SHORT-TERM EFFECTS OF TREATMENT WITH 300 MG ORAL-DOSE DIETHYLCARBAMAZINE ON NOCTURNALLY PERIODIC *WUCHERERIA BANCROFTI* MICROFILAREMIA AND ANTIGENEMIA

Chumsin Siriaut[1], Adisak Bhumiratana[1], Surachart Koyadun[2], Kowit Anurat[3] and Pratana Satitvipawee[4]

[1]Department of Parasitology, [4]Department of Biostatistics, Faculty of Public Health, Mahidol University, Bangkok; [2]Office of Disease Prevention and Control 11 (Nakhon Si Thammarat), Department of Disease Control, Ministry of Public Health, Nakhon Si Thammarat; [3]Vector Borne Disease Control Center 11.5 (Ranong), Ranong, Thailand

Abstract. Seven microfilaremic Myanmar patients were treated with a single 300 mg dose of diethylcarbamazine (DEC) orally, as part of a case-finding survey in Ranong Province, Southern Thailand. This was conducted in order to evaluate the short-term effects of single-dose DEC on *Wuchereria bancrofti* microfilaremia and antigenemia during a 12-week course of treatment. Analysis of microfilarial periodicity on initial treatment revealed the microfilarial peak density (*k*) was at 52 minutes after midnight (0052). The periodicity index was then 103.26%. Single-dose DEC treatment did not affect the *k* values. A linear model of *W. bancrofti* microfilarial density reduction predicts a sharp decrease in the mean microfilarial density 2 weeks after DEC intake (Z=-2.197, p=0.028). Over a longer period, a non-linear model predicts an increase in the mean microfilarial density to pre-treatment levels, having little or no macrofilaricidal effects. We reconfirmed the existence of nocturnally periodic *W. bancrofti* infection in Myanmar migrants in Ranong Province, and the short-term microfilaricidal activity of 300 mg single-dose DEC treatment used for biannual mass treatment and the DEC provocative test. Without an adequate DEC treatment dose, recrudescence can occur. A rational approach to the management of introduced nocturnally periodic *W. bancrofti* in Myanmar migrants, who came for short periods of stay in transmission-prone areas, is needed.

INTRODUCTION

Emergence of bancroftian filariasis in Thailand, caused by nocturnally periodic *Wuchereria bancrofti*, has occurred in cross-border Myanmar migrants. This mosquito-borne infection is acquired outside of Thailand. It is transmitted by the bite of the nocturnal feeding mosquito, *Culex quinquefasciatus* (Phantana *et al*, 1996a; Sitthai and Thammapalo, 1998; Triteeraprapab *et al*, 2000). Both experimental-based and field-based studies demonstrate this vector harbors nocturnally periodic *W. bancrofti* microfilaria, as well as its developmental larvae (L1, L2 and L3) (Baruah and Rai, 2000; Gunasekarun *et al*, 2000). In Thailand, where breeding grounds for *Cx. quinquefasciatus* are widely distributed, there have been no reported studies of the infectivity rates in the vector population under field conditions.

High point estimates of infection prevalence, *ie* the number of microfilaremic individuals (% microfilarial positive rate - MPR) have been made using cross-sectional night blood surveys (Swaddhiwudhipong *et al*, 1996; Phoopattanakool, 1997; Sitthai and Thammapalo, 1998). These estimates have been used to implicate Myanmar immigrants as the source for introduced *W. bancrofti* infection. Therefore, when the current magnitude of this disease needs to be assessed, Myanmar immigrants to Thailand are considered the sentinel population for surveillance (Anonymous, 2004).

