A RANDOMIZED CLINICAL TRIAL COMPARING SINGLE- AND MULTI-DOSE COMBINATION THERAPY WITH DIETHYLCARBAMAZINE AND ALBENDAZOLE FOR TREATMENT OF BANCROFTIAN FILARIASIS

MAGED EL SETOUHY, REDA M. R. RAMZY, Ehab S. AHMED, AMR M. KANDIL, OMAR HUSSAIN, HODA A. FARID, HANAN HELMY, AND GARY J. WEIL

Research and Training Center on Vectors of Diseases, Ain Shams University, Cairo, Egypt; Department of Community, Environmental, and Occupational Medicine, and Department of Radiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt; Barnes-Jewish Hospital and Washington University School of Medicine, St. Louis, Missouri

Abstract. The Global Program for Elimination of Lymphatic Filariasis calls for mass drug administration for endemic populations outside of sub-Saharan Africa with a single dose of diethylcarbamazine (DEC) and albendazole (Alb) annually for 4–6 years. Single-dose DEC/Alb dramatically reduces blood microfilaria (MF) counts, but most treated subjects fail to completely clear MF after a single dose. A more effective regimen might reduce the number of years required for elimination programs. We performed a randomized clinical trial in Egyptian adults with asymptomatic microfilaraemia to compare treatment with seven daily doses of oral DEC (6 mg/kg) and Alb (400 mg) with a single dose of the same combination. We also studied the effect of re-treatment with single-dose DEC/Alb 12 months after the first treatment course. Multi-dose DEC/Alb was significantly more effective than single-dose therapy for reducing and clearing microfilaraemia (mean reduction in MF/ml relative to pretreatment counts at 12 months, 99.6% versus 85.7%, with complete clearance in 75% versus 23.1%). The two regimens had similar activity against adult filarial worms, as indicated by serial ultrasound assessments. Neither regimen resulted in complete clearance of filarial antigenemia. There was no difference in adverse events, which were mild to moderate. Blood microfilaria and parasite antigen clearance rates increased following re-treatment. Multi-dose DEC/Alb may be a useful option for filariasis elimination programs, especially in the first year (when enthusiasm for mass drug administration and coverage rates are high), to quickly reduce community MF loads and transmission rates.

INTRODUCTION

Approximately 100 million people are infected with Wuchereria bancrofti, a mosquito-transmitted nematode parasite that causes deforming lymphatic filariasis in the tropics.1 Improved therapies and diagnostic methods have led to new thinking about lymphatic filariasis and the realization that it should be possible to interrupt transmission and eliminate this major public health problem by repeated, annual cycles of mass drug administration (MDA) with new, single-dose combination regimens.2–5 There is little doubt that combination therapy represents a significant advance in treatment of filariasis.6 A single dose of diethylcarbamazine with albendazole (DEC/Alb) results in dramatic and sustained reductions in blood microfilaria (MF) counts7 and is at least as effective as the previously widely used regimen of 6 mg/kg of DEC daily for 12 days. Single-dose therapy is easier to take and administer, and this should improve compliance and treatment coverage rates in MDA programs. However, clinical trial results and early experience from MDA programs suggest that there is room for further improvement in therapeutic regimens for MDA. For example, two clinical trials have reported that a majority of subjects had persistent low-level microfilaraemia one year following treatment with a single dose of Alb with either DEC or ivermectin.8,9 Other studies have shown that mosquitoes fed on subjects with persistent, low-level microfilaraemia following therapy ingest MF and produce infective filarial larvae.10 The issue of treatment coverage must also be considered. No MDA program achieves complete coverage, and 80% coverage of eligible subjects (nonpregnant, more than two years of age, with no serious acute or chronic illness) is sometimes quoted as a goal for MDA programs;11 actual coverage rates are often much lower than this.12 To some extent, the problems of incomplete MF clearance and treatment coverage can be solved by the recommended practice of continuing MDA programs for 4–6 years in endemic areas. However, early results from some MDA programs suggest that initial enthusiasm for treatment, with high coverage rates, has been difficult to sustain beyond the first years of the programs.13 Elimination programs could be shortened considerably by availability of practical regimens that either killed all adult filarial worms or completely cleared MF from the blood of most MF carriers. Unfortunately, existing regimens do not achieve either of these objectives.

