

## A Critical Appraisal of Molecular Xenomonitoring as a Tool for Assessing Progress toward Elimination of Lymphatic Filariasis

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**Abstract.** We used molecular xenomonitoring (MX, detection of filarial DNA in mosquitoes) to evaluate the impact of mass drug administration (MDA) in sentinel locations in Egypt with high (11.5%) and low (4.1%) baseline microfilaria prevalence rates. Blood-fed *Culex pipiens* were pooled by household and tested for *Wuchereria bancrofti* DNA by PCR. There was no significant relationship between the infection status of household residents and parasite DNA status of mosquitoes from the same houses. After 5 MDA rounds, parasite DNA rates in mosquitoes in high- and low-prevalence areas were reduced by 93.8% and 100% to 0.19% (95% CI: 0.076–0.382%) and 0% (95% CI: 0–0.045%), respectively. These changes were consistent with decreases in microfilaria prevalence rates in these sites; they provide insight regarding the minimal mosquito DNA rates necessary for sustained transmission of filariasis in Egypt. We conclude that MX is a powerful tool for monitoring the impact of MDA on filariasis endemicity and transmission.

### INTRODUCTION

Lymphatic filariasis (LF) is a major tropical disease with an estimated 120 million people infected in 83 countries and some 1,300 million at risk of acquiring the infection.<sup>1</sup> The World Health Assembly resolved to eliminate lymphatic filariasis as a public health problem in 1997.<sup>2</sup> The Global Program for Elimination of Lymphatic Filariasis (GPELF) is based on a strategy of mass drug administration (MDA) of 4–6 annual rounds of antifilarial medications with the goal of reducing the reservoir of blood microfilariae (MF) below the level required for transmission by mosquitoes.<sup>3</sup>

Filariasis is focally endemic in Egypt. The mosquito vector responsible for transmission of filariasis in Egypt is *Culex pipiens*. This mosquito is widely distributed and extremely abundant throughout the country. It is mainly anthropophilic, endophilic, and endophagic.<sup>4</sup> Prior to the initiation of MDA in 2000, ~137,000 people in 181 villages and towns were infected with *Wuchereria bancrofti*, and 2.7 million were at risk of acquiring the infection.<sup>5,6</sup> Egypt was one of the first countries that initiated a National Program for Elimination of Lymphatic Filariasis (NPELF) based on WHO guidelines. The program comprised 5 annual rounds of MDA with diethylcarbamazine (DEC, 6 mg/kg) and albendazole (400 mg) in all known endemic areas in the country. MDA was distributed in September of each year from 2000 through 2004 to all eligible people in endemic areas (excluding pregnant women and children < 2 years of age). Reported coverage rates for the program exceeded 90% in all years. Approximately 2.7 million people were targeted in the 5th round of MDA (100% of the at-risk population in 2004).<sup>6</sup>

Molecular xenomonitoring (MX) employs PCR to detect filarial DNA in wild-caught mosquitoes. Prior studies have suggested the potential of this method for assessing the success of filariasis elimination.<sup>5,7</sup> Our early studies showed that MX could be used to estimate the relative prevalence of *W. bancrofti* infection in villages with high and low filariasis prevalence rates.<sup>8</sup> These studies used the *SspI* PCR assay to test pools of indoor-resting *Cx. pipiens*. Although dissection

can be used to detect filarial parasites in mosquitoes, MX becomes particularly valuable after implementation of MDA programs, when mosquito infection rates are reduced to levels that cannot be accurately assessed by dissection. MX has the potential to be a sensitive method for detecting persistent filarial parasites in communities. It should also provide an indirect indication of the potential for ongoing filariasis transmission.<sup>9,10</sup>

The purpose of this study was to evaluate MX as a tool for assessing the impact of a national filariasis elimination program in well-characterized sentinel sites. Our results show that MX can be a practical and efficient tool for assessing the impact of MDA in communities; it may also be useful for determining endpoints for LF elimination programs.

### METHODS

**Study sites.** Mosquito collections were carried out in 2 sentinel study sites at approximately the same time as blood surveys that were also conducted by our group. The study sites included a high-prevalence area in Giza governorate (Giz) and a low-prevalence area in Qalubia governorate (Qal) that had MF prevalence rates of 11.5% and 3.1% and community MF loads of 0.534 and 0.114, respectively, just prior to the first round of MDA in 2000.<sup>11</sup> The Giz study area (45 km south of Cairo) included the 2 contiguous villages of Kafr Bahary (KB) with 1,057 houses and Kafr Qebly (KQ) with 1,014 houses. These villages had no significant antifilarial treatment prior to initiation of MDA. The Qal study area included two adjacent villages (Kafr Tahoria, KT, with 208 houses and Tahoria, TH, with 852 houses) in the southeastern Nile delta ~35 km NE of Cairo. The Qal villages were more typical of localities included in the Egyptian NPELF than the Giz villages because they had low infection rates and a history of treatment of filariasis prior to initiation of the national program in 2000. We have reported results of prior studies of filariasis in the Qal villages.<sup>12,13</sup> KT was mass-treated with DEC (6 mg/kg) in 1998; ~20% of TH residents were screened in the same year, and infected subjects were selectively treated with DEC. Population estimates for KB, KQ, and TH are between 5,000 and 6,000; ~1,500 people live in KT. Study sites were mapped, and houses were sequentially numbered. Households were randomly selected for assessment of filarial