Diethylcarbamazine (DEC) is an antifilarial

Correspondence: Asst Prof Adisak Bhumiratana, Department of Parasitology, Faculty of Public Health, Mahidol University, 420/1 Rajavithi Road, Bangkok 10400, Thailand.
Tel: +66 (0) 2644-5130, 2354-8543-9 ext 1202; Fax: +66 (0) 2644-5130
E-mail: phabr@mahidol.ac.th

drug that has been used for the treatment of lymphatic filariasis in Thailand (Suvannadabba, 1993; Filariasis Division, 1998, 2000). A reduction in microfilaremia prevalence of the nocturnal subperiodic *W. bancrofti* and both diurnal and nocturnal subperiodic *Brugia malayi* has resulted from selective treatment with DEC 6 mg/kg oral-dose. A single oral dose of DEC 6 mg/kg has been recommended for use in prevention and control of imported bancroftian filariasis in Myanmar immigrants (Filariasis Division, 1998, 2000). In a hospital-based study (Wongcharoenyong *et al*, 1997), the drug had short-term microfilaricidal activity, with rapid killing of microfilariae (Mf) in the blood. This drug has never been evaluated for its effects on microfilaremia and antigenemia in a community-based treatment program. Active *W. bancrofti* infection (*ie* microfilaremia and/or antigenemia) is considered to be more prevalent in the at-risk Myanmar immigrants who stay in Thailand for only a short period (Koyadun *et al*, 2003; Bhumiratana *et al*, 2004). The prevalence of infection will remain stable if there is no repeat treatment with DEC 6 mg/kg. The response to DEC is dose-dependent, based on the susceptibility of the local group. Therefore, the benefits of single-dose DEC 6 mg/kg for the control of infection in Myanmar migrants is in doubt.

The National Program to Eliminate Lymphatic Filariasis (PELF) during fiscal years 2002-2006 (Filariasis Division, 2000), a mass drug administration (MDA) program, has used 300 mg oral-dose DEC to interrupt transmission in prone areas. The DEC regimens are recommended for use in biannual mass treatment programs (Koyadun *et al*, 2003) and in DEC provocative day tests (Bhumiratana *et al*, 2004). An understanding of the efficacy of 300 mg oral-dose DEC in treating Myanmar microfilaremics is helpful for the PELF's implementers to optimize MDA's effectiveness at the provincial level. In the present study, we describe the existence of nocturnally periodic *W. bancrofti*.

MATERIALS AND METHODS

Selection of subjects and blood examination

During case finding surveys in Ranong Province in 2003, *W. bancrofti* microfilaremic subjects (Table 1) were selected from cross-border Myanmar migrants aged ≥10 years. Night fingerprick blood specimens were obtained in duplicate, and laboratory-confirmed using Giemsa-stained thick smears. The microfilaremic individuals gave informed consent and followed-up in 14 days at the Vector Borne Disease Control Unit (VBDU). Ethical clearance was approved by the Institutional Review Board, Mahidol University. Prior to treatment, blood was collected 7 times over a 24-hour period: 0900, 1300, 1700, 2100, 0100, 0500 and 0900. Giemsa-stained 3-line thick smears in individuals, as shown in Fig 1, were performed in duplicate. For quality control, two separate observers (C Siriaut and A Bhumiratana) were used for microscopic examination.

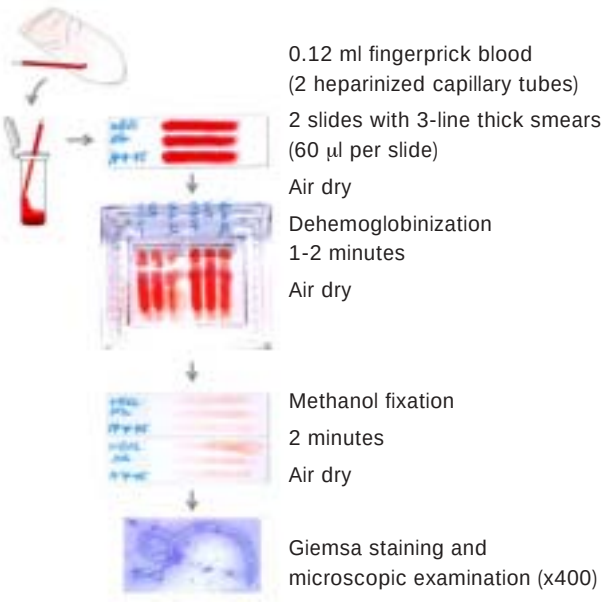

Fig 1–Procedure for 3-line thick smear with a modification of a representative sample.

