BORDER AND IMPORTED BANCROFTIAN FILARIASES: BASELINE SEROPREVALENCE IN SENTINEL POPULATIONS EXPOSED TO INFECTIONS WITH *WUCHERERIA BANCROFTI* AND CONCOMITANT HIV AT THE START OF DIETHYLCARBAMAZINE MASS TREATMENT IN THAILAND

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Abstract. Border bancroftian filariasis caused by *Wuchereria bancrofti* nocturnally subperiodic mainly exists in Karens residing alongside the Thailand-Myanmar border. Imported bancroftian filariasis caused by *W. bancrofti* nocturnally periodic mainly exists in cross-border Myanmar migrants. We analyzed seroprevalence data based on *W. bancrofti* adult worm antigen (Ag) loads and human immunodeficiency virus (HIV) immunoglobulins in the sentinel population samples which were studied prior to the start of the diethylcarbamazine (DEC) mass treatment phase in the PELF during fiscal years 2002-2006. In the Karens, the cumulative infection prevalence (36.8% serological antigen positivity or SAP) was specific for age (p<0.001) but universal for gender (p=0.77). The infection intensity (median Ag load=60,827 antigen units or AU/ml) was specific for age (p=0.031) and for males (p=0.016). In the Myanmars, infection prevalence (24.0% SAP) was universal for age (p=0.961) and for gender (p=0.676). The infection intensity (median Ag load=19,068 AU/ml) was universal for age (p=0.433) but specific for females (p=0.027). Overall, the Ag loads between the groups were significantly different (p=0.014). In analysis of concomitant HIV and *W. bancrofti* infections, 7 (3.2%) Myanmars infected with HIV 1 and 3 (5.7%) with concomitant infections, subjected to biannual DEC treatment with 300 mg oral-dose FILADEC, were prevalent. The antigenemia clearance in the concomitant infections (r=-0.732, p=0.039) as well as in the single *W. bancrofti* infection (r=-0.781, p=0.022) was correlated with time required to clear antigenemias. We reemphasize that *W. bancrofti* adult worm Ag loads in the sentinel population samples would be beneficial for the PELF's implementers at the provincial level to probe the disease burdens in target areas and to evaluate and monitor the DEC treatment efficacy and effectiveness in those sentinel populations, including those with concomitant HIV eligible for the DEC mass treatment phase in the PELF.

INTRODUCTION

Wuchereria bancrofti, a filarial nematode parasite of Superfamily Filarioidea, mainly exists in Myanmar and in remote areas along the Thailand-Myanmar border, where an estimated 50 million people are at risk (Anonymous, 2001; WHO, 2002). *W. bancrofti* nocturnally subperiodic causing border bancroftian filariasis affects more than a hundred thousands people in endemic communities close to the Thailand-Myanmar border, where mosquito-borne transmission is considered to be uncertain (Filariasis Division, 2000, 2001; Bhumiratana *et al*, 2002). The border bancroftian filariasis with focal distribution in Thailand has been confined to the border areas in Tak, Kanchanaburi, and Mae Hong Son Provinces (Harinasuta *et al*, 1970a,b;

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Filariasis Division, 1998). *W. bancrofti* nocturnally periodic causing imported bancroftian filariasis mainly exists in at-risk Myanmar migrants (CDCD, 2001) who naturally acquire mosquito-borne transmission outside Thailand. The imported bancroftian filariasis is considered as potential for emergence of introduced infection within the at-risk local Thai population in areas where abundant breeding grounds of the potent vector, *Culex quinquefasciatus*, are widely distributed (Filariasis Divison, 2000, 2001). In the National Program to Eliminate Lymphatic Filariasis (PELF) during fiscal years 2002-2006 (Filariasis Division, 2000, 2001), the goal has been to interrupt transmission and eliminate human infection by mass chemotherapy with diethylcarbamazine (DEC) and albendazole in those target populations on a wide scale (Ottesen *et al*, 1997, 1999; WHO, 1999, 2000, 2001).

In endemic areas where mosquito-borne transmission is certain, *W. bancrofti* infection causes a wide spectrum of clinical manifestations, which are characterized by endemic normals, asymptomatic microfilaremia, acute lymphatic inflammation, and chronic lymphatic pathology (WHO, 1992). Individuals with naturally prolonged infection or exposure to infective bites of third-stage larvae (L3) may have developed hypo-/hyper-immune responsiveness (Ottesen, 1992). The explanatory model of infection involves acquired immunity against established lymphatic-dwelling worms in early stages of infection (Bundy *et al*, 1991; Grenfell *et al*, 1991; Mitchell, 1991; Grenfell and Micheal, 1992). Prior epidemiological studies underscored that microfilaremic infections and disease prevalences in border Bancroftian filariasis were not correlated. Cumulative infection prevalence in the affected Karen communities with uncertain transmission of *W. bancrofti* nocturnally subperiodic was noted to increase with increasing age (Bhumiratana *et al*, 2002). The population after 15 years of age may have experienced reinfection, generating proportionately adult worm burdens in individuals. In Myanmars, microfilaremia and/or antigenemia prevalence, in the at-risk age group ≥15 years, was significantly lower than that of the endemic Karens, indicative of the dilutional effect of the at-risk Myanmar

migrants under surveillance among the migrant population of Thailand (our unpublished data). As compares well with previous findings in India (Srividya *et al*, 1991), adulthood infection was recognized as asymptomatic microfilaremia and chronic lymphatic pathology, such as hydroceles, common in males older than 30 years. Microfilaremic and/or antigenemic infection prevalence was dynamic, *ie* showed an age-specific infection pattern, but was universal for gender. Underlying factors that contribute to increased risks of bancroftian filariases have been considered due either to their movement from one endemic and/or prone area to another, or to immigration of microfilaremic persons who might have experienced no history of selective treatment with 6 mg/kg DEC given once daily for 12 consecutive days (Filariasis Division, 1998). These phenomena have been considered to cause the current magnitude of the disease and the distribution in transmission-prone areas.

In addition, in border health issues, the impact of increased burdens of potentially infectious diseases, such as human immunodeficiency virus (HIV), tuberculosis (TB), sexually-transmitted diseases (STD), hepatitis viruses, lymphatic filariasis, and malaria, have been considered to be due to cross-border migration of those sentinel populations in disease transmission (Anonymous, 2004). Among those causing important public health and economic burdens, the incidence of HIV infection in those sentinel populations has been observed to be particularly high among the Myanmar migrant workers. Concomitant parasitic infections are common in them (Anonymous, 2004) but there are no observations of host-parasite relationships in those harboring helminthes and viral infection, specifically the co-evolutionary ecology, immunology and epidemiology of interactions between *W. bancrofti* and HIV. Little is known about interactions of HIV-infected or immunocompromized persons who develop *W. bancrofti* infection of the lymphatics or have a cryptic infection. There are two intriguing hypotheses that, having acquired infection with the L3 stage, the clustering of *W. bancrofti* infection in immunocompromized hosts occurs regularly when predisposed persons harboring adult worm burdens experi-

ence repeated infections in endemic conditions, or irregularly in those harboring low degrees of adult worm burdens. If repeated infections in adults with concomitant HIV and worms are influenced by insufficient and/or deficient dual mechanisms of immune response in affected persons the concomitant infections may or may not have established adult worm burdens or microfilaremic burdens in the population. The adult worm burdens in such concomitant HIV and *W. bancrofti* infections may be clinically important for diagnosis and treatment. Its epidemiological implications may be related to the spread and severity of *W. bancrofti* infection and the susceptibility to DEC treatment in those target populations eligible for DEC mass treatment in the PELF. Concomitant infections or *W. bancrofti* infection alone in sentinel populations and the factors that contribute to cyclic prevalence remain to be established.

In our study, using cumulative longitudinal seroprevalence data which relied on the determination of *W. bancrofti* adult worm circulating antigens and HIV immunoglobulins in those sentinel population samples tested, we describe *W. bancrofti* infection prevalence and intensity, and more interestingly, the incidence of concomitant HIV and *W. bancrofti* infections at the start of DEC mass treatment in the PELF. Based on *W. bancrofti* antigenemia clearance in response to DEC treatment, the concomitant infections in Myanmars, as well as in *W. bancrofti* infection alone, were biannually treated with a 300 mg oral-dose of FILADEC (Koyadun *et al*, 2003), as recommended for use in biannual DEC mass treatment of eligible Myanmar migrants in the PELF.

MATERIALS AND METHODS

Preparation and laboratory-confirmed classification of endemic population samples

The endemic population samples were categorized into two groups: *W. bancrofti*-endemic local Karens and cross-border Myanmar migrants. All were collected by the active case detection approach. Ethical clearance was approved by the Institutional Review Board at the Faculty of Public Health, Mahidol University. In-

formation, such as age, gender, previous and current exposures to bancroftian filariasis, concomitant infections, and history of DEC treatment, were recorded in assistance with the local Karen or migratory Myanmar translators from sites of survey.

