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Towards eliminating lymphatic filariasis in Papua New Guinea: impact of annual single-dose mass treatment on transmission of *Wuchereria bancrofti* in East Sepik Province

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SUMMARY

The impact of annual single-dose community-wide treatment on the transmission of *Wuchereria bancrofti* was investigated in 5 villages in the East Sepik Province where pretreatment prevalence of microfilaraemia ranged from 34% to 73%. *Anopheles punctulatus* and *An. koliensis* were the only carriers of the parasite. 3 villages received diethylcarbamazine citrate (DEC) in combination with ivermectin (IVR) and 2 received DEC alone. The rate and intensity of microfilaraemia were both reduced in all 5 villages. Reduction in prevalence was between 43% and 67% in the DEC+IVR study villages and between 24% and 27% in the DEC alone villages. Density was reduced by between 81% and 95% in the DEC+IVR villages and between 69% and 74% in the DEC alone villages. Breaks in perennial transmission (failure to detect infective mosquitoes in four or more consecutive monthly collections) occurred in all 3 communities treated with DEC+IVR. Transmission was almost completely interrupted in 2 villages, where infective mosquitoes were not detected during 11 of the 12 months following treatment. We concluded that repeated annual single-dose community-wide treatment with DEC+IVR could lead to complete interruption of transmission and ultimately elimination of lymphatic filariasis.

Introduction

Lymphatic filariasis is a major cause of acute and chronic morbidity in the tropical and subtropical areas of Asia, Africa, the Western Pacific and some parts of the Americas. Over 20% of the world's population now live in areas where they are at risk of infection with filarial parasites. Of the estimated 128 million cases of lymphatic filariasis, 91% are caused by *Wuchereria bancrofti* while *Brugia malayi* and *Brugia timori* infections account for the other 9% (1). These lymphatic-dwelling parasites cause damage to the lymphatic system which leads to lymphoedema, genital pathology (especially hydroceles) and elephantiasis. Infection is initiated by the deposition of the third-stage infective larvae (L3) on the skin of the human host following a bite by an infective mosquito. The larvae penetrate the skin at the site of the bite and migrate to the lymphatic system where they mature into male and female adult worms. The lymphatic-dwelling filariae are diecious and undergo ovoviviparous reproduction resulting in the release from the females of microfilariae, which circulate in the bloodstream. The life cycle is completed when these microfilariae are ingested by mosquito vectors during a bloodmeal. In the vector the microfilariae penetrate the gut wall, migrate to the flight muscles and develop to infective larvae (L3).

Filariasis control programs aimed at interrupting transmission of *Wuchereria bancrofti* have relied entirely or mainly on mass drug administration at the community

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level. For nearly 50 years diethylcarbamazine citrate (DEC) has been the drug of choice, which has produced significant reductions in the intensity of microfilaraemia in some countries. However, many people remain microfilaraemic even after taking full courses of the drug and in areas where culicine mosquitoes are vectors these individuals serve as reservoirs for further transmission to continue (2). Recent studies have shown that treatment with ivermectin (IVR) leads to greater and more sustained reductions in microfilaraemia than DEC (3-5). Analyses of vector-parasite relationships have predicted that reductions in vector density and intensity of microfilaraemia below certain thresholds could lead to breaks in transmission and ultimately eradication of anopheline-transmitted filariasis (6,7). In countries like Solomon Islands and Togo, where anopheline mosquitoes were the main vectors of *W. bancrofti*, transmission was completely interrupted through reduction in vector density by spraying of residual insecticides aimed at eradicating malaria (8,9). These breaks in transmission have been shown as examples of facilitation (7). However, reduction in the microfilarial reservoir through community-wide mass chemotherapy has been proposed as a more practical and effective means of achieving breaks in transmission.

This paper describes the impact of annual single-dose community-wide treatment with DEC and DEC+IVR on the prevalence and intensity of microfilaraemia of anopheline-transmitted *W. bancrofti* in the East Sepik Province, Papua New Guinea. The treatment effect on transmission and prospects for achieving the World Health Organization goal of elimination are discussed.

