# THE IMPACT OF A NATIONAL PROGRAM TO ELIMINATE LYMPHATIC FILARIASIS IN SELECTED MYANMAR IMMIGRANT COMMUNITIES IN BANGKOK AND RANONG PROVINCE, THAILAND

Wichai Satimai[1], C Jiraamonnimit[1], S Thammapalo[2], W Choochote[3], P Luenee[4], JJ Boitano[5] and S Wongkamchai[5]

[1]Bureau of Vector-Borne Disease, Department of Disease Control, Nonthaburi; [2]Office of Disease Prevention and Control # 12, Songkhla; [3]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai; [4]Department of Clinical Pathology, [5]Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

**Abstract.** Some immigrants from Myanmar to Thailand have brought *Wuchereria bancrofti* infections with them, causing a community health problem for Thai citizens. The seroprevalence of bancroftian filariasis was detected in 438 and 512 Myanmar immigrants residing in Bangkok and Ranong Provinces, respectively, along with 81 Thai citizens living in Bangkok. The immunochromatograpy card test was positive in 5 Myanmar immigrants living in Bangkok and 1 living in Ranong for a prevalence of 0.63%. Antifilarial IgG4 antibodies were found in 21 Myanmar immigrants living in Bangkok and 14 living in Ranong for a prevalence of 3.68%. None of the samples from Thai citizens were positive with either test. These prevalence rates are lower than those observed between 2001 and 2005. The Thai mass drug administration program to eliminate lymphatic filariasis among Myanmar immigrants appears to be a successful public health strategy.

**Keywords:** *Wuchereria bancrofti,* Myanmar immigrants, ICT antigens, IgG4 antibodies

## INTRODUCTION

In 1997 the WHO set forth the goal to completely climinate lymphatic filariasis (LF) by the year 2020. The world-wide population at risk for LF was estimated to be 1.254 million people in December 2006; 83 countries world-wide were endemic and the highest proportion of cases (64%) were in Southeast Asia, with 9 of 11 countries being endemic (WHO, 2006). In 2009, Thailand was one of 3 countries with a prevalence of microfilaremia of under 1%, making it eligible for post-mass drug administration (post-MDA) surveillance and certification in the process of eliminating LF (WHO, 2010). Two of the major goals of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) are (a) to reduce and eliminate transmission of this disease and (b) to obviate the suffering of affected individuals by controlling morbidity (Ottesen and Weil, 2004). The Thai provinces

Correspondence: Dr Sirichit Wongkamchai, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok Noi, Bangkok 10700, Thailand.
Tel: + 66 (0) 2419 6468; Fax: + 66 (0) 2411 2084

on the Thai-Myanmar border endemic for bancroftian filariasis in the Northwest are Mae Hong Son, Tak and Kanchanaburi and in South, it is Ranong; further south but not on the border, Phang-nga Province is also endemic (Nuchprayoon *et al*, 2003a; Koyadun and Bhumiratna, 2005; Bhumiratana *et al*, 2010). Thailand's national PELF began in 2002 and consisted of five annual rounds of mass drug administration (MDA) of either diethylcarbamazine (DEC) and albendazole or ivermectin.

Myanmar, situated to the west of Thailand, has reported 69% of their 65 districts are LF endemic and 45.6 million are at risk for this infection. The Myanmar MDA program commenced in the year 2001 (Bureau of Vector Borne Diseases, 2009), but not without challenges, such as insufficient funds for field operations and inadequate monitoring. Exacerbating an already difficult situation was the delay in 2006 of receiving DEC resulting in reduced MDA coverage of only 11.87 million individuals residing in Sagaing Division, Magway Division and Rakhine State (WHO, 2009). In 2009, Myanmar reported treating 15.7 million people out of a targeted population of 17.7 million (WHO, 2010).

During the past 10 years, it has been estimated that more than one million Myanmar migrant workers have settled in large urban areas of Thailand situated in Bangkok, Samut Sakhon, Tak and Ranong Provinces (Triteeraprapab *et al*, 2000). There have been a number of diseases associated with migrant workers (Lam, 1998), including typhoid, tuberculosis, leprosy, malaria, HIV-AIDS and the nocturnal periodic strain of *W. bancrofti* transmitted by *Culex quinquefasciatus*, which is abundant in many of these urban centers. It was recently determined the Thai version of this species is permissive for the development of Myanmar *W. bancrofti* to infective third-stage larvae (Nuchprayoon, 2009), which is infectious to humans and may enter the human body with some migration to the lymphatic system for subsequent maturation. The national program's efforts to control lymphatic filariasis included not only Thai citizens living in endemic areas and in the areas surrounding migrant Myanmar communities but also migrant Myanmar workers, themselves.

An essential component of any program to eliminate LF is effective monitoring of transmission, which includes MDA cessation and certification of elimination by the WHO.

The decision to discontinue MDA does not require the complete absence of filarial parasites but rather the reduction of parasite numbers to such low quantities that transmission will cease (Duerr, 2005). Prevalence of infection is the principal impact factor which may be assessed by any or all the following: microfilaremia (thick blood smear or the DEC provocation test followed by Knott's concentration technique), positive rates of circulating filarial antigens (as seen by ICT or Og4C3 ELISA) and IgG4 antibody positivity (Gyapong, 2004). The target rate set by the WHO is to reduce MF prevalence to <0.1% of the general population or <1:3,000 of children born after initiation of MDA if additional MDA is not acceptable to all (Weil, 2005). It has been suggested that the number of MDA rounds employed in a specific country/area should not be fixed (one number does not fit all), but will vary depending on compliance rates, efficiency of the MDA regimen, baseline infection rates and vector capacity (Michael *et al*, 2006; El-Setouhy *et al*, 2007). These data are necessary to monitor human infectivity rates and degree of parasitic elimination. A suc-

Table 1
Characteristics of study population.

| Province | Study population | Number of blood samples (%) | Age range (years) | Males *n* (%) | Females *n* (%) |
|---|---|---|---|---|---|
| Bangkok | Myanmar immigrants | 437 (42.5) | 7-55 | 290 (66.2) | 147 (33.8) |
| | Thais | 81 (7.9) | 12-79 | 47 (58) | 34 (42) |
| Ranong | Myanmar immigrants | