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Evaluation of effectiveness of diethylcarbamazine/albendazole combination in reduction of *Wuchereria bancrofti* infection using multiple infection parameters

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ABSTRACT

Objectives: To evaluate the effect of multiple rounds of annual single dose of DEC (6 mg/kg) or albendazole (400 mg) given alone or in combination on *Wuchereria bancrofti* microfilaraemia, anti-filarial IgG1 and IgG4 and antigenaemia.
Methods: A total of 170 participants were randomly assigned to albendazole ($n = 62$), DEC ($n = 54$), and DEC plus albendazole (DEC/ALB) combination ($n = 54$). Blood samples were collected at pre-treatment in 1998, at 1 week and 6 months after the first treatment and thereafter before subsequent treatments in 1999 and 2000. Effects of treatment on *W. bancrofti* infection were determined by changes in levels of microfilaraemia, antifilarial antibodies and circulating filarial antigen.
Results: Comparison of geometric mean microfilariae intensities between DEC/ALB combination and DEC or albendazole single therapy groups after two rounds of annual treatment and 24 months follow-up showed that combination therapy resulted in a greater reduction of microfilaraemia than single therapy with either albendazole ($p < 0.001$) or DEC alone ($p = 0.146$). The overall levels of anti-filarial antibodies decreased significantly ($p = 0.028$ for IgG1 and $p < 0.043$ for IgG4) in all treatment groups at 24 months follow-up. Additionally, overall reduction in geometric mean circulating filarial antigen levels at 24 months was 44%, 60% and 85% for albendazole, DEC and DEC/ALB groups, respectively.
Conclusions: These study findings suggest that albendazole improved efficacy of DEC and mass administration of a combination of the two drugs would therefore enhance the interruption of transmission of *W. bancrofti* in endemic areas. This information has important implications for the ongoing Global Program for Elimination of Lymphatic Filariasis.

1. Introduction

An estimated 120 million people living in the tropics are infected with *Wuchereria bancrofti*, a mosquito-transmitted nematode parasite that causes a debilitating form of lymphatic filariasis (LF) and at least one billion people are at risk of this disease worldwide (Michael et al., 1996; The Carter Center, 2002). In Kenya, the disease is endemic in the Coast Province where approximately 3 million people are estimated to be at risk of infection. Previous epidemiological studies have reported microfilaraemia prevalence of 15–25% in the coastal areas (Estambale et al., 1994; Wamae et al., 1998; Njenga et al., 2000; Mukoko et al., 2004) and antigenaemia prevalence above 35% (Wamae et al., 1998; Njenga and Wamae, 2001). For a long time the disease remained uncontrolled in many endemic areas due to lack of suitable diagnostic tools and treatment regimens. However, advances in chemotherapeutic strategies, diagnostic tools and innovative interventions have led to the optimism that LF can be eliminated as a public health problem in all areas of endemicity.

Diethylcarbamazine (DEC) is an effective microfilaricide (Florencio and Peixoto, 2003) and partially effective macrofilaricide (Noroes et al., 1997) which has led to its use as the standard treatment for LF since mid 1940s. During the earlier days the recommended treatment regimen consisted of a 12-day course of DEC (6 mg/kg body weight). However, research has previously demonstrated that single-dose treatment with DEC at 6 mg/kg body weight has comparable long-term microfilaricidal efficacy to the 12-day course of treatment which was inconvenient for community-wide chemotherapy campaigns (Ottesen, 2000).