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infections using EpiInfo software, version 6.<sup>14</sup> Approximately 10–20% of the houses in these villages were studied each year.

**Blood collection and screening for *W. bancrofti* infection.** Population surveys were conducted 2–4 months prior to the first round of MDA and repeated each year just before the next round of MDA for 5 consecutive years as previously described.<sup>11</sup> Briefly, subjects were tested for circulating filarial antigen (CFA) with a rapid-format card test; persons with positive antigen tests were tested for microfilaremia by membrane filtration of 1 mL of night blood. Persons with negative antigen tests were considered to be amicrofilaremic.<sup>15</sup>

**Mosquito collections.** Mosquitoes were collected in 150–200 houses per year in each of the 2 study areas, with new houses sampled each year. Collections were carried out from May to August for 6 successive years (from 2000 to 2005), 1–4 months pre-MDA, and 8–11 months after each of 5 annual rounds of MDA. Individual houses were visited on a weekly basis for 1 month (4–5 visits per house). Sampling was conducted in each village approximately the same time each year. Trained field technicians aspirated resting mosquitoes on walls and beneath furniture in bedrooms from 10 PM to 1 AM (about 10 minutes/house). Female *Cx. pipiens* were counted and classified according to their abdominal appearance as blood-fed, gravid, or empty (neither blood-fed nor gravid) and transferred into labeled 1.5-mL Eppendorf tubes that were stored at –70°C for later detection of *W. bancrofti* DNA. Only blood-fed and gravid mosquitoes were retained for PCR analysis; empty mosquitoes were discarded. Mosquitoes from houses with > 25 fed or gravid females were divided into equal pools for PCR testing.

**Detection of *W. bancrofti* DNA in *Cx. pipiens*.** We used a standard PCR protocol for detecting *W. bancrofti* DNA in mosquitoes.<sup>9</sup> This method uses NV-1 and NV-2 primers to amplify the *SspI* DNA repeat sequence.<sup>16</sup> The test detects 1 pg of genomic *W. bancrofti* DNA, which is < 1% of the DNA content of a single microfilaria. The 188-bp amplified product was detected by agarose gel electrophoresis. Each PCR run included 2 types of negative control (DNA from unfed *Cx. pipiens* and a no-template control). Three types of positive controls were also tested in each PCR run: a dissected mosquito that contained 1 or more filarial worms, DNA from non-blood-fed mosquitoes that was spiked with purified *W. bancrofti* DNA, and purified *SspI*-DNA PCR product.

**Estimation of filarial DNA rates in resting mosquitoes.** Parasite DNA rates in mosquitoes (maximum likelihood with 95% CI) were estimated by PoolScreen 2.0 software as previously described.<sup>17</sup> We also analyzed PCR results in terms of the percent of positive pools and percent of houses with positive mosquitoes. The distribution of filarial infections within each study site was assessed by scoring houses as being in the “core” or “periphery” of the village. Peripheral houses face vacant lots or agricultural land on at least one side, whereas core houses are surrounded by other houses.

**Statistical analysis.** Data were analyzed with SPSS 11.0 software (SPSS, Inc., Chicago, IL). Proportions were compared by  $\chi^2$  or Fisher exact tests. The Mann–Whitney *U* test was used to compare group means for nonparametric data.

**Ethical clearance.** This study was reviewed and approved by institutional review boards at Ain Shams University and at Washington University School of Medicine. The Egyptian Ministry of Health and Population also approved the study.

## RESULTS

**General description of study areas.** There were 1,060 houses in the Qal study area. Figure 1 shows that the 130 houses surveyed before the first round of MDA in TH were distributed throughout the village. The major mosquito breeding sites identified in TH were 2 cesspools in the northern and NE edges of the village. The major mosquito breeding sites in KT were 2 abandoned wells near the edge of the village in the northeast sector. The Giz study area consisted of 2,071 houses. The main mosquito breeding site in Giz is a polluted drainage canal that runs along the east side of the study area.

