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EFFECTS OF COMBINED DIETHYLCARBAMAZINE AND ALBENDAZOLE TREATMENT OF BANCROFTIAN FILARIASIS ON PARASITE UPTAKE AND DEVELOPMENT IN *CULEX PIPIENS* L.

HODA A. FARID, RAGAA E. HAMMAD, MARAH M. HASSAN, REDA M. R. RAMZY, MAGED EL SETOUHY, AND GARY J. WEIL*

Research and Training Center on Vectors of Diseases, Ain Shams University, Cairo, Egypt; Infectious Disease Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri

Abstract. We studied effects of combined diethylcarbamazine (DEC) and albendazole (ALB) treatment on *Wuchereria bancrofti* microfilaria (MF) uptake and development of infective larvae (L3) in *Culex pipiens*. Consenting Egyptian adults with microfilaremia (MF > 300/mL) were treated with one or seven daily doses of DEC/ALB. Laboratory-reared mosquitoes were fed on subjects before and after treatment. MF uptake and infectivity (assessed by mosquito dissection) were reduced by 89.6% and 82.9%, respectively, 12 months after single-dose treatment and by 96.2% and 99.7%, respectively, after multi-dose treatment. The L3:mosquito ratio decreased by 88% to 0.082 after single-dose treatment and by 99.8% to 0.001 after multi-dose treatment. If high coverage rates can be achieved for several annual cycles, mass drug administration (MDA) with DEC/ALB has the potential to decrease transmission to unsustainable levels and eliminate filariasis in populations. Multi-dose MDA (especially in the first year) might interrupt transmission with fewer cycles than single-dose treatment.

INTRODUCTION

Lymphatic filariasis (LF) is a serious, mosquito-borne illness that affects some 120 million people in 80 countries; more than one billion people reside in endemic areas and are at risk of becoming infected with the nematode parasites that cause LF (*Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*).[1] Several features of LF make this disease a good candidate for global elimination. First, there is no significant animal reservoir, and the infecting organisms cannot survive outside of their human and mosquito hosts. Second, the infection is inefficiently transmitted; many infective bites are required to establish new infections. Third, improved diagnostic tests have greatly simplified the process of identifying and mapping endemic areas.[2] Fourth, there are now single-dose oral treatments that safely and effectively reduce microfilaria (MF) counts to very low levels.[3,4] Based on these and other considerations, the World Health Assembly adopted a resolution calling for global elimination of LF as a public health problem by the year 2020, and this resolution led to launching of the Global Program for Elimination of Lymphatic Filariasis (GPELF) in 2000.[1,5] This program is based on the premise that mass administration of antifilarial medications (mass drug administration or MDA) can reduce the reservoir of MF to levels that cannot sustain transmission of the infection. In practice, national filariasis elimination programs generally aim to treat at least 80% of endemic populations with a single-dose of treatment annually for five years. Available treatments are only partly effective against adult filarial worms, which are believed to live for 5–7 years. Therefore, the five-year duration for MDA programs was chosen to minimize chances for adult parasites to survive MDA and renew the reservoir of MF in human populations needed for re-establishment of transmission. Repeated cycles of MDA also increase the percentage of endemic populations that receive any treatment, which further reduces availability of MF for transmission.

The recommended annual regimen for MDA in areas without coexisting onchocerciasis or loiasis is a single dose of diethylcarbamazine citrate (DEC) plus albendazole (ALB). This regimen is clinically safe and well tolerated, and it reduces levels of *W. bancrofti* MF for up to two years post-treatment.[3,4,6,7] However, prior studies have shown that low-level residual microfilaremia persists in most patients after a single dose of DEC/ALB.[4] The impact of this treatment on parasite uptake and development by mosquitoes has not been previously studied.