Materials and Methods

Study population

The study was carried out in 5 villages located within 20 km of the Dreikikir government station in the East Sepik Province. Tropical humid climate prevails throughout the year in this area where rainfall is seasonal, with most rain occurring between December and June (wet season). The remaining months constitute the dry season. The average annual rainfall is 1600 mm and relative humidity varies between 80 and 100%. Lymphatic filariasis is endemic in these villages and anopheline mosquitoes are the main vectors of *W. bancrofti* (10,11).

A baseline entomological survey in the area had demonstrated pronounced geographical

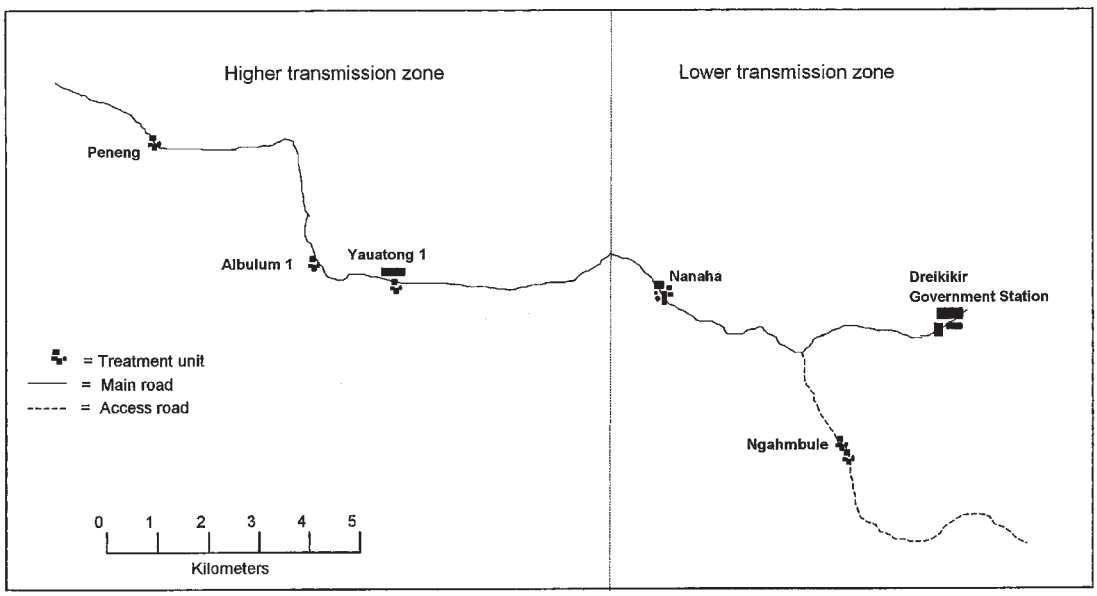

Figure 1. Map of the study area showing Dreikikir government station and the five study villages where the communities were treated.

variation in vector density, with villages located more than 10 km from the Dreikikir government station experiencing more mosquito bites than nearby ones (Figure 1). Many of the distant villages are situated very close to fast-moving, open rivers and streams. On the other hand, villages near the Dreikikir station tend to be located on ridges with shaded rivers and streams running metres below in much cooler environments. This difference in ecology was clearly reflected in the predominance of the shade-loving *Anopheles koliensis* species in villages within 10 km of Dreikikir station. A line showing the limit of distribution of *An. koliensis* (Figure 1) separated the lower transmission zone from the higher. The treatment given in 3 of the 5 study villages, Albulum 1, Nanaha and Peneng, was DEC in combination with ivermectin while the other 2, Yauatong 1 and Ngahmbule, received DEC alone.

Parasitological examinations and treatment

Microfilaraemia was quantified from a 1 ml blood sample obtained between 2200 and 0200 hours. Nuclepore filtration and counting of mf were performed as described by Desowitz and Hitchcock (12) and results were expressed as mf per ml blood. After blood had been collected, those eligible for treatment were treated with DEC only (6 mg/kg) or DEC (6 mg/kg) + IVR (400 µg/kg).