Albendazole was first shown to be effective against lymphatic filariasis by a study conducted in laboratory animals infected with *Brugia malayi* (Mak et al., 1984). Subsequently, several clinical

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trials have reported persistently low-levels of microfilaraemia in a majority of subjects one year following treatment with a single dose of albendazole combined with either DEC or ivermectin (Ismail et al., 1998, 2001; El Setouhy et al., 2004). The recognition that two-drug single dose treatment strategies are significantly more effective than treatment with either drug alone has been a major advancement in the development of control strategies for LF (Moulia-Pelat et al., 1995; Gyapong et al., 2005; Molyneux and Zagaria, 2002; Addiss et al., 1997). Another scientific advancement has been the development of a drug delivery approach known as community-directed treatment (Com-DT) where selected community members are trained to be Community Drug Distributors (CDDs). A multi-country study on validation of Com-DT approach, first developed for onchocerciasis, established that the strategy is also feasible for delivery of single-dose anti-filarial treatments to LF endemic communities in sub-Saharan Africa (Gyapong et al., 2001; Wamae et al., 2006). Based on significant developments primarily in Com-DT approach against LF and a related filarial infection, onchocerciasis, the World Health Assembly (WHA) in 1997 resolved to eliminate LF as a public health problem (Resolution WHA50.29, 1997). Following the WHA resolution, the WHO launched the global programme to eliminate lymphatic filariasis (GPELF) in 2000 (Molyneux and Zagaria, 2002). The GPELF uses the WHO recommended strategy of mass drug administration (MDA) of DEC plus albendazole, DEC-fortified salt or, where onchocerciasis is co-endemic with LF, ivermectin plus albendazole to interrupt transmission of infection so as to achieve the elimination goal (Ottesen, 2000).

Although WHO recommends co-administration of albendazole with either diethylcarbamazine (DEC) or ivermectin for elimination of LF (2000) (Ottesen, 2000; Molyneux and Zagaria, 2002), there are contradicting views on the role of albendazole in enhancement of the macrofilaricidal and microfilaricidal effects of DEC or ivermectin. For example, a meta-analysis done to assess the efficacy and safety of two-drug regimens used in LF elimination programs concluded that the suppression of microfilaraemia is significantly enhanced by the addition of albendazole to DEC or ivermectin compared to either drug alone (Gyapong et al., 2005). However, a Cochrane review that assessed the effectiveness of albendazole alone or in combination with either DEC or ivermectin concluded that there is insufficient evidence to confirm or refute that albendazole alone, or co-administered with DEC or ivermectin, has an effect on LF (Critchley et al., 2005). We thus analyzed data collected in our 1998–2000 randomized controlled trial in Msambweni District, south coastal Kenya, to evaluate the effectiveness of multiple DEC/ALB while compared to single dose annual DEC (6 mg/kg) and albendazole (400 mg) on multiple parameters; namely *W. bancrofti* microfilaraemia, antigenaemia and anti-filarial IgG.

2. Methods

2.1. Study area

This study was conducted in Muhaka area in Msambweni district, south coastal Kenya (the district was carved out of the greater Kwale district in 2007). The study protocol was reviewed, cleared and given ethical approval by both the Scientific Steering Committee and Ethical Review Committees of the Kenya Medical Research Institute (KEMRI).

2.2. Study design, sample collection and treatment

This study was a three treatment arms randomized–controlled trial without a placebo arm. Preliminary screening of households for microfilaraemia was done by collection of finger prick blood samples from consenting persons between 20:30 and 00:00 h (consent/assent of parents/guardians was sought for children). The finger prick blood samples were examined for microfilariae (MF) using the counting chamber method (McMahon et al., 1979). A household was selected to participate in the study if at least one member was found to be microfilaraemic. All consenting members of the household who met inclusion criteria (age 5 years or more and not severely ill or pregnant) were requested to participate in the study. A total of 64 households were randomly assigned to three treatment groups, namely, DEC (6 mg/kg body weight) alone, albendazole (400 mg) alone (ALB), and DEC plus albendazole (DEC/ALB) combination.

Three rounds of annual treatment were administered to consenting individuals in the study households in 1998, 1999, and 2000. After the pre-treatment blood sample collection for baseline examinations, follow-up specimens were collected again at 2 weeks and 6 months after the first treatment and before each subsequent round of treatment at 12 months and 24 months. Examination of finger prick blood specimens for MF was done using the counting chamber method. Additionally, a random sub-sample of 110 (43 on ALB, 32 on DEC and 35 on DEC/ALB) of venous samples were selected from the 170 stored sera and plasma specimens prepared for assays of anti-filarial IgG1 and IgG4 assays (indicators of filarial infection status and/or exposure to mosquito-borne larvae), and circulating filarial antigen (CFA), a marker for adult filarial worm infection intensity (Weil et al., 1991; Ramzy et al., 1991).