Bancroftian filariasis is focally endemic in Egypt, and the population at risk has been estimated to be approximately two million.[8] The principal vector responsible for transmission of filariasis in Egypt is *Culex pipiens pipiens*.[9] Culicines are relatively efficient vectors of *W. bancrofti*.[10] The thresholds for MF prevalence and MF counts in blood necessary for transmission by culicines are largely unknown. We have recently reported that only about one-third of *W. bancrofti* MF ingested by *Cx. pipiens* mature to the infective stage (L3).[11] We found that while *Cx. pipiens* sometimes ingest MF when they feed on infected subjects with negative 50 μL thick blood smears, such feedings produce very few L3. That study also showed that single-dose DEC treatment of MF carriers dramatically decreased MF uptake and L3 development in *Cx. pipiens*. When one considers that GPELF (which calls for delivery of MDA to millions of people in scores of countries) is based on the premise that MDA will interrupt transmission of filariasis, it is surprising that there are no published data on the effects of combination treatment regimens recommended by GPELF on parameters of transmission by vector mosquitoes. Therefore, the present study was designed to investigate effects of combined DEC and ALB treatment of subjects with bancroftian filariasis on MF uptake and L3 development in *Cx. pipiens*. We have also compared effects of single-dose and multi-dose treatments on these parameters.

MATERIALS AND METHODS

Selection of *W. bancrofti*-infected subjects. Human subjects for mosquito studies were recruited from a two-arm clinical treatment trial carried out by our team as previously

* Address correspondence to Gary J. Weil, Infectious Diseases Division, Washington University School of Medicine, Box 8051, 660 S. Euclid Ave., St. Louis, MO 63110. E-mail: gweil@im.wustl.edu

described.[12] Eligible subjects (healthy adult MF carriers with at least 80 MF/mL of venous blood) were treated with one oral dose of 6 mg/kg of DEC (Pharmamed Ltd., Zejtun, Malta) and 400 mg of ALB (Zentel®; GlaxoSmithKline, Uxbridge, United Kingdom) or seven daily doses of the same combined regimen. None of these subjects had received antifilarial therapy for at least one year prior to the initiation of the trial. Study subjects were assigned to treatment groups by stratified randomization; strata used were MF/ML counts, sex, and age. One subject in the multi-dose treatment group received a single dose of DEC with ALB approximately one month following the study treatment during a government mass treatment program. This did not materially affect our results, and we have elected to include data from this individual in this report (intention to treat analysis).

Mosquito exposure to *W. bancrofti* carriers. Mosquito feeding studies were performed 2–3 weeks before treatment and repeated 3, 6, 9, and 12 months after treatment. *Culex pipiens* pupae were collected from natural breeding sites in Qalubiya Governorate and transported to an indoor insectary with controlled temperature (26 ± 2°C), relative humidity = 70–80%, and a 16:8 photoperiod. Emerging mosquitoes were maintained on a 10% sugar solution until 24 hours before blood feeding. Three-to-five day old mosquitoes (approximately 200 females) were transported at night in a 30 × 30 × 30 cm mesh-screened wooden cage to the field site for exposure to MF carriers. The selected volunteer was asked to rest for 30 minutes and then introduce his forearm into the cage for another 30 minutes. To coincide with the periodicity of the parasite, blood meals were offered to mosquitoes for 30 minutes between 10:00 PM and midnight. A finger prick thick blood film (50 μL) was prepared immediately prior to mosquito feeding for later staining with Giemsa and microscopic examination.

Measurement of mosquito vector competence. For MF uptake studies, an aliquot of blood-engorged females were cold-killed immediately after feeding and stored at −70°C. Later, ingested blood meals were thawed, dissected onto glass slides, lysed in tap water, and microscopically examined for the presence and number of MF. Microfilaria uptake rates (percentage of mosquitoes with MF) and infection intensities (MF/mosquito) were recorded.

To assess the development of L3 in mosquitoes, other blood-fed females were maintained on a carbohydrate diet for 13 days (the extrinsic incubation period for *W. bancrofti* in these mosquitoes in our insectary), dissected, and microscopically examined for the presence and number of L3. The infectivity rate (percentage of mosquitoes with L3) and L3/mosquito were recorded. The L3 yield was calculated for mosquitoes fed on each donor, and defined as L3/mosquito divided by MF/mosquito. Yield was not calculated in cases with no MF ingested. Yield was assigned a value of 1.00 when L3/mosquito was greater than MF/mosquito. Laboratory staff responsible for dissections and entomologists evaluating the effects of the treatments were unaware of treatment group assignments for the human subjects.

