EVALUATION OF A SINGLE ORAL DOSE OF DIETHYLCARBAMAZINE 300 MG AS PROVOCATIVE TEST AND SIMULTANEOUS TREATMENT IN MYANMAR MIGRANT WORKERS WITH *WUCHERERIA BANCROFTI* INFECTION IN THAILAND

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Abstract. We assessed the efficiency of oral diethylcarbamazine (DEC) 300 mg as a provocative test on blood examination 30 minutes after administration, while gauging the overall infection rate in Myanmar migrant workers with *Wuchereria bancrofti* infection who enrolled for work permits in Thailand in 2002, using circulating filarial antigens (CFA) assays, the NOW® ICT Filariasis card test and the Og4C3 ELISA as reference. Overall infection rates of 0.3% (95% CI=0-0.7%), 4.2% (95% CI=1.8-6.5%) and 5.9% (95% CI=3.2-8.7%) by three diagnostic tests, respectively, were observed. Among three different location groups of Myanmar population sample tested, there were no statistically significant differences in the overall infection detection rates. When either the ICT card test or the Og4C3 ELISA was used as a reference, the specificity and positive predictive value of the DEC-provocative day test was the same, 100%. The sensitivities were 25.0% (95% CI = 0.5-49.5%) and 17.6% (95% CI = 0-35.8%) on the ICT and ELISA tests, respectively. The negative predictive values were 96.8% (95% CI = 94.8-98.9%) and 95.1% (95% CI = 92.6-97.6%), respectively. In three microfilaremic persons followed-up monitored at 8-weeks DEC post-provocation, there were 6 x 10^{-1} and 7 x 10^{-1} decreases in microfilaremia and antigenemia. These findings suggested that, unlike the CFA assays, the DEC-provocative day test is unsuitable for the diagnosis of active *W. bancrofti* infection in the population tested, and for gauging current infection prevalence. The treatment would likely be beneficial to reduce microfilaremia and antigenemia.

INTRODUCTION

Diethylcarbamazine (DEC), 1-diethylcarbamyl-4-methylpiperazine, is a microfilaricide (WHO, 1992; 1994). In the filariasis control program in Thailand, which aims to reduce the number of microfilaremic cases per annum, the drug has been used for the treatment of lymphatic filariasis for the past thirty years. Biannual treatments with single oral doses of DEC citrate, *ie* 6 mg/kg body weight given once daily for 12 consecutive days (for *Wuchereria bancrofti*), or 6 mg/kg given once daily for 6 consecutive days (for *Brugia malayi*) and repeatedly twice a year for 2 consecutive years, are recommended as a standard treatment for lymphatic filariasis, as the most effective strategy for the control of lymphatic filariasis (WHO, 1992; 1994; Suvannadabba, 1993). Declines in lymphatic filariasis, *ie* reduction in microfilaremia prevalence of *W. bancrofti* and *B. malayi*, have been achieved as a result of this DEC regimen.

The infection with the nocturnally periodic form of *W. bancrofti* imported by cross-border Myanmar migrant workers is an emerging disease in Thailand (CDC, 2001). An accurate estimate of infection prevalence, by night blood survey, is unknown, but cross-sectional surveys report a more than 1% microfilarial positive rate (MPR) (Phantana *et al*, 1996; Swaddhiwudhipong *et al*, 1996; Sitthai and Thammapalo, 1998). Imported bancroftian filariasis has become a health

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issue. Mass drug administration (MDA) (CDC, 2001) needs to be applied to interrupt transmission within the Myanmar population at risk. A DEC regimen of 6 mg/kg single oral-dose is recommended to treat those who are infected (Wongcharoenyong *et al*, 1997). The short period DEC regimen reduces microfilarial density. A single provocation of DEC 2 mg/kg followed by a peripheral blood smear for microfilariae (MF) 45 minutes later has been recommended for daytime diagnosis (Phantana *et al*, 1997).