**Mosquito collection and the influence of the number of mosquitoes per pool on detection of *W. bancrofti*.** *Cx. pipiens* comprised > 99% of indoor-resting mosquitoes collected in both study areas. Other species included *Ochlerotatus caspius*, *Anopheles tenebrosus*, and *Anopheles pharoensis*. In the months just prior to the first round of MDA, 6,571 female *Cx. pipiens* were collected from 223 houses in Giz in 946 house-nights ( $7.0 \pm 5.6$  [SD] mosquitoes per house-night). Of these, ~50.0% were blood-fed or gravid ( $14.7 \pm 8.7$  per house collected in 4 or 5 nights). In Qal, 2,671 *Cx. pipiens* females were captured from 179 houses in 728 house-nights ( $3.7 \pm 3.0$  per house-night), and 44.9% of the females were blood-fed or gravid ( $6.6 \pm 4.4$  per house collected in 4 or 5 nights). Thus mosquito densities were higher in Giz than in Qal ( $P < 0.001$ ). The number of mosquitoes collected per house-night tended to increase slightly over the course of the study (data not shown). Therefore, decreased infection rates in humans and mosquitoes after MDA did not result from changes in mosquito densities in the study areas.

Figure 2 shows the relationship between the number of mosquitoes tested per house and the percent of houses with mosquitoes that contained *W. bancrofti* DNA for each year of the study. The pre-MDA data show that the percent of positive pools increased with pool size in both study areas. The proportion of positive pools decreased after MDA for both large and small mosquito pools. These differences were significant for pool sizes of 10 or more mosquitoes in both areas ( $P \leq 0.01$ ). We consider pools with < 10 mosquitoes to be suboptimal for MX assessment of filarial DNA in mosquitoes by household.

Figure 3 shows frequency distributions for the number of blood-fed or gravid mosquitoes collected in houses in the 2 study areas, pre-MDA. Despite 4–5 visits per house, < 10 females were recovered in many of the houses in both study areas (74.3% of 179 houses studied pre-MDA in Qal, and 35.0% of 223 houses in Giz). This is a significant limitation for using the percent of positive houses to follow parasite DNA rates in mosquitoes in endemic areas. In contrast, PoolScreen 2.0 takes pool size into account for estimation of parasite DNA rates in mosquitoes. However, analysis of PCR results by household should provide information on the general location of mosquitoes with parasite DNA in a study area.

**Impact of MDA on parasite DNA in household mosquito collections (poolwise analysis).** Five rounds of MDA significantly reduced the frequency of positive mosquito pools in both study areas ( $P \leq 0.001$ ) for pools with  $\geq 5$  females (Figure 4) and also for houses with pools of  $\geq 10$  females (data not shown). Parasite DNA rates in mosquitoes de-