The efficacy of the treatment regimens at each time point was assessed three ways. The first was by changes in absolute numbers of blood smear MF counts and parameters of mosquito vector competence. The second was by changes in blood smear MF counts and parameters of mosquito vector competence relative to pre-treatment values. Relative values were calculated for each subject by dividing post-treatment values by pre-treatment values and multiplying by 100. In cases where MF counts, MF uptake or infectivity increased after treatment, relative values were considered to be 100% (i.e., no reduction). The third was by clearance rates for MF counts, MF uptake, or infectivity, which were defined as the percentages of treated subjects with negative blood smears, who failed to transmit MF to blood-fed mosquitoes, or who failed to generate infective mosquitoes, respectively. The primary endpoints for group comparisons in this study were predefined to be complete absence of MF uptake and absence of infective larvae in mosquitoes 13 days after feeding. Secondary endpoints were percentage reductions in MF uptake and L3 production relative to pre-treatment values.

Ethical clearance. This study was reviewed and approved by institutional review boards at Washington University School of Medicine and at Ain Shams University. Written informed consent was obtained from all subjects enrolled in the study.

Data analysis. Database management and analysis were performed with SPSS 11.1 software (SPSS Science, Chicago, IL). The significance of differences in proportions was tested by chi-square or Fisher's exact tests. The Mann-Whitney U test for nonparametric data was used to analyze differences between independent group means of MF counts (thick smear), percentages of MF uptake, and L3 infectivity, MF/mosquito, and L3/mosquito. Paired comparisons over time of absolute numbers of MF counts, rates and intensities of MF uptake, and infectivity within each treatment group were performed by the nonparametric Wilcoxon signed ranks test. Error values shown are SD except as noted. Correlations between variables were assessed by computing Spearman's (r_s).

RESULTS

Twenty-nine subjects were enrolled in the study. The two treatment groups were comparable in terms of pre-treatment filter MF counts by membrane filtration, sex ratio, and age ($P = 0.727$, $P = 0.812$, and $P = 0.983$, respectively) (Table 1). In addition, subjects in the two treatment groups had comparable MF counts by smear immediately before exposure to mosquitoes (Table 2). A total of 4,084 mosquito blood meals

TABLE 1

Pre-treatment characteristics of *Wuchereria bancrofti*–infected subjects treated with either single- or multi-dose diethylcarbamazine and albendazole

		Microfilaria/mL				Age (years)		
Treatment	No. studied	Range	Mean ± SD*	Median	Male:female*	Range	Mean ± SD*	Median
Single-dose	14	453–3,720	1,051.9 ± 843.8	747.5	9:5	18–67	35.0 ± 17.9	24.5
Multi-dose	15	340–4,531	1,064.0 ± 1,047.7	717.0	9:6	18–42	30.3 ± 8.2	31.0

* $P > 0.05$ for differences between treatment groups by Mann-Whitney test for means and chi-square test for proportions.

TABLE 2

Wuchereria bancrofti infection* in human subjects and in blood-fed *Culex pipiens* before and after treatment with single- or multi-dose diethylcarbamazine and albendazole