Sampling and processing of mosquitoes

Human-biting mosquitoes were caught by two adult residents using the all-night landing catch method as described by Bockarie et al. (10). Hungry mosquitoes were captured between 1800 and 0600 hours as they attempted to feed on humans seated on benches near houses, with feet and legs bared to the knee. Each of the 5 villages was divided into 4 sections and mosquito collectors rotated through the sections on different nights for 4 nights each month for 24 months. Mosquitoes were identified in the field and stored separately in 70% ethanol for transport to the laboratory, where they were later stained for filarial parasites using Mayer's acid haemalum (13). Specimens were individually dissected to determine infection with filarial larvae.

Entomological measures of transmission intensity

Monthly biting rate (MBR), the estimated number of mosquitoes caught landing on a collector who worked every night (1800 - 0600 hours) for one calendar month, and monthly transmission potential (MTP), the number of infective third-stage larvae of *W. bancrofti* which would be inoculated into the same person, were calculated using the formulae of Walsh et al. (14). Monthly infective biting rate (MIBR), the number of infective bites the collector would receive, was obtained by multiplying MBR by the monthly infective rate – the proportion of mosquitoes with one or more L3. Annual biting rate (ABR), annual infective biting rate (AIBR) and annual transmission potential (ATP) were obtained by summing the respective monthly indices for 12 consecutive months.

Statistical analysis

To measure the intensity of microfilarial counts we used the Williams mean (15), which is one less than the geometric mean. Changes in prevalence were expressed as percentage differences from the pretreatment rates. The significance of differences in mosquito infection rates was evaluated by the chi-squared test. The significance of correlation between different indices was tested using the Student t test.

Results

Impact of treatment on microfilaraemia

Before treatment, both prevalence and intensity of microfilaraemia were higher in villages in the higher transmission zone than in the lower. Mf rate and mf intensity were both reduced in all 5 villages (Table 1). The reduction in prevalence was between 43% and 67% in the DEC+IVR study villages and between 24% and 27% in the DEC villages. Density was reduced by between 81% and 95% in the DEC+IVR villages and between 69% and 74% in the DEC villages.

Effect of treatment on vector infective rates and transmission intensity

A total of 26,641 *Anopheles* mosquitoes, all belonging to the *Anopheles punctulatus*

TABLE 1

WUCHERERIA BANCROFTI MICROFILARAEMIA PREVALENCE (%) AND INTENSITY (MF/ML) BEFORE (1994) AND AFTER (1995) ONE SINGLE-DOSE MASS TREATMENT IN 5 COMMUNITIES IN EAST SEPIK PROVINCE

Treatment	No examined		% of people with microfilaraemia			Intensity of microfilaraemia (mf/ml)		
Community	**1994**	**1995**	**1994**	**1995**	**% reduction**	**1994**	**1995**	**% reduction**
DEC+Ivermectin								
Albulum 1	60	69	73.3	37.7	49	26.2	5.1	81
Nanaha	238	207	48.3	27.5	43	12.2	1.6	87
Peneng	65	88	61.5	20.5	67	26.6	1.3	95
DEC only								
Yauatong 1	143	110	72.0	52.7	27	49.2	12.6	74
Ngahmbule	343	300	34.4	26.3	24	5.1	1.6	69

complex, were caught during 499 person-nights of landing catches carried out in 5 villages between September 1993 and September 1995. These included 24,448 *An. punctulatus* and 2193 *An. koliensis*, with 99% of the latter coming from the low transmission zone. A total of 17,999 mosquitoes were dissected: 10,237 before and 7762 after treatment. No mosquitoes were collected in October 1993 for logistic reasons.

Table 2 gives pre- and post-treatment annual infective rates of *An. punctulatus* s.l. and mean number of L3 per infective mosquito for all 5 villages. Pretreatment infective rates varied from 0.5% in Ngahmbule to 2.7% in Peneng. The overall pretreatment infective rate for the 3 villages (Albulum 1, Nanaha and Peneng) treated with DEC+IVR (1.7%) was statistically similar to the overall rate (1.8%) for the 2 villages (Yauatong 1 and Ngahmbule) treated with DEC only (χ^2=0.36, p=0.546). Treatment resulted in reductions in infective rates ranging from 56% in Ngahmbule to 96% in Peneng and the differences in the pre- and post-treatment infective rates were statistically significant in all communities except Ngahmbule. However, overall post-treatment infective rates for communities given the combination treatment was significantly lower than communities given DEC only (χ^2=4.59, p=0.03, df=1).