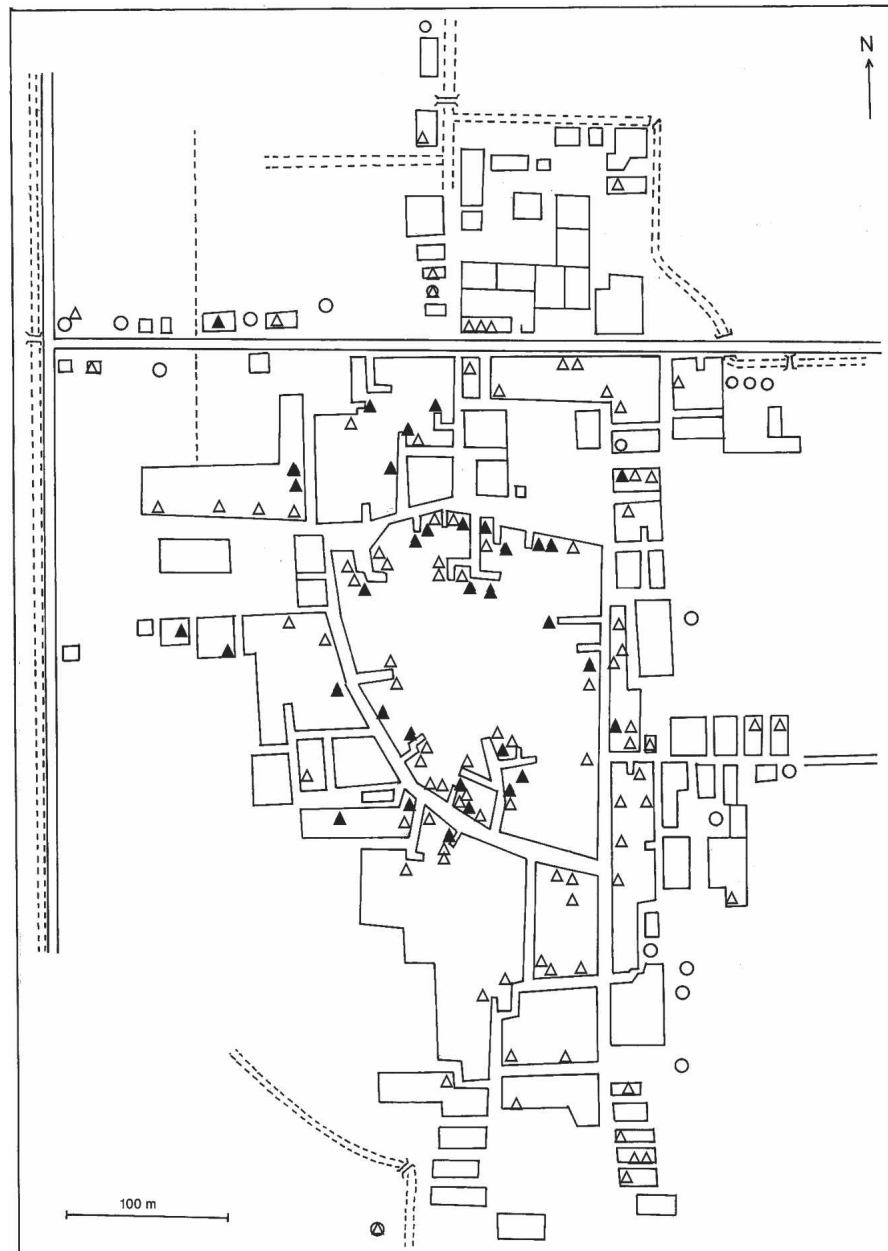


FIGURE 1. Map of Tahoria village in the Qal study area, showing households where mosquitoes were collected for PCR testing prior to the first round of mass drug administration (MDA): ▲, houses with mosquitoes positive for filarial DNA by PCR; △, houses with mosquitoes negative for filarial DNA by PCR; housing blocks are shown as squares; dashed lines represent irrigation and drainage canals.

creased more quickly in Qal (with the low baseline MF prevalence rate) than in Giz.

**Impact of MDA on filarial DNA rates in mosquitoes (pool-wise and PoolScreen analyses).** Table 1 shows parasite DNA rates in mosquitoes in the 2 study areas before and after MDA. This analysis includes all mosquito pools. Baseline infection rates were similar in the 2 areas prior to MDA. Parasite DNA rates in mosquitoes and the percentage of positive pools declined more rapidly after MDA in Qal than in Giz. Five rounds of MDA decreased the parasite DNA rate in Giz mosquitoes by 93.8%, from 3.066% to 0.190%, and the percentage of mosquito pools with parasite DNA decreased by 90.2%. The MF prevalence rate in humans in Giz decreased from 11.5% to 1.2% by membrane filter during this time

(89.6% decrease); the post-MDA MF rate by 50- $\mu$ L thick smear was 0.3%.

Five rounds of MDA decreased the parasite DNA rate in mosquitoes, percent positive mosquito pools, and MF prevalence by 100% in Qal. Indeed, only a few positive mosquito pools or MF carriers were detected in Qal after the second round of MDA.

**Effect of location in the village on mosquito and human infection rates.** Prior studies in Egypt have suggested that filariasis infection rates are higher in peripheral houses (houses that face vacant or agricultural land) than in core houses (houses that are surrounded by other houses).<sup>18</sup> Therefore, we compared human infection rates and parasite DNA rates in mosquitoes in peripheral and core houses pre-MDA in Giz

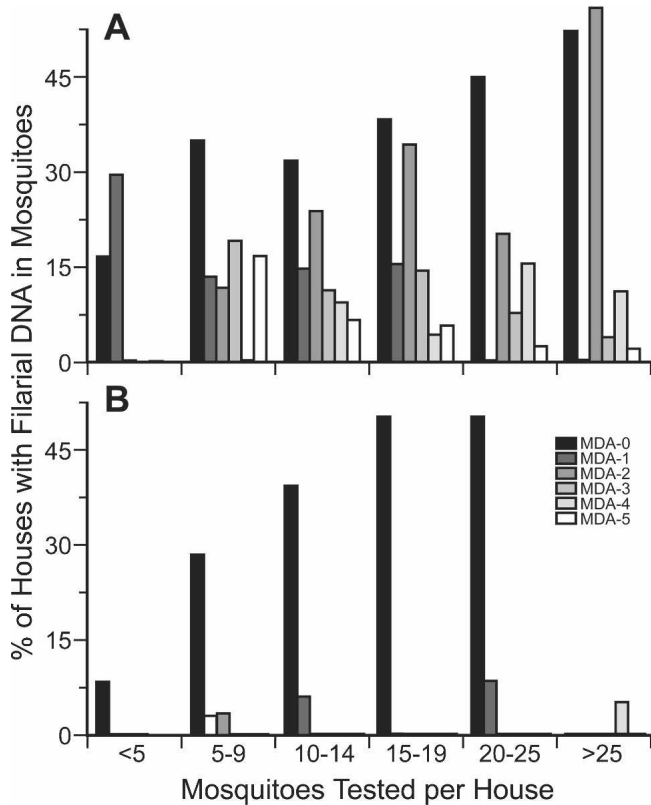


FIGURE 2. Effect of mosquito sample size on the percentage of houses with *Wuchereria bancrofti* DNA in *Culex pipiens* in the Giz (A) and Qal (B) study areas. Histograms show results obtained before the first round of mass drug administration (MDA) and after each round of MDA.