By cross-sectional community surveys between 1998 and 1999, 212 Karens (or group A): males (M)=125 and females (F)=87, 6 to 69 years of age, residing in endemic villages in Tak Province, Northwestern Thailand, with more than 1% microfilarial positive rates reported (Bhumiratana, 2000), were selected. Microfilaria detection by night time finger-prick bloods using a conventional capillary tube technique (WHO, 1992; Filariasis Division, 1998; Bhumiratana *et al*, 1999) was done to identify microfilaremic individuals. Venous blood was collected between 19 00 and 24 00 hours. The sera were prepared, transferred to the laboratory, and stored at -20ºC until use. For laboratory-confirmed classification, the duplicate night blood specimens in individuals were examined by a Giemsa-stained thick smear (WHO, 1992; Filariasis Division, 1998; Bhumiratana *et al*, 1999) for specific identification of microfilaria and microfilarial counts. Some microfilaremia samples (n=3) were filtered with a Nucleopore® polycarbonate membrane (5 μm, 25 mmø) (WHO, 1992; Filariasis Division, 1998). The filters were separately placed into 15-ml centrifuge tubes containing 5 ml of sterile filtered RPMI 1640 medium in the presence of 25 mM HEPES pH 7.4, 32 mM Na_2HCO_3, 0.02% gentamicin, supplemented with 10-15% human serum. All tubes were kept in the ice box for days under field conditions, and transferred afterwards to the laboratory.

One hundred and seventy-two single stool samples were collected, fixed with 10% formalin, transferred to the laboratory, and examined by the formalin-ether sedimentation technique (Bhumiratana *et al*, 1999) to verify concomitant helminthic infection. The samples were categorized into three groups as follows: 1) 70 with single infections: Hookworms (Hw) (n=59); *Ascaris lumbricoides* (n=5); *Opisthorchis viverrini* (n=5); and *Taenia* spp (n = 1); 2) 29 with multiple infections: Hw, *Opisthorchis* (n=8); Hw, *Ascaris* (n=7); Hw, *Trichuris trichiura* (n=3); Hw, *Ascaris*,

Trichuris (n=2); Hw, *Opisthorchis*, minute intestinal flukes (MIF) (n=2); Hw, MIF (n=1); *Ascaris*, *Opisthorchis* (n=1); *Opisthorchis*, MIF (n=1); Hw, *Ascaris*, *Opisthorchis* (n=1); Hw, *Ascaris*, *Taenia* (n=1); Hw, *Trichuris*, *Opisthorchis* (n=1); and *Trichuris*, *Opisthorchis*, MIF (n=1); and 3) 73 negative by stool examination.

By cross-sectional surveys between 1999 and 2001, 221 other cross-border Myanmar migrants (or group B): M=147 and F=74, 13 to 55 years of age, residing for less than a two-year period of residency in urban areas of southern, northern and central Thailand, were selected. For the laboratory-confirmed microfilaremic Myanmar samples, night venous bloods of some microfilaremic samples (n=2) were collected for both microfilaria examination and serum preparation, and filtered as before. The other samples of intravenously collected EDTA-blood, were obtained from individuals after a 30-minute provocation with DEC, 300 mg oral-dose FILADEC tablet (Koyadun *et al*, 2003). These were collected during the day time for plasma preparation, then transferred to the laboratory, and stored at -20ºC until use. The microfilaria were examined afterwards by Knott's concentration technique (WHO, 1992; Filariasis Division, 1998), and by Giemsa-stained thick smear as before.

Preparation and laboratory-confirmed classification of non-endemic population samples

The 143 prospective plasma samples, used as negative controls, were collected from a *W. bancrofti* non-endemic Thai population during case finding surveys of asymptomatic and symptomatic HIV infected individuals (with/without lung pathology) and non-HIV infected individuals with lung pathology as follows. Ninety-three HIV plasma samples (or group C), including 80 with known CD4+ counts, were collected from patients who had been reported by an anonymous AID case reporting system at the Army Institute of Pathology, Pramongkutkao Medical Center, Bangkok, between 1999 and 2000. Nineteen HIV/AIDS patients (or group D), as well as 31 non-HIV patients (or group E), were all selected from Northeastern Thailand between 2001 and 2002. At the OPD of the Srinagarind Hospital, Khon Kaen University, in association with Dr

Wisut Sukeepaisarncharoen, and at the 6th Zonal Tuberculosis Control, Khon Kean Province, in association with Dr Nonglak Tessana, they were recruited following either chest x-ray radiography, sputum examination with acid-fast-bacilli (AFB) staining, or HIV antibody testing. The group D included 10 HIV, non-TB patients: paratracheal node enlargement (n=1), diffuse interstitial infiltration (n=1), non-diffuse interstitial infiltration (n=1); 4 HIV, non-TB patients: lymphadenopathy (n=3), lymphadenopathy and pleural effusion (n=1); and 5 HIV, TB patients (all AFB positive): cavitary lung (n=3); and non-cavitary lung (n=2). Group E included 13 non-HIV, TB patients (all AFB-negative): cavitary lung (n=4), non-cavitary lung (n=9); and 18 non-HIV, non-TB patients (all AFB-negative): pneumonia (n=7), pleural effusion (n=4), chronic obstructive pulmonary disease (n=1), bronchitis (n=1), lung cancer (n=1), lung mass (n=1), and pulmonary nodules (n=3).

The other negative control sera (n=90), both prospective and retrospective, were selected either from *W. bancrofti*-non-endemic Thai population samples during case finding surveys of asymptomatic and symptomatic helminthic infections, between 1998 and 2001, or from the serum bank as follows. Fifty-three normal sera (or group G) were prepared from persons negative on stool examination: having no history of helminthic and/or protozoal infections; having no travel history to endemic areas of *W. bancrofti* (Northwestern Thailand) or *Brugia malayi* (Southern Thailand). The 32 sera of the helminth-infected persons (or group F) including *Gnathostoma* (n=5), *Opisthorchis* (n=5), *Schistosoma* (n=5), *Strongyloides* (n=10), hookworm infection (n=6), and multiple infections with Hw, *Opisthorchis* and *Echinostoma malayanum* (n=1), were collected from Northern and NorthEastern Thailand. The 5 other sera of *B. malayi*-infected persons (or group F) were also collected from Southern Thailand. The samples were all transferred to the laboratory and stored at -20ºC until use.

W. bancrofti antigen detection

For the diagnosis of active *W. bancrofti* infection (Fig 1), two commercially available antigen (Ag) capture detection methods were used based on monoclonal antibodies (MAbs) specific

for *W. bancrofti* adult worm circulating antigens (WHO, 1999), which included the qualitative format as ICT Filariasis (AMRAD ICT, French's Forest, NSW, Australia) with whole blood, serum or plasma samples, and the quantitative and qualitative format, Og4C3 ELISA (Tropical Biotechnology, Townsville, Queensland, Australia) using serum or plasma samples. For the ICT Filariasis, which is based on a AD12 MAb (Weil and Liftis, 1978; Weil *et al*, 1997), all the intravenously EDTA-blood test samples and negative controls (~100 µl each), except for 20 sera of the *Strongyloides*, *Gnathostoma* and *Schistosoma* infections, were used. The Ag detection with the ICT Filariasis and interpretation of test results were performed according to the methods described elsewhere (Bhumiratana, 2000; Bhumiratana *et al*, 2002). For the Og4C3 ELISA, which is based on an Og4C3 MAb (More and Copeman, 1990; Chanteau *et al*, 1994), 100-µl frozen serum or plasma test samples in individuals and negative controls (totally 666) were used. The Og4C3 ELISA was performed with automatic processing (*ie* dispensing of the reagent and washing) using a Behring ELISA Processor (BEP) II Plus (Dade Berhing, Marburg, Germany) for menu programing. The operations (steps 1-3) were performed according to the manufacturer, but reading test results (test wells and controls) was performed with a EL312e Microplate, BIO-KINETICS READER (Bio-Tek Instruments, USA). The thawed samples were fourfold diluted, with sample diluent, in microcentrifuge tubes, and then boiled at 98ºC for 5 minutes. The boiled samples were spun down at 10,000*g* for 5 minutes, and the supernatants (50 µl each) were added into test wells (80 samples tested per ELISA plate) in a U-bottom polystyrene 96-well microtiter plate pre-coated with the Og4C3 MAb. For controls, the same 50 µl of each standard antigen (*Onchocerca gibsoni* standard Ags no. 1-7) and conjugate control per well were added into the control wells in duplicate. The reactions were incubated overnight in a dark and humid container at room temperature, and washed three times with 300 µl/well of wash buffer (step 1). The 50-µl thirty-fold diluted rabbit anti-*O. gibsoni* Ab (1:120) (antibody sandwich) was added to all the wells (step 1), incubated for one hour at room temperature in a dark container,

and then washed three times as before (step 2). For color development, 50-µl thirty-fold diluted goat anti-rabbit immunoglobulin G (IgG) conjugated to horseradish peroxidase (1:120) was added to all the wells (step 2), and then performed as before (step 3). The chromogen, 100-µl ABTS: 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonate), was added to all the wells (step 3). After a maximum of one hour of reaction incubated at room temperature in a dark container, the reactions were immediately read at a wavelength of 405 nm (A_{405}).