The registration of Myanmar migrant workers for work permits is carried out at the provincial level (Srismith, 1998; Koyadun *et al*, 2003). A large number are subjected to a hospital-based health survey for infectious diseases and drug abuse (Srismith, 1998). Surveillance for bancroftian filariasis is performed by single oral administration of 300 mg DEC and examining the venous bloods 30 minutes after daytime provocation (Srismith, 1998; Filariasis Division, 2000; Koyadun *et al*, 2003). It has been suggested that this DEC provocative day test has the benefit of simultaneous treatment of bancroftian filariasis. This has not been evaluated. The DEC provocative test was analyzed here in terms of its efficiency in gauging infection rates and treatment for imported bancroftian filariasis.

DEC-provocative day blood samples of Myanmar migrant workers, which were collected during the foreign migrant workers' registration and hospital-based health survey in Provinces in Northern, Central and Southern Thailand, were used. Its performance efficiency was assessed using two other commercially available circulating filarial antigens (CFA) assays, which were an immunochromatographic test, NOW® ICT Filariasis card test (formerly ICT Filariasis) (Weil and Liftis, 1987; Weil *et al*, 1987; 1997), and an enzyme-linked immunosorbent assay (ELISA), Og4C3 ELISA (More and Copeman, 1990; Chanteau *et al*, 1994), as reference.

MATERIALS AND METHODS

DEC-provocative day blood sample collection and preparation

During hospital-based surveys in Phang-Nga Province (Southern) and Suphanburi Province (Central) between August and October 2002, two groups of registered Myanmar migrant workers were recruited. In the Tak Province (Northern), unregistered Myanmar migrant workers were also selected. A total of 860 adults of both sexes: 435 (Southern), 338 (Central), and 87 (Northern) were given an oral dose of DEC, 300 mg FILADEC tablet (Pond's Chemical Thailand ROP, Bangkok, Thailand). The DEC regimen was from the guidelines of the MDA in the National Program to Eliminate Lymphatic Filariasis (PELF). Ethical clearance and approval for the study was obtained from the hospitals. Thirty minutes after the DEC provocation test, 3.0 ml intravenously EDTA-blood samples in individuals were obtained for Mf and CFA. The samples were then transferred to the laboratory and refrigerated at 4ºC until use.

Microfilaremia and antigenemia examination

The Knott's concentration technique (WHO, 1992) was used to detect Mf. In each centrifuge tube, the nine parts of the 1% formalin solution were added to the blood (1.0 ml each), mixed thoroughly, and spun down at 5,000*g* for 5 minutes. After decanting the hemolysate, the small amount of sediment was microscopically examined for Mf with 100x magnification. Specific identification of Giemsa's stained Mf and microfilarial counts were done afterwards. All microfilaria-positive samples were evaluated for CFA.

In each of two groups (Central and Southern), the same 100 plasma samples (0.1 ml each) including the microfilaria-positive samples, were evaluated for CFA by the NOW® ICT Filariasis (Binax, Portland, Maine, USA) and by the Og4C3 ELISA (JCU Tropical Biotechnology, Townsville, Queensland, Australia). In the Northern group, all 87 plasma samples (0.1 ml each) were examined by the CFA assays. The diagnostic test procedures and interpretation of test results have been described elsewhere (Bhumiratana, 2000; Bhumiratana *et al*, 2002; Koyadun *et al*, 2003). For validation of the CFA assays, any discordant sample (defined as negative with the NOW® ICT Filariasis but positive with the Og4C3 ELISA) was retested with the NOW® ICT Filariasis using the same pretreated samples (0.1 ml) prepared for the Og4C3 ELISA. An arbitrary antigen titer (≥120 antigen units or AU/ml) of the concordant and discordant samples was considered positive

with the Og4C3 ELISA (Koyadun *et al*, 2003).

The Mf and CFA present in all microfilaremic persons were follow-up monitored at the DEC-post-provocation, at 2, 4, and 8 weeks. Night venous blood samples between 2100 and 2200 hours were examined according to the methods mentioned above.