Although the World Health Organization has recommended single-dose DEC/Alb as a preferred combination for repeated, annual MDA to hundreds of millions of people residing in filariasis-endemic areas,3,14 relatively few patients (mostly men in Sri Lanka) have been carefully studied to assess the safety and efficacy of this combination for treatment of bancroftian filariasis.7 In addition, no studies of multi-dose DEC/Alb treatment or DEC/Alb re-treatment of Bancroftian filariasis have been reported to date. Therefore, the goals of this study were to restudy the safety and efficacy of single-dose DEC/Alb in a different patient population, to compare single-dose DEC/Alb therapy with seven daily doses of the same regimen, and to study effects of re-treatment after one year.

MATERIALS AND METHODS

Patient selection. Potential study participants with W. bancrofti microfilaraemia were identified in the course of night blood surveys for filariasis in an endemic village in Badrasheen district, Giza Governorate, approximately 45 km south of Cairo, Egypt. Subjects who met inclusion criteria (men and women, 18 years of age or older, with night blood microfilaria counts > 80 MF/mL) were invited to participate in the study. Written informed consent was required for participation in the study, before pretreatment safety screening.
tests were performed. Subjects with evidence of clinical filariasis by physical examination (hydrocele or lymphedema), a history of treatment of filariasis in the past 12 months, disability (inability to work), or serious concurrent illness requiring chronic medication were excluded from the study. Other exclusions included acute febrile illness, renal disease (creatinine > 2 mg/dL), liver disease (serum bilirubin > 2 mg/dL or alanine aminotransferase [ALT] > 80 IU/L), pregnancy, or lactation.

Randomization and therapy. Eligible subjects were randomly assigned to treatment groups with block stratification for sex and blood microfilaria count. The trial was not blinded. Study subjects were treated with either a single oral dose of 6 mg/kg of diethylcarbamazine citrate (Pharmamed, Zejtun, Malta) plus 400 mg of albendazole (GlaxoSmithKline, Uxbridge, United Kingdom) or with the same medications daily for seven days. All treatment was directly observed by study personnel.

End points and sample size considerations. The primary endpoint for this study was predefined to be the rate of complete clearance of microfilaremia 12 months after treatment (defined as zero MF in 1 mL of venous blood collected at night), and the study was powered for this end point. This end point was chosen because complete MF clearance is an important goal for filariasis elimination programs that aim to interrupt transmission of the infection. We expected 25% of subjects to clear MF 12 months after treatment with single-dose Alb/DEC (based on prior studies).8,9 We hypothesized that the seven-day treatment would completely clear MF in 75% of the subjects. This would be a biologically and epidemiologically significant difference. We needed 18 evaluable subjects to have 90% power to detect a significant difference. We needed 18 evaluable subjects to clear MF 12 months after treatment with single-dose Alb/DEC (based on prior studies).8,9 We hypothesized that the seven-day treatment would completely clear MF in 75% of the subjects. This would be a biologically and epidemiologically significant difference. We needed 18 evaluable subjects (with follow-up at 12 months after treatment) per group to have 90% power to detect a significant difference (P < 0.05) between the treatment groups. Therefore, we decided to enroll at least 25 subjects per treatment group to allow for some dropouts. We also designated complete cure (defined as complete clearance of microfilariaemia and filarial antigenemia 12 months after treatment) as a predefined secondary end point for the study. The study protocol specified that group differences would be assessed based on an intention to treat analysis.

Tests for W. bancrofti infection. Venous blood samples were collected between the hours of 9:00 PM and 1:00 AM before and after treatment for parasitology and serology studies. Microfilariae were detected by membrane filtration (5 μM; Nuclepore Corp., Pleasanton, CA) of 1 mL of venous blood and microscopic examination of stained filters. Filarial antigenemia, a marker for adult worm infection intensity, was detected in the field with finger prick blood samples with the AMRAD ICT Filariasis Test (AMRAD ICT, French’s Forest, New South Wales, Australia) according to the manufacturer’s instructions. This test is a rapid format immunochromatography “card test”.13 Card test results were read visually in the field after 15 minutes and reviewed the next day in the laboratory. Equivocal card results were considered to be negative.

