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Editorial
Molecular Xenomonitoring of Lymphatic Filariasis

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Traditionally, estimates of the prevalence of lymphatic filariasis (LF) infection in its obligatory insect vector have been based on the results of labor-intensive, tedious, and time-consuming individual dissection of thousands of wild-caught female mosquitoes. In recent years, several polymerase chain reaction (PCR)–based assays have been shown to provide more rapid, sensitive, and species-specific methods of detecting LF in mosquitoes.[1–3] Molecular xenomonitoring is a term used to describe the detection of filarial DNA in mosquitoes.[1,2,4–6] It has been proposed as a sensitive marker for assessing the endemicity of LF and a useful tool for evaluating the success and progress of control programs.[2] Publication of an article in this issue of the journal by Farid and others[5] working in Egypt confirms that a PCR-based method for detecting LF in mosquitoes can be used successfully to evaluate national filariasis control programs.

Lymphatic filariasis is a major cause of acute and chronic morbidity of the lymphatic system of the extremities and male genitalia. The disease affects primarily poor populations who live in filariasis-endemic areas of the tropics and subtropics. An estimated 120 million persons are currently infected with LF in 83 countries, and some 1.3 billion are at risk of acquiring the infection. Most cases are caused by *Wuchereria bancrofti*, and *Brugia malayi* and *B. timori* infections account for less than 10%.[7] The World Health Assembly resolved in 1997 to eliminate LF as a public health problem. The Global Program to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000, with the principal objective of breaking mosquito transmission of LF. In September 2000, the Egyptian government initiated a national program to eliminate LF in line with GPELF. The strategy is to carry out mass drug administration (MDA) with five annual treatment rounds of single-dose diethylcarbamazine citrate plus albendazole to decrease the human reservoir of microfilariae below the level that is required for continuing transmission of LF infective larvae in the local mosquito population. Mass drug administration proceeded on a house-to-house basis, with directly observed ingestion of the drugs when possible, to protect 2.5 million people living in 181 filariasis-endemic villages.

In a recent assessment of the impact of five yearly rounds of MDA on LF in four sentinel villages, Ramzy and others[4] showed that in most localities MDA may have interrupted transmission through reduction in community microfilaria load. The decision about whether to stop or to continue MDA is critical for program managers. If MDA is stopped prematurely, several years may pass before continuing transmission is discovered; re-starting MDA may be extremely difficult for logistical and financial reasons. To date the main measures used to assess the impact of MDA have relied upon detection of parasites, parasite antigen, or antibodies against parasites in humans. Although these indicators decrease, they may persist in humans after transmission has been interrupted. Detection of LF microfilaria in mosquitoes indicates the existence of a reservoir of microfilaria in the local human and the presence of infective third-stage larva unequivocally signifies and quantifies transmission potential. Although molecular diagnostic tools now available do not distinguish between microfilaria and infective larvae (this would require detection of RNA or protein expressed exclusively by infective larvae or microfilaria), it is impractical and imprecise in most circumstances to rely on traditional mosquito dissection and microscopic detection methods to follow the progress of MDA, especially when infection rates are low after several rounds of MDA.

Farid and others[5] describe the application of a PCR-based entomologic method for assessing the impact of MDA on transmission of LF by *Culex* mosquitoes. Microfilaria rates in the human population were determined by membrane filtration of 1 mL of night blood, and a PCR assay was used to test pools of mosquitoes for infection with *W. bancrofti*. Studies were performed in Egyptian governorates with high and low endemicity and microfilaria-positive rates of 11.4% and 4.1%, respectively. Corresponding pre-treatment LF infection rates in mosquitoes were 3.07% and 1.76%, respectively. After five rounds of MDA, parasite DNA rates in mosquitoes was reduced to 0% in the low prevalence area. However, in the high-prevalence area where the post-MDA microfilaria positive rate in humans was 0.19%, LF-positive mosquitoes were sometimes detected by xenomonitoring in households where none of the residents were microfilaria positive. Alternatively, no PCR-positive mosquitoes were detected in some houses with microfilaria-positive residents. Many aspects of molecular xenomonitoring critical to evaluating it as a tool for assessing progress towards elimination of the disease are discussed in the report by Farid and others, including sampling strategies and interpretation of a positive PCR result for mosquito pools.

Demonstration of the utility of a highly sensitive method to show that five rounds of MDA can lead to the interruption of transmission in a *Culex-W. bancrofti* system is encouraging. The similarity between the pre-treatment mosquito infection rates in the areas of high and low endemicity areas suggests that xenomonitoring may not be appropriate for comparing filariasis endemicity levels in different areas where *Culex* mosquitoes are vectors. *Culex* species, which exhibit the phenomenon of limitation, appear to be able to pick up microfilaria from individuals who are microfilaria negative by membrane filtration. This may partly explain why positive mosquitoes were sometimes detected in households where none of the tested residents were positive. The observation that no PCR-positive mosquitoes were detected occasionally in houses with microfilaria-positive residents may be explained by non-random feeding by mosquitoes.

Recent studies show that LF DNA can be detected in both vector and non-vector mosquitoes for two weeks or longer after they ingest microfilaria positive blood.[6] Thus, although

molecular xenomonitoring may be sensitive for indirectly detecting LF in human populations, positive test results for parasite DNA in mosquitoes do not necessarily prove that transmission is ongoing in the area. New rapid, sensitive, and specific methods are required for detection of mosquitoes that harbor infective larvae.

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