Table 3 describes pre- and post-treatment MBRs, MIBRs and MTPs for the three communities treated with DEC +IVR. In all 3 villages, post-treatment AIBRs and ATPs were <25% of the pretreatment values, giving percentage reductions in AIBR of 85%, 87% and 96% in Albulum 1, Nanaha and Peneng respectively. The corresponding reductions in ATPs were 84%, 76% and 99%. In both Nanaha and Peneng ABR increased slightly after treatment but in Albulum 1 ABR decreased by 57%.

Breaks in perennial transmission, as assessed by the failure to detect infective mosquitoes in four or more consecutive monthly collections, occurred in all 3 communities treated with DEC+IVR. Transmission was almost completely interrupted in Nanaha and Peneng where infective mosquitoes were not detected during 11 of the 12 months following treatment. Before treatment, infective mosquitoes were never absent continuously for more than two months in any of these communities; they were observed during 10, 8 and 9 months in Albulum 1, Nanaha and Peneng respectively.

Table 4 gives the pre- and post-treatment monthly transmission indices for Yauatong 1 and Ngahmbule. Percentage reductions in AIBR and ATP in both communities treated with DEC only were lower than in the 3 communities treated with DEC+IVR. In Yauatong 1, AIBR decreased by 75% and ATP by 79%; the corresponding percentage reductions in Ngahmbule were 55% and 76% respectively. In all 5 entomology villages treatment resulted in an immediate and sustained reduction in MIBRs and MTPs with the exception of Albulum 1 where there was a delay of 2 months before a reduction was recorded.

Relationships between entomological and parasitological findings

Pretreatment ATP correlated positively with pretreatment mf rate (r=0.84, p<0.05) and pretreatment mf intensity (r=0.95, p<0.02). Also post-treatment ATP correlated positively with post-treatment mf rate (r=0.98, p<0.005) and post-treatment mf intensity (r=0.99, p<0.005). Similar significantly positive correlations existed between AIBR, mf prevalence and mf intensity both before and after treatment. There was not, however, a significant correlation between both measures of transmission intensity and infective rate.

Before treatment, the mean number of L3 per infective mosquito correlated positively with mf intensity (r=0.95, p<0.02) and mf rate but the correlation with mf rate (r=0.82) was not statistically significant. However, after treatment the mean number of L3 per infective mosquito correlated significantly with mf intensity (r=0.93, p<0.02) and mf rate (r=0.96, p<0.01). Percentage reduction in infective rates correlated positively with percentage reduction in mf intensity (r=0.94, p<0.02) but its correlation with percentage reduction in mf prevalence (r=0.80) was not statistically significant.

The effect of reduction of mf intensity on the uptake and development of microfilariae by

TABLE 2

INFECTIVE RATES (%) OF *ANOPHELES PUNCTULATUS* GROUP AND MEAN NUMBER OF L3 PER INFECTIVE MOSQUITO BEFORE AND AFTER SINGLE-DOSE COMMUNITY-WIDE TREATMENT WITH DIETHYLCARBAMAZINE (DEC) OR DEC IN COMBINATION WITH IVERMECTIN (IVR) IN 5 COMMUNITIES IN EAST SEPIK PROVINCE

Treatment community	Percentage of mosquitoes with L3						Number of L3 per infective mosquito			
	Before treatment		After treatment		% reduction	χ^2	Before treatment		After treatment	
	N	%	N	%			Maximum	Mean	Maximum	Mean
DEC+IVR										
Albulum 1	3910	1.38	1799	0.44	68	10.06*	2.61	11	2.63	9
Nanaha	1195	1.84	1532	0.13	93	22.52**	2.50	6	4.50	8
Peneng	929	2.69	908	0.11	96	21.92**	2.96	15	1.00	1
Total	6034	1.67	4239	0.26	84	46.19**	2.67	15	2.82	9
DEC only										
Yauatong 1	3638	2.03	3097	0.61	70	24.79**	3.36	14	2.89	8
Ngahmbule	565	0.53	426	0.24	56	0.05***	2.00	3	1.00	1
Total	4203	1.83	3523	0.57	69	24.71**	3.31	14	2.80	8

