Tropical Medicine and International Health

VOLUME 8 NO 12 PP 1082–1092 DECEMBER 2003

The impact of six rounds of single-dose mass administration of diethylcarbamazine or ivermectin on the transmission of *Wuchereria bancrofti* by *Culex quinquefasciatus* and its implications for lymphatic filariasis elimination programmes

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Summary Lymphatic filariasis (LF) is targeted for global elimination. Transmission interruption through repeated annual single-dose mass administration of anti-filarial drugs is the mainstay of the LF elimination strategy. This study examined the ability of six rounds of mass administration of diethylcarbamazine (DEC) or ivermectin (IVM) to interrupt transmission of *Wuchereria bancrofti* by *Culex quinquefasciatus*, the predominant parasite and vector species, respectively. After six rounds of mass drug administration (MDA), received by 54–75% of the eligible population (≥15 kg body weight), the resting vector infection and infectivity rates fell by 83% and 79% in the DEC arm, 85% and 84% in the IVM arm and 31% and 45% in the placebo arm, respectively. The landing vector infection and infectivity rates fell by 83% and 94% in the DEC arm, 63% and 75% in the IVM arm and 1% each in the placebo arm, respectively. The filarial larval load per resting mosquito declined by 92% and 93% and per landing mosquito by 83% and 69% in the DEC and IVM arms, respectively. The annual infective biting rate (AIBR) fell from 735 to 93 (87%) in the DEC arm, 422 to 102 (76%) in the IVM arm and 472 to 398 (16%) in the placebo arm. The annual transmission potential (ATP) declined from 2514 to 125 (95%), 1212 to 241 (80%) and 1547 to 1402 (9%) in the DEC, IVM and placebo arms, respectively. However, mosquitoes with infection [microfilaria/larva 1/larva 2 (Mf/L1/L2)] were found in all study villages. Three of five villages in the IVM arm and two of five in the DEC arm recorded no resting mosquitoes with infective-stage (L3) larva. Although the ATP, after six rounds of MDA, fell substantially and remained at 125 and 241 in the DEC and IVM arms, respectively, the cumulative exposure to infective stage larvae (ATP) during the treatment period of 6 years was as high as 2995 in the DEC arm and 1522 in the IVM arm, because of considerable level of transmission during the initial (1–3) rounds of MDA. We conclude that (i) six rounds of MDA, even with 54–75% treatment coverage, can reduce LF transmission very appreciably; (ii) better treatment coverage and a few more rounds of MDA may achieve total interruption of transmission; (iii) high vector densities may partly nullify the reductions achieved in vector infection and infectivity rates by MDA and (iv) achievement of 'true zero' Mf prevalence in communities and 0% infection rate (mosquitoes with Mf/L1/L2) in mosquitoes may be necessary to totally interrupt *Culex*-transmitted LF.

keywords lymphatic filariasis, *Wuchereria bancrofti*, *Culex quinquefasciatus*, transmission, control, elimination, India

Introduction

Lymphatic filariasis (LF) is an infectious disease caused by lymph-dwelling nematode parasites and transmitted by mosquitoes. A total of 1.1 billion people living in tropical countries are vulnerable to LF infection (Michael *et al.* 1996) and nearly one-fourth (Das *et al.* 2001a) of them may already have infection. Globally, LF is the second leading cause of disability (WHO 1995). In India alone, the disease causes an annual economic loss of nearly US$ 1 billion (Ramaiah *et al.* 2000). Recognizing the disease burden and public health importance, a global programme to eliminate LF was launched in 1997. The mainstay of this programme is to interrupt transmission of infection by vectors by diminishing the parasite population in human hosts through annual mass administration of single-dose diethylcarbamazine (DEC) or ivermectin (IVM) in combination with albendazole (ALB) (Ottesen 2000). Although

addition of ALB was reported to yield better clearance of microfilaraemia and other benefits such as clearance of intestinal helminths in treated communities (Ottesen *et al.* 1999), DEC or IVM constitute the principal anti-filarial drugs in combination treatment. Clinical (Cao *et al.* 1997; Ottesen *et al.* 1997) and community trials (Meyrowitsch *et al.* 1996; Bockarie *et al.* 1998; Gyapong 2000; Ramaiah *et al.* 2002) showed that single-dose DEC or IVM significantly reduces microfilaria (Mf) prevalence and intensity in human populations. Because of a strong relationship between Mf prevalence and intensity in human beings and Mf intake and their development in vector mosquitoes (McGreevy *et al.* 1982; Subramanian *et al.* 1998), the lower Mf prevalence and intensity in treated communities is likely to result in reduction or interruption of transmission, leading to prevention of new infections. This, combined with the gradual fading of existing infections because of repeated mass treatment, may lead to elimination of LF. Hence, total interruption of transmission is crucial to achieve LF elimination. However, information on the potential of annual mass drug administration (MDA) to interrupt transmission is scanty. In this study, we examine the impact made by six rounds of mass administration of DEC or IVM, hypothesized to be adequate to eliminate LF (Ottesen 2000), on transmission of *Wuchereria bancrofti* by *Culex quinquefasciatus*. *Wuchereria bancrofti* transmitted by *C. quinquefasciatus* is responsible for more than 50% of LF infections worldwide (Southgate 1984). Hence, the results of the study may have significant implications for LF control/elimination programmes in many endemic regions.