and Qal (Table 2). Human infection and parasite DNA rates in mosquitoes were essentially the same in core and peripheral houses in Giz. Human infection rates were higher in peripheral houses in the Qal study area, although only the filarial antigen test difference was statistically significant. The parasite DNA rate in Qal mosquitoes was higher in core houses, but this difference was not statistically significant. Thus, household location (core versus periphery) was not a major risk factor for filariasis in these study areas.

**Relationship between filarial DNA in mosquitoes and human filariasis in households.** Households with one or more MF carriers before MDA did not have significantly higher filarial DNA rates in mosquitoes than houses without MF carriers (Table 3). Mosquitoes with parasite DNA were sometimes detected in households where none of the tested residents had MF, whereas no PCR-positive mosquitoes were detected in some houses with MF-positive residents. After MDA-5, MF-positive people were detected in 5 houses in Giz, but no filarial DNA was detected in mosquitoes collected in these houses. No MF carriers and no DNA-positive mosquitoes were detected in Qal houses after MDA-5.

DISCUSSION

This study was conducted in 2 areas in Egypt in the context of its NPELF. The Qal study area had a low filariasis prevalence rate before the initiation of MDA in September 2000.

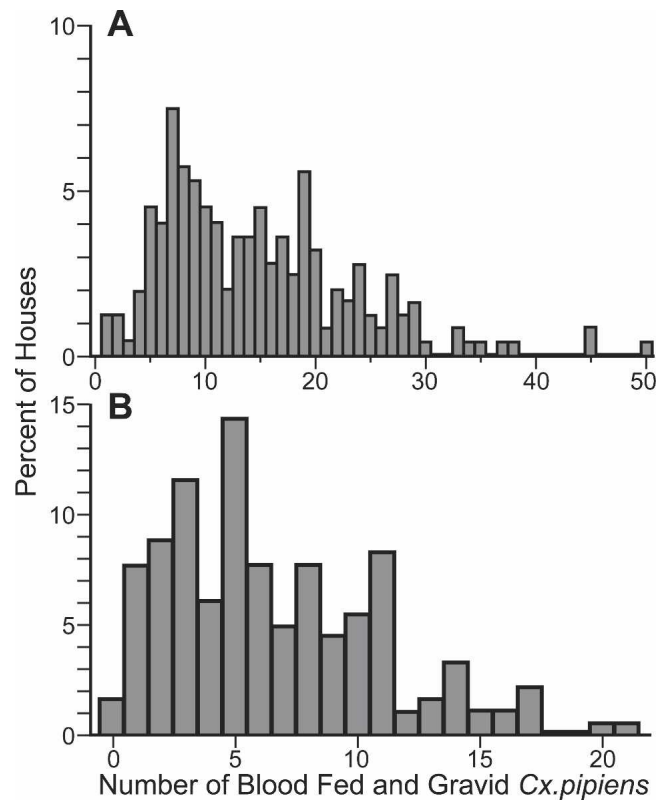


FIGURE 3. Frequency distributions for the number of houses with different numbers of gravid or blood-fed *Culex pipiens* captured before treatment in the Giz (A) and Qal (B) study areas.

This area was fairly typical of filariasis-endemic villages in Egypt just prior to the NPELF. In contrast, Giz had very high filariasis endemicity (for Egypt) pre-MDA. This was probably due to a lack of prior systematic treatment in the area, high mosquito densities, and perhaps other uncharacterized local conditions favorable for transmission.

We have previously shown that MX is useful for comparing filariasis endemicity levels in different areas.<sup>8</sup> Several prior studies have used PoolScreen to estimate filarial DNA rates

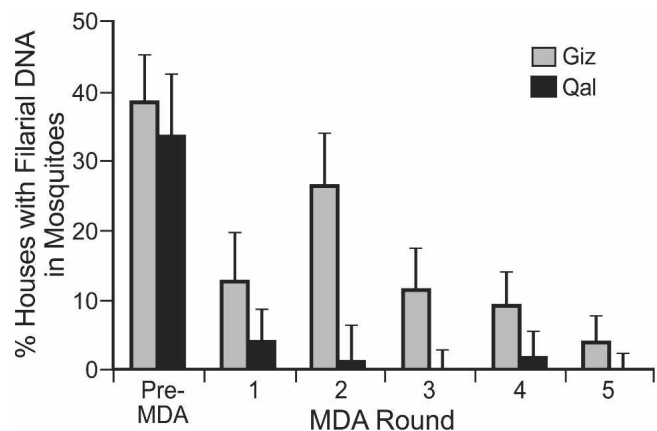


FIGURE 4. Impact of mass drug administration on the percent of houses with *Wuchereria bancrofti* DNA in *Culex pipiens* in the Giz (gray bars) and Qal (black bars) study areas. Data are shown for households with mosquito pool sizes  $\geq 5$ ; error bars show 95% confidence limits.