* = $p<0.002$
** = $p<0.0001$
*** = $p>0.05$

TABLE 3

MONTHLY BITING RATES (MBR), MONTHLY INFECTIVE BITING RATES (MIBR) AND MONTHLY TRANSMISSION POTENTIALS (MTP) OF *ANOPHELES PUNCTULATUS* S.L. BEFORE AND AFTER ONE ANNUAL SINGLE-DOSE COMMUNITY-WIDE TREATMENT WITH DIETHYLCARBAMAZINE PLUS IVERMECTIN IN 3 COMMUNITIES IN EAST SEPIK PROVINCE

Month	Albulum 1			Nanaha			Peneng		
	MBR	MIBR	MTP	MBR	MIBR	MTP	MBR	MIBR	MTP
Before treatment 1993/1994									
September	8325	74	135	694	0	0	1178	30	47
October	*	*	*	*	*	*	*	*	*
November	14520	60	157	2895	15	15	675	9	9
December	2519	135	477	674	31	74	1077	9	9
January	70	0	0	1178	0	0	23	0	0
February	1876	49	195	497	11	32	371	14	29
March	527	13	25	388	21	53	39	0	0
April	180	0	0	713	0	0	225	0	0
May	2829	13	13	1008	7	16	1147	42	308
June	5470	39	78	1283	0	0	1238	31	82
July	3178	85	222	1372	42	151	884	53	141
August	1349	86	172	279	20	32	1232	21	64
September	1485	11	11	630	20	31	105	15	15
Total	42328	565	1485	11611	167	404	8194	224	704
After treatment 1994/1995									
October	2689	11	32	1984	0	0	1175	0	0
November	2588	41	133	675	0	0	480	0	0
December	1230	0	0	730	0	0	837	0	0
January	1240	0	0	977	0	0	915	0	0
February	595	0	0	2170	0	0	364	8	8
March	659	0	0	2170	0	0	442	0	0
April	405	8	8	1403	0	0	143	0	0
May	264	0	0	1519	22	98	140	0	0
June	345	16	49	1523	0	0	480	0	0
July	1000	0	0	829	0	0	853	0	0
August	3906	9	9	705	0	0	1023	0	0
September	3105	0	0	1133	0	0	1650	0	0
Total	18026	85	231	15818	22	98	9102	8	8
% reduction		85	84		87	76		96	99

* = No data collected

TABLE 4

MONTHLY BITING RATES (MBR), MONTHLY INFECTIVE BITING RATES (MIBR) AND MONTHLY TRANSMISSION POTENTIALS (MTP) OF *ANOPHELES PUNCTULATUS* S.L. BEFORE AND AFTER ONE SINGLE-DOSE COMMUNITY-WIDE TREATMENT WITH DIETHYLCARBAMAZINE IN 2 COMMUNITIES IN EAST SEPIK PROVINCE

Month	Yauatong 1			Ngahmbule		
	MBR	**MIBR**	**MTP**	**MBR**	**MIBR**	**MTP**
Before treatment 1993/1994						
July				248	0	0
August				446	7	7
September	5171	64	153	1489	6	17
October	*	*	*	*	*	*
November	6383	38	38	1118	0	0
December	1767	168	450	70	0	0
January	589	23	102	0	0	0
February	2023	0	0	14	0	0
March	946	63	180	62	0	0
April	1635	34	157	248	0	0
May	2821	72	391	132	0	0
June	6563	57	230	255	0	0
July	4204	111	305	264	11	21
August	2220	57	261			
September	2730	55	251			
Total	37052	742	2518	4346	24	45
After treatment 1994/1995						
August				62	0	0
September				113	0	0
October	5069	28	83	2527	0	0
November	1628	10	10	338	11	11
December	1581	0	0	0	0	0
January	1984	19	66	248	0	0
February	1589	18	27	98	0	0
March	775	0	0	147	0	0
April	1470	0	0	83	0	0
May	2736	28	156	70	0	0
June	705	0	0	30	0	0
July	3410	18	46	240	0	0
August	5665	31	72			
September	3480	30	59			
Total	30092	182	519	3956	11	11
% reduction		75	79		55	76