Study area

The study was conducted in 15 villages in Villupuram district in Tamil Nadu, south India. The population of the villages ranges from 517 to 3321. The major occupations of the villagers are agriculture and weaving. The study villages are separated by a few kilometres from one another. They constitute independent transmission zones as the vector species *C. quinquefasciatus* does not fly more than a few hundred metres in inhabited areas (Subra 1980). Most of the houses are made up of thatched roof and mud walls, which provide ideal resting places for the vector mosquito species *C. quinquefasciatus*. Wastewater collections in and around the houses are the major breeding habitats for the vector mosquito. No vector control measures were implemented in the rural areas including the study villages and very few rural households use personal protection measures against mosquitoes. No other anti-filarial measures were implemented in the study villages prior to this study. The villages are endemic for bancroftian filariasis with pre-control Mf rate ranging from 6.8% to 20.2% (Ramaiah *et al.* 2002).

Study design

The study was double blind and placebo controlled and implemented in 15 villages (Das *et al.* 2001b; Ramaiah *et al.* 2002). The 15 study villages were grouped into three arms and each arm consisted of five villages. The three arms were randomly allocated to three treatment groups – DEC or IVM or placebo. DEC was administered to the study population at the dose of 6 mg/kg body weight and IVM at 400 mcg/kg. The study comprised of 1-year pre-intervention data collection (1993–94), intervention with six rounds of MDA (1994–2000) and 1-year post-intervention assessment. The initial two rounds of MDA were carried out at a half-yearly intervals and subsequent rounds at 12–15-month intervals (Figure 1). DEC, IVM and placebo (casein) were packaged into

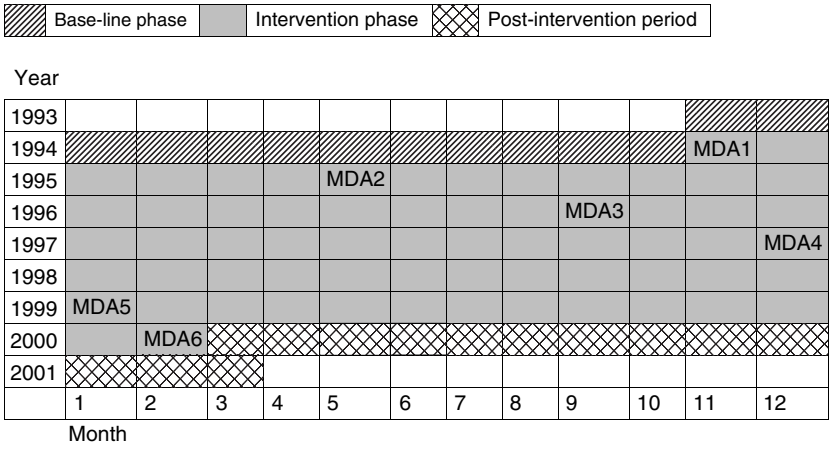

Figure 1 Study phases, months, years and intervals at which mass drug administration (MDA) was started in the study villages. Mf surveys were carried out a week prior to each round of MDA in each village. Resting and biting collections were carried out every month in all study villages throughout the three phases of the study.

identical capsules. The study was conducted in a double-blind fashion during the initial two rounds and after the second MDA, the drug codes were opened to assess the benefits of MDA. Subsequently, the study was continued in a single-blind form. No placebo tablets were given during the third to sixth round of treatment. However we continued to monitor the mosquito population to assess the changes in vector infection and infectivity rates and other transmission parameters. The Mf carriers detected during the parasitological surveys (Ramaiah *et al.* 2002) in all study arms were treated with single dose of DEC (6 mg/kg body weight). Children under 15 kg body weight were excluded from treatment in the IVM arm because the drug had never been used before at community level in India. For comparison, they were excluded in the DEC and placebo arms also. Pregnant women, lactating mothers and seriously ill people were also excluded from treatment.

Material and methods

Entomological evaluation

Entomological evaluation of MDA was carried out through collection of indoor resting mosquitoes in all 15 study villages and all-night man landing mosquitoes in six villages, two each in three study arms, at monthly intervals throughout the study period (Figure 1). Resting mosquitoes were collected using mechanical aspirators in 12 fixed houses spending three man-hours in each of the 15 villages. Night man landing mosquitoes were collected by an insect collector on a human volunteer in a fixed house for 12 h (18.00 to 06.00 hours) in each of the six villages. The landing mosquitoes were located using torch lights and trapped into test tubes.

The collected mosquitoes were transported on the same day to the laboratory. All the collected or a maximum of 100 indoor resting *C. quinquefasciatus* mosquitoes per village and all the collected or a maximum of 25 landing mosquitoes collected per hour were dissected to determine the filarial infection rates. The stage and the number of filarial parasites present in the infected mosquitoes were recorded. The infection rate was calculated as the percentage of dissected mosquitoes positive for any filarial parasite stage (Mf/L1/L2/L3 or infective stage) and infectivity rate as percentage positive only for L3. The infection and infectivity rates were calculated based on the aggregate number of mosquitoes collected and dissected from 12 resting and landing collections prior to the first MDA (pre-intervention period); six collections after the first MDA and 12 collections each after the second, third, fourth, fifth and sixth round of MDA (intervention and post-intervention periods) (Figure 1). Three indices – annual infective biting rate (AIBR), annual transmission potential (ATP) and transmission intensity index (TII) – were used to monitor the changes in the intensity of transmission. The monthly transmission potential (MTP) is derived as the monthly biting rate × total number of L3/total number of landing mosquitoes dissected. The monthly biting rate is obtained by multiplying the number of landing mosquitoes per person per night (in monthly night-landing mosquito catches) with the number of days in a month. ATP is the sum of 12 MTPs in a year (WHO 1977). TII is the product of resting density × proportion of mosquitoes with L3 × average number of L3/infective mosquito (Krishna Rao *et al.* 1981).

Treatment coverage

The details on treatment coverage have been recorded during all the six rounds of treatment by the drug distributors. Among the eligible population, 49–84% received treatment during different rounds of treatment in different study villages ($n = 15$ villages). The overall proportion of the eligible population who received treatment ranged from 54% to 73% in the DEC arm and 57% to 75% in the IVM arm during the six rounds of treatment, while 4.8% and 5.6%, respectively, did not receive even a single treatment (systematic non-compliance) of the six treatments offered.

Statistical analysis

The significance of reduction in infection and infectivity rates and the proportion of mosquitoes with high parasite load from pre- to post-intervention period was tested by chi-squared test. The significance of reduction in intensity of parasite load in the vector mosquitoes was assessed by Student's *t*-test. *Z* interaction test from a log-linear model was applied to test the relative difference in the change from pre- to post-intervention period in infection and infectivity rates and the proportion of mosquitoes with higher parasite count between placebo and DEC or IVM arms.

Results

Infection and infectivity rate of resting vector population

A total of 49 714 resting and 27 311 landing vector mosquitoes were dissected during the study period to assess the changes, caused by six rounds of MDA, in vector infection and infectivity rate and transmission dynamics of LF (Table 1). The pre-intervention infection rate of resting

Table 1 Resting and landing *Culex quinquefasciatus* mosquitoes dissected to determine the infection and infectivity rates and assess the impact of MDA

Study arm	No. of mosquitoes dissected							
	Pre-MDA period	Post-I round	Post-II round	Post-III round	Post-IV round	Post-V round	Post-VI round	Total
Resting *C. quinquefasciatus*								
DEC	3520	1710	3181	2373	3051	2742	3275	19 852
IVM	2689	1040	2735	2536	2546	3183	2921	17 650
Placebo	1941	1144	2195	1853	1823	1524	1732	12 212
Total	8150	3894	8111	6762	7420	7449	7928	49 714
Landing *C. quinquefasciatus*								
DEC	2059	1472	1241	784	1119	2483	3324	12 482
IVM	1313	761	362	669	831	1467	1143	6546
Placebo	1824	451	1437	675	1161	1364	1371	8283
Total	5196	2684	3040	2128	3111	5314	5838	27 311

C. quinquefasciatus ranged from 8.9% to 29.2% in the 15 study villages and the average rate in the three study arms was similar and around 18.5% (Figure 2). From the pre-intervention level of 18.4%, the infection rate in the DEC arm showed gradual decline with each round of MDA and stood at 2.7% after the fifth round. No further decline was observed after the sixth round. The infection rate in the IVM arm declined sharply from the pre-intervention level of 18.7% to 5.5% with the first round of MDA itself. Thereafter the rate declined only gradually, reaching 2.7% after the sixth round. After six rounds of MDA, the decline in average infection rate was 83% in the DEC arm ($P < 0.001$) (range = 66–91%; $n = 5$ villages) and 85% in the IVM arm ($P < 0.001$) (range = 79–98%; $n = 5$ villages), compared with 31% in the placebo arm ($P < 0.001$) (range, an increase of 16% to 92% decline; $n = 5$ villages) (Figure 2). Note that none of the villages became completely free from infected mosquitoes and the maximum reduction of 98% (15.7% to 0.4%) was observed in one village (Thenputhur) in the IVM arm.

The pre-intervention infectivity rate of *C. quinquefasciatus* ranged from 0.22% to 4.0% in different study villages (Figure 3) and it was <2.0% in 11 of 15 villages. The average pre-intervention infectivity rate was 0.99%, 1.71% and 1.91% in the DEC, IVM and placebo arm, respectively. Following six rounds of MDA, it declined by 79% in the DEC arm ($P < 0.001$) (range = 34–100%; $n = 5$ villages), 84% in the IVM arm ($P < 0.001$) (range = 60–100%; $n = 5$ villages) and 45% in the placebo arm ($P < 0.02$) (range = 20–100%; $n = 5$ villages) (Figure 3). Like the infection rate, the infectivity rate fell very sharply after the first round of MDA itself in the IVM arm. After six rounds of MDA, three of five villages in the IVM arm and two of five in the DEC arm showed 0% infectivity rate. One village (Muppili) in the DEC arm showed zero infectivity continuously after the fourth round of MDA (Figure 3).

The difference in the decline in average infection rate from pre-treatment to post-sixth MDA period, between the placebo and DEC or IVM arms was highly significant ($P < 0.01$). While the difference in the decline in infectivity rate between the placebo and IVM arms was significant ($P < 0.05$), it was not so between the placebo and DEC arms ($P > 0.05$). However, the difference in the decline in infection or infectivity rate between the DEC and IVM arms was not significant ($P > 0.05$).