Data analysis and statistical methods

In order to assess overall infection rates (%) among the three location groups of the Myanmar migrant workers by the three diagnostic tests with statistical significance, the Kruskal-Wallis test ($p < 0.05$) was used (Knapp and Miller, 1992; Sheskin, 2000). For indices of agreement in the measurement of *W. bancrofti* infection, the κ-test was used and efficiency, such as sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) with 95% confidence intervals (CI) for the DEC-provocative day test were assessed (Knapp and Miller, 1992), using the CFA assays as a reference. Differences in infection rates by the CFA assays were described using the χ^2-test or Fisher's exact test where appropriate ($p < 0.05$). In order to assess the short-term effect of 300 mg single oral-dose DEC provocation in the microfilaremics, the antigen titers with ranges and mean ± standard deviations were analyzed for residual antigenemia (%). Residual microfilaremia (Mf per ml) was recorded, since no data for initial microfilarial density at night was taken.

RESULTS

Of the 860 persons who were tested with the DEC-provocative day test, the microfilaremic infection rate was 0.3% (95% CI = 0-0.7%) (Table 1), with no statistically significant difference among the different location groups ($\chi^2 = 1.05$, df = 2, p = 0.59). Using the Og4C3 ELISA, the overall antigenemia rate was 5.9% (95% CI = 3.2-8.7%). With the ICT card test, the rate was 4.2% (95% CI = 1.8-6.5%) (Table 1). There was a statistically significant difference between the antigenemia rates (Fisher's exact test, $p < 0.001$). Among the groups, there were no differences in antigenemia prevalence with the ICT card test ($\chi^2 = 3.09$, df = 2, p = 0.21) or the Og4C3 ELISA ($\chi^2 = 2.63$, df = 2, p = 0.27).

Using either the ICT card test or the Og4C3 ELISA as a reference (Table 2), the specificity of the DEC-provocative day test was 100%, and the sensitivities were 25% and 17.6%, respectively, while the κ-test was 0.39 and 0.29, respectively. Using the Og4C3 ELISA as a reference, the sensitivity, specificity, NPV, and PPV of the ICT card test were 70.6% (95% CI = 68.4-72.8%), 100%, 98.2% (95% CI = 96.6-99.8%), and 100%, respectively, with good agreement (the κ-test, 0.82) (data not shown).

There were 17 antigenemic persons: 16 males aged 17 to 40 years and one 42-yr-old female (Table 1 and Fig 1). Among the Northern, Central and Southern groups (Fig 1), median antigen titers (25^{th}, 75^{th} percentiles) were 26187 AU/ml (11504, 43825), 92925 AU/ml (18496, 121972), and 21737 AU/ml (10106, 61907), respectively (data not shown). There were 5 discordant samples (ICT-negative but Og4C3 ELISA-positive) of varying antigen titers, 2924-13983 AU/ml, compared with 12 concordant

Table 1
Infection rates (%) with the three diagnostic tests among the three location groups.

Group	No. of persons	No. of persons[a] positive (%)		
		DEC-provocative day test	NOW® ICT Filariasis	Og4C3 ELISA
Northern	87	0/87 (0)	2/87 (2.3)	4/87 (4.6)
Central	338	[b]2/338 (0.6)	3/100 (3.0)	4/100 (4.0)
Southern	435	[c]1/435 (0.2)	7/100 (7.0)	9/100 (9.0)
Total	860	3/860 (0.3)	12/287 (4.2)	17/287 (5.9)

[a]All persons positive with the DEC-provocative day test were used for the CFA assays.
[b]Microfilarial counts (Mf per ml) were 2 to 9, [c]Microfilarial count (Mf per ml) was 12.

Table 2
Test results of the three diagnostic tests among the three location groups.