TABLE 1

Impact of 5 annual rounds of mass drug administration (MDA-0 to MDA-5) of single-dose DEC/Alb on *Wuchereria bancrofti* DNA rates in *Culex pipiens* collected in the Giz and Qal study areas

| Study area | MDA round | No. tested | No. of pools | Mosquitoes          |                  | PoolScreen mosquito infection estimates |           |
|------------|-----------|------------|--------------|---------------------|------------------|---|-----------|
|            |           |            |              | Mosq./pool $\pm$ SD | % Infected pools | %                                       | 95% CI    |
| Giz        | MDA-0     | 3,270      | 248          | 13.2 $\pm$ 5.8      | 33.5             | 3.07                                    | 2.38–3.88 |
|            | MDA-1     | 1,446      | 155          | 9.3 $\pm$ 5.4       | 15.5             | 1.76                                    | 1.08–2.68 |
|            | MDA-2     | 2,364      | 155          | 15.3 $\pm$ 5.1      | 24.5             | 1.84                                    | 1.25–2.58 |
|            | MDA-3     | 2,543      | 174          | 14.6 $\pm$ 5.0      | 9.8              | 0.70                                    | 0.38–1.14 |
|            | MDA-4     | 3,970      | 252          | 15.8 $\pm$ 4.5      | 7.1              | 0.47                                    | 0.27–0.77 |
| Qal        | MDA-5     | 4,273      | 245          | 17.4 $\pm$ 4.3      | 3.3              | 0.19                                    | 0.08–0.38 |
|            | MDA-0     | 1,197      | 179          | 6.7 $\pm$ 4.3       | 24.6             | 4.37                                    | 3.07–5.99 |
|            | MDA-1     | 1,832      | 153          | 12.0 $\pm$ 5.2      | 3.3              | 0.28                                    | 0.08–0.66 |
|            | MDA-2     | 1,374      | 136          | 10.1 $\pm$ 6.4      | 0.7              | 0.07                                    | 0.00–0.38 |
|            | MDA-3     | 2,110      | 151          | 14.0 $\pm$ 5.0      | 0.0              | 0.00                                    | 0.00–0.09 |
|            | MDA-4     | 3,730      | 220          | 17.0 $\pm$ 3.9      | 1.4              | 0.08                                    | 0.02–0.24 |
|            | MDA-5     | 4,258      | 237          | 18.0 $\pm$ 3.7      | 0.0              | 0.00                                    | 0.00–0.05 |

in culicines. For example, Goodman and co-workers, using 20- $\mu$ L thick smears,<sup>10</sup> found a filarial DNA rate in mosquitoes of 7.2% in an area in Haiti where 11.0% of the people had microfilaria. A study from Egypt reported a filarial DNA rate in mosquitoes of 8.1% by MX with PoolScreen when the MF prevalence rate by 50- $\mu$ L smear was 9.5%.<sup>17</sup> Lower parasite DNA rates in mosquitoes in the current study are generally consistent with these published results, given that the MF rates in the present study are based on membrane filtration of venous blood rather than thick smear. We were surprised to see equivalent pre-MDA parasite DNA rates in mosquitoes in Giz and Qal because of the difference in MF rates in these areas. Limitation (enhanced ability of *Culex* mosquitoes to ingest MF at low blood MF levels) may have contributed to this finding.<sup>19,20</sup> We doubt that infection rates in Qal were inflated by contamination in the laboratory, because we included negative controls in each PCR run. It is possible that mosquitoes were older (with more prior feedings on average) in Qal than in Giz. A prior study by our group showed that differences in rates of daily mosquito survival and survival to infectivity might explain local differences in filariasis endemicity.<sup>21</sup> Because PCR can detect parasite DNA taken up at any time during a mosquito's life, age could certainly affect parasite DNA rates in mosquitoes.<sup>22</sup> We did not assess mosquito parity in this study. In any case, we believe that comparison of parasite DNA rates in selected areas over time (as in this study) is more useful than comparisons between villages at a single point in time.