2.3. Anti-filarial IgG1 and IgG4 assays

Measurement of anti-filarial immunoglobulin G1 and G4 (IgG1 and IgG4) was done using previously described methods. (Njenga et al., 2007; Hitch et al., 1991) Briefly, micro-titre plates (Immunolon 2 HB, Dynex Technologies, VA, U.S.A.) were coated with 50 μl/well of 2 μg/ml of *B. malayi* antigen. After blocking and washing steps, 50 μl/well serum specimens diluted 1:50 were added followed by a biotin-conjugated anti-human IgG monoclonal antibody. After another washing step, streptavidin conjugated to alkaline phosphatase was added and finally the plate developed by addition of p-nitro phenyl phosphate. In each ELISA plate, serum standards with known anti-filarial IgG1 and IgG4 isotype levels were included and used to determine antibody levels in the test specimens. The plates were read using an ELISA reader (UVmax, Molecular Devices, U.S.A.).

2.4. Circulating filarial antigen assays

Assays for CFA were done using the Og4C3 monoclonal antibody-based commercial ELISA kit (TropBio Pty Ltd, Queensland, Australia). The assays were performed using plasma specimens diluted at 1:10 following the manufacturer's instructions and plates were read using an ELISA reader (UVmax, Molecular Devices, U.S.A.). Standards provided in the kit were included in each ELISA plate and the readings used to develop a standard curve that was applied to assign arbitrary antigen units to the test specimens, as previously described (Njenga et al., 2007).

2.5. Data analysis

Database management and statistical analyses were done using STATA™ statistical software program (College Station, TX). Microfilariae counts were expressed as MF/ml by multiplying absolute counts in 100 μl of finger prick blood by 10. Geometric mean intensity (GMI) was calculated by adding 1 to MF/ml counts before log transformation of the data and subtracting 1 from the antilog of the mean of the log transformed data: $(\{\text{antilog}\,[\sum \log\,(x+1)/n]\})-1$, where x was the count and n the number of persons included).

Table 1
Baseline characteristics of the study population, Muhaka, south coastal Kenya, 1998–2000.

	Treatment groups				
	Albendazole	Diethylcarbamazine	DEC/ALB	All groups	*p*
MF positive (*n*)	30 (62)	26 (54)	25 (54)	81 (170)	
CFA positive (*n*)	29 (43)	25 (32)	21 (35)	77 (110)	
IgG1 positive (*n*)	41 (43)	30 (32)	34 (35)	105 (110)	
IgG4 positive (*n*)	42 (43)	30 (32)	34 (35)	106 (110)	
Households (%)	22 (34)	20 (31)	22(34)	64 (100)	
Age, median (Q1, Q3)[a]	23 (12, 35)	26 (15, 50)	19 (12, 38)	23 (12, 40)	0.148
Sex *N* (%)					
Males	33 (53)	31 (57)	30 (56)	94 (55)	0.597
Females	29 (47)	23 (43)	24 (44)	76 (45)	
MF[b] (*n* = 81)	5.8	5.7	5.8	5.8	0.97
CFA[c] (*n* = 77)	6.3	7.0	6.9	6.7	0.52
IgG1[d] (*n* = 105)	6.3	6.1	5.9	6.1	0.27
IgG4[e] (*n* = 106)	5.5	6.2	5.5	5.7	0.19

[a] Q1, Q3: lower and upper quartiles.
[b] MF: mean natural log microfilaria per millilitre of blood.
[c] CFA: mean natural log CFA in arbitrary units per millilitre of plasma.
[d] IgG1: mean natural log IgG1 in μg per millilitre of plasma.
[e] IgG4: mean natural log IgG4 in μg per millilitre of plasma.