DEC-provocative day test	NOW® ICT Filariasis[a]			Og4C3 ELISA[b]		
	Positive	Negative	Total	Positive	Negative	Total
Positive	3	0	3	3	0	3
Negative	9	275	284	14	270	284
Total	12	275	287	17	270	287

[a]Sensitivity, 25.0% (95% CI = 0.5-49.5%); Specificity, 100%; NPV, 96.8% (95% CI = 94.8-98.9%); PPV, 100%; κ, 39.0%.
[b]Sensitivity, 17.6% (95% CI = 0-35.8%); Specificity, 100%; NPV, 95.1% (95% CI = 92.6-97.6%); PPV, 100%; κ, 28.7%.

Table 3
Parasitological and serological responses to the 300 mg single oral-dose diethylcarbamazine provocation in the three microfilaremic persons.

Follow-ups	Microfilaremia (Mf per ml)		Antigenemia (x 10^3 AU/ml)		
	Range	Mean ± SD	Range	Mean ± SD	Residuals (%)
Week 0[a]	2 - 12	7.7 ± 4.2	121 - 122	121.7 ± 0.5	100
Week 2	20 - 45	28.7 ± 11.6	107 - 120	115.0 ± 5.7	94.5
Week 4	11 - 39	22.7 ± 11.9	95 - 99	96.7 ± 1.7	79.5
Week 8	10 - 27	17.3 ± 7.1	85 - 92	88.0 ± 3.6	72.3

[a]Using day blood samples

samples of varying antigen titers, 14110-122417 AU/ml (Fig 1). Of the 5 discordant samples whose fresh plasma samples were ICT-negative, there were 3 weakly positive samples with antigen titers of 6102-13983 AU/ml when the pretreated samples were retested with the ICT card test (Fig 2). The other 2 samples, with antigen titers of 2924-3051 AU/ml, were negative.

Only 3 microfilaremic males aged 19 to 24 years were followed up 8 weeks post-DEC provocation (Table 3). All had parasitological and serological responses to the DEC regimen. Excluding the initial average microfilaremia, the mean microfilaremia (Mf/ml) declined slightly (6 x 10^{-1} fold) 6 weeks after administration. The antigenemias (AU/ml) monitored at the initial treatment were 122417, 122035, and 120637, respectively, similar to the microfilaremic serum controls (Fig 1). The mean antigenemias also declined slightly. At 8 weeks, the mean antigenemia (%) had decreased 72.3 or 7 x 10^{-1} fold.

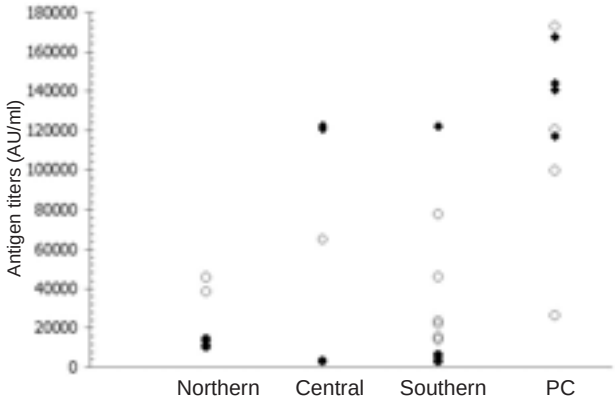

Fig 1–Antigen titers (AU/ml) of serum positive controls (PC) of the Myanmar migrant workers and of the 17 plasma samples among the three groups both concordant (♦ = microfilaremic; o = amicrofilaremic) and discordant (• = amicrofilaremic) with the CFA assays.