Ultrasound studies. Effects of therapy on adult worms were also directly assessed by ultrasound as previously described.16 The sonologist was blinded with regard to treatment group assignments. Ultrasound findings after treatment were compared with pretreatment results to assess loss of motile filarial worms.17 The primary end point for this sub-study was predefined to be loss of all motile worms in subjects who had motile worms before therapy. The secondary end point was the percentage decrease (by group) in the number of foci (or “nests”) observed with motile adult filarial worms.

Incidence. Prior studies showed that MF incidence in the study area was very low in recent years (less than 1% per year).18 In addition, all endemic areas in Egypt (including our study area) were mass-treated with DEC/Alb approximately one month after our subjects were treated in this study. Therefore, the data analysis plan for this study assumed that there were no incident infections during the follow-up period.

Assessment of adverse events (AEs). Study personnel visited subjects in their homes on days 2, 7, and 14, at 4 weeks, and 3 months after the first treatment dose to record the presence and severity of AEs and to provide symptomatic treatment of AEs. Complete blood counts, urinalysis, and serum bilirubin, ALT, and creatinine tests were performed one and four weeks after treatment. A pre-printed toxicity table, modified for the study from a standard reference,19 was used to grade the severity of AEs.

Re-treatment. All subjects were treated with a single oral dose of 6 mg/kg of DEC and 400 mg of Alb one year after the first round of treatment. Adverse event monitoring following re-treatment was performed on days 2 and 7 after treatment. Blood samples were collected 6 and 12 months after re-treatment to assess the effects of re-treatment on MF counts and filarial antigenemia.

Ethical clearance. This study was reviewed and approved by institutional review boards at Washington University School of Medicine and at Ain Shams University. The project was also monitored by an independent data safety and monitoring board (Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD USA). The funding source also reviewed the study protocol but had no other role in the project.

Data analysis. Database management and statistical analyses were performed with a statistical software package (SPSS, Chicago, IL). Geometric mean MF counts were calculated by adding 1 to MF counts before log transformation of the data and subtracting 1 from the antilog of the mean of the log-transformed data. Relative MF levels (expressed as % of the pretreatment level) were calculated for each subject by dividing post-treatment MF counts by the pretreatment value and multiplying by 100. Proportions were compared by chi-square analysis or Fisher’s exact test (two-tailed). The Mann-Whitney U test was used to assess the significance of group differences for continuous variables. We also performed a mixed models analysis with subject as the random effect to assess overall changes in relative MF levels and MF clearance rates between treatment groups over time, controlling for age, sex, and baseline microfilaria count.20

RESULTS

Treatment assignment and follow-up. Fifty-eight eligible subjects consented to participate in the study. Pretreatment clinical and laboratory parameters for the treatment groups are summarized in Table 1. The two groups were comparable in terms of age, sex, and infection intensity. All subjects completed the assigned therapy in August 2000. Cooperation with follow-up was excellent (≥ 93% at all time points through 12
months after treatment and 86% at 24 months). One subject (a female in the single-dose treatment group) refused blood draws after the one week post-treatment time point. She was followed clinically and included in the assessment of adverse events. Eleven subjects received an extra dose of DEC/Alb approximately one month after they received the study treatment. Six of these subjects were in the multi-dose treatment group, and five were in the single-dose group. This extra treatment dose was provided by government health officials as part of the Egypt’s National Filariasis Elimination Program. Results presented below represent an intention-to-treat analysis, as stipulated by our study protocol. However, essentially the same results were obtained when the analysis was restricted to subjects who did not receive the extra treatment dose.

**Effects of therapy on microfilaria counts.** Microfilaria counts decreased dramatically in both treatment groups by one month after therapy, and these decreases were sustained until subjects were re-treated at 12 months (Table 2). Decreases in MF counts (Figure 1, top panel) and rates of complete MF clearance (Figure 1, bottom panel) were significantly greater in the multi-dose treatment group at all times after treatment.