The same procedure was done for the antibody and CFA measurements. Approximately normal distribution was achieved for the four outcome variables. Relative reduction in the levels of MF, IgG1, IgG4 and CFA (expressed as % of the pretreatment level) was calculated for the three treatment regimens. For comparison of baseline sample characteristics between the three treatments groups, proportions were compared by chi-squared analysis (two-tailed) for categorical variables while baseline means were compared using one-way analysis of variance (ANOVA) for continuous variables (Table 1).

For the outcome analyses, an intention-to-treat basis was used as stipulated by our study protocol. Multilevel mixed-effects regression models were used to compare changes in log MF, log IgG1, log IgG4 and log CFA with time between the three treatments. The mixed-effects model was chosen to accommodate the correlation due to repeated measures of the outcome variables within each study subject and also clustering within each household. Subject and household were used as the random-effects variables in the models. The models were also adjusted for age, sex and the interaction between follow-up visit and treatment regimen (the fixed-effects). Since the study subjects were randomized into the three treatments and the repeated measures were unevenly spaced in time the exchangeable correlation structure was adopted when developing the multilevel mixed-effects regression models.

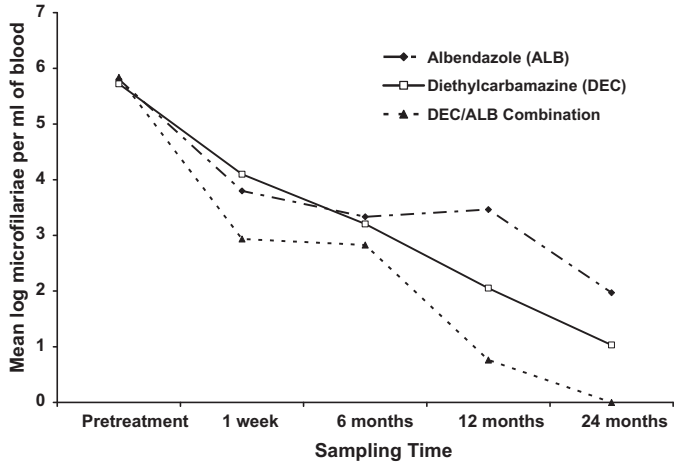

Fig. 1. The effects of ALB (*N* = 30), DEC (*N* = 26) and DEC/ALB combination treatment (*N* = 25) in the clearance of *W. bancrofti* microfilaraemia in Muhaka, south coastal Kenya (1998–2000). [The levels of microfilaraemia at 24 months follow-up represent the effect of two rounds of annual treatment.]

3. Results

3.1. Treatment assignment and follow-up

Among 205 persons whose households were randomly assigned to the three drug regimens, 176 (85.9%) gave a blood specimen at pre-treatment. However, six persons did not meet the inclusion criteria for treatment (pregnant females). For the 170 participants that gave blood samples and met the criteria for treatment 62, 54, and 54 individuals were randomly assigned to ALB alone, DEC alone and DEC/ALB combination arms, respectively. Out of these 170 participants, 81(47.6%) were positive for MF at baseline, 30 in the ALB group, 26 in the DEC group and 25 in the DEC/ALB group. The three groups were comparable in terms of age, sex, and infection intensity at baseline (Table 1).

3.2. Effects of treatment on microfilaraemia

Although there was no significant difference in geometric mean microfilaraemia among the three treatment groups at pre-treatment ($p = 0.97$), none of the persons receiving DEC/ALB combination had detectable microfilaraemia at 24 months follow-up (Fig. 1). In general, at two years of follow-up the decrease in geometric mean MF count was very high for all the 3 treatment groups, 98%, 99% and 100% for ALB, DEC and DEC/ALB groups, respectively. Microfilariae counts decreased dramatically in all three treatment groups by one week after the start of treatment, and these decreases were sustained throughout the following 12 months, after which the participants received a second dose of treatment.