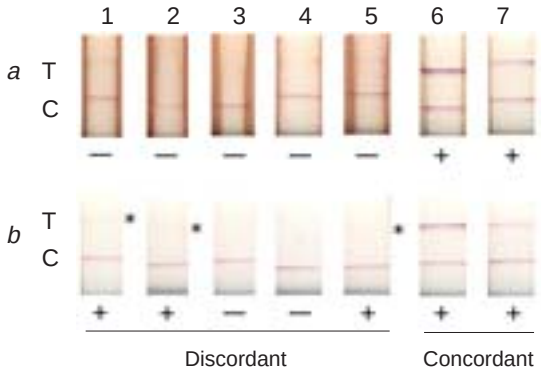

Fig 2–Five antigen-positive samples discordant with the CFA assays. Both fresh (*a)* and pretreated (*b*) plasma samples of the discordant samples: 1-2, Northern; 3, Central; 4-5, Southern, were used in comparison with concordant samples: 6, microfilaremic; 7, amicrofilaremic. The ICT test results (–, negative; +, positive; T, test; C, control) were shown as: weakly positive (*), pale pink line (T) forming after 15 minutes but within 45 minutes of closing the card; strongly positive, deep pink line (T) forming within 15 minutes of closing the card.

DISCUSSION

The DEC-provocative day test has been proposed for the detection of *W. bancrofti* Mf in endemic areas where the nocturnally periodic form is present, and community surveys at night are impossible (WHO, 1992). The technique relies on a single oral dose of DEC to increase the microfilarial density in day blood samples of patients infected with the nocturnally periodic *W. bancrofti* in endemic parts of the world (Sasa *et al*, 1963; Sullivan and Hembree, 1970; Manson-Bahr and Wijers, 1972; Rajapaske, 1974; Wijeyaratne *et al*, 1982; WHO, 1992). Previous trials of the oral DEC provocative test given to the Myanmar population show it can estimate infection rates (or MPR) (Phantana *et al*, 1997); but it has not been used for rapid diagnosis (Srismith, 1998). In our study, we evaluated the efficiency of the DEC- provocative day test, *ie* giving a 300 mg FILADEC tablet then examining the blood 30 minutes after administration, for use in a hospital-based health survey for bancroftian filariasis (Srismith, 1998; Koyadun *et al*, 2003). In particular, we evaluated its value in gauging current infection rates, since this DEC regimen is run at the level of the health care providers; which deliver annual DEC mass treatment (Koyadun *et al*, 2003) to interrupt the transmission of imported bancroftian filariasis, and reduce infection prevalence in Myanmar migrant workers.

We demonstrated, using the CFA assays, ICT card test and Og4C3 ELISA, as references, the efficiency of the DEC-provocative day test (sensitivity and NPV) is low. The specificity and PPV were the same, 100%. The provocative test resulted in point estimates of overall infection rates up to 20-fold lower than those found with the CFA assays. These false negative rates can indicate containment of microfilaremia with the DEC mass treatment program (Koyadun *et al*, 2003). Our results showed microfilaremia rates lower than those observed before the PELF implementation. Most workers with work permits had a previous history of DEC treatment at registration, and many were diagnosed as amicrofilaremic (data not shown). In this study, three microfilaremic persons with the first registration were diagnosed with this technique. Between the registered and unregistered groups, the misdiagnosed persons might be due either to the sensitivity of the test or the infection status of the study group. In other words, the sensitivity of the provocative technique relies on population sample size and microfilaremic numbers with increased Mf density.

Among the different location groups, high antigenemia rates in the southern group were observed. This compared well with previous antigen screening with the ICT card test in the Phang-Nga Province (Koyadun *et al*, 2003). Keeratihuttayakorn (2002) observed lower antigenemia rates (4%) in Myanmar migrants aged ≥15 yrs in Ranong Province. The CFA assays gave higher estimates of the infection prevalence. Having a high sensitivity and specificity, similar to the Og4C3 ELISA (WHO, 1999), the ICT card test showed good agreement (the κ-test, 0.8); and a low level of 1.7% discrepancy in this study. Of the five antigen-positive samples which were discordant with the CFA assays (Table 1 and Fig 2), the ICT card test performed well with retesting (3 out of 5 samples). These samples had low antigen titers. In other words, discordant samples (amicrofilaremic antigenemics) had antigen titers