Time†	No.*	MF/50 μL	Infection rates (%)		Infection intensity	
			MF uptake	Infectivity	MF/100 mosquitoes	L3/100 mosquitoes
Pre-treatment						
Single-dose	14	49.9 ± 23.2	49.8 ± 20.4	33.9 ± 15.1	195.9 ± 169.4	66.8 ± 47.7
Multi-dose	15	42.7 ± 43.5	52.4 ± 25.2	30.5 ± 15.9	255.5 ± 3.454	64.0 ± 47.7
P‡		0.138	0.827	0.600	0.827	0.861
3 months post-treatment						
Single-dose	14	25.1 ± 38.2	20.9 ± 20.6	3.1 ± 4.2	44.2 ± 46.1	6.2 ± 9.2
Multi-dose	15	1.1 ± 2.1	0.8 ± 3.1	0.0 ± 0.0	2.0 ± 7.8	0.0 ± 0.0
P‡		0.000	0.000	0.002	0.000	0.002
6 months post-treatment						
Single-dose	14	17.0 ± 22.7	12.8 ± 14.0	12.5 ± 14.4	19.6 ± 25.2	24.2 ± 32.5
Multi-dose	12	1.9 ± 5.4	1.1 ± 1.9	0.8 ± 1.3	1.3 ± 1.9	1.0 ± 2.0
P‡		0.005	0.005	0.004	0.006	0.004
9 months post-treatment						
Single-dose	14	11.7 ± 16.1	11.7 ± 13.9	7.8 ± 9.8	16.0 ± 22.6	14.0 ± 20.7
Multi-dose	13	1.2 ± 3.6	0.2 ± 0.7	0.2 ± 0.6	0.2 ± 0.6	0.2 ± 0.6
P‡		0.002	0.000	0.002	0.000	0.002
12 months post-treatment						
Single-dose	14	9.9 ± 14.2	5.2 ± 7.9	5.8 ± 6.9	6.1 ± 10.5	8.2 ± 10.9
Multi-dose	12	0.3 ± 0.5	2.0 ± 3.7	0.09 ± 0.2	2.0 ± 3.7	0.1 ± 0.2
P‡		0.012	0.317	0.002	0.317	0.002

* Data shown are means ± SD. No. = number of human subjects tested; MF = microfilaria; L3 = infective larvae.

† All post-treatment values in both treatment groups were significantly lower than pre-treatment values ($P \leq 0.009$ and $P \leq 0.003$, by analysis of variance for the single-dose and multi-dose treatment groups, respectively).

‡ The Mann-Whitney U test was used for group comparisons.

from 137 feeds were dissected for MF uptake, with 29.8 ± 6.7 (median = 30) females dissected per feed. Likewise, 18,780 females were dissected for infectivity, with 137.1 ± 82.9 mosquitoes dissected per feed (median = 112). Pre-treatment rates and intensities of MF uptake and infectivity for mosquitoes did not vary significantly between treatment groups (Table 2).

Relationships between blood microfilaremia and parameters of *W. bancrofti* infection in mosquitoes. Before treatment, rates of MF uptake (Figure 1A) and infectivity (Figure 1B) were significantly correlated with MF counts in thick blood smears. There was a tendency for yield to increase with lower MF counts (pre-treatment data), but this tendency was not statistically significant. Uptake of MF by mosquitoes remained highly correlated with MF counts in blood smears at all time points after treatment ($r_s \geq 0.68$, $P < 0.001$). Mosquito infectivity was moderately correlated three months after therapy ($r_s = 0.45$, $P < 0.014$), but this correlation became stronger at later time points ($r_s \geq 0.66$, $P < 0.001$).

The L3 yield data were calculated for all subjects with positive MF smears (both groups combined). The L3 yields at 0, 3, 6, 9, and 12 months were 0.44 ± 0.28, 0.14 ± 0.24, 0.55 ± 0.44, 0.66 ± 0.35, and 0.55 ± 0.47, respectively. The decrease in yield between times 0 and 3 months is significant ($P = 0.001$); this may reflect a temporary post-treatment effect on MF. The L3 yields were not significantly correlated with smear MF counts before treatment or three months after treatment ($r_s = -0.17$, $P = 0.384$ and $r_s = 0.20$, $P = 0.540$, respectively). However, at all later time points, when MF counts in blood smears were reduced, yields were significantly correlated with smear MF counts ($P \leq 0.05$).