Prior studies have reported clustering of mosquitoes and human filarial infections at the edges of villages where larval breeding sites are concentrated and where residents are exposed to more mosquito bites.<sup>18,23,24</sup> Although the main mosquito breeding sites were in peripheral locations in both of our study sites, we did not observe significantly higher parasite DNA rates in mosquitoes, higher human infection rates, or higher mosquito abundance (data not shown) in peripheral houses in this study.

The Egyptian NPELF achieved high MDA coverage rates, and this had a dramatic impact on MF rates in humans.<sup>11</sup> Our study shows that MDA also had a dramatic impact on parasite DNA in mosquitoes whether this is expressed as the percent of mosquitoes with parasite DNA using PoolScreen or as the percent of houses with parasite DNA in mosquitoes. MDA had a more rapid impact on both measures of parasite DNA in mosquitoes in Qal, where MF prevalence rates and counts had already been decreased by treatment prior to initiation of MDA in 2000.

Researchers in Papua New Guinea and Haiti have reported significant declines in *W. bancrofti* infection rates in mosquitoes by dissection after MDA.<sup>10,25</sup> However, this is the first study that has demonstrated dramatic decreases and clearance of filarial DNA in mosquitoes after MDA using MX. Very large numbers of mosquitoes have to be tested to detect low residual filarial infection rates in mosquitoes after MDA and to show the absence of filarial infections with any degree of certainty. MX is superior to dissection for this purpose

TABLE 2

Human infection rates and parasite DNA rates in *Culex pipiens* from the Giza (Giz) and Qalubiya (Qal) study areas before mass drug administration

| Study area | Location* | Residents |                      |                       | Mosquitoes   |                     |
|------------|-----------|-----------|----------------------|-----------------------|--------------|---------------------|
|            |           | No.       | ICT card, % Positive | MF filter, % Positive | No. of pools | % Infected (95% CI) |
| Giz        | Periphery | 517       | 18.4                 | 10.6                  | 126          | 3.12 (2.20–4.28)    |
|            | Core      | 322       | 18.6                 | 12.1                  | 83           | 3.76 (2.43–5.50)    |
|            | <i>P</i>  |           | 0.998                | 0.585                 |              |                     |
| Qal        | Periphery | 310       | 18.7                 | 3.5                   | 64           | 3.48 (1.79–5.94)    |
|            | Core      | 193       | 8.3                  | 2.1                   | 46           | 6.54 (3.39–11.11)   |
|            | <i>P</i>  |           | 0.005                | 0.356                 |              |                     |

\* "Periphery" refers to houses that faced vacant land on at least one side. "Core" houses were surrounded by other houses on all sides.

TABLE 3

Relationship between rates of filarial DNA in *Culex pipiens* and human infections in Giz and Qal households before mass drug administration

| Study area | Household status* | No. of houses tested | No. tested | No. of pools | Mosquitoes          |                                   |   |            |
|------------|-------------------|----------------------|------------|--------------|---------------------|-----------------------------------|---|------------|
|            |                   |                      |            |              | Mosq./pool $\pm$ SD | % Pools positive for filarial DNA | PoolScreen mosquito infection estimates |            |
|            |                   |                      |            |              |                     |                                   | %                                       | 95% CI     |
| Giz        | MF-negative       | 127                  | 1,797      | 138          | 13.0 $\pm$ 5.8      | 32.6                              | 3.0                                     | 2.14–4.16  |
|            | MF-positive       | 61                   | 948        | 70           | 13.5 $\pm$ 5.9      | 41.4                              | 3.9                                     | 2.49–5.72  |
| Qal        | MF-negative       | 112                  | 772        | 112          | 6.9 $\pm$ 4.5       | 27.7                              | 4.9                                     | 3.22–7.15  |
|            | MF-positive       | 14                   | 84         | 14           | 6.0 $\pm$ 3.8       | 21.4                              | 4.3                                     | 0.83–12.03 |

\* "MF-negative" means that no microfilaria carriers were identified in the household sample; "MF-positive" means that at least 1 person tested in the house had microfilaremia.

because of its high sensitivity and its high-throughput capability. For example, we detected a residual parasite DNA rate of 0.19% (CI: 0.076–0.38) in Giz mosquitoes after MDA-5 with 4,273 mosquitoes in 245 pools. Confidence limits for parasite DNA rates in mosquitoes become smaller with larger mosquito samples. However, as with dissection, there are practical limits to the number of mosquitoes that can be captured and tested by PCR.