A mixed models analysis of relative MF counts over time with subject as the random effect revealed highly significant effects of treatment group ($P < 0.001$) and baseline MF count ($P < 0.001$). Age ($P = 0.28$) and sex ($P = 0.83$) were not significant factors influencing relative MF after treatment. A mixed generalized linear model was also used to analyze MF clearance (also setting subject as the random effect). Again, treatment group ($P < 0.001$) and baseline MF count ($P = 0.011$) were significant factors while sex ($P = 0.593$) and age ($P = 0.69$) were not significant.

**Effects of therapy on adult worm viability.** Fifty-six of 58 subjects (96.6%) had filarial antigenemia detectable by the ICT antigen card test prior to treatment. Antigen lines in card tests tended to be less intense with post-treatment blood samples. Six of 52 subjects (11.5%, 3 in each treatment group) had negative card test results 12 months after treatment.

Motile adult filarial worms were detected by ultrasound in a majority of subjects prior to treatment (Table 1). This finding was more common in men than in women (28 of 36, [78%] versus 5 of 22 [22.7%]). Most worms seen in men were located in dilated scrotal lymphatic vessels. Worms in women were located in dilated lymphatic vessels draining the extremities. Inactivation of all visible worm nests was achieved 12 months after treatment in 12 of 15 subjects tested on both occasions in the single-dose treatment group and in 12 of 14 subjects in the multi-dose group. A majority of motile worm nests were inactivated in both treatment groups by 12 months after treatment (overall 57 of 63, [90.5%]; single dose, 27 of 31 [87.1%];

![Figure 1. Top. Effects of single dose (circles) or multi-dose (triangles) diethylcarbamazine with albendazole on blood microfilaria counts relative to pretreatment counts. Data shown are the mean ± SE. Differences at all time points after treatment are statistically significant ($P < 0.01$). Bottom. Efficacy of these regimens for achieving complete clearance of microfilariaemia. Differences at all time points after treatment are statistically significant ($P < 0.01$). Microfilaria clearance was achieved 12 months after treatment (the predefined primary endpoint for the study) in 6 (23.1%) of 26 subjects in the single-dose group (95% confidence interval [CI] = 9.8–44.1%) and in 21 (75%) of 28 subjects in the multi-dose group (95% CI = 54.8–88.6%).](image)

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Single-dose group</th>
<th>Multi-dose group</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SE)</td>
<td>36.7 (16.1)</td>
<td>30.4 (10.5)</td>
<td>0.14</td>
</tr>
<tr>
<td>Sex</td>
<td>18 M, 10 F</td>
<td>18 M, 12 F</td>
<td>0.95</td>
</tr>
<tr>
<td>GM MF/mL (range)</td>
<td>359.1 (90–3,720)</td>
<td>399.9 (100–4,531)</td>
<td>0.72</td>
</tr>
<tr>
<td>Presence of motile adult worms by US</td>
<td>16/28 (57.1%)</td>
<td>17/30 (56.7%)</td>
<td>0.97</td>
</tr>
<tr>
<td>Presence of motile adult worms by ICT antigen card test</td>
<td>27/28 (96.4%)</td>
<td>29/30 (96.7%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*GM = geometric mean; MF = microfilariae; US = ultrasound.
† Mann-Whitney test.
‡ By chi-square analysis (Fisher’s exact test for filarial antigenemia).

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-treatment</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
<th>9 Months</th>
<th>12 Months</th>
<th>18 Months</th>
<th>24 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose</td>
<td>359.1 (90–3,720)</td>
<td>58.3 (0–820)</td>
<td>31.3 (0–1,300)</td>
<td>37.0 (0–700)</td>
<td>18.4 (0–843)</td>
<td>14.4 (0–1,250)</td>
<td>5.0 (0–117)</td>
<td>2.4 (0–182)</td>
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<tr>
<td>Multidose</td>
<td>399.9 (100–4,531)</td>
<td>0.9 (0–17)</td>
<td>0.6 (0–19)</td>
<td>1.0 (0–17)</td>
<td>0.5 (0–20)</td>
<td>0.6 (0–93)</td>
<td>0.3 (0–122)</td>
<td>0.2 (0–70)</td>
</tr>
<tr>
<td>$P$</td>
<td>0.715</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* Subjects were treated with single-dose or multi-dose DEC/Alb at time zero and re-treated with single-dose DEC/Alb 12 months later.
† Data shown are geometric means (range).
‡ By Mann-Whitney U test.
multi-dose 30 of 31 [93.8%]; group difference not statistically significant).