DEC treatment administration and monitoring

The patients were each given 300 mg DEC citrate, orally (FILADEC - Pond's Chemical Thailand ROP, Bangkok, Thailand), after the 24-hour blood collection cycle was completed. The patients were monitored post-treatment at 1, 2, 4, 8 and 12 weeks. At each follow-up appointment finger-prick blood specimens were collected at

Table 1
Demographics and epidemiology of the 7 male microfilaremic study subjects form Ranong Province, 2003.

Subject	Age (yrs)	Residence in Myanmar (Township)	Length of residence in Thailand (months)	Work permit[a]	Type of migration[b]	History of DEC treatment[c]
M1	23	Moulmein	24	YES	Long-term	YES
M2	18	Moulmein	6	YES	Periodic	NO
M3	26	Moulmein	7	NO	Periodic	NO
M4	42	Moulmein	4	NO	Periodic	NO
M5	20	Kawthoung	3	NO	Seasonal	NO
M6	20	Rangoon	4	YES	Periodic	NO
M7	26	Moulmein	7	NO	Seasonal	NO

[a]Work permit is defined as permission for foreign employees to work and live under legal conditions in the permitted province, given after registration and a hospital-based health survey.
[b]The types of migration are divided into daily movement, seasonal migration, periodic migration, and long-term migration, as described elsewhere (WHO, 2000).
[c]Persons who had been treated with DEC during the pevious 2 years, in either Ranong Province or elsewhere.

2100, 0100, 0500 and 0900. The Giemsa-stained 3-line thick smears and microscopic examinations were done as before. For the qualitative antigenemia evaluation, 100 μl of blood was collected at 0900, before and after treatment in each individual, and was used for the detection of *W. bancrofti* adult worm circulating antigen using the NOW® ICT Filariasis test kit (Binax, Portland, Maine, USA). Residual antigenemias were monitored post-treatment at 4, 8 and 12 weeks. The diagnostic procedures and interpretations of the test results were performed using methods described elsewhere (Bhumiratana *et al*, 2002, 2004). For quality control, 2 observers were used (S Koyadun and A Bhumiratana).

Data analysis

The blood samples, taken every 4 hours during the 24-hour sampling period prior to DEC treatment, were evaluated for baseline mean microfilarial density (Y). Arithmetic means (Mf/60 μl blood) were calculated. According to the mathematical method described by Aikat and Das (1977), a simple harmonic-wave equation was used to calculate the time (hour) of microfilarial peak density (*k*) and periodicity index (*D*) during the 24-hour testing period. Microfilarial density is presented by a circadian cycle (cos15h and sin15 h) calculated from the *W. bancrofti* microfilarial counts in the peripheral blood samples (n) collected specific points in time (h) which has a maximum density of $\tan 15k = c/b$, where b is $2/n\Sigma Y\cos 15h$ and c is $2/n\Sigma Y\sin 15h$. The periodicity index (D) represents the relative amplitude of the wave: $(a/m) \times 100$, where a is the amplitude (the difference in density between the peak and the mean) and m is the mean density. According to Sasa and Tanaka (1972, 1974), D-values indicate that *W. bancrofti* infections of the nocturnally periodic ($D \geq 70$) and nocturnally subperiodic forms (approximately 50) occur in the microfilaremics.

Microfilaremia levels were determined after DEC treatment to evaluate its short-term effects on the mean microfilarial density (Y) and the maximum microfilarial density (k). The Wilcoxon signed ranks test for paired data observed before and after DEC treatment was used to compare the values being repeatedly measured. Tests of normality of those values were not statistically significant. Significance ($p<0.05$) based on negative or positive rank was two-tail analyzed.