At this stage of the GPELF, when a number of countries have completed 5 or more rounds of MDA, there is heightened interest in endpoints or targets that program managers can use to decide when to stop MDA.<sup>26</sup> A recent paper suggested a post-MDA target of 0.5% MF for culicine-transmitted filariasis.<sup>27</sup> Based on our experiences with MX in Egypt, we believe it is reasonable for MDA programs to aim for residual parasite DNA rates in mosquitoes with an upper confidence limit of 0.25%. This is a conservative target that might be lower than necessary. In practical terms, this target is met if no more than 3 of 200 pools of 15 fed or gravid mosquitoes are positive by PCR. This proposed target applies to *Culex* mosquitoes. Other targets will be needed for areas where filariasis is transmitted by *Anopheles* or *Aedes* mosquitoes. The parasite DNA rate in Giz mosquitoes was just over our proposed target after MDA-5 (0.38%), when the MF rate in Giz was 1.2% by filter and 0.3% by 50- $\mu$ L blood smear. Parasite DNA rates met this target in Qal after MDA rounds 3–5, when MF rates in the population were well under 0.5% by filter. The target might also have been achieved in Qal after MDA-2 (when the residual MF rate was 0.6% by filter) if more mosquitoes had been tested. The small number of mosquitoes and pools tested that year limited the power of MX to show that the target had been reached. Of course, low residual rates of parasite DNA in mosquitoes do not necessarily mean that filariasis transmission is ongoing in an area. Prior studies by our group showed that mosquitoes fed on people with very low MF levels sometimes ingested MF but rarely produced infective larvae.<sup>28</sup> These mosquitoes would have been scored as positive by PCR. More data are needed on relationships between MF rates and other parameters after MDA to firm up targets for MDA programs.

More work is needed to develop better guidelines for mosquito sampling for monitoring by dissection or MX. A WHO workshop report recommended collection of at least 1,000 mosquitoes (10–50 mosquitoes per house, with 100–250 households) per sentinel site prior to MDA.<sup>5</sup> More mosquitoes are needed to detect parasites by MX or dissection when infection rates are reduced by MDA.<sup>5,29,30</sup> Our results confirm that large mosquito samples are critical for detecting *W. bancrofti* infection during late stages of a MDA program. Pool infection rates were higher for larger pools. This empha-

sizes the advantage of following PoolScreen estimates of DNA rates in mosquitoes over the simpler parameter "percent of positive pools."

We were able to obtain sufficient samples of resting mosquitoes for MX studies in this research project. However, the mosquito capture method we used is labor intensive and intrusive to village residents; our experienced field teams collected only  $3.8 \pm 3.5$  (median 3) blood-fed or gravid mosquitoes per house-night. This method is probably not practical for large-scale evaluation of national elimination programs. Gravid traps, which capture blood fed culicine mosquitoes in the act of oviposition, might be more practical for conducting MX on a large scale. Although filarial DNA rates in gravid trap mosquitoes tend to be somewhat lower than those in resting mosquitoes (Gad AM and others, unpublished data), we have found that gravid traps are much more efficient than aspiration of resting mosquitoes for collecting large numbers of *Cx. pipiens* for MX.

Improvements are also needed on technical aspects of MX for it to be practical for use in large-scale filariasis elimination programs. DNA isolation and PCR methods used in this study are labor-intensive and inefficient. New methods for isolation of DNA from mosquitoes and real-time PCR (with better sensitivity and throughput capacity relative to conventional PCR) promise to greatly improve our capacity to test large numbers of mosquitoes.<sup>31</sup> Work is also ongoing to further improve the statistical analysis and interpretation of MX data.

We found no significant relationship between filarial infections in humans and filarial DNA in mosquitoes in individual households. Filarial DNA was sometimes detected in mosquitoes collected in houses with no MF carriers and sometimes not detected in houses with MF carriers. Thus, MX was not useful for identification of households with MF carriers in this study. Such households may be more efficiently detected by pooling night blood samples by household for PCR testing in areas where filarial DNA is detected in mosquitoes. Complex blood feeding behavior may have contributed to the discrepancy between MF and MX test results. MF carriers may have been away from their houses when mosquitoes were blood feeding, or they may have not served as a blood source for collected mosquitoes. Positive mosquitoes may have taken blood from microfilaremic visitors in or near houses where they were captured, or they may have ingested MF in prior feedings. They also may have fed on microfilaremic household members who did not have blood collected for MF testing. Endophagic mosquitoes like *Cx. pipiens* rest indoors to release excess fluid from ingested blood for 6–8 hours after feeding, and they fly away once this process is completed.<sup>32,33</sup> However, studies in Egypt and India have shown that blood-

fed mosquitoes sometimes move between houses.<sup>8,34,35</sup> Therefore, the lack of concordance between the presence of MF carriers and DNA-positive mosquitoes in the same houses is not too surprising.

In conclusion, this study has provided interesting data on the use of MX in the context of filariasis elimination programs. Our data show that MX is a powerful tool for assessing the impact of MDA, and we have suggested a MX-based target that might be used with other targets such as MF rate for filariasis elimination programs in areas where filariasis is transmitted by *Culex* mosquitoes. We have also highlighted limitations of current methods and suggested changes that may make MX more practical for large-scale use.

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