A multilevel mixed-effects regression model of log MF count per millilitre of blood over time was constructed with subject and household as the random-effects and adjusted for age, sex and the interaction between follow-up visit and treatment regimen. The model revealed significant reduction of MF count with treatment over time ($p < 0.001$) in all treatment groups and at all time points. Age ($p = 0.688$) and sex ($p = 0.163$) were not significant factors influencing the reduction of microfilaria levels with time. There was a significantly greater reduction in MF count in the DEC/ALB group compared to the ALB group (geometric mean difference 17, 95% confidence interval 3–81, $p < 0.001$). Similarly, there was greater

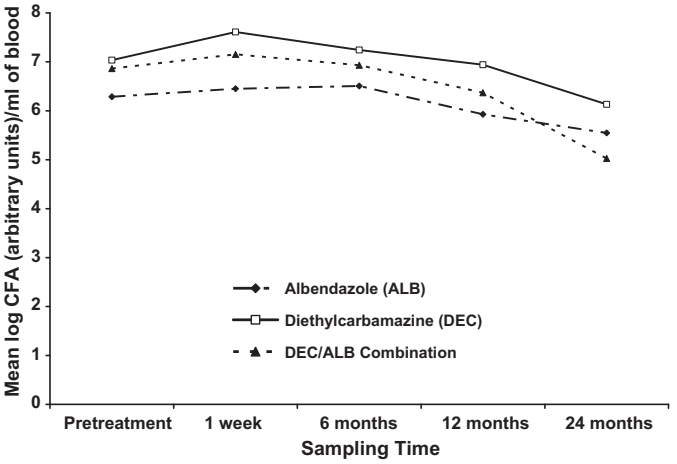

Fig. 2. The effects of ALB ($N = 30$), DEC ($N = 26$) and DEC/ALB combination treatment ($N = 21$) in the clearance of *W. bancrofti* circulating filarial antigens (CFA) in Muhaka, south coastal Kenya (1998–2000). [The levels of CFA at 24 months follow-up represent the effect of two rounds of annual treatment.]

reduction in MF count in the DEC/ALB group compared to the DEC group although the difference was not statistically significant (geometric mean difference 2.9, 95% confidence interval 1.5 to 12.9, $p = 0.146$).

3.3. Effects of treatment on anti-filarial antibodies

To evaluate the impact of the three treatment regimens on anti-filarial antibodies, we assessed changes in the levels of IgG1 and IgG4 during the study period in a total of 110 plasma specimens (ALB = 43; DEC = 32; DEC/ALB = 35). There was an initial increase in anti-filarial IgG1 levels one week and six months after the first treatment in the ALB group and at one week in the DEC/ALB group. However, all the treatment regimens resulted in an overall decrease in IgG1 and IgG4 at 2 years. When compared to pre-treatment levels, the overall reduction in mean IgG1 levels at 2 years was 38%, 45% and 52% and that for IgG4 was 40%, 68% and 64% for ALB, DEC and DEC/ALB groups, respectively.

Multilevel mixed-effects regression models were constructed for changes in both log IgG1 and log IgG4 levels over time with subject and household as the cluster variables. The models were adjusted for age, sex and the interaction between follow-up visit and treatment regimen. The first model revealed overall reduction of anti-filarial IgG1 ($p < 0.028$) in all treatment groups at 2 years of follow-up. However, there was no significant difference in the reduction of IgG1 among the three treatment groups. The second model revealed overall reduction of anti-filarial IgG4 ($p < 0.043$) in all treatment groups at 2 years of follow-up. Similarly, there was no significant difference in the reduction of IgG4 among the three treatment groups.

3.4. Effects of treatment on antigenaemia

To evaluate the impact of the three treatment regimens on antigenaemia, we assessed changes in the levels of CFA during the study period in a total of 91 available plasma specimens, 77 of which were positive for CFA. All the treatment regimens resulted in an overall decrease in CFA. When compared to pre-treatment levels, the overall reduction in mean CFA levels at 2 years was 34%, 60% and 85% for ALB, DEC and DEC/ALB groups, respectively (Fig. 2).