RESULTS

Before DEC treatment

The microfilaremia prevalence rate observed

Table 2
Analysis of *Wuchereria bancrofti* microfilarial periodicity in the 7 microfilaremic subjects prior to treatment.

Time of blood collection (hours)	cos15h	sin15h	Y[a]	Y^2	Ycos15h	Ysin15h
0900	-0.7071	0.7071	21	441	-14.8491	14.8491
1300	-0.9659	-0.2588	13	169	-12.5567	-3.3644
1700	-0.2588	-0.9659	44	1936	-11.3872	-42.4996
2100	0.7071	-0.7071	113	12769	79.9023	-79.9023
0100	0.9659	0.2588	185	34225	178.6915	47.878
0500	0.2588	0.9659	125	15625	32.35	120.7375
Σ			501	65165	252.1508	57.6983

[a]sum of mean counts [microfilarial density (Mf/60 μl) = total number of microfilariae observed in duplicate sildes/2] for each subject at each time-point [n = 6, the time of the day (h) of blood samples collected].

in all 7 subjects prior to DEC treatment, measured at 2100, 0100 and 0500, was 100%. The maximum microfilarial density in subject M5 was 62.5 Mf/60 μl, which was measured at 0100. The prevalence rate at 0900 was 28.6%. The mean microfilarial densities at 0900 between the day of starting blood collection (2.9 Mf/60 μl) and the next day (1.6 Mf/60 μl) in all the subjects did not significantly differ (Z=-0.816, p=0.414). The microfilarial periodicity was analyzed using combined microfilarial densities (Y) for the 7 microfilaremic subjects at each time-point (Table 2). The mathematical equations were computed as follows:

$m = 1/n\Sigma Y = 1/6 \times 501 = 83.5$

$b = 2/n\Sigma Y\cos 15h = 2/6 \times 252.1508 = 84.0502$

$c = 2/n\Sigma Y\sin 15h = 2/6 \times 57.6983 = 19.2327$

$a^2 = b^2 + c^2 = 7434.3328$, therefore $a = 86.2225$

$\tan 15k = c/b$ or $k = 1/15 \times \tan^{-1} 0.2288 = 1/15 \times 12.8887 = 0.86$

The calculated time of maximum microfilarial density (k) was 0.86, or at 00:52. The periodicity index (D) was computed with the following formula:

$D = (a/m) \times 100 = 86.2225/83.5 \times 100 = 103.26\ \%$

In order to analyze observed and theoretical values for *W. bancrofti* microfilaremia present in all the 7 subjects (Table 3), a sum of the mean counts (Mf/60 μl) (Y_a) for each subject at each time-point, as shown in Table 2, were mathematically expressed with the following formula (I):

$$Y_a = m_a + b_a\cos 15h + c_a\sin 15h \quad \text{..... (I)}$$

Where;

$m_a = 1/n\Sigma Y_a = 1/6 \times 501 = 83.5$

$b_a = 2/n\Sigma Y_a\cos 15h = 2/6 \times 252.1508 = 84.0502$

$c_a = 2/n\Sigma Y_a\sin 15h = 2/6 \times 57.6983 = 19.2327$

Therefore, $Y_a = 83.5 + 84.0502\cos 15h + 19.2327\sin 15h$

For calculating mean counts (Y_b), the equation was computed with the following formula (II):

$$Y_b = m_b + b_b\cos 15h + c_b\sin 15h \quad \text{..... (II)}$$

Where;

$m_b = 1/n\Sigma Y_b = 1/6 \times 71.29 = 11.8816$

$b_b = 2/n\Sigma Y_b\cos 15h = 2/6 \times 35.9708 = 11.9902$

$c_b = 2/n\Sigma Y_b\sin 15h = 2/6 \times 8.3021 = 2.7673$

Therefore, $Y_b = 11.8816 + 11.9902\cos 15h + 2.7673\sin 15h$.