Anti-HIV antibody detection

Two commercially available qualitative anti-HIV Ab detection methods were used (Weber *et al*, 2002; CDC, 2003), which were based on detecting HIV immunoglobulins in the tested serum and plasma samples reacted with recombinant proteins and synthetic peptides of HIV types 1 (HIV 1) and 2 (HIV 2). These were the anti-HIV ELISA, Enzygnost® HIV Integral (Dade Behring, Marburg, Germany), and the Western blot, HIV Blot 2.2 (Genelabs® Diagnostics, Singapore Science Park, Singapore). The 3 groups (*ie* concordant-positive, discordant, and concordant-negative) of single samples (not diluted) (Fig 1) were tested using the Enzygnost HIV Integral, which allows one to determine HIV immunoglobulins that can bind to the *Escherichia coli* recombinant proteins/synthetic peptides of HIV 1 gp41, HIV 2 gp43 and HIV 1 (subtype O) gp41 pre-coated onto the wells of the ELISA microtiter plate, and any HIV p24 Ags that can bind to the rabbit anti-HIV p24 polyclonal antibody (PAb) pre-coated onto the wells. The anti-HIV ELISA was performed according to the methods described by the manufacturer, with full-automatic processing (*ie* dispensing, washing and evaluation) using the same instrument, BEP II Plus. For the test samples (91 samples tested per 96-well microtiter plate), 100 µl of undiluted samples was added into each well coated with the solid phase, containing 25 µl of sample buffer. For the controls (5 control wells) containing the same 25 µl/well of sample buffer, 100 µl/well of HIV negative control containing human serum without HIV Ag and Abs to HIV 1, HIV 2, and HIV 1 (subtype O) Ags, was added into 3 wells. The 100 µl/well of HIV positive con-

trol containing heat-treated human serum with Abs to HIV 1 Ags was added into 2 wells. The reactions were incubated at 37ºC for 30 minutes in a dark container and immediately washed four times with 300 µl/well of wash buffer (step 1). The 100 µl/well of HIV conjugate I solution containing biotin-conjugated recombinant proteins/synthetic peptides of HIV 1, HIV 2, and HIV 1 (subtype O) (antigen sandwich), as well as mouse MAb to HIV p24 (antibody sandwich), was added, and the reactions were incubated as before. In step 2, the reactions were washed as before, and 100 µl/well of HIV conjugate II solution containing streptavidin conjugated to peroxidase (POD) was added, and the reactions were then incubated as before. In step 3, the reactions were washed as before, and 25 µl/well of working chromogen solution containing TMB: 3,3′,5,5′-Tetramethylbenzidine was added into each well. The reactions were incubated at room temperature for 30 minutes in a dark container, and then stopped by adding 75 µl/well of stopping solution POD. The reactions were read within one hour at dual wavelengths, at 450 nm and for the reference wavelength at 650 nm. All reactive samples of the concordant-positive and discordant samples (both a_1 and b_1) (Fig. 1) were subjected to the HIV Blot 2.2, which was performed and interpreted in accordance with the manufacturer (Genelabs Diagnostics).

Evaluation of test results of *W. bancrofti* and HIV infections

In analysis of *W. bancrofti* (Wb) antigenemic infection profiles, serological antigen positivity (SAP) of the Wb-endemic groups (relative to Wb-non-endemic Thais as negative controls) were validated and evaluated as shown in Fig 1. Their principal characteristics, such as age, gender and concurrent infection status with microfilaremia or helminths, were described using descrip-
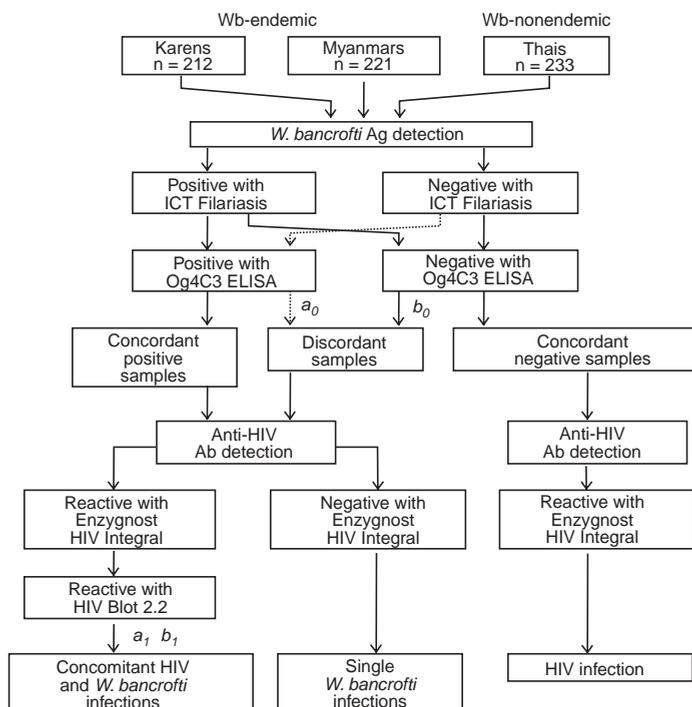

Fig 1–An algorithm for evaluation of the test results with concomitant HIV and *W. bancrofti* infections, and single *W. bancrofti* or HIV infection. Index of suspicion was high when any discordant sample (b_0) negative with the Og4C3 ELISA, but reactive with the Enzygnost HIV Integral and the HIV Blot 2.2 (b_1), was considered to have HIV and filarial infection with an existing predisposition. Some discordant samples (a_0) of varying Ag titers reactive with the Enzygnost HIV Integral and the HIV Blot 2.2 (a_1), as well as some concordant-negative samples reactive with the Enzygnost HIV Integral, were tested using the HIV Blot 2.2.
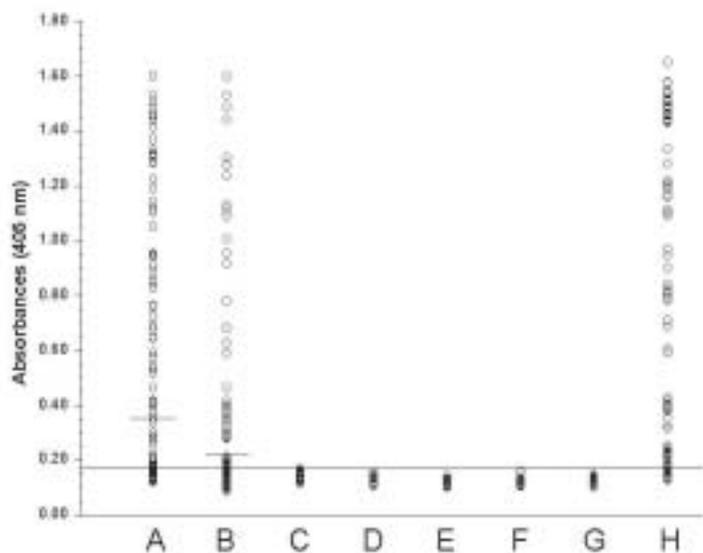
Fig 2–A scatter plot of test samples and controls with absorbances (o) measured at 405 nm, which were determined by the Og4C3 ELISA. The legends showed 7 groups: A, 212 Karens ($\bar{A}$ ± SD = 0.357 ± 0.399); B, 221 Myanmars ($\bar{A}$ ± SD = 0.226 ± 0.275); C, 93 Wb-non-endemic Thais with HIV ($\bar{A}$ ± SD = 0.139 ± 0.012); D, Wb-non-endemic Thais ($\bar{A}$ ± SD = 0.128 ± 0.015) both 14 HIV, non-TB and 5 HIV, TB (AFB positive); E, Wb-non-endemic Thais ($\bar{A}$ ± SD = 0.113 ± 0.009) both 13 non-HIV, TB (AFB negative) and 18 non-HIV, non-TB; F, 37 Wb-non-endemic Thais with the other helminthic infections ($\bar{A}$ ± SD = 0.121 ± 0.011); G, 53 Wb-non-endemic normal Thais ($\bar{A}$ ± SD = 0.122 ± 0.009); H, *O. gibsoni* standard antigens no. 1 to 7 and conjugate controls (12 control reactions per each). Wb-endemic samples with $\bar{A}$ (bars) yielded significant absorbances above the borderline ($\bar{A}_{cut-off}$=0.169).

tive statistics. In the Og4C3 ELISA, a linear regression of reciprocal anti-log Ag titers (Ag units or AU) (X) of *O. gibsoni* standard Ags no. 2 to 7 (32 to 32,000 AU) as positive controls and absorbances measured at 405 nm ($\bar{A}_{405}$) (Y) was mathematically expressed by the equation: $Y=b_0+b_1X$, or $Y=-0.527+0.477X$. Determination of the arbitrary Ag titers (AU/ml) of the positive samples was computed as follows: a sample $\bar{A}_{405}$ minus $\bar{A}_{cut-off}$ was multiplied by an arbitrary Ag titer (equal to 1589 AU) of *O. gibsoni* standard Ags (X) at an A_{405} equal to 1.0 (Y) and by a dilution factor (equal to 80). A sample was considered positive if the absorbance was greater than a mean absorbance ($\bar{A}$) of negative controls (n=233) plus 3 standard deviations ($\bar{A}_{cut-off}$).

