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; Sithai and Thammapalo, 1998; TTrapecha 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) (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 (Swadhiwudhipong et al, 1996; Phoopattanakool, 1997; Sithai 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).
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 (Wongcha-roenyong 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 Prov-ince 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.

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
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 \( \mu l \) 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 (\( \text{Mf}/60 \mu l\) 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 (\( \cos 15h \) and \( \sin 15 h \)) 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 \( 2/n\Sigma \cos 15h \) and \( c \) is \( 2/n\Sigma \sin 15h \). 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...
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Analysis of Wuchereria bancrofti microfilarial periodicity in the 7 microfilaremic subjects prior to treatment.

<table>
<thead>
<tr>
<th>Time of blood collection (hours)</th>
<th>cos15h</th>
<th>sin15h</th>
<th>$Y^a$</th>
<th>$Y^2$</th>
<th>Ycos15h</th>
<th>Ysin15h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0900</td>
<td>-0.7071</td>
<td>0.7071</td>
<td>21</td>
<td>441</td>
<td>-14.8491</td>
<td>14.8491</td>
</tr>
<tr>
<td>1300</td>
<td>-0.9659</td>
<td>-0.2588</td>
<td>13</td>
<td>169</td>
<td>-17.5567</td>
<td>-3.3644</td>
</tr>
<tr>
<td>1700</td>
<td>-0.2588</td>
<td>-0.9659</td>
<td>44</td>
<td>1936</td>
<td>-17.3872</td>
<td>-4.2496</td>
</tr>
<tr>
<td>2100</td>
<td>0.7071</td>
<td>-0.7071</td>
<td>113</td>
<td>12769</td>
<td>79.9023</td>
<td>79.9023</td>
</tr>
<tr>
<td>0100</td>
<td>0.9659</td>
<td>0.2588</td>
<td>185</td>
<td>34225</td>
<td>178.6915</td>
<td>47.878</td>
</tr>
<tr>
<td>0500</td>
<td>0.2588</td>
<td>0.9659</td>
<td>125</td>
<td>15625</td>
<td>32.35</td>
<td>120.7375</td>
</tr>
<tr>
<td>$\Sigma$</td>
<td>501</td>
<td>65165</td>
<td>252.1508</td>
<td>57.6983</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$sum of mean counts [microfilarial density (Mf/60 $\mu$l) = total number of microfilariae observed in duplicate slides/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 $\mu$l) and the next day (1.6 Mf/60 $\mu$l) 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:

$$m = \frac{1}{n} \Sigma Y = \frac{1}{6} \times 501 = 83.5$$

$$b = \frac{2}{n} \Sigma Y \cos 15h = \frac{2}{6} \times 252.1508 = 84.0502$$

$$c = \frac{2}{n} \Sigma Y \sin 15h = \frac{2}{6} \times 57.6983 = 19.2327$$

$$a^2 = b^2 + c^2 = 7434.3328$$

$$\therefore a = 86.2225$$

$$\tan 15k = c/b$$ 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 microfilaria present in all the 7 subjects (Table 3), a sum of the mean counts (Mf/60 $\mu$l) ($Y_a$) for each subject at each time-point, as shown in Table 2, were mathematically expressed with the following formula (l):

$$Y_a = m_a + b_a \cos 15h + c_a \sin 15h \ldots \ldots \ldots \ldots \ldots (I)$$

Where:

$$m_a = \frac{1}{n} \Sigma Y_a = \frac{1}{6} \times 501 = 83.5$$

$$b_a = \frac{2}{n} \Sigma Y_a \cos 15h = \frac{2}{6} \times 252.1508 = 84.0502$$

$$c_a = \frac{2}{n} \Sigma Y_a \sin 15h = \frac{2}{6} \times 57.6983 = 19.2327$$

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 (Il):

$$Y_b = m_b + b_b \cos 15h + c_b \sin 15h \ldots \ldots \ldots \ldots \ldots (II)$$

Where:

$$m_b = \frac{1}{n} \Sigma Y_b = \frac{1}{6} \times 71.29 = 11.8816$$

$$b_b = \frac{2}{n} \Sigma Y_b \cos 15h = \frac{2}{6} \times 35.9708 = 11.9902$$

$$c_b = \frac{2}{n} \Sigma Y_b \sin 15h = \frac{2}{6} \times 8.3021 = 2.7673$$

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

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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 (e.g. cephalic space, cuticular striation, nerve ring, excretory pore, excretory cells, central viscus, germinal cells and anal pore) are well established. Its
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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%. Sittahai and Thammaphalo (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 microfilaricides 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 microfilariae 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; Mouila-Pelat et al, 1994). A linear model of W. bancrofti microfilarial density reduction according to time was predicted with the assumption that microfilarial...
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 (Mouila-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 microfilaria reduction by 300 mg oral-dose DEC occurs for as long as 2 to 4 weeks after ingestion. However, the microfilaria tended to 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-

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**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.
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 microfilaria rate (up to 16%) was seen for at least 7 months post-treatment, compared to the pre-treatment level (25% microfilaria 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 receiving 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 microfilaria 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 microfilaria and antigenemia (Table 5 and Fig 3). It is unwise to suggest that the levels of microfilaria 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 occur 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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