**Adverse events.** Adverse events of mild-to-moderate severity were common following therapy in both treatment groups, with no significant difference in event frequencies between the two treatment groups (Table 3). The most frequent adverse events observed were fever, headache, and myalgia. These symptoms usually resolved within 2–3 days. Subjective fever, myalgia, and headache were more common in people with high blood MF counts, and headache was significantly more common in women than in men. Serum creatinine, bilirubin, and ALT levels were stable in all patients tested one and four weeks following treatment. Scrotal discomfort peaked at one week after treatment, and one man had persistent, mild scrotal discomfort four weeks after treatment. No severe or serious adverse events were observed; indeed, all adverse events were scored as grade 1 or 2 events. That is to say, no AEs were severe enough to interfere with activities of daily living.

**Re-treatment results.** Cumulative effects of both rounds of treatment on MF are shown by treatment group in Figure 1 (18 and 24 month time points). Note that MF reduction and clearance rates 12 months after multi-dose DEC/Alb were superior to those seen 12 months after two annual single-dose treatments. All but one of the subjects in the multi-dose group in year one cleared MF by 12 months after re-treatment with single-dose DEC/Alb.

Twenty-seven of the re-treated subjects had persistent microfilaremia (mean geometric 104.3 MF/mL, range = 1–1,250) at the 12-month time point, just prior to re-treatment. Mean reductions in MF count (relative to the 12 month levels) for 25 subjects studied 6 and 12 months following re-treatment were 67% (SE = 9%) and 89% (SE = 4%), respectively. Total MF clearance was achieved 6 and 12 months following re-treatment in 9 (36%) of 25 and 15 (60%) of 25 of these subjects, respectively.

Ultrasound results showed further clearance of adult worms following re-treatment. Five subjects had motile worms visible 12 months after the first round of treatment (one nest in each subject). Two of these subjects had persistent motile worms visible 12 months after re-treatment (one subject in each of the original treatment groups). The 24-month data show that two rounds of treatment inactivated 56 of 58 worm nests (96.6%) in 25 subjects who had motile worms pretreatment and who were retested at 24 months. Twenty-three of these subjects (92%) had total inactivation of motile worms.

Many subjects had complete clearance of filarial antigenemia by the ICT card test 12 months after re-treatment (11 of 22 originally seropositive subjects treated with single-dose DEC/Alb in year 1; 9 of 26 originally seropositive subjects treated with multi-dose DEC/Alb in year 1, difference in clearance rates is not significant). Interestingly, only 2 of 20 subjects who cleared antigenemia had persistent microfilaremia (range = 20–56 MF/mL) 12 months after re-treatment, while 8 of 28 subjects with persistent antigenemia at this time had persistent microfilaremia (range = 4–182 MF/ml). This difference in MF persistence rates was not statistically significant ($P = 0.16$, by Fisher’s exact test).

Adverse events in the week following re-treatment of 51 subjects (33 men and 18 women) were greatly reduced compared with those observed following the first round (fever 9%, headache 5%, myalgia 7%, scrotal pain one week after treatment 0%) ($P < 0.02$, by Fisher’s exact test).

**DISCUSSION**

This study has generated significant new information on the use of combination DEC/Alb therapy for bancroftian filariasis. Single-dose DEC/Alb was highly effective for reducing blood MF counts, with a 96% reduction in geometric mean MF/mL 12 months after treatment. However, as in prior studies, most single-dose DEC/Alb recipients had persistent, low-level microfilaremia 12 months after treatment. Multi-dose DEC/Alb therapy was much more effective than single-dose treatment for reducing and clearing MF. Entomology results will be reported separately, but parallel results favoring multi-dose treatment were observed in studies of the effects of DEC/Alb therapy on MF ingestion and production of infective larvae by mosquitoes fed on these subjects.