Effects of treatment on blood MF counts, MF uptake, and infectivity in mosquitoes. Absolute and relative data are shown in Table 2 and Figure 2, respectively. Changes over time within and between groups are more easily seen in Fig-

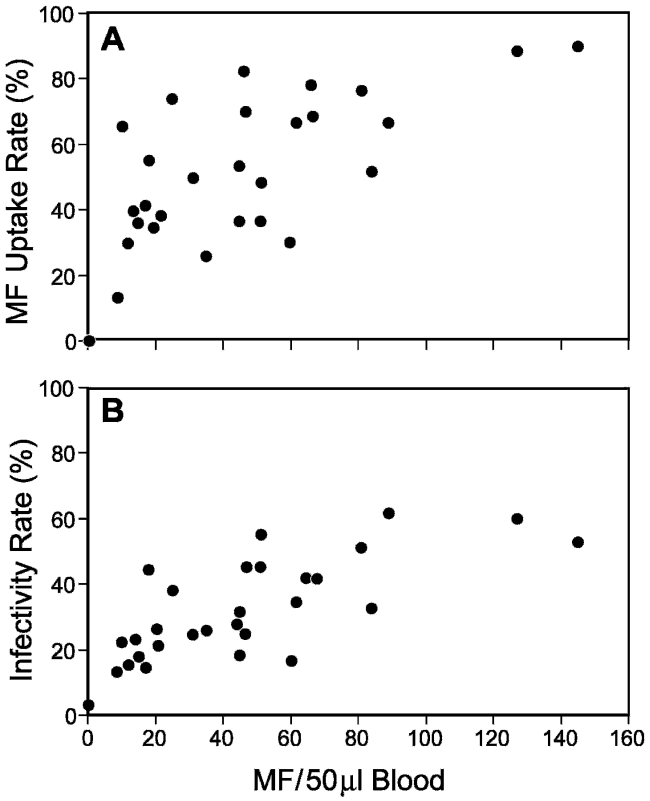

FIGURE 1. Relationships between night blood microfilaria (MF) counts by thick smear in human subjects and MF uptake rates (**A**) and infectivity rates (**B**) in *Culex pipiens* fed on these subjects. Spearman's r_s for MF count versus MF uptake rate, MF count versus infectivity rate, and MF uptake rate versus infectivity rate were 0.84, 0.79, and 0.81, respectively.

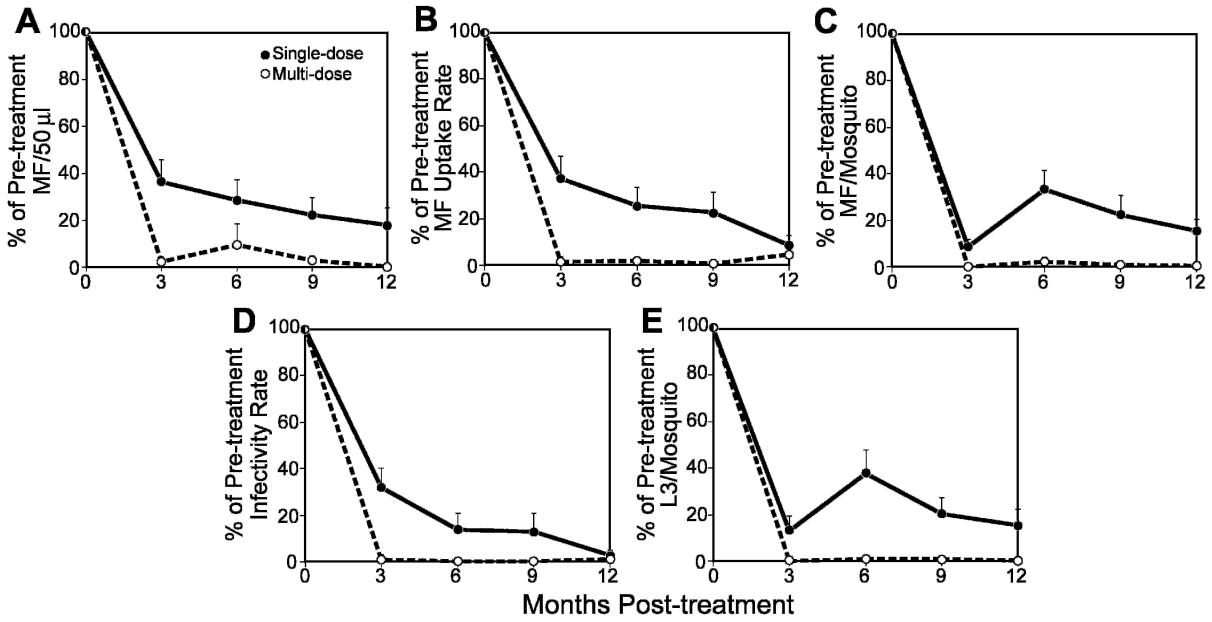

FIGURE 2. Changes in various parameters following single-dose (●) or multi-dose (○) treatment of human subjects with diethylcarbamazine and albendazole. Data points (mean and SE) are shown as percentages relative to pre-treatment values for night blood microfilaria (MF) counts by thick smear (**A**), MF uptake rates (**B**), MF per mosquito (**C**), infectivity rates (**D**), and infective larvae (L3) per mosquito (**E**).