Differences in SAPs (%) by the Og4C3 ELISA or by the ICT Filariasis between the endemic groups were tested by the χ^2 test or Fisher's exact test as appropriate ($p<0.05$) (Sheskin, 2000). Differences in Ag titers or *W. bancrofti* adult worm Ag loads between the endemic groups, or between either genders or age groups of the endemic groups, were tested by the unpaired Mann-Whitney *U* test or Kruskal Wallis test as appropriate ($p<0.05$) (Sheskin, 2000). For the test performance efficiency, the ICT Filariasis was assessed using the Og4C3 ELISA as a reference and, for indices of agreement, the κ-test was used, as well as the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) (Knapp and Miller, 1992). For the test performance discrepancy, discordant samples (both a_0 and b_0) (Fig 1) between the endemic groups were qualitatively and quantitatively assessed in a similar manner as in the χ^2 test, or the Mann-Whitney *U* test.

In analysis of HIV infection profiles, the antibodies to HIV 1 and HIV 2 infections in the 3 characterized groups of the test samples (Wb-endemic) and the negative controls (Wb-nonendemic) were also validated and evaluated (Fig 1). In the Enzygnost HIV Integral, validation of the test relied on absorbance readings for the control sera for calculating the mean values: $0.01 \leq \bar{A}_{neg} \leq 0.15$ and $\bar{A}_{pos} \geq 0.7$. The cut-off limit was calculated by the mean absorbances of the negative controls ($\bar{A}_{neg}$) plus a maximum negative value, 0.45. The test samples were grouped as follows: Negative, $\bar{A}_{sample}<$cut-off - 0.05; Reactive, $\bar{A}_{sample}>$cut-off; Equivocal, cut-off -0.05$\leq \bar{A}_{sample} \leq$cut-off. Agreement of test results by the Enzygnost HIV Integral and the HIV Blot 2.2 was performed using the 3 characterized groups of the Wb-endemic and Wb-nonendemic test samples as shown in Fig 1. Criteria of Enzygnost HIV Integral test results repeatedly reactive with the HIV Blot 2.2 were defined according to the Centers for Disease

Control and Prevention (CDC, 1989) and World Health Organization (WHO, 1997).

Analysis of the incidence of concomitant HIV and *W. bancrofti* infections

To verify the incidence of the concomitant HIV and *W. bancrofti* infections, the Friedman two-way analysis of variance (p<0.05) (Sheskin, 2000) was used to describe significant *W. bancrofti* antigenemia clearance after being given treatment twice a year (6-month interval) with the 300 mg oral-dose FILADEC tablet. Whereas the Spearmann rank correlation coefficient *r* (p<0.05) (Sheskin, 2000) was used to estimate the association between Ag loads (AU/ml) and time (months) required to clear antigenemia between the concomitant infections and the single *W. bancrofti* infection.

RESULTS

W. bancrofti infection profiles in the sentinel populations

Of the 666 samples serologically determined by the Og4C3 ELISA, 131 (19.7%) endemic samples including 78 (36.8%) of the 212 Karens and 53 (24.0%) of the 221 Myanmars (Table 1) showed significant absorbances above the borderline (Fig 2). The negative controls (groups C to G) were all negative with the test. With the ICT Filariasis, there were 86 (19.9%) endemic samples, including 56 (26.4%) Karens and 30 (13.6%) Myanmars (Table 1). There was one Wb-non-endemic sample positive with the ICT Filariasis (b_0) (Fig 1). Table 1 shows significant differences in the SAPs in the 433 samples by the two Ag detection methods (χ^2=247.40, p<0.001). Even in the groups there was a significant difference in the SAP in the Karens (χ^2=130.74, p<0.001) and in the Myanmars (χ^2=110.03, p<0.001) (Table 1). However, the 45 (10.4%) discordant samples, negative with the ICT Filariasis but positive with the Og4C3 ELISA (a_0) (Fig 1), included 22 (10.4%) Karens and 23 (10.4%) Myanmars (Tables 1 and 2), with no statistical significance (χ^2=3.23, p=0.072). To determine *W. bancrofti* infection profiles that were considered to be valid, good agreement between the test results of the two Ag detection methods was observed (κ=0.75) (Table 2) by exclud-
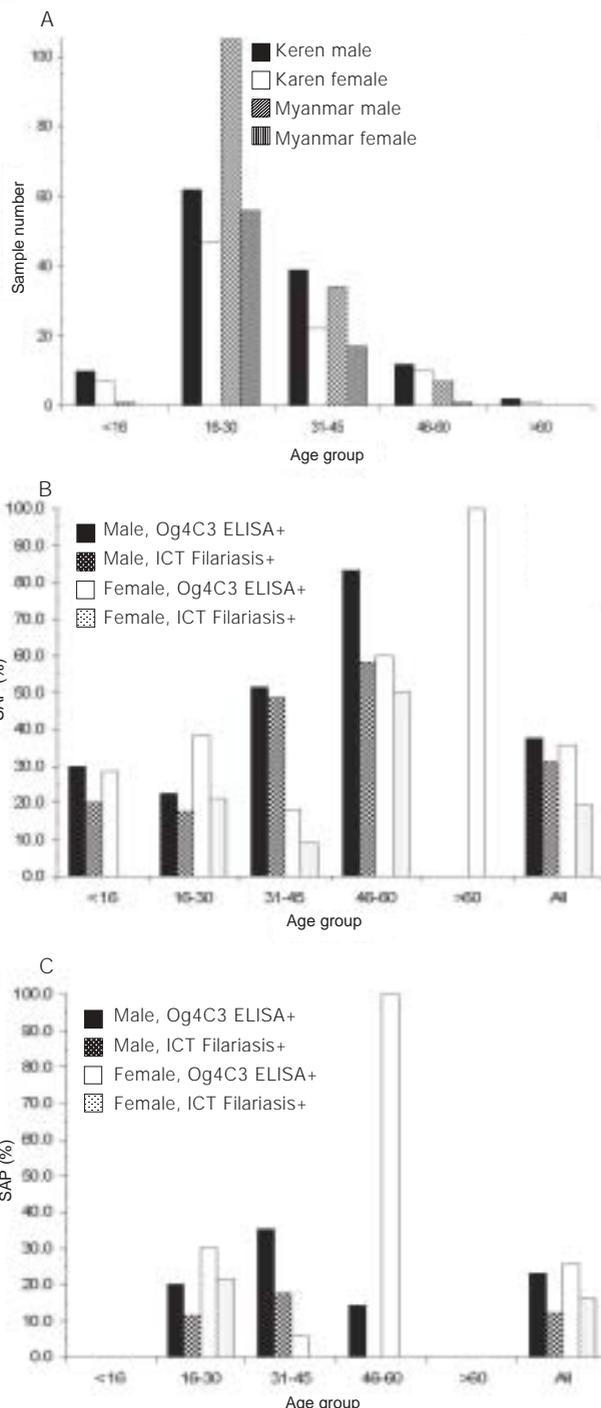
Fig 3–The *W. bancrofti* antigenemic infection in relation to age and gender between the endemic groups (A) showing the SAPs (%) that were determined by the two Ag detection methods (ICT Filariasis+ or Og4C3 ELISA+ = positive) in the Karens (B) and the Myanmars (C).
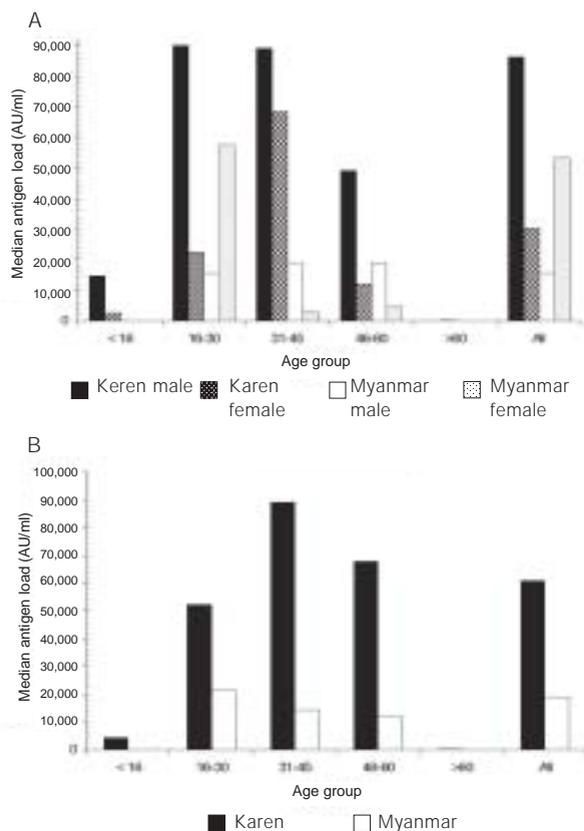
A