Infection and infectivity rate of landing vector population

The pre-intervention average infection rate of landing mosquitoes was 14.3% in the DEC as well as the placebo arms and 10.5% in the IVM arm. After six rounds of MDA, the average infection rate fell from 14.3% to 2.4% (83%) ($P < 0.001$) and 10.5% to 3.9% (63%) ($P < 0.001$) in the DEC and IVM arms, respectively, compared with 14.3% to 14.2% (1%) ($P > 0.05$) in the placebo arm. Infected mosquitoes continued to persist in all the six monitored villages (Table 2).

The pre-intervention average infectivity rate was 2.38%, 2.13% and 1.70% in the DEC, IVM and placebo arms, respectively. After six rounds of MDA, the infectivity rate was reduced by 94% ($P < 0.001$) in the DEC arm and 75% ($P < 0.001$) in the IVM arm, compared with 1% ($P > 0.05$) in the placebo arm. After five rounds of MDA, one of the two monitored villages (Thenputhur) in the IVM arm became and remained free from infective landing mosquitoes (Table 2).

The difference in the decline in infection and infectivity rate from pre- to post-treatment period, between the placebo and DEC or IVM arms was highly significant ($P < 001$). Between the DEC and IVM arms also, the difference in the change in infection rate was significant, but, not in infectivity rate ($P > 0.05$).

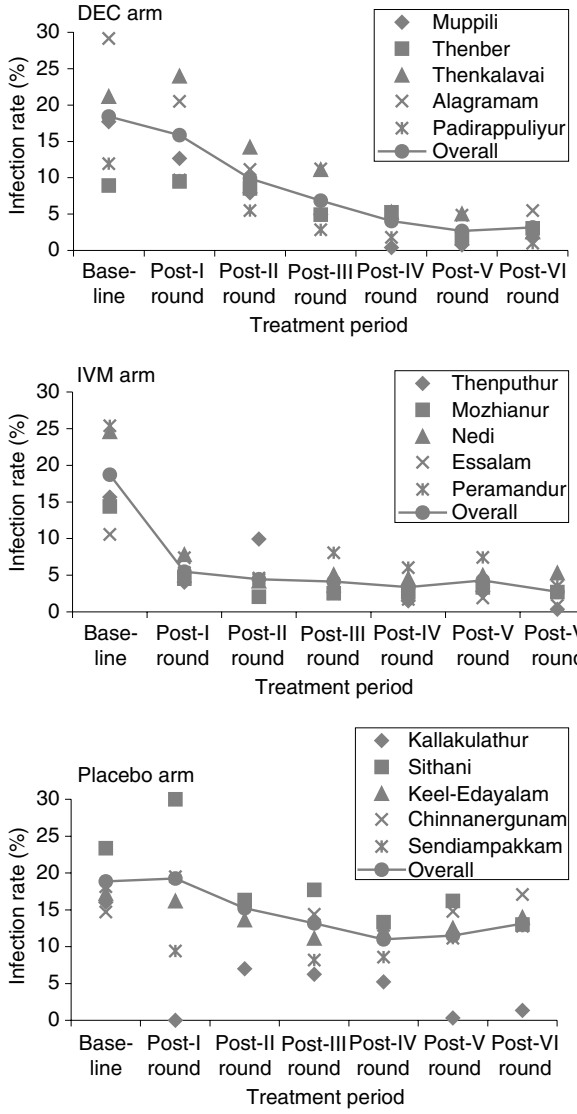

Figure 2 Effect of six rounds of MDA on resting vector infection rate.

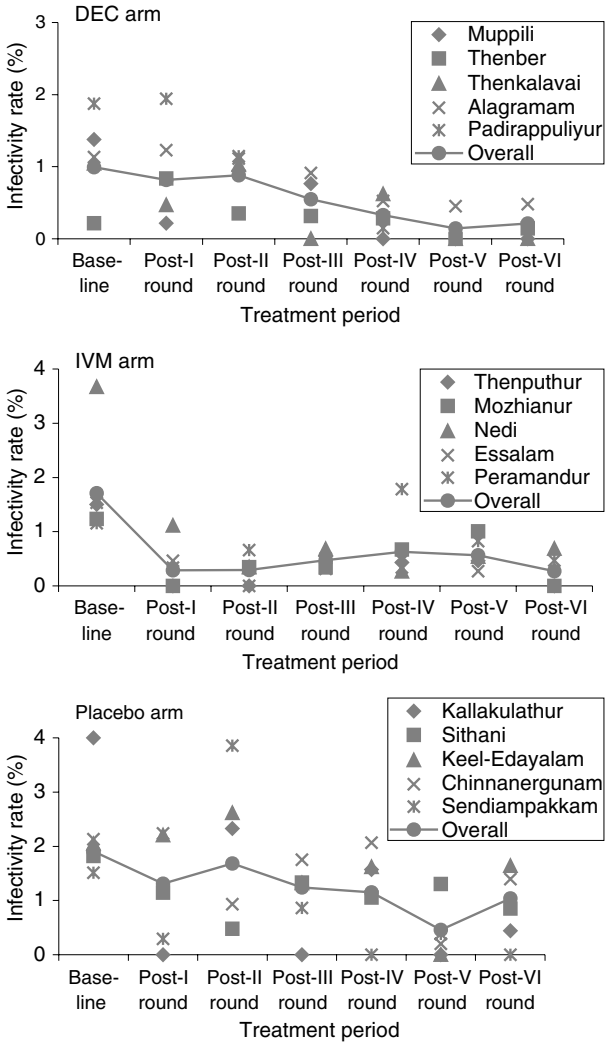

Figure 3 Effect of six rounds of MDA on resting vector infectivity rate.