A multilevel mixed-effects regression model of log CFA levels over time was constructed with subject and household as the cluster variables. The model was adjusted for age, sex and the interaction between follow-up visit and treatment regimen. The model revealed significant reduction of CFA ($p < 0.001$) in all treatment groups at 2 years of follow-up. Age ($p = 0.744$) and sex ($p = 0.700$) were not significant factors influencing the reduction of CFA levels with time. We also found that DEC/ALB combination treatment reduced the CFA significantly more than ALB treatment alone on the final follow-up visit (geometric mean difference 4.4, 95% confidence interval 1.7–11.7, $p = 0.003$). DEC/ALB combination treatment was also significantly more effective than DEC alone (geometric mean difference 4.4, 95% confidence interval 0.6–9.67, $p = 0.049$).

3.5. Adverse events

The medication ALB, DEC and the combination of both were all well tolerated. Of the 170 participants who received initial treatment, none reported any complaints associated with the medication. The patients were seen again after one week during blood collection and they all remained in good condition.

4. Discussion

This study was conducted to evaluate the effectiveness of DEC/ALB combination in comparison to single dose annual DEC (6 mg/kg) and albendazole (400 mg) given alone on markers of *W. bancrofti* infection namely microfilaraemia, filarial antigenaemia and anti-filarial antibodies. When administered as single-dose annual chemotherapy, all the three treatments showed a relatively high effectiveness in reduction of microfilarial levels in the current study. The implication of this finding is that all the three treatments have anti-filarial effects and are useful in the current global efforts to eliminate lymphatic filariasis. At the 24 months follow-up, the reduction of microfilariae was higher in the DEC/ALB combination treatment group compared to ALB or DEC as single drug therapy groups. Moreover, although the pre-treatment microfilariae levels were similar for the 3 groups, DEC/ALB combination resulted in lower microfilarial densities at all the follow-up time-points. This observation is similar to the findings of a community based study in Papua New Guinea (Weil et al., 2008) in which two annual mass administrations of DEC/albendazole combination led to over 90% reduction of microfilaria rates. DEC/ALB combination mass administration in Egypt also demonstrated significant reduction of microfilaraemia among treated communities (Ramzy et al., 2006). However, these two studies did not compare the effects of each of the two drugs in isolation and they were conducted in areas with lower pretreatment microfilaria prevalence unlike in the present study. A study involving school children in Haiti, also showed that the combination chemotherapy of DEC/ALB resulted in higher reduction of microfilarial density compared to DEC alone (Fox et al., 2005). Thus in addition to the important ancillary benefits of albendazole while in combination regimens in decreasing the intensity of intestinal helminthes (Mani et al., 2004), the results of our study demonstrated that addition of the drug to DEC results in improved effectiveness on reduction of microfilaraemia.

A review to assess the efficacy and safety of two-drug regimens by Gyapong et al. (2005) concluded that suppression of microfilaraemia is enhanced by the addition of albendazole to DEC or ivermectin compared to either drug alone. However, while a 2005 review (Critchley et al., 2005) concluded that there is insufficient information to refute or confirm that albendazole alone or in combination with DEC or ivermectin has an effect on LF, randomized trials have continued to show significantly greater MF reduction in DEC/ALB combination treatment groups compared to either drug alone (Rajendran et al., 2004; Mani et al., 2004; El Setouhy et al., 2004; Rizzo et al., 2007). Recent reports on the effectiveness of DEC/ALB combination in filariasis elimination programs linked to

the Global Program for Elimination of Lymphatic Filariasis (GPELF) from several regions including south Pacific, Indonesia, Egypt and Kenya suggest that the combination treatment has relatively high effectiveness in reduction of microfilaraemia (Helmy et al., 2006; Oqueka et al., 2005; Njenga et al., 2008; El Setouhy et al., 2004). Furthermore, drug regimen has been listed as one of the most important determinants affecting the outcome GPELF programs (Kyelem et al., 2008). Thus our study results not only appear to support the first review but also provide additional information that may help in making of programmatic decisions regarding the role of albendazole in the interruption of transmission of *W. bancrofti* infection within the context of GPELF in non LF-onchocerciasis co-endemic settings.