In other words, similar to the total counts, the observed and theoretical (Y_a) values for the mean counts were not significantly different (Z=-0.676, p=0.499). The harmonic wave is shown in Fig 2.

After DEC treatment

The initial and post-treatment microfilarial

Table 3
Observed and theoretical values for *Wuchereria bancrofti* microfilarial counts and mean densities prior to treatment, given over a 24-hour period.

Time of blood collection (hours)	Total microfilarial counts (Y_a)		Mean microfilarial counts (Y_b)	
	Observed	Theoretical[e]	Observed	Theoretical[f]
0900	21	37.67	2.93	5.36
1300	13	-3.32	1.86	-0.42
1700	44	43.17	6.21	6.11
2100	113	129.33	16.07	18.40
0100	185	169.66	26.36	24.18
0500	125	123.83	17.86	17.66
0900	12	37.67	1.64	5.36

The formulas (I and II) were derived :
[e]$Y_a = 83.5 + 84.0502\cos 15h + 19.2327\sin 15h$; [f]$Y_b = 11.8816 + 11.9902\cos 15h + 2.7673\sin 15h$, respectively.

Table 4
Calculated peak density times and periodic indicies based on the circadian cycles for *Wuchereria bancrofti* in 7 infected patients before and after treatment.

Follow-ups[a] (weeks)	Time of *k* hours after midnight (actual time)	*D* (%)
0	0.86 (0052)	103.26
1	0.90 (0054)	154.92
2	1.59 (0136)	133.44
4	0.33 (0019)	159.13
8	0.96 (0058)	144.61
12 [b]	0.92 (0055)	146.67

Abbreviation: *k* = calculated peak density; *D* = periodicity index.
[a]At each follow-up appointment, fingerprick blood was obtained at 4 different points in time as described in the text. This was used to determine the sum of the mean counts (Mf/60 μl) for each subject.
[b]Not including patient M7 who dropped out of the study before these samples were collected.

densities, both observed and theoretical, were not significantly different (p>0.05) (Table 3 and Fig 2). The *k* values, before and after treatment, were not significantly different (Z=-0.944, p=0.345) (Table 4). The microfilarial densities at 2 weeks were decreased (up to 29%) compared to the pre-treatment densities (Table 5). An increase to pre-treatment levels was noted by 4 to 12 weeks. There were no significant differences between pre-treatment densities and post-treatment densities at weeks: 1 (Z=-0.931, p=0.352), 4 (Z=-0.169, p=0.866), 8 (Z=-0.676, p=0.499), and 12 (Z=-0.105, p=0.917). The only significant difference between pre- and post-treatment densities was noted at week 2 (Z=-2.197, p=0.028). Week 2 densities were also significantly different from weeks 4 and 12 (Z=-2.201, p= 0.028).

Subject M3 had no microfilaremia at week 2 post-treatment. Subject M4 had a high density level at week 1 post-treatment. In subject M1, the highest microfilaremia level was seen at week 12 post-treatment. All the subjects were positive for *W. bancrofti* soluble antigen by the NOW® ICT Filariasis test kit (Fig 3) both before and after treatment. Subject M3 did have a decrease in *W. bancrofti* microfilaremia (Table 5) and antigenemia (Fig 3) during the 12-week follow-up period.

DISCUSSION

In the parts of Thailand where nocturnally periodic *W. bancrofti* infection occurs, Myanmar male migrant workers were often found to have microfilaremia. The 7 subjects in our study had *W. bancrofti* Mf whose morphologic characteristics on peripheral blood smear (*eg* cephalic space, cuticular striation, nerve ring, excretory pore, excretory cells, central viscus, germinal cells and anal pore) are well established. Its

Table 5
Parasitological response to 300 mg oral-dose diethylcarbamazine in the 7 microfilaremic subjects.