Parasite load

The average number of filarial larvae (Mf/L1/L2/L3) per dissected resting mosquito declined from a pre-intervention level of 2.39 to 0.18 (92%) during the post-sixth MDA period in the DEC arm ($P < 0.05$), 2.12 to 0.15 (93%) in the IVM arm ($P < 0.05$), compared with 2.12 to 0.94 (56%) in the placebo arm. Among landing mosquitoes it declined by 83% (1.07 to 0.18; $P < 0.01$), 69% (0.65 to 0.20; $P < 0.01$) and 21% (1.46 to 1.15; $P > 0.05$) in the DEC, IVM and placebo arm, respectively. Following six rounds of MDA, the proportion of mosquitoes with higher numbers of filarial larvae (>20) per dissected resting and landing mosquito fell by 97% ($P < 0.05$) and 80% ($P < 0.05$), respectively, in the DEC arm and by 96% ($P < 0.05$) and 75% ($P < 0.05$) in the IVM arm (Table 3).

Transmission parameters

The average AIBR, following six rounds of MDA, fell from the pre-intervention level of 735 to 93 (87%) in the DEC arm, 422 to 102 (76%) in the IVM arm, compared with 472 to 398 (16%) in the placebo arm. The average

Table 2 Changes in landing vector infection and infectivity rate (%) following six rounds of mass administration of DEC or IVM or placebo

Study arm	Village	Pre-MDA period	Post-I round	Post-II round	Post-III round	Post-IV round	Post-V round	Post-VI round	Percentage reduction*
Infection rate									
DEC	Muppili	12.6	8.6	3.2	2.7	1.0	2.4	0.7	94
	Alagramam	17.5	9.5	7.0	5.6	5.2	3.2	3.5	80
	Average	14.3	9.0	5.8	4.3	2.6	2.8	2.4	83
IVM	Thenputhur	8.5	4.2	3.3	1.8	0.7	0.4	0.3	96
	Nedi	19.4	8.7	7.2	2.7	6.2	4.5	5.2	73
	Average	10.5	4.5	5.2	2.4	4.3	3.1	3.9	63
Placebo	Sithani	12.3	11.9	9.3	3.7	5.4	6.5	9.4	24
	Chinnanergunam	17.7	15.4	15.2	16.3	15.8	13.6	15.9	10
	Average	14.3	14.0	12.9	9.3	9.0	11.2	14.2	1
Infectivity rate									
DEC	Muppili	2.01	1.57	1.05	0.60	0.00	0.28	0.07	96
	Alagramam	3.08	0.78	0.93	0.44	0.24	0.21	0.21	93
	Average	2.38	1.22	0.97	0.51	0.09	0.24	0.15	94
IVM	Theputhur	1.59	0.84	0.55	0.00	0.35	0.00	0.00	100
	Nedi	4.55	0.00	0.56	0.22	1.10	0.52	0.70	85
	Average	2.13	0.79	0.55	0.15	0.84	0.34	0.52	75
Placebo	Sithani	1.20	2.16	1.64	0.27	0.66	1.09	1.08	11
	Chinnanergunam	2.57	1.13	1.46	2.33	2.50	1.33	1.90	26
	Average	1.70	1.55	1.53	1.19	1.29	1.25	1.68	1

* From pre-MDA to post-sixth round of MDA.

Table 3 Proportion of mosquitoes with different number of filarial larvae during pre- and post-sixth MDA periods in the DEC, IVM and placebo arms

No. of filarial larvae	Percentage of mosquitoes								
	Pre-MDA period			Post-sixth MDA period			Percentage reduction*		
	DEC	IVM	Placebo	DEC	IVM	Placebo	DEC	IVM	Placebo
Resting mosquitoes									
1–5	9.7	10.0	9.3	2.0	2.0	8.4	79	80	10
6–10	3.5	3.2	4.2	0.8	0.3	2.2	77	90	48
11–20	2.2	2.7	2.6	0.3	0.3	1.8	86	89	31
>20	3.0	2.8	2.8	0.1	0.1	0.7	97	96	75
Landing mosquitoes									
1–5	8.5	6.8	8.3	1.5	2.6	8.9	82	61	+7
6–10	2.8	2.0	2.6	0.4	1.0	2.2	85	50	15
11–20	2.0	1.3	1.4	0.3	0.2	1.9	85	85	+35
>20	1.0	0.4	2.0	0.2	0.1	1.2	80	75	40

* From pre-MDA to post-sixth round of MDA.

ATP declined by as much as 95% in the DEC arm, 80% in the IVM arm compared with 9% in the placebo arm (Table 4; Figure 4). Although the initial four rounds of treatment reduced ATP appreciably, some amount of transmission did take place, particularly in one village each in DEC and IVM arms. This led to a cumulative exposure to infective larvae (ATP) of 1434–3005 in the four monitored villages treated with DEC or IVM, during the six rounds of MDA. Even after six rounds of MDA the ATP remained at as high as 191 in one of the two villages monitored in the DEC arm and 482 in one of the two villages in the IVM arm. One village (Thenputhur) in the IVM arm showed and remained at zero ATP after five rounds of MDA (Table 4). The TII declined by 86%, 59% and 71% in the DEC, IVM and placebo arms, respectively (Figure 4).