of ≤14000 AU/ml, whereas the concordant ones (*ie* both amicrofilaremic and microfilaremic antigenemics) had higher titers. The findings compared well with a previous study of Myanmars and Karens that had CFA of varying antigen titers. Some samples had high antigen titers with a negative ICT card test, but were positive when pretreated samples were used (Bhumiratana *et al*, 2004, our unpublished data). The most likely explanation for this is the CFA detection with the ICT card test, which is based on the specific AD12 monoclonal antibody (MAb) showed a limited detection window. Extensive observations with samples from other parasitic infections showed no cross-reaction with the AD12 MAb (Weil *et al*, 1987; 1997; Bhumiratana *et al*, 1999). The epitopes of the CFA, 200 kDa in the native form (Weil and Liftis, 1987; Weil *et al*, 1987), in some fresh plasma or serum samples might be masked with non-specific circulating antibodies in the samples in the presence of the DEC. This causes no 200 kDa CFA complexed with the specific polyclonal antibody (PAb) conjugated to the gold particle and hence only the C formed. In similar fashion, when the whole blood samples were used, the ICT card test can misdiagnose some microfilaremic samples in the absence of the DEC (Pani *et al*, 2000). In those three discordant samples whose pretreated samples were positive with the ICT card test, the 200 kDa CFA (heat stable form) possessed the epitope that could be complexed with the PAb gold conjugate in the absence of non-specific circulating antibodies. Given a small amount of the CFA present in the sample, the heat stable antigen-PAb gold conjugate complex was captured by the specific AD12 MAb (referred to as T) and hence the two T and C formed. In the ELISA, pretreatment of the plasma or serum samples in the acid solution by boiling increased the sensitivity of the Og4C3 MAb that specifically captures the heat stable CFA, 50-60 and 130 kDas (More and Copeman, 1990; Chanteau *et al*, 1994). In principle, the discordant samples with the CFA assays, which had the antigen titers significantly lower than that of the concordant samples, were due to a low detectable quantity of the CFA present in the plasma samples in the study. The ICT card test can misdiagnose some antigen-positive samples (*ie* either amicrofilaremic or microfilaremic antigenemics) (Pani *et al*, 2000; Bhumiratana *et al*, 2004, unpublished data). The presence of the DEC may have caused problems for this test. When a large number of samples need to be analyzed and compared with the Og4C3 ELISA, it might cause type-one-error interference with the interpretation of the test results in general. Findings suggested that the presence of the CFA in day blood samples is a surrogate measure for estimates of the infection. In the diagnosis of active infection in the Myanmar migrants via the hospital-based health survey, the rapid simple-to-use ICT card test performed well with fresh plasma. In the PELF, the ICT card test is suitable for screening the CFA in daytime finger-prick blood samples, and monitoring and evaluation of the efficacy of DEC mass therapy. In crosscheck points, the Og4C3 ELISA, available at the public health reference laboratory, is a more appropriate tool for evaluating and monitoring the short- and long-term effects of the MDA in the at-risk Myanmar populations in the target areas.

The DEC regimen resulted in the short-term effects of decreasing in microfilaremia and antigenemia in the 3 microfilaremics. The 300 mg single oral-dose DEC showed microfilaricidal activity shortly after ingestion. The long-term effects of a DEC-provocative dose (100 mg oral-dose) on the reduction of microfilaremia prevalence in the endemic population has been seen (Simonsen *et al*, 1997). A reduction in antigenemia to 72% was seen at the 8-weeks post-treatment. There is little evidence to evaluate regarding the long-term effects on microfilaremia and antigenemia due to subject migration. It would be important to follow up at 3 and 6 months post DEC provocation to determine if there is benefit to using this regimen under the PELF in target areas.

In summary, our findings suggest that the DEC-provocative day test is unsuitable for the diagnosis of *W. bancrofti* infection in the cross-border Myanmar migrant workers via the hospital-based health survey and for gauging current infection prevalence. The DEC provocative day test can permit point estimates of infection prevalence, which may underestimate active *W. bancrofti* infection. Treatment with a single-dose of DEC 300 mg orally has beneficial effects on the reduction of microfilaremia and/or antigenemia prevalence, and thereby can interrupt

transmission of bancroftian filariasis in transmission-prone areas of Thailand.

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