Subject	Mean microfilarial density (Mf/60 μl blood)[a] in weeks post-DEC-treatment (% residual microfilaremia)					
	0	1	2	4	8	12
M1	31.5	13.0	5.0	32.0	17.5	68.0
M2	42.5	17.0	21.0	21.0	11.5	24.0
M3	9.5	8.0	0.0	19.5	2.0	7.0
M4	7.5	9.0	12.5	26.5	19.0	27.0
M5	62.5	15.5	10.5	33.5	33.5	15.5
M6	10.5	12.5	2.0	15.5	27.0	23.0
M7	20.5	26.5	4.5	17.5	30.0	ND
All	26.4 (100)	14.5 (54.9)	7.9 (29.9)	23.6 (89.4)	20.1 (76.1)	27.4 (103.8)

Abbreviation: Mf = microfilariae, ND = no data available for Mf examination.
[a]Monitored at 1:00 AM.

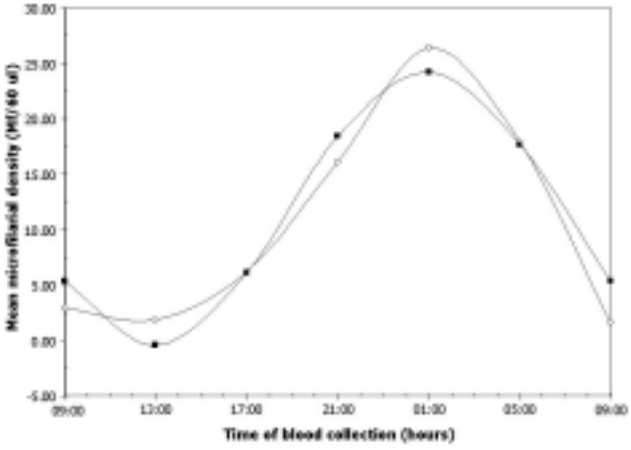

Fig 2–The observed (○) and theoretical (■) values for mean microfilarial densities determined prior to treatment with single oral-dose diethylcarbamazine, over a 24-hour period.

morphologic characteristics and cyclic appearance in the peripheral blood distinguish it from that of the local isolates of nocturnally subperiodic *W. bancrofti* (Harinasuta *et al*, 1970; Phantana *et al*, 1996b). All the subjects in our study had a peak microfilarial density (*k*) time of 0052, where the *D* was greater than 100%. Phantana *et al* (1996a) observed in selected parts of Thailand, a *k* at 0110 and a *D* of 132.7%. Sitthai and Thammapalo (1998) also observed in Ranong Province a *k* at 0118 and a *D* of 134.4%. In general, although Myanmar microfilaremics were found in different locations in Thailand, the *W. bancrofti* microfilarial periodicity was the same. Our study reconfirmed nocturnally periodic *W. bancrofti* in 7 Myanmar migrants in the Ranong Province.

Microfilarial peak density (*k*) before and after the treatment was analyzed to see whether a single dose of DEC 300 mg orally can affect the *k* values. The *k* values observed at 2 and 4 weeks post-treatment were at 0136 and 0019, respectively, slightly differed from pre-treatment. However, the *k* values were not affected by the treatment. In the absence of DEC, nocturnally periodic *W. bancrofti* microfilaremics did not have a change in their *k* values (Fontes *et al*, 2000). In our study, a single dose of DEC 300 mg orally had no effect on microfilarial peak density. The circadian cycle of the *W. bancrofti* parasite in human hosts is clinically unimportant for treatment, but important for its epidemiological implications. Thick blood smears for microfilaremia are more sensitive the closer in time they are taken to the peak hour. The nocturnal periodicity of *W. bancrofti* implicates *Cx. quinquefasciatus* as the potential vector in this area.