Table 4 Changes in annual infective biting rate (AIBR) and annual transmission potential (ATP) following six rounds of mass administration of DEC or IVM or placebo

		Observation period								
Study arm	Village	Pre-MDA period	Post-I round	Post-II round	Post-III round	Post-IV round	Post-V round	Post-VI round	Percentage reduction*	Cumulative†
AIBR										
DEC	Muppili	803	392	120	61	0	93	30	96	696
	Alagramam	667	145	240	59	30	111	157	76	742
	Average	735	269	180	60	15	102	93	87	719
IVM	Theputhur	513	174	30	0	30	0	0	100	234
	Nedi	330	0	30	34	192	153	203	38	612
	Average	422	87	30	17	111	77	102	76	424
Placebo	Sithani	426	121	280	31	150	155	121	72	858
	Chinnanergunam	517	87	411	216	302	429	676	−31	2121
	Average	472	104	345	124	226	292	398	16	1489
ATP										
DEC	Muppili	2337	2532	207	92	0	93	60	97	2984
	Alagramam	2690	852	1576	59	90	237	191	93	3005
	Average	2514	1692	892	76	45	165	125	95	2995
IVM	Theputhur	1303	1344	60	0	30	0	0	100	1434
	Nedi	1120	0	30	101	288	708	482	57	1609
	Average	1212	672	45	51	159	354	241	80	1522
Placebo	Sithani	1874	300	468	62	864	372	211	89	2277
	Chinnanergunam	1220	1452	1474	804	1311	1442	2593	−113	9076
	Average	1547	876	971	433	1087	907	1402	9	5676

* From pre-MDA to post-sixth round of MDA.
† During six rounds of MDA period.

Transmission levels and prevalence of Mf in villages with very low infectivity rate

A total of five villages – two in the DEC arm and three in the IVM arm – became free from resting mosquitoes with L3, following six rounds of MDA. Both these villages in the DEC arm and one (Thenputhur) of three villages in the IVM arm, also showed zero Mf prevalence (Table 5). However, all the five villages continued to have mosquitoes with Mf/L1/L2. Notably, one village (Thenputhur) recorded zero-infectivity rate in resting as well as landing mosquitoes and also zero Mf prevalence, indicating near total interruption of transmission. Data from one village (Muppili) show that mosquitoes with L3 can be found even when the infection (Mf/L1/L2) rate is as low as 0.7% (Table 5).

Discussion

Transmission interruption is the principal goal of annual MDA under LF elimination programmes. It was surmised that six rounds of MDA have the potential to reduce the parasite load in human population to negligible levels and each of the six rounds may, as shown in clinical trials (Cao *et al.* 1997), suppress microfilaraemia very drastically, leading to reduction or interruption of transmission over a 6-year period (Ottesen *et al.* 1999; Ottesen 2000). This paper addresses levels of reduction or interruption in transmission achieved by six rounds of MDA.

Six rounds of mass treatment in the present study villages with DEC or IVM, at 54–75% treatment coverage of the eligible population (≥15 kg body weight), reduced the community Mf prevalence by 86% and 72% and Mf intensity by 91% and 84%, respectively (Ramaiah *et al.* 2002). As a consequence, the resting and landing vector infection and infectivity rates fell by 79–94% in the DEC arm and 63–85% in the IVM arm (Figures 2–3; Table 2). AIBR and ATP, which are more direct measurements of transmission, fell by 87% and 95% in the DEC arm and by 76% and 80% in the IVM arm, respectively (Figure 4). These results are impressive compared with the results of a previous study, in which four rounds of DEC administration reduced resting vector infection rate and infectivity rate by 25% and 51%, respectively (Balakrishnan *et al.* 1992). The reduction reported in *Anopheles*-transmitted *W. bancrofti* in Papua New Guinea (PNG) is much more impressive – one treatment with DEC alone reduced AIBR by 54–75% and ATP by 76–79%

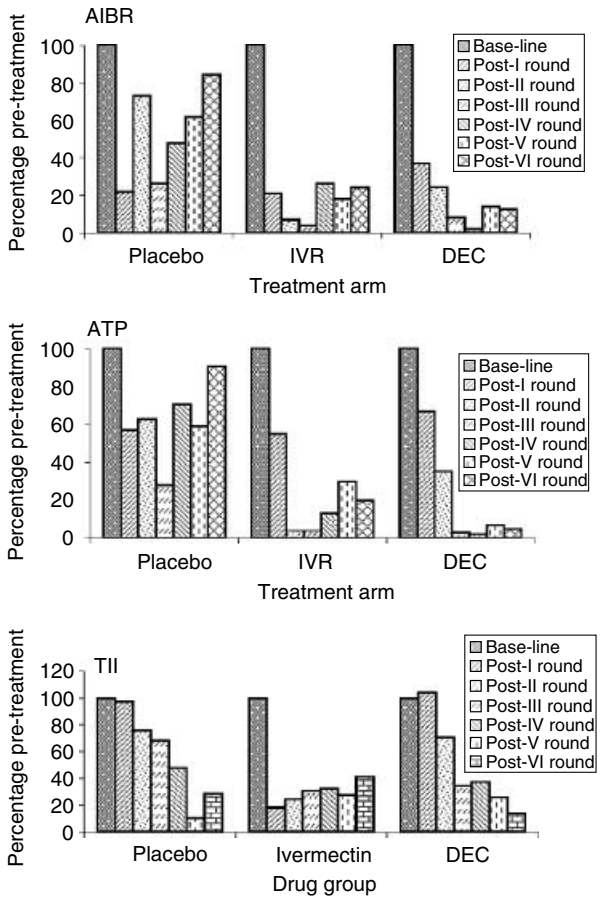

Figure 4 Percentage reduction in transmission parameters following six rounds of mass administration of placebo or ivermectin or DEC.