B

Fig 4–The *W. bancrofti* infection intensity, median antigen load (AU/ml), between the endemic groups (A) in relation to age and gender (B).

ing one Wb-non-endemic sample positive with the ICT Filariasis (b_0). Using the two Ag detection methods for the 666 samples, there were 86 (12.9%) concordant-positive samples, 46 (6.9%) discordant samples, and 534 (80.2%) concordant-negative samples.

In relation to age and gender (Fig 3A), the overall SAPs in the Karen females: 87 F aged 7 to 64 years, (mean ± SD = 29.5 ± 11.1 years) showed 31 F (35.6% with the Og4C3 ELISA) aged 15 to 64 years (mean ± SD = 31.3 ± 13.0 years), or 17 F (19.5% with the ICT Filariasis), with statistical significance (χ^2=38.17, p<0.001) (Fig 3B). Of the 125 Karen males aged 6 to 69 years (mean±SD=29.5±12.4 years), there were 47 M (37.6% with the Og4C3 ELISA) aged 15 to 60 years (mean ± SD = 34.9 ± 12.6 years), and 39 M (31.2% with the ICT Filariasis), with statistical significance (χ^2=94.08, p<0.001). In other words, between genders of all age groups (Fig

3B), there was no significant difference in the SAPs with the Og4C3 ELISA (χ^2=0.08, p=0.77) or the ICT Filariasis (χ^2=3.59, p=0.058). In relation to age, excluding age group >60 years, there was significant difference in the SAPs with the Og4C3 ELISA (χ^2=15.52, p<0.001) and with the ICT Filariasis (χ^2=11.35, p=0.01) (Fig 3B).

In similar fashion, the Myanmars: 74 F aged 16 to 50 years (mean±SD=26.4±8.7 years) and 147 M aged 13 to 55 years (mean±SD=29.8±9.0 years) (Fig 3A) were serologically determined. In the Myanmar females, the SAPs showed 19 F (25.7% with the Og4C3 ELISA) aged 16 to 50 years (mean±SD=24.6±8.5 years), or 12 F (16.2% with the ICT Filariasis). In the Myanmar males, the SAPs showed 34 M (23.1% with the Og4C3 ELISA) aged 16 to 50 years (mean ± SD=28.9 ± 9.3 years), or 18 M (12.2% with the ICT Filariasis) (Fig 3C). For gender, there was the same significant difference in the SAPs with the two tests (Fisher's exact test, p<0.001) (Fig 3C). Among the age groups, excluding <16 and >60 years, there was no significant difference in the SAPs with the Og4C3 ELISA (χ^2=0.79, p=0.961) or the ICT Filariasis (χ^2=1.64, p=0.441) (Fig 3C). The SAPs had no significant difference between genders with the Og4C3 ELISA (χ^2 = 0.18, p = 0.676) or the ICT Filariasis (χ^2=0.66, p=0.416) (Fig 3C).

In analysis of *W. bancrofti* infection intensity in the endemic, median Ag load (AU/ml) was used. In the antigenemic Karens, including 47 M with Ag loads of 1,017-197,290 AU/ml (median=86,442 AU/ml) and 31 F with Ag loads of 127-181,654 AU/ml (median=30,382 AU/ml), the infection intensity was high (range=127-197,290 AU/ml, median=60,827 AU/ml) (Fig 4A). There was significant difference in the antigen loads between the Karen males and females (p=0.016) (Fig 4B). Among the age groups within the Karens, there was significant difference in the Ag loads (p=0.031). There was no correlation between antigen loads and ages (r=0.2, p=0.079). In the antigenemic Myanmars, including 34 M with antigen loads of 127-181,654 AU/ml (median=15,572 AU/ml) and 19 F with Ag loads of 763-172,883 AU/ml (median=53,390 AU/ml), a lower infection intensity was seen (range=127-181,654 AU/ml, median=19,068

Table 1
Test performance discrepancy of the two Ag detection methods with the endemic groups.

| Source | Samples tested | Samples positive (% SAP) | | Samples with test performance discrepancy (%) |
		ICT Filariasis	Og4C3 ELISA	
Karens	212[d]	56 (26.4)[a]	78 (36.8)[a]	22 (10.4)*
Myanmars	221	30 (13.6)[b]	53 (24.0)[b]	23 (10.4)*
Total	433	86 (19.9)[c]	131 (30.2)[c]	45 (10.4)

[a] $p < 0.001$, [b] $p < 0.001$, [c] $p < 0.001$, * $p = 0.072$.
[d]For eosinophil counts (%): range=1-36%, 18 samples presented normal eosinophil counts varying from 1% to 3% and the others with >3%. For the concomitant helminths (*ie* 4 groups of single, multiple, negative, and not-done), the SAPs in all groups significantly different with the ICT Filariasis (χ^2 = 9.62, p = 0.022), but not with the Og4C3 ELISA (χ^2 = 6.73, p = 0.081). Within the groups by the different Ag detection methods, the SAPs are shown for the multiple and the negative significantly different (Fisher's exact test, p<0.001), as well as the single (χ^2=54.98, p<0.001) and of the not-done (χ^2=21.54, p<0.001) findings.

Table 2
Test performance efficiency of the two Ag detection methods with the 665 samples.*

| ICT Filariasis | Og4C3 ELISA[a] | | |
	Positive	Negative	Total
Positive	86[b]	0	86
Negative	45[c]	534	579
Total	131	534[d]	665

*Excluding one discordant sample (T1).
[a]Sensitivity=65.6% (95%CI=57.5-73.7%), Specificity=100%, NPV=92.2% (95%CI=90.0-94.4%), PPV=100%; κ=75%.
Three groups determined after the two Ag detection methods included concordant-positive[b], discordant[c], and concordant-negative[d] samples.

AU/ml) (Fig 4A). There was a significant difference in the Ag loads between the genders (p=0.027) (Fig 4A). Among the 3 age groups within the Myanmars, there was no significant difference in the Ag loads (p=0.433). Overall, the Ag loads between the endemic groups were significantly different (p=0.014) (Fig 4A).

In addition, regarding the microfilaremic infection status, infection intensity was observed between amicrofilaremic and microfilaremic groups of the Karens and the Myanmars. Of the 78 antigenemic Karens, there were 13 with microfilaremia (range=97,501-181,654 AU/ml, median=158,392 AU/ml) and 65 with amicrofilaremia (range=127-197,290 AU/ml, median=43,729 AU/ml). Of the 54 antigenemic Myanmars, there were 7 with microfilaremia (range = 57,712-167,417 AU/ml, median = 140,468 AU/ml) and 46 with amicrofilaremia (range = 127-181,654 AU/ml, median=15,572 AU/ml). In the endemic groups, microfilaremic infection tended to have Ag loads significantly higher than amicrofilaremic infection (p<0.001). Moreover, there was no significant difference (p=0.742) in the Ag loads of the discordant samples (a_0) between the Karens (range=127-31,399 AU/ml, median=2,924 AU/ml) and the Myanmars (range=127-57,712 AU/ml, median=2,670 AU/ml) (Table 1).