In principle, anti-filarial antibody responses can serve as very sensitive markers of filarial exposure and transmission (Washington et al., 2004). Furthermore, monitoring of antibody responses provides an indication of transmission (Weil et al., 2008). The initial increase in anti-filarial antibody levels observed in this study after the first treatment is most likely due to filarial antigens released from dying or dead microfilariae or adult worms. Since the increase was observed in all the 3 treatment groups, this further corroborates our conclusion that all the 3 treatments have an effect on clearance of *W. bancrofti* infection. The significant reduction in anti-filarial antibody levels by 24 months follow-up supports the view that anti-filarial antibody-based tests may have a role in long-term monitoring of programs to eliminate LF, but their application to precise program monitoring still needs further development (Lammie et al., 2004; Tisch et al., 2008; Weil et al., 2008).

Although decrease in levels of CFA was generally observed in all groups, only the DEC/ALB arm showed a significant decrease in antigenaemia at 24 months follow-up when paired comparisons were done. Circulating filarial antigen is released by adult worms (Weil et al., 1991), and may thus be used as a proxy for assessing effect of treatment on adult worms. In the current study, addition of albendazole to DEC showed significant improved effectiveness against adult worms, the source of microfilariae. The combination therapy of DEC/ALB was found to achieve a three fold higher reduction in the prevalence of antigenaemia compared to DEC alone in a study conducted in Tamil Nadu, south India (Rajendran et al., 2006). Results of a pilot filariasis elimination research project in Kenya indicate that the prevalence of parasite antigenaemia decreased by 43.5% after two annual mass drug administrations (MDA) of DEC/ALB (Njenga et al., 2008). Therefore, lack of clear improved effectiveness of DEC/ALB combination compared to DEC alone in suppression of microfilaraemia in our study may suggest that combining DEC with albendazole is inconsequential. However, significant reduction of antigenaemia as observed in this study supports the use of DEC/ALB combination in GPELF. In addition, albendazole provides de-worming benefits to endemic communities which may improve adherence to treatment and thus result in higher therapeutic coverage, which is a critical factor in filariasis elimination (Oqueka et al., 2005; Yongyuth et al., 2006; Fox et al., 2005). Moreover, co-occurrence of soil-transmitted helminthes in similar settings that are currently targeted in the integrated control of neglected tropical diseases get a cost effective "piggy-back ride" on GPELF.

Our study had several limitations. Firstly, it is possible that persistence of low level microfilaraemia undetectable by the conventional counting chamber technique used in this study may have affected our results, for example, by classifying the antigenaemia positives as "mf true negatives". However, this would have affected all the treatment groups and would therefore not have significantly affected our results. Secondly, this study was not designed to directly detect the macrofilaricidal effects of DEC, ALB or DEC/ALB combination particularly since there was no ultrasonographic assessment of adult worms. However, all three treatment regimens demonstrated modest, but significant reductions in CFA levels two years post-treatment which is suggestive of a macrofilaricidal effect of the three treatment regimens.

In conclusion, our study results support the main strategy of GPELF by suggesting that sustained annual MDA with repeated doses of DEC/ALB could enhance reduction of microfilaraemia in endemic communities in similar settings leading to the interruption of LF transmission. In the WHO–AFRO Region, co-administration of DEC/ALB is currently recommended for use in countries such as the Comoros, Kenya, Madagascar, Zambia and Zimbabwe where onchocerciasis is not co-endemic. To the best of our knowledge our study presents the first unique data based on multiple parameters of lymphatic filariasis infection from sub-Saharan Africa. It would be interesting to see how results from other non-ochocerciasis endemic countries and countries using DEC alone such as Brazil compare to those from onchocerciasis endemic countries where albendazole is used in combination with ivermectin. Besides, to make general predictions, computer simulations rely on data from various studies and different geographical regions.

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