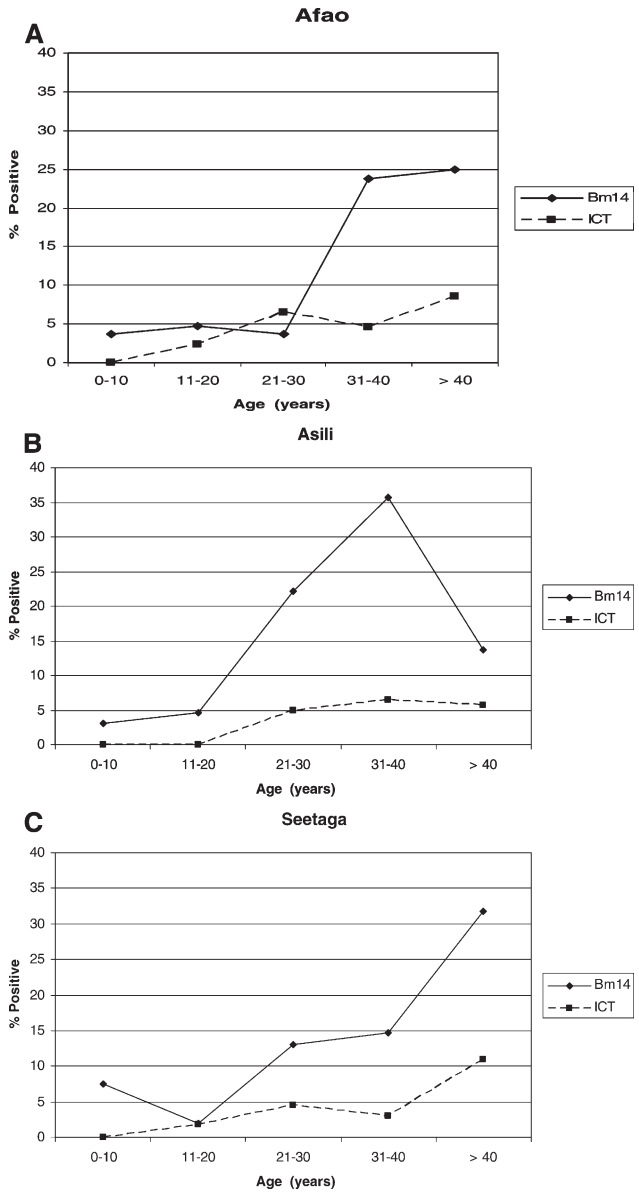

FIGURE 1. Age-specific prevalence of antigenemia (ICT), and anti-filarial antibody (Bm14) are plotted with squares and diamonds respectively for A) Afao, B) Asili and C) Seetaga.

Because *W. bancrofti*-infected mosquitoes were trapped in all three villages,[14] we have to consider, at a minimum, that a potential for transmission exists. Thus, antifilarial antibody responses may serve as an indicator of recent or ongoing transmission. Our study was cross-sectional in design and thus we can not establish a definitive association between the antibody responses in children and the infected mosquitoes that we trapped. However, work in Egypt and Papua New Guinea provides additional support for the conclusion that monitoring Bm14 antibody responses in children is a sensitive tool for assessing transmission.[17,24] Additional studies will be needed to define the sensitivity as well as the relative cost effectiveness of entomologic and serologic monitoring strategies. More monitoring experience will be required to determine the programmatic significance of trapping infected mosquitoes or identifying antibody-positive children. Introduction of a rapid format antibody test would also greatly enhance the utility of these tests for monitoring LF in the program context.

The implications of our observations for other LF programs in the Pacific are not clear. It is important to acknowledge that the detection of infected mosquitoes is indicative of potential transmission, but does not prove that people are being exposed to infective stage larvae or that levels of larval exposure are adequate to sustain transmission. Nonetheless, given past concerns about the potential for long-term persistence of transmission of LF in areas where *Aedes* spp. are vectors, a conservative interpretation of the entomologic and serologic findings seems warranted and these findings, in addition to an antigen prevalence of approximately 4% in these 3 villages, would argue that MDA should be continued in American Samoa.[13,25] Whether the PacELF target of 1% antigen prevalence is an appropriate stopping point for MDA will only be resolved by implementing rigorous post-MDA surveillance. Our observation of an association between antifilarial antibody responses in children and detection of infected mosquitoes suggests that either one of these tools could be used as the basis of a post-MDA surveillance strategy. In a recent editorial, Huppatz and others suggested that rapid antigen assays could be used to test children to monitor residual transmission.[26] Our data argue that the sensitivity of this surveillance strategy could be enhanced by measuring antifilarial antibody in children, rather than antigen.

Only one microfilaria-positive person was detected in any of the three villages. Nonetheless, *W. bancrofti*-infected mosquitoes

were collected in all three sites. This finding may reflect the use of a relatively insensitive method to detect microfilariae in antigen-positive persons, our failure to prepare blood slides from persons with negative ICT results, or the small number of people we were not able to test. However, there may be alternative explanations for the detection of infected mosquitoes in communities where we failed to identify microfilaremic persons. Because *W. bancrofti* is diurnally sub-periodic in American Samoa, and *Ae. polynesiensis* is a daytime feeder, mosquitoes in villages without detectable microfilaremia (Asili and Seetaga) may have acquired their infections by feeding on microfilaremic persons who were not village residents, but who visited or worked in these villages during the daytime. If this explanation is correct, it will be difficult to establish a spatial link between antibody-positive children and active transmission and this will pose a special challenge for the development of surveillance systems in areas where transmission is sub-periodic. It is important to investigate this relationship in the context of post-MDA surveillance activities.

Our results indicate that the impact of MDA on LF in American Samoa was less than previously reported.[11] Antigen prevalence in Asili, Afao and Seetaga was higher (approximately 4%) than in sentinel sites monitored as part of the LF program (< 1%). This finding is not surprising because greater levels of program activity in sentinel sites increase awareness of LF and subsequently, MDA participation. Conversely, all three villages selected for this study were small rural villages and therefore, may not be representative of the entire population of American Samoa. Consequently, we are not able to use these results as an indication of the overall infection level. An island-wide prevalence survey is necessary before programmatic decisions can be made either with respect to stopping MDA entirely or modifying the MDA strategy to target specific populations or communities that continue to represent potential reservoirs of infection. Nonetheless, these results demonstrate the value of spot check sites as recommended in the Program Manager's Guidelines for monitoring LF programs.[27]

In summary, in American Samoa, antifilarial antibody assays and xenomonitoring are useful tools to identify areas with potential transmission of lymphatic filariasis. Additional studies will be needed to determine the feasibility and cost effectiveness of these approaches. Understanding the limitations of available monitoring tools and the differences in the epidemiology of LF transmission is critical for making appropriate programmatic decisions, and this research should be a priority for the global program to eliminate LF.

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