Concomitant HIV and *W. bancrofti* infection profiles

The 3 groups (concordant-positive, discordant, and concordant-negative) of the 666 samples were used to determine HIV immunoglobulins. Of the 86 concordant-positive samples (*W. bancrofti* antigenemias) (Table 2) including the 56 Karens ($\bar{A}_{sample}$±SD=0.046±0.016) and the 30 Myanmars ($\bar{A}_{sample\ (n=29)}$±SD=0.036±0.009), there was only one amicrofilaremic antigenemic Myanmar female (M3) reactive with the Enzygnost HIV Integral (Table 3). Of the 46 discordant samples (*W. bancrofti* antigenemias) (Table 2), 22 Karens ($\bar{A}_{sample}$±SD=0.058±0.02), 23 Myanmars ($\bar{A}_{sample\ (n=20)}$±SD=0.04±0.016) and one Thai (T1) in group C , there were 2
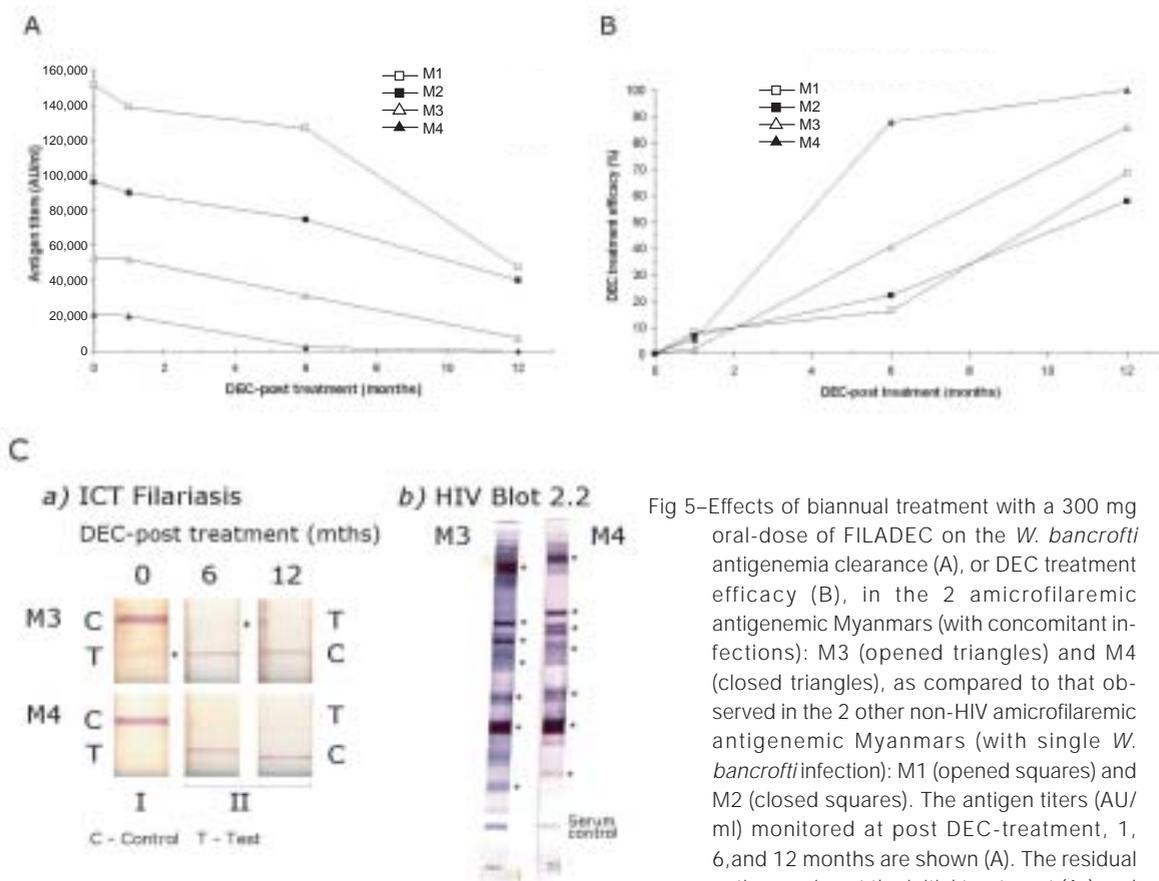
Fig 5–Effects of biannual treatment with a 300 mg oral-dose of FILADEC on the *W. bancrofti* antigenemia clearance (A), or DEC treatment efficacy (B), in the 2 amicrofilaremic antigenemic Myanmars (with concomitant infections): M3 (opened triangles) and M4 (closed triangles), as compared to that observed in the 2 other non-HIV amicrofilaremic antigenemic Myanmars (with single *W. bancrofti* infection): M1 (opened squares) and M2 (closed squares). The antigen titers (AU/ml) monitored at post DEC-treatment, 1, 6,and 12 months are shown (A). The residual antigenemias at the initial treatment (A_0) and in response to DEC treatment (A_T) were used for the calculation of the long-term DEC efficacy with macrofilaricidal activity (%) as follows: A_0-A_T/A_0x100 (B). In panel C, using the former ICT Filariasis (I) or the latest NOW® ICT Filariasis (II) (Binax, Portland, Maine, USA) (*a*), the test results of the M3 and M4 observed at 3-point intervals of follow-up periods are shown. In the M3, the asterisks indicate ICT Filariais-weakly positive on the initial treatment, as the others were considered negative with the test. For the Enzynost HIV Integral and the HIV BLOT 2.2 (*b*) the HIV immunoglobulins in them that reacted with reactive antigen bands (relative to strong reactive control) as denoted by the asterisks were detected at the initial treatment and at 12-months post-treatment (data not shown).

amicrofilaremic antigenemic Myanmars (M4 and M5) reactive with the test, as well as the T1 (Table 3). Of the 534 concordant-negative samples, 134 Karens ($\bar{A}_{sample}$±SD=0.046±0.019) and168 Myanmars ($\bar{A}_{sample\,(n=164)}$±SD=0.04±0.017), there were 4 amicrofilaremic non-antigenemic Myanmar males reactive on the test. Three (5.7%) of the *W. bancrofti* antigenemic Myanmars with HIV immunoglobulins (Table 3 and Fig 5), and 7 (3.2%) of the Myanmars with HIV immunoglobulins alone were found in this study. All 7 Myanmars with HIV infection ($\bar{A}_{sample}$ greater than 2.5) were confirmed by the HIV Blot 2.2 to have

antibodies to HIV 1 (Fig 5), as well as one discordant sample (T1) (Fig 6). M3 and M4 were treated twice a year with a 300 mg oral-dose of FILADEC, compared to the non-HIV amicrofilaremic antigenemic Myanmars (M1 and M2) (Table 3 and Fig 5). During a 12-month follow-up after the course of treatment, the residual antigenemias in them were detected with the Og4C3 ELISA before and after treatment (Fig 5A). A significant decrease in the residual antigenemias in all cases was noted (χ_r^2=12.0, p=0.007) (Fig 5A). Increases in the DEC treatment efficacy in all cases were observed and, at

Table 3
W. bancrofti and HIV infection profiles in some concordant-negative, concordant-positive, and discordant samples with a history of DEC treatment.

Case no.	Age (years)	Sex	History of DEC treatment profiles[a]	Diagnosis of *W. bancrofti* infection			HIV Ab detection[d]
				Mf detection[b]	Ag detection		
					ICT Filariasis[c]	Og4C3 ELISA (AU/ml)	
K1	33	F	Discharged	Neg	Neg	Neg	Neg
K2	24	F	Discharged	Neg	Neg	Neg	Neg
K3	69	M	Discharged	Neg	Neg	Neg	Neg
K4	31	M	Discharged	Neg	Pos++	87,459	Neg
K5	50	F	Discharged	Neg	Pos++	37,246	Neg
K6	51	M	Discharged	Neg	Pos++	51,484	Neg
K7	42	M	Discharged	Neg	Pos+	8,009	Neg
K8	15	M	Followed-up	Neg	Pos+	14,746	Neg
K9	33	M	Followed-up	Pos	Pos++	142,120	Neg
K10	48	F	Followed-up	Pos	Pos++	158,392	Neg
K11	46	M	Followed-up	Neg	Pos++	98,772	Neg
K12	45	M	Followed-up	Neg	Pos++	144,408	Neg
M1	17	F	No	Neg	Pos++	151,400	Neg
M2	36	M	No	Neg	Pos++	96,230	Neg
M3	25	F	No	Neg	Pos++	53,390	Pos++
M4	25	M	No	Neg	Neg	21,229	Pos++
M5	18	F	No	Neg	Neg	2,034	Pos++
M6	18	F	Provocative	Pos	Neg	57,712	Neg
T1	26	M	No	ND	Pos++	Neg	Pos++

Abbreviation; Ab=antibody, Ag=antigen, AU/ml=antigen units per milliliter, F=female, M=male, ND=not done, Neg=negative, Pos=positive.
[a]Discharged persons (K1 to K7) were given a full course of biannual selective treatment with 6 mg/kg oral-dose DEC given once daily for 12 consecutive days, and repeated twice a year for 2 consecutive years. Followed-up persons (K8 to K12) were given DEC doses: 4 for the K8; the same 2 for the K9 to the K11; and only one dose for the K12. The DEC-provocative person (M6) was given a 300 mg single oral-dose of FILADEC via hospital-based active surveillance for burdens of infectious diseases and drug abuses in registered foreign laborers' registration and health survey.
[b]Microfilaremic persons positive with the thick smear had Mf densities (Mf/ml) of 142 (K9) and 42 (K10). But in the M6, with the DEC-provocative day test, the Mf density in day blood was 4 Mf/ml.
[c]For a sample positive with the ICT Filariasis: Pos+=weakly positive after 15 minutes but within 45 minutes of closing the card; Pos++=strongly positive within 15 minutes.
[d]For a sample positive with the Enzygnost HIV Integral: Pos++=strongly positive with an absorbance value greater than 2.5.