Single oral-dose DEC 6 mg/kg has been reported to immediately decrease *W. bancrofti* microfilarial density (Rajapake, 1974; Moulia-Pelat *et al*, 1994). A linear model of *W. bancrofti* microfilarial density reduction according to time was predicted with the assumption that microfi-

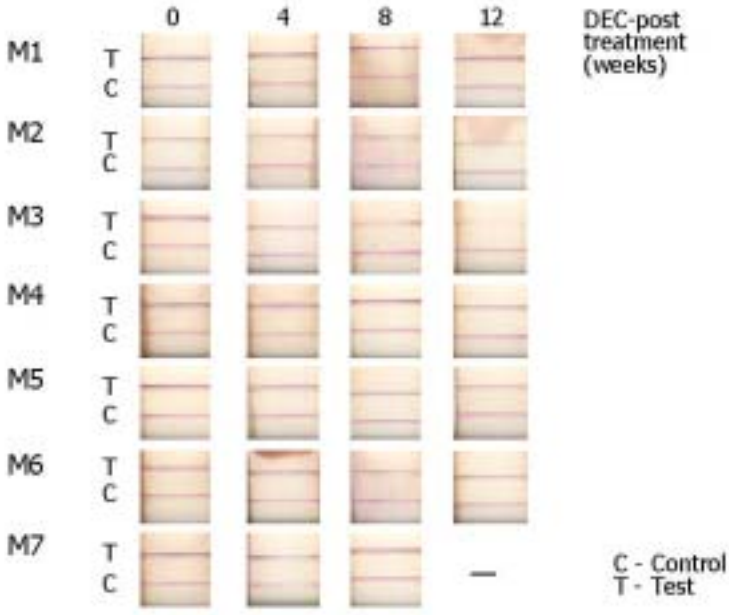

Fig 3–A schematic presentation of the NOW® ICT Filariasis test kit with detectable soluble antigen in the 7 microfilaremics before and after treatment. The hyphen indicates no data available for antigenemia evaluation.

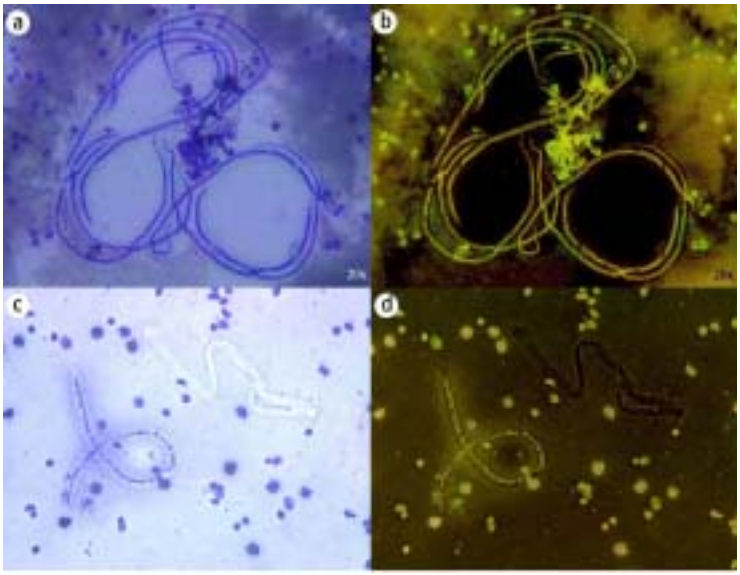

Fig 4–Photomicrographs of disintegrating microfilariae present in subject M6 4 weeks after treatment. Clustered microfilariae (a and b) and shed microfilariae (c and d) at 200X magnification. Note that b and d are negative images of a and c, respectively.

larial density in untreated subjects is stable. A sharp decrease in microfilarial density was observed. Thirty minutes after oral administration, a 30% reduction from pre-treatment level was noted, but 1 to 96 hours later the microfilarial density was only 6% lower than the pre-treatment level (Moulia-Pelat *et al*, 1994). This confirmed an immediate microfilaricidal effect of single dose DEC treatment, with the peak plasma concentration occurring 1 to 2 hours after drug intake (WHO, 1992). Wongcharoenyong *et al* (1997) demonstrated, by using blood samples (Mf/20 μl) collected around 2200, that DEC 6 mg/kg single oral-dose had a short-term effect on nocturnally periodic *W. bancrofti* in Myanmar migrants in Ranong Province. The microfilarial densities decreased 5.9% for 2 days after treatment. However, there was an increase in microfilaremia within 3 days to 6 months post-treatment in those patients.