ure 2, which plots data relative to pre-treatment values. Following DEC/ALB treatment, both treatment groups showed substantial reductions in all parameters. However, multi-dose treatment was more effective than single-dose treatment in reducing all parameters at all times until 12 months after treatment, when differences in MF uptake and MF/mosquito were not significant. Note that relative infectivity in the single-dose treatment group was significantly lower three months after treatment than at later time points. By the 12-month time point, relative infectivity rates were 15.2 ± 17.6 and 0.3 ± 0.7% of pre-treatment values for the single-dose and multi-dose treatment groups, respectively.

Poor (or delayed) responses to treatment. Responses to treatment were somewhat variable in different subjects. We considered subjects whose MF counts, MF uptake, or infectivity decreased by < 50% relative to pre-treatment values to be poor responders (Table 3). There were more poor responders in the single-dose treatment group, but this difference decreased over time. Most poor responses were only temporary. That is to say, they were actually delayed responses to treatment. Only one of 26 people studied in either group was a poor responder 12 months after treatment. This person (a 24-year-old man who was a single-dose treatment recipient) had pre-treatment MF counts of 45 per 50 µL and 1,375 per mL by thick smear and membrane filtration, respectively. His MF counts 12 months post-treatment were 48 per 50 µL by smear and 264 per mL by filter. The reduction by filter was 81%. Thus, it is likely that his pre-treatment MF count by smear was spuriously low. Infectivity values for this subject were 28.5% before treatment and 17.0% at 12 months. Thus, although his MF count (by membrane filtration) decreased after treatment, his residual microfilaremia at 12 months was sufficient to give him a poor response regarding infectivity.

TABLE 3

Number of poor (or delayed) responses* to treatment with single- or multi-dose diethylcarbamazine and albendazole

Time post-treatment	No. of subjects tested	Smear	MF uptake	Infectivity
3 months				
Single-dose	14	3	4	0
Multi-dose	15	1	0	0
6 months				
Single-dose	14	2	3	5
Multi-dose	12	1	1	0
9 months				
Single-dose	14	1	2	1
Multi-dose	13	0	0	0
12 months				
Single-dose	14	1	0	1
Multi-dose	12	0	0	0

* Subjects with poor (or delayed) responses to treatment had < 50% reductions in microfilaria (MF) count by thick smear, MF uptake rate, and/or infectivity rate (relative to pre-treatment values).

Clearance rates. Clearance data strongly favor the multi-dose treatment group (Table 4). However, neither treatment regimen completely cleared blood MF, MF uptake, or L3 development in all subjects. Complete clearance of infectivity was transiently observed three months after multi-dose treatment.

Vector competence of mosquitoes fed on MF smear-positive and smear-negative subjects. Table 5 compares vector competence data obtained from subjects with positive and negative MF smears after treatment, regardless of treatment group. Rates and intensities of MF uptake by mosquitoes for smear-negative subjects were significantly lower than those for smear-positive subjects at all time points after therapy with DEC/ALB. Infectivity and L3/mosquito were also significantly lower in mosquitoes fed on smear-negative subjects at all time points except three months post-treatment. Indeed, very few L3 were recovered from mosquitoes fed on smear-negative subjects.