12-months post-treatment, DEC efficacy (85% to 100%) in M3 and M4 was higher than that observed in the M1 and M2 (57% to 68%) (Fig 5B). In other words, the Ag clearance in the concomitant infections was correlated with the time required to clear antigenemias ($r=-0.732$, p=0.039), as well as in the single *W. bancrofti* infection ($r = -0.781$, p = 0.022). In addition, in M3 the antigenemia at 12 months post-treatment was positive with the ICT Filariasis and, in the M4, it was negative with this test before and after treatment (Fig 5C).

DISCUSSION

There is increasing evidence that bancroftian filariasis exists in sentinel populations in Thailand due to cross-border migration. Such people are predisposed to concomitant infections with two or more infectious agents, which
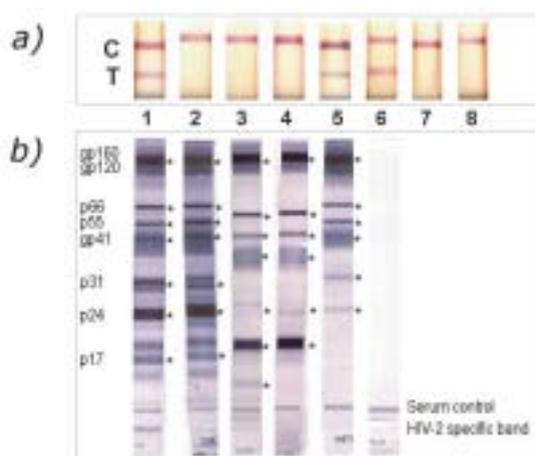
Fig 6–A 26-year old Thai male (T1) with HIV immuno-globulins and circulating filarial antigens who dropped out of DEC treatment. Using ICT Filariasis (*a*), the legends of the strips shown are as follows: 1) Wb-endemic serum with high density of >9,900 microfilariae per ml; 2-5) the same as in *b*; 6) dog serum with *Dirofilaria immitis* microfilariae; 7) dog serum with *D. repens* microfilariae; 8) normal dog serum. Using the HIV Blot 2.2 (*b*), the legends of the strips shown are as follows: 1) strongly reactive control serum; 2-4) HIV plasma samples of group C with varying CD4+ counts of 1, 16, and 32%, respectively; 5) T1 (of discordant samples) positive with the ICT Filariasis but negative with the Og4C3 ELISA; 6) negative control serum. The asterisks indicate reactive antigen bands to be considered valid when interpreting the pattern (relative to the strongly reactive control that is reactive for antibodies to HIV 1 and HIV 2).

may affect border health development (Anonymous, 2004), particularly community-directed treatment programs to eliminate lymphatic filariasis in Thailand. Understanding their role in disease transmission is necessary for infection control personnel, epidemiologists and scientists to figure out the modes of transmission and the severity of infection in the at-risk population under surveillance. Bancroftian filariasis in the local Karens in the border areas has been considered a source of transmission to schoolchildren and high risk groups. Imported bancroftian filariasis in Myanmar migrants in transmission-prone areas in Thailand has been considered a source of transmission in schoolchildren and persons with existing predispositions, such as

impaired immunity. In our study, we demonstrated that, based on the *W. bancrofti* adult worm Ag loads, bancroftian filariases occurred frequently within the endemic groups studied prior to the start of DEC mass treatment in the PELF. Using baseline seroprevalence data, a high infection prevalence was found to occur in both endemic groups. Heretofore, Myanmar migrant workers had been considered a source of re-emergence of transmission. HIV persons co-infected with *W. bancrofti* were studied to evaluate their susceptibility to DEC.

At the start of the DEC mass treatment phase in the PELF, the baseline seroprevalence data in the endemic groups were evaluated using two commercially available Ag detection methods used in the global program to eliminate lymphatic filariasis (GELF) (WHO, 1999). This program has not only proposed the determination of the overall infection prevalence in the population with, or at risk for infection, but also the monitoring and evaluation of the effectiveness of therapy in areas targeted for mass treatment (WHO, 1999, 2001, 2002). Prior studies demonstrated that, regardless of microfilaremic infection, cryptic infection, or other clinical manifestations, a high degree of sensitivity and specificity of the Ag detection methods has been validated to detect *W. bancrofti*-infected individuals in endemic areas (Itoh *et al*, 1996; Ngugen *et al*, 1999; Simonsen and Dunyo, 1999; Ramzy *et al*, 2000), or in areas prone to transmission of *W. bancrofti* (Omar *et al*, 2000; Koyadun *et al*, 2003). The ICT card test is based on the specific AD12 MAb that recognizes excretory-secretory (ES) Ag present in humans infected with *W. bancrofti* (Weil and Liftis, 1987; Weil *et al*, 1997). The Og4C3 ELISA is based on the specific Og4C3 MAb that reacts with ES Ags of *W. bancrofti*, 50-60 kDa and >130 kDa (More and Copeman, 1990). Two MAbs have no cross-epitopes on these ES Ags, hence the detection of *W. bancrofti* adult worm ES Ags present in human blood is relied on quality control and the diagnostic procedures of the tests. We demonstrated that, regardless of the Thai HIV sample (T1), the specificity of the ICT card test was 100%. The false-negative rate (34.4%) was high using the test samples of the cross-border

Myanmar migrants and the endemic Karens residing along the Thai-Myanmar border (Table 2). The sensitivity and specificity of the ICT card test was similar to the Og4C3 ELISA (Weil *et al*, 1997; Ngugen *et al*, 1999; Ramzy *et al*, 2000): high, using microscopy as a reference (Bhumiratana *et al*, 1999; Pani *et al*, 2000). The sensitivity of the Og4C3 ELISA decreased when using microfilaremic samples having ultra-low microfilarial densities (Rocha *et al*, 1996). The sensitivity of the ICT card test relied on microfilarial densities (Pani *et al*, 2000). In other words, with some endemic samples, the ICT card test had false-negative test results when it was performed with whole blood, serum, or plasma samples (usually not required for pretreatment) of either microfilaremic or amicrofilaremic patients. Contrary to prior studies (Itoh *et al*, 1996; Ngugen *et al*, 1999; Simonsen *et al*, 1999; Omar *et al*, 2000; Ramzy *et al*, 2000; Koyadun *et al*, 2003), there is discrepancy between the ICT card test and the Og4C3 ELISA, as shown in Table 1. Amicrofilaremic findings had an overall 10% discordance between the endemic groups or within the groups. The discordant samples in the endemic groups had the same low Ag load intensity 7- to 20-fold lower than the concordant-positive samples. This study found that in the discordant Myanmar samples (a_0), only one microfilaremic sample (M6) having an initial Ag load of 57,712 AU/ml (A_{405} = 0.623) was negative with the ICT card test (Table 3) but the pretreated one (0.1 ml) prepared for the Og4C3 ELISA was positive with the ICT card test (data not shown). After receiving the DEC-provocative dose, the untreated sample had an Ag load of 31,780 AU/ml (A_{405}=0.419). At 2-weeks post-treatment, it was still negative with the ICT card test, but the pretreated sample was positive with the test (data not shown). The 5 other untreated serum samples of the Ag-positive Karens (amicrofilaremic) had varying Ag loads of 25,297-31,399 AU/ml (A_{405} values of 0.368-0.416), and were negative with the ICT card test, but the pretreated samples were positive with the test (data not shown). Except for M6, which had an eosinophil count of 7% and whose stool specimens was not obtained, all subjects aged 22 to 56 years had eosinophil levels varying from 3% to 20%: 3 were helminth-negative (5% to 10%