In contrast to MF results, single and multi-dose DEC/Alb seemed to have similar partial killing effects on adult filarial worms, based on direct visualization of adult worms by ultrasound and reductions in the degree of positivity in filarial antigen card test results. The ultrasound data suggest that a majority of adult worms were killed by the first round of DEC/Alb therapy. However, antigen test results suggest that some adult parasites survived the first round of treatment with these regimens in most subjects.

Single-dose and multi-dose DEC/Alb therapy were well tolerated by men and women, and study subjects did not experience severe or serious adverse events. It is possible that a larger study might have identified differences in AEs between the study groups; our study was not powered to detect subtle group differences in rates or severity of AEs. Similarly, our results do not guarantee that no serious AEs would be seen in large-scale treatment programs with these regimens. While it is not practical to provide active follow-up for all participants in MDA programs, such programs must provide a system for detection of AEs and for referral of subjects for evaluation.
and treatment of AEs following MDA.\textsuperscript{14} This provides relief to those who experience AEs after treatment, and it is an important public relations tool to help ensure continued cooperation with MDA over the years needed to achieve filariasis elimination.

Re-treatment was effective at further reducing MF counts in people with residual microfilaraemia following the first round of treatment; similar results have been reported after DEC/Alb re-treatment of patients with brugian filariasis.\textsuperscript{21} Thus, there was no evidence that subjects who failed to clear MF after the first round of treatment harbored resistant parasites. It is interesting that many subjects (20 of 48 subjects who were originally seropositive [41.67\%]) had negative filarial antigen test results 12 months following re-treatment with DEC/Alb. This rate of antigen clearance is much higher than those observed in prior studies of repeated treatment with DEC or ivermectin.\textsuperscript{22,23} We believe that complete clearance of parasite antigenemia usually indicates clearance of all adult filarial worms. Although occasional subjects who cleared filarial antigenemia had persistent, low-level microfilaraemia, our results suggest that it may be useful to follow serial filarial antigen prevalence rates as a means of monitoring the effect of MDA on filariasis endemicity in communities and regions.

Our study has several other potentially important implications for the Global Program for Elimination of Lymphatic Filariasis (GPELF). First, our results support the main strategy of GPELF, because they suggest that MDA with repeated annual doses of DEC/Alb could lead to elimination of lymphatic filariasis in some endemic areas. Our results also suggest that multi-dose treatment may be a useful option for filariasis elimination programs, since this can be more effective than single-dose treatment of rapidly reducing and clearing microfilaraemia. Seven-day treatment courses may not be practical for MDA, and additional research is needed to study shorter multi-dose regimens. Multi-dose therapy might be especially useful in areas with high baseline infection prevalence rates and intensities and high transmission rates. Filariasis elimination program coordinators working in such areas might choose to use multi-dose MDA in the first year of elimination programs, when enthusiasm for MDA and coverage rates are high. Multi-dose MDA is likely to quickly reduce community MF loads and transmission rates to levels that can be further reduced to zero by subsequent rounds of single-dose treatment. This approach might reduce the number of years of MDA required for filariasis elimination.

Received July 2, 2003. Accepted for publication September 8, 2003.

Acknowledgments: We are grateful for technical assistance provided by the field research teams and laboratory staff at the Research and Training Center on Vectors of Diseases at Ain Shams University. The late Professor Rifky Faris helped to plan this study. Dr. William Shannon at Washington University provided expert statistical assistance.

Disclosure: The authors wish to disclose that the AMRAD ICT filariasis test used in this study employs materials licensed from Barnes-Jewish Hospital. This information is provided in the interest of full disclosure and not because the authors consider this to be a conflict of interest.

Financial support: This work was supported by National Institutes of Health grant AI-35855.

Authors’ addresses: Maged El Setouhy and Omar Hussain, Faculty of Medicine, Ain Shams University, Abbassia, Cairo, Egypt, Telephone: 20-2-6853276, Fax: 20-2-4837888. Reda M. R. Ramzy, Ehab S. Ahmed, Amr M. Kandil, Hoda A. Farid, and Hanan Helmy, 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. Gary J. Weil, Infectious Diseases Division, Box 8051, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, Telephone: 314-454-7782, Fax: 314-454-5293, E-mail: gweil@im.wustl.edu.

Reprint requests: Gary J. Weil, Infectious Diseases Division, Box 8051, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110.

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