* = No data collected

anopheline mosquitoes is clearly illustrated by the variation in monthly infection and infective rates of *An. punctulatus* before and after treatment in Nanaha community (Figure 2). Amongst entomology villages where transmission was perennial before treatment Nanaha had the lowest pretreatment mf rate (48%) and mf intensity (12.2 mf/ml). Nevertheless, infected mosquitoes were seen in all 12 monthly catches performed before treatment. However, during the 3 months following treatment, of 313 mosquitoes dissected none was found to be infected. In comparison, a total of 270 mosquitoes dissected during the 3 months before treatment produced infection and infective rates of 9% and 3% respectively. After December 1995, mosquitoes were again able to pick up microfilariae but no L3 was seen in 704 mosquitoes dissected between January and April 1995 and only 4 mosquitoes contained developing larvae. Infective mosquitoes were observed only in May 1995 and the infection rates during this and later months indicated an increase in the uptake of microfilariae.

Discussion

Dramatic reductions in the intensity of microfilaraemia were observed in all 5 villages following treatment with DEC alone or DEC+IVR suggesting that annual single-dose community-wide treatment using either regimen is effective for the control of lymphatic filariasis in highly endemic areas. Ivermectin in combination with DEC was shown to be more effective than DEC given alone in reducing both prevalence and intensity of microfilaraemia, as has been reported from the studies based on selected individuals. Earlier clinical trials involving individuals with high levels of microfilaraemia from the present study population showed that single-dose treatment with DEC (6 mg/kg) or ivermectin (420 µg/kg) produced only mild adverse reactions and led to over 85% reduction in microfilaraemia up to 18 months after drug administration (5).

Remarkable reductions in AIBR and ATP were shown in all 5 communities and DEC in combination with ivermectin appeared to be more effective than DEC alone in reducing transmission intensity. The sudden and sustained reduction in monthly infective rates, MIBRs and MTPs following treatment clearly showed that treatment was the most important factor determining changes in transmission intensity. The high positive correlation between patent infection and entomological