(Bockarie *et al.* 1998). In the PNG study, one round of MDA with DEC reduced Mf intensity by 64–75%. Better reduction seen in transmission in PNG may partly be due to the facilitation phenomenon exhibited by anopheline vectors, in which the well-developed pharyngeal armature damage the mf when they are ingested. At low densities, most of the Mf are damaged and very few are left to infect the mosquitoes. In contrast, the limitation phenomenon is seen in culicine vectors, which can pick up Mf even from ultra-low density Mf carriers and become infected (Webber 1991; Southgate & Bryan 1992). Hence, in culicine-transmitted LF, relatively more rounds of MDA are necessary and one or two rounds of MDA may not have as dramatic an impact as in *Anopheles*-transmitted LF. However, comparison of the present and PNG study may not be very sound because of the differences between the two studies in human population size per village (average size 159 in PNG study compared with 1788 in this study), pre-intervention Mf rates (32–88% *vs.* 6.8–20.2%), treatment coverage (87.6% *vs.* 54–75%) (Bockarie *et al.* 1998; Ramaiah *et al.* 2002). Besides, there may be differences in the susceptibility of parasite to the drug.

Despite appreciable reduction in vector infection and transmission rates, infected resting and landing mosquitoes (with Mf/L1/L2 stage) were found in all villages and infection rate ranged from 0.3% to 5.5% in the DEC or IVM treated villages, after six rounds of MDA (Table 2, Figure 2). This is not surprising because nearly 2% of the population in the DEC arm and 4.1% in the IVM arm, who were shown to harbour Mf after MDA (Ramaiah *et al.* 2002), might have contributed to mosquito infection. Although infected resting mosquitoes (with Mf/L1/L2 stage) were found in all 15 villages, five of these villages (two in the DEC arm and three in the IVM arm), with an infection rate of 0.4–2.5%, recorded no mosquito with L3 (Figures 2 and 3). This may be because of the fact that a large proportion of Mf ingested by vector mosquitoes fails to develop into L3. For example,

Table 5 Transmission parameters observed during post-sixth treatment period in villages with zero infectivity rate (%) in resting mosquitoes

Study village arm	Resting vectors		Landing vectors					
	Infection rate	Infectivity rate	Infection rate	Infectivity rate	AIBR	ATP	Mf rate*	Mf intensity*
DEC								
Muppili	1.7	0.00	0.70	0.07	30	60	0.00	0.00
Thenkalavai	2.5	0.00	ND	ND	ND	ND	0.00	0.00
IVM								
Thenputhur	0.4	0.00	0.30	0.00	0	0	0.00	0.00
Mozhianur	2.7	0.00	ND	ND	ND	ND	2.10	0.08
Essalam	0.9	0.00	ND	ND	ND	ND	2.40	0.05

* *Source*: Ramaiah *et al.* (2002). Mf rate and intensity was assessed by examining 60 mm^3 of finger prick blood.
ND: not done.

15% of *C. quinquefasciatus* that fed on low-density Mf carriers ingested Mf, but, only 0.6% developed them into L3 (McGreevy *et al.* 1982). Although no mosquito was found with L3, presence of mosquitoes with pre-L3 stages viz., Mf/L1/L2 may also indicate low levels of transmission, as observed in the village Muppili, where landing mosquitoes with L3 were encountered, although the infection rate (with Mf/L1/L2) was as low as 0.4% and 0.7% in resting and landing mosquitoes, respectively (Table 5). Hence, total interruption of transmission may be possible only at 0% infection rate. However, it is not known whether an inefficient vector like *C. quinquefasciatus* (Hairston & de Meillon 1968) can transmit new infections in communities in a situation where mosquitoes with Mf/L1/L2 are found but mosquitoes with L3 are either absent or rare.

The data presented in Table 5 suggest that the zero Mf prevalence observed in some villages (Muppili, Thenkalavai and Thenputhur), may not be true because 0.4–2.5% of resting mosquitoes were found infected in these villages and landing mosquitoes with L3 were also found in one village (Muppili). Presence of infected mosquitoes in villages with zero Mf prevalence, after six rounds MDA, suggests existence of low-density Mf carriers, who may not be detected by the standard finger-prick blood sampling technique (Desowitz & Southgate 1973; McMahon *et al.* 1979; Southgate 1992) or by sampling only 7% of the population as followed in this study (Ramaiah *et al.* 2002). *C. quinquefasciatus* is capable of picking up Mf from such Mf carriers and develop them into L3 (Table 5) (Bryan & Southgate 1976; McGreevy *et al.* 1982). Therefore, only at 'true' zero community Mf prevalence, total interruption of transmission may be possible.