TABLE 4

Clearance rates* for *Wuchereria bancrofti*–infected subjects treated with single- or multi-dose diethylcarbamazine and albendazole

Time post-treatment	No. tested	MF/50 μL	MF uptake	Infectivity
3 months				
Single	14	7.1	21.4	50.0
Multiple	15	66.7	93.3	100
P†		0.001	< 0.001	0.002
6 months				
Single	14	21.4	21.4	21.4
Multiple	12	66.7	66.7	58.3
P†		0.020	0.020	0.105
9 months				
Single	14	21.4	21.4	28.6
Multiple	13	84.6	92.3	84.6
P†		0.001	< 0.001	0.003
12 months				
Single	14	35.7	57.1	28.6
Multiple	12	75.0	75.0	83.3
P†		0.045	0.429	0.005

* Data shown are percentages of subjects with either total clearance of microfilaria (MF) by thick blood smear, zero MF uptake by mosquitoes, or zero infective larvae recovered from mosquitoes.

† Group differences were assessed by chi-square test or Fisher's exact test.

DISCUSSION

We have previously reported effects of therapy with single-dose DEC on MF uptake and L3 production in *Cx. pipiens*.[11] The present project studied people with higher MF counts who were treated with two different DEC/ALB treatment regimens; subjects were restudied on multiple occasions over 12 months following treatment. A number of groups have reported effects of DEC/ALB on microfilaremia and adult worms in bancroftian filariasis.[3,4,12–17] However, this is the first study of the impact of DEC/ALB on MF uptake and L3 production by mosquitoes.

The relationships between MF counts in blood smears and MF uptake or infectivity observed prior to treatment in this study were consistent with results in our previous study.[11] The L3 yield prior to treatment was slightly higher in this study (0.44) than in our previous study (0.29). The low L3 yield three months after treatment suggests the possibility that DEC/ALB had a post-treatment effect on MF that impaired their ability to survive or develop in mosquitoes. This effect did not persist at later time points. Indeed, increased yield values observed at 6–12 months after treatment may reflect very low MF counts at those time points. This would be consistent with limitation, as previously observed in *Cx. pipiens*.[10,18–21] This tendency toward increased efficiency of L3 production at low MF counts would tend to make it more difficult to interrupt filariasis transmission in areas with *Culex* transmission.

Treatment with single-dose DEC/ALB induced dramatic and sustained reductions in blood MF counts and parameters of mosquito infection that lasted for at least one year after treatment. Although single-dose treatment did not completely clear MF from human blood or totally block MF uptake and infectivity in mosquitoes in most cases, it is quite possible that multiple cycles of this treatment will be sufficient to eliminate filariasis transmission in disease-endemic areas such as Egypt. Indeed, we have previously suggested that repeated MDA with DEC alone might be sufficient to interrupt transmission in areas such as Egypt with low baseline infection prevalence rates and intensities.[11,22,23]

Multi-dose DEC/ALB treatment was significantly more effective than single-dose treatment in suppressing and sustaining reductions in MF in blood smears and MF uptake and development in mosquitoes. This was true at almost all time points, whether one looks at absolute numbers, relative reductions, or total clearance rates.

Our study identified a group of relatively poor or delayed responders to DEC/ALB treatment. Most of these people were in the single-dose treatment group, and most turned out to be just delayed or late responders. Only one subject was classified as a poor responder 12 months after treatment, and this subject showed a good reduction in MF counts by membrane filtration. Thus, there was no significant evidence of resistance to DEC/ALB in the present study. Larger studies and ongoing surveillance will be needed to look for emergence of resistance to DEC/ALB as GPELF progresses.

TABLE 5

Wuchereria bancrofti infection of *Culex pipiens* fed on subjects with microfilaria (MF)–positive or –negative blood smears following diethylcarbamazine and albendazole treatment*