In our study, all the microfilaremics had a decrease in their mean microfilarial densities near peak hour (0100) soon after the DEC intake, but an increase in the mean microfilarial density was observed afterwards. A linear model of *W. bancrofti* microfilarial density reduction was predicted for the 2 weeks after drug intake ($p=0.035$) (data not shown). The efficacy of microfilaremia reduction by 300 mg oral-dose DEC occurs for as long as 2 to 4 weeks after ingestion. However, the microfilaremia tended to be recur thereafter. There were no differences in the microfilarial densities between pre-treatment and those at 4 to 12 weeks post-treatment. DEC 300 mg single oral-dose is recommended for both the DEC provocative day test and si-

multaneous treatment (Bhumiratana *et al*, 2004) in Myanmar migrants with a short period of stay in the area. A non-linear model for *W. bancrofti* microfilarial density will occur over time unless there is repeat DEC treatment.

Hakim *et al* (1995) demonstrated that mass treatment with single oral-dose DEC 6 mg/kg given annually for 2 years, was equally effective in controlling nocturnally periodic brugian filariasis along the Thailand-Malaysia border as DEC 36 mg/kg divided over 6 doses. In the first round of the biannual mass treatment with DEC 6 mg/kg, a significant reduction in the microfilaremia rate (up to 16%) was seen for at least 7 months post-treatment, compared to the pre-treatment level (25% microfilaremia rate). However, 12 months post-treatment, an increased rate of up to 60% of the pre-treatment level was seen. In this trial of single-dose DEC mass treatment of brugian filariasis, a long-term microfilaricidal effect may occur when the treatment is repeated over sufficiently long periods of time (Ottesen *et al*, 1997). A similar phenomenon may have occurred in the Myanmar migrants recieving biannual DEC mass treatment in the PELF program.

In spite of the lack of a direct relationship between microfilarial loads and adult worm loads in infected patients, DEC resulted in a decrease in microfilarial density and has short-term effects on the retention of microfilariae in the uterus of the adult female worm. In our study, the appearance of Giemsa-stained microfilaria on smears between the first and the fourth week after DEC was abnormal. Mostly disintegrating microfilariae were seen in the 7 subjects (Fig 3). Over a period of 12 weeks post-treatment, most of the 7 subjects did not respond well to DEC by showing a sharp decrease in antigenemia. Low quantities of antigenemia were detectable in subjects M2 and M3 12 weeks post-treatment. The decreases in microfilaremia and antigenemia in subject M3 (Table 5 and Fig 3) may be related to low adult worm loads. The subject responded well to DEC over the short-term. In addition, subject M1, with a previous history of treatment with both biannual DEC mass treatment and DEC provocation, did have rather high levels of microfilaremia and/or antigenemia (Table 5 and Fig 3). It is unwise to suggest that the levels of microfilaremia and antigenemia in the subjects with a history of DEC treatment reflect resistance. It was not possible to rule out variation in susceptibility to single-dose DEC treatment in the group. Detectable antigen levels were equivalent at pre-treatment and one month post-treatment (data not shown). Soluble antigen was equally detected in all the subjects (except M7) 16 weeks post-treatment. Our findings suggest that single-dose DEC had little or no immediate effects on *W. bancrofti* antigenemia in the group.

In Southern Thailand, where there are large numbers of Myanmar migrants eligible for the DEC treatment regimen (Wongcharoenyong *et al*, 1997; Koyadun *et al*, 2003; Bhumiratana *et al*, 2004), recrudescence may occurr unless a sufficiently prolonged DEC delivery process is effectively available at the provincial level. It is necessary for the PELF's implementers at the provincial level to design measures for controlling *W. bancrofti* infection in Myanmar migrants with short periods of stay in the target areas.

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