AIBR and ATP indicate entomological inoculation rates. In one of the two villages in the IVM arm (Thenputhur), ATP fell to 0 after five rounds of MDA (Table 4). In this village, the Mf rate was also 0, the resting mosquitoes did not show L3 stage and infection rate of resting and landing mosquitoes was close to 0 (Table 5). Thus, this village provides the strongest evidence for total interruption of transmission of infection. Similarly in one village in the DEC arm, the ATP fell to 60, which is below the level of 100 required for causing new infections (Ramaiah *et al.* 1994). However, one village each (Alagramam and Nedi) in the DEC and IVM arms continued to show substantial inoculation rates, even after six rounds of MDA. Both of these villages had relatively high pre-treatment Mf prevalence and this may be one of the reasons for prevalence of higher Mf rate even after six rounds of MDA (Ramaiah *et al.* 2002) and consequent higher inoculation rates. The infectivity rate of landing mosquitoes in these two villages, after achieving a reduction of 93% and 85%, remained at 0.21% (Alagramam) and 0.70% (Nedi) (Table 2) and the vector annual biting rate was 75 616 and 28 846, respectively (K.D. Ramaiah unpublished data). These data suggest that although annual MDA reduces the proportion of mosquitoes with L3 by more than 85%, there may still be a considerable number of infective mosquitoes and substantial amount of transmission, because of high annual biting rates. Therefore, community-wide vector control and/or personal protection measures should be encouraged, in addition to MDA, in order to achieve better results. Unlike AIBR and ATP, the TII stood at a very low level in the placebo arm also (Figure 4). This is partly because the resting density, which is the most important constituent of TII, was much less (9.8 per man-hour) in the placebo arm compared with that in the DEC (23.7 per man-hour) or IVM (20.8 per man-hour) arm (K.D. Ramaiah unpublished data). The infection and infectivity rates of resting mosquitoes declined by 30% and 45% in placebo arm, respectively (Figures 2 and 3). This may partly be due to the treatment of Mf carriers, detected during blood surveys, carried out after each round of MDA. However, no such decline was observed in infection and infectivity rates of landing mosquitoes (Table 2). It is difficult to give the reasons for this discrepancy between the resting and landing mosquitoes and decide the merit of the resting and landing collection to evaluate the effectiveness of intervention measures. If resources permit, both resting and landing collections should be carried out to complement each other. However, several years of repeated landing collections are cumbersome and costly.

Based on clinical trials (Cao *et al.* 1997), one may expect that the first round of MDA itself will substantially reduce the human microfilaraemia levels and thereby transmission of infection and the suppression of microfilaraemia through MDA for 4–6 years, which is equivalent to the fecundic life span of *W. bancrofti* (Vanamail *et al.* 1996), is sufficient to prevent establishment of new infection (Ottesen 2000). However, the reduction in Mf prevalence and transmission levels was substantial, with 54–75% treatment coverage, only after three to four rounds of MDA, particularly in the DEC arm (Tables 2 and 4; Figures 2–4). Prior to the fourth round, the average cumulative exposure to L3 (ATP) amounted to as high as 2659 and 768 in DEC and IVM arms, respectively (Table 4), at which levels new infections can occur (Ramaiah *et al.* 2003). Therefore, the process of total interruption of transmission to eliminate LF involves two steps: (i) to suppress blood microfilaraemia and reduce the transmission levels to below the level of 100 ATP (Ramaiah *et al.* 1994), at which the probability of new infections may become very low, with initial rounds of MDA and (ii) to achieve 'true' zero Mf

prevalence in communities and total interruption of transmission and prevent new infections with further rounds of MDA. Computer models are required to predict the number of MDAs required to accomplish each of these two steps. Nevertheless, the number of MDAs required may be influenced tremendously by treatment coverage levels.

From clinical (Cao *et al.* 1997) and field studies (Ramaiah *et al.* 2002), it has been concluded that DEC is slightly better than IVM against infection in the human population. As far as transmission is concerned, at the end of six rounds of treatment DEC achieved better reduction in ATP than IVM. However, in the IVM arm, the reduction was steep after the first round of treatment itself, whereas it was gradual with each round of treatment in the DEC arm. Therefore, the cumulative ATP over a 5-year treatment period observed in the IVM arm was only half of that observed in the DEC arm (Table 4). This highlights the role of IVM in relatively quickly reducing the intensity of transmission, leading to lower cumulative exposure.

We conclude that repeated annual MDA, even with moderate treatment coverage, has excellent potential to reduce LF transmission drastically. However, total interruption of transmission may require high treatment coverage levels, more than six rounds of MDA and complete elimination of microfilaraemia in the community. High densities of vector species like *C. quinquefasciatus* may perpetuate transmission even when vector infectivity rates are at very low level. Hence, vector control, along with MDA, needs to be encouraged and may significantly contribute to elimination of LF. While our results pertain to administration of a single drug (DEC or IVM alone), the global programme recommended two-drug regimen of DEC + ALB may facilitate better treatment coverage because of the additional health benefits of ALB, leading to rapid control/elimination of LF.

Acknowledgements

The study received financial support from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (Grant No. 920702). The authors gratefully acknowledge the extensive fieldwork carried out by the staff of the Applied Field Research division of the VCRC. They thank Dr Ravi Rengachari, VCRC, for his constant support. Our thanks are due to Drs P. Krishnamoorthy and D.J. Augustin, Directorate of Public Health and Preventive Medicine, Chennai, Government of Tamil Nadu, India. They thankfully acknowledge the support extended to the study by Drs Hans Remme, Eric Ottesen and V. Kumaraswamy.

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