Mosquito infection	Months post-treatment	Smear-positive (no.) mean ± SD	Smear-negative (no.) mean ± SD	*P*†
% MF uptake	3	(18) 15.0 ± 19.5	(11) 3.3 ± 10.8	0.016
	6	(15) 12.5 ± 13.5	(11) 0.6 ± 1.4	< 0.001
	9	(13) 11.6 ± 14.6	(14) 1.1 ± 2.7	0.006
	12	(12) 7.2 ± 7.8	(14) 0.7 ± 2.7	0.003
MF/100 mosquitoes	3	(18) 30.7 ± 41.8	(11) 8.7 ± 29.0	0.016
	6	(15) 18.8 ± 24.4	(11) 0.6 ± 1.4	< 0.001
	9	(13) 16.2 ± 23.6	(14) 1.1 ± 2.7	0.006
	12	(12) 8.3 ± 10.5	(14) 0.7 ± 2.7	0.003
% infectivity	3	(18) 1.7 ± 2.8	(11) 1.2 ± 4.0	0.209
	6	(15) 11.9 ± 14.1	(11) 0.5 ± 1.2	0.001
	9	(13) 8.0 ± 10.2	(14) 0.6 ± 1.3	0.005
	12	(12) 6.7 ± 7.2	(14) 0.2 ± 0.4	0.001
L3/100 mosquitoes	3	(18) 3.5 ± 7.0	(11) 2.2 ± 7.2	0.198
	6	(15) 23.0 ± 31.7	(11) 0.6 ± 1.2	0.001
	9	(13) 14.5 ± 21.4	(14) 0.7 ± 1.9	0.007
	12	(12) 9.4 ± 11.3	(14) 0.2 ± 0.4	0.001

* No. = number of subjects tested; L3 = infective larvae.

† By Mann-Whitney U test.

We previously reported that MF were sometimes ingested by *Culex* mosquitoes that had fed on smear-negative MF carriers. However, since very few of the ingested MF developed to the infective stage, we concluded that low-level and ultralow-level MF carriers are unlikely to sustain filariasis transmission under natural conditions.[11] Results in the present study reinforce this conclusion. Although mosquitoes ingested MF from some subjects who had negative MF smears 12 months after DEC/ALB treatment, only 0.2% of mosquitoes that fed on such subjects produced L3. This supports the conclusion in our previous report that the presence of MF in thick smears in a population is a functional threshold for L3 production and transmission by *Culex* mosquitoes. Therefore, we believe that filariasis elimination programs should aim to reduce MF rates by thick smear to close to zero, but not waste time and resources trying to detect ultra-low MF carriers by membrane filtration.

Our study was performed with *Cx. pipiens.* While it is likely that similar results would be obtained with other *Culex* species, this should be tested. Results might be very different with more distantly related mosquitoes. Therefore, we believe that parallel studies of effects of treatment on MF uptake and L3 production should be performed with other major filariasis vector mosquitoes such as *Cx. quinquefasciatus* and species of *Anopheles* and *Aedes.*

We conclude this report with three points. First, although our results suggest that mass treatment with single-dose DEC/ALB (as recommended by GPELF) should greatly decrease transmission of bancroftian filariasis, multi-dose treatment was much more effective than single-dose treatment in lowering blood MF counts and reducing MF uptake and L3 production by mosquitoes. Therefore, we believe that program managers should consider using a multi-dose DEC/ALB regimen for the first round of MDA in filariasis elimination programs, especially in areas with high baseline infection rates and intensities. The increased expense and work involved might be worthwhile if this approach can interrupt transmission and eliminate filariasis with fewer rounds of treatment than repeated cycles of single-dose MDA. Second, our study showed that mosquitoes are very efficient at ingesting MF from low-count MF carriers. This supports the idea of using molecular xenodiagnosis as an alternative to blood MF surveys as a means of monitoring progress in filariasis elimination programs. Third, we strongly believe that more mosquito research is needed to improve the knowledge base for filariasis elimination programs. It is a mistake to focus solely on filarial parasites in the human host.

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Authors' addresses: Hoda A. Farid, Ragaa E. Hammad, Marah M. Hassan, and Reda M. R. Ramzy, Research and Training Center on Vectors of Diseases, Faculty of Science Building, Ain Shams University, Abbassia, Cairo 11566, Egypt, Telephone and Fax, 20-2-683-9622; Maged El Setouhy, Faculty of Medicine, Ain Shams University, Abbassia, Cairo, Egypt. Telephone and Fax, 20-2-683-9622; Gary J. Weil, Infectious Diseases Division, Box 8051, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, Telephone: 314-454-7782, Fax 314-454-5293, E-mail: gweil@im.wustl.edu.

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