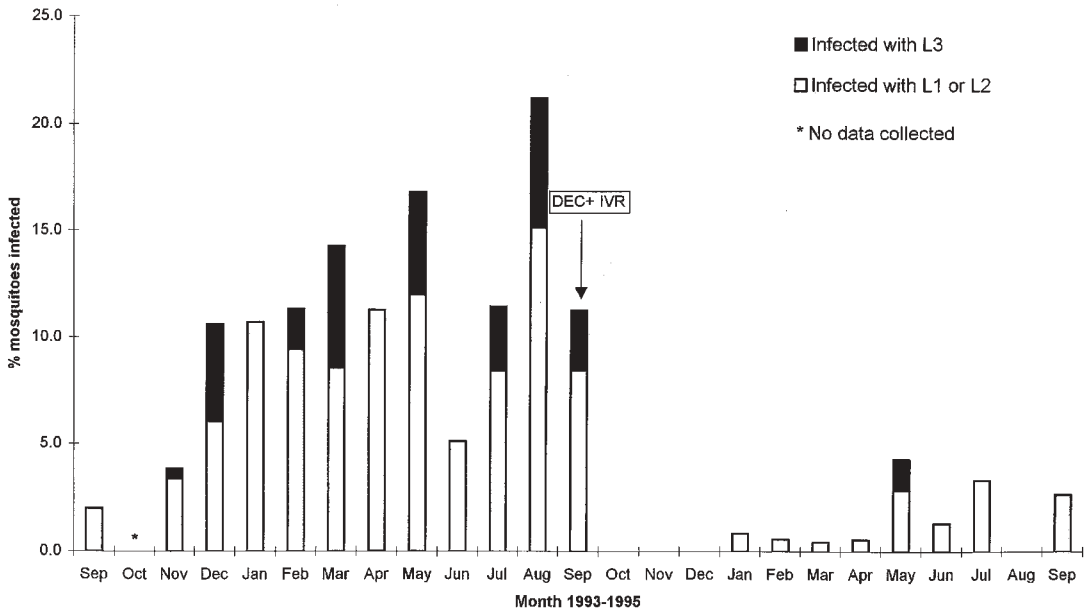

Figure 2. Effect of treatment-induced reduction in intensity of *Wuchereria bancrofti* microfilaraemia on uptake and development of microfilariae to infective larvae in wild-caught *Anopheles punctulatus* in Nanaha village.

measures of transmission intensity (AIBR and ATP), both before and after treatment, confirms our previous findings in the area (11). This suggests that ATP, which has been successfully used for many years in monitoring onchocerciasis control, could also be used in monitoring control of lymphatic filariasis.

Reductions in ABR in Albulum 1 and Yauatong 1 after treatment were due to an unusually high pretreatment ABR arising from an upsurge in mosquito density during a very severe dry season that ended in November 1993. During the dry season many rivers almost dried up, giving rise to numerous breeding sites for *An. punctulatus*. The effect of this on mosquito density depended on the proximity of a community to an open river. Communities in the lower transmission zone and Peneng, which were not located close to rivers or streams, were therefore not affected in the same way. It is, however, clear from the changes in MTPs and MIBRs that the reduction in mosquito density after treatment was not a very important factor in the reductions in transmission in these two communities.

The pretreatment prevalence of microfilaraemia in Peneng (62%) was higher than the 50% reported for Makunduchi in Zanzibar where integrated control of filariasis involving multiple-dose DEC mass treatment (72 mg/kg) and vector control using polystyrene beads in wet pit latrines reduced ABR by 98%, infective rate by 83% and AIBR by 99.7% (16). Despite a higher ABR following treatment in Peneng, the reductions in infective rate and AIBR were similar to reductions found in Makunduchi suggesting that annual single-dose mass treatment with DEC plus ivermectin without vector control could be as effective as the integrated control measures involving vector control.

Despite the remarkable effect mass chemotherapy had on filariasis endemicity in the present study, the prevalence of microfilaraemia varied between 21% and 53% one year after treatment. This has raised concerns that single-dose treatments may result in a high prevalence of low-density mf carriers that could serve as reservoirs for very efficient vectors like *An. punctulatus*. Nevertheless, transmission was almost completely interrupted following treatment in Nanaha and Peneng, where transmission had been perennial before treatment. One possible explanation could be the lowering of mf intensity below a threshold value leading to breaks in transmission as predicted by the phenomenon of facilitation (6,7). The concepts of facilitation and limitation which describe the relationships between mf intake and L3 yield arose from experimental studies (17,18); the positive correlation between mf intensity and mean number of L3 per infective mosquito established in this study is the first based on field studies involving wild-caught mosquitoes. This relationship and the high numbers of infective larvae seen in infective mosquitoes in communities of high mf intensity are in accordance with facilitation. Failure to detect infected mosquitoes for 3 months after treatment in Nanaha (Figure 2) is in line with reports that ivermectin often causes microfilaria counts to fall to zero but they begin to rise again after approximately 3 months (19). Failure to detect infective mosquitoes for 4 months after microfilaria counts began to rise also clearly suggests that anopheline mosquitoes are not efficient vectors when mf intensity is low. What has previously been shown as examples of facilitation have been breaks in transmission following reductions in vector density. However, in a critical review of the validity of this much argued phenomenon Wada et al. (20) concluded that evidence for the existence of facilitation should have to be discussed in relation to chemotherapy rather than vector control. This study presents the first evidence of breaks in transmission following chemotherapy alone in areas of high vector density and perennial transmission. It could therefore be concluded that repeated annual single-dose community-wide treatment with DEC+IVR could lead to complete interruption of transmission and ultimately elimination of lymphatic filariasis.

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