eosinophils), one had no stool specimen, and one the highest eosinophil count, and was HW-positive. When the endemic Karen samples were parasitologically and serologically examined for concomitant helminths (4 groups: single, multiple, negative, and not-done) and eosinophil counts, the SAPs in all the groups were significantly different with the ICT card test, but not with the Og4C3 ELISA. The concordant-positive Karen samples were more likely to show that, among the 3 groups of single, multiple, and negative findings, eosinophil counts and adult worm Ag loads were not correlated. In the discordant Karen samples (a_0) excluding the 6 in which stool specimens were not done, 7 discordant samples showed 4 single infected with HW or OV, with 3% to 20% eosinophils, and 3 multiple infected, with HW and flukes with 7% to 11% eosinophils. The 9 other discordant samples which were helminth-negative had eosinophil counts varying from 4% to 19%. Because of the antigenicity of the helminths, the concomitant helminthic infections were considered as generating the antigenic variation of their ES Ags and surface Ags. The nature of immunity to extracellular helminths depends on function of the Th_2 cells. Their cytokine production will lead to activation of B cells, antibody production, and proliferation and differentiation of eosinophils (Cox, 2001). In addition to human IgG, IgM and IgA Abs to the concomitant helminths, Ab production of specific and nonspecific IgE is commonly observed. These phenomena suggest that in the native form ES Ag may be complexed with specific and nonspecific Abs in some infected individuals harboring very high Ag loads, which are distinguishable from excess Ag-Ab immune complexes. The Abs may mask the epitopes of the PAb specific for the 200 kDa ES Ag. The PAb gold conjugate with antigen free always gives a valid control line when using untreated Ag-positive serum or plasma samples. When using the pretreated Ag-positive samples, the 200 kDa ES Ag (in heat-stable form) complexed with the PAb gold conjugate was captured by the specific AD12 MAb and hence the two valid test and control lines. The ICT card test with the discordant samples of both endemic groups, even in the M4 having much higher Ag loads, is likely to give a false-negative result. These find-

ings suggest that the detection limit of the ICT card test in this study was 127-25,297 AU/ml. On the other hand, the sensitivity of the ICT card test increased with increasing Ag loads. In epidemiological assessment, infection harboring adult worm Ag loads in individuals with natural infection or with DEC suppression would be a drawback of the ICT card test for use in the field conditions and in the public health reference laboratory in Thailand. The sentinel population with a history of DEC treatment, as shown in Table 3, can be evaluated and monitored by the Ag detection methods in order to identify the clearance of *W. bancrofti* infection (as in K1 to K3) or active *W. bancrofti* infection during (as in the K8 to the K12) and after (as in the K4 to the K7) a full course of the treatment. The ICT card test needs to be reevaluated regarding its quality, the validity of the test, and discrepancies when a large number of the sentinel population is sampled. We had no data regarding the reliability of the ICT-test in regards to intra- and inter-observer variation.

In analysis of *W. bancrofti* infection profiles with the SAPs between the endemic groups, it was clear that the epidemiological focus of the bancroftian filariases had an age-specific infection pattern. In the Karens, cumulative infection prevalence and intensity, as shown in Fig 4B, did not change. In the population after 50 years, a low level of infection prevalence was noted. For genders, the infection prevalence in the Karen males was similar to the Karen females, as shown in Fig 3B. The infection intensity in the Karen males was higher than in the Karen females, as shown in Fig 4A. It was unclear if the cumulative infection prevalence and intensity observed in the Myanmar migrants was related to age, gender, or infection status, since the sample was not representative of the entire population. In the at-risk Myanmar migrants with the SAPs, as shown in Fig 3C, in imported bancroftian filariasis, the infection prevalence in Myanmar males was similar to Myanmar females. Contrary to the Karens, the infection intensity in the Myanmar females rather than in the Myanmar males was relatively higher. We had no additional data supporting that high infection intensity in the Myanmar females might suggest an occupational risk for Bancroftian filariasis in the town-

ships of Myanmar, where female workers may have experienced infection exposure in the community. In border bancroftian filariasis, Karen males had more infection exposure to L3 than Karen females did, which suggests that they may have been exposed outside the community. Consequently, they may have experienced repeated infections, generating the microfilaremias and amicrofilaremias in the population. This could be explained by a model of infection dynamics in nature that, in endemic groups, the infection intensity in microfilaremic persons rather than in amicrofilaremic persons was higher. The Ag load intensity in the Karens was 4-fold higher and in the Myanmars 9-fold higher. In the analysis of *W. bancrofti* infection profiles in those sentinel populations exposed to concomitant HIV, we found that current situation of HIV infection in the Karens showed an HIV seronegative baseline. In the Myanmar migrants, 7 persons with HIV 1 or 2 (M3 to M4) were infected with *W. bancrofti* and had HIV Abs to p24 and p31 (Fig 5C), which are known to decrease during the course of AIDS. It was interesting to note that in those with concomitant infections, the adult worm Ag loads were lower than expected. In the M3 and M4 (of the discordant Myanmar samples), it was clear that, without the course of AIDS, the *W. bancrofti* was susceptible to the biannual DEC treatment with the 300 mg oral-dose of FILADEC. Compared to the single *W. bancrofti* infection in the M1 and the M2 groups with higher initial Ag loads, reduction of the adult worm Ag loads in the concomitant infections was correlated with time required to clear antigenemias. Those with concomitant infections were more likely to show a high degree of DEC treatment efficacy at the 12-months post-treatment. They tolerated the DEC treatment well. The Ag clearance in all cases were affected by the macrofilariacide (McCarthy *et al*, 1995; Koyadun *et al*, 2003). In spite of the lack of understanding of immunity to *W. bancrofti* infection with concomitant HIV, which may be differentiated from opportunistic parasitoses with helminths, such as with strongyloidiasis causing hyperinfection (Ambroise-Thomas, 2001), and immune response to the DEC treatment, the treatment efficacy and tolerability in concomitant infections accounts for the direct macrofilaricidal

activity of the DEC that can be given to the eligible Myanmar migrants with or without HIV. In other words, those with concomitant infections in the sentinel populations can be effectively treated with DEC in the DEC mass treatment phase of the PELF.

In addition, we found that the Ag detection methods performed well with plasma samples of the non-endemic Thai HIV/non-HIV patients with/without lung pathology, which were all negative, the T1 (b_0) which was ICT card test-positive (Fig 6A) but Og4C3 ELISA-negative (A_{405} = 0.141). We can not rule out the possibility of false positivity on the ICT card test based on the AD12 MAb specific for the *W. bancrofti* ES Ag at 200 kDa that is raised against *D. immitis* (Weil and Liftis, 1987). Similar to dog serum with *D. immitis*, it reacted strongly with the ES Ag present in the T1 sample, whereas on a serum sample of a *D. repens*-infected dog, it did not react. The Og4C3 MAb against *O. gibsoni* (More and Copeman, 1990) did not react with the ES Ag on the T1 sample. It is unwise to suggest that, perhaps with the course of AIDS, active *W. bancrofti* infection was present in the T1 harboring small amounts of the Abs to p24 and p31 (b_1). If, on the other hand, an opportunistic infection of the animal filarial parasites occurs, the ES Ag with the high daily-outputs present in the T1 sample, could be misdiagnosed with the ICT card test. This suggests filarial zoonosis in an immunocompromised host in the population tested may be a sporadic pattern of zoonotic filarial infection (Flieder and Moran, 1999) more than a cryptic filarial case (Ong and Doyle, 1998). We could not retest this T1 sample either with the same Ag detection methods or with ELISAs for *Dirofilaria* spp. and investigate the incidence of a zoonotic filarial infection with the DEC-therapeutic diagnosis (Glickman *et al*, 1986; Jelinek, 1996). However, it is possible that such a zoonotic filarial infection occurring in the T1, but rarely in the population, was due to mosquito-borne transmission of *D. immitis*, which is widely distributed in Thailand. In transmission-prone areas, *W. bancrofti* Ag screening by the ICT card test with daytime finger-pricked blood samples in the sentinel populations under surveillance in the DEC mass treatment phase in the PELF needs to be theoretically and logistically analyzed when such at-risk Thai persons are used for foci investigation of transmission.

In conclusion, initial assessment of the PELF, to recognize the *W. bancrofti* adult worm Ag loads in the sentinel population is valuable to estimate infection prevalence and identify areas to target for treatment. Its clinically diagnostic and epidemiological implications will have an important role in the evaluation and monitoring of DEC treatment efficacy in those sentinel populations, including those with concurrent HIV infection eligible for DEC mass treatment in the PELF. Based on baseline seroprevalence data, immunoepidemiological study of *W. bancrofti* infection that focuses on age-and sex-specific infection prevalence and intensity in those target populations should improve our understanding of the immunity to concomitant infections, and the immune response to DEC treatment in order to optimize and improve coverage and compliance with the DEC mass treatment program.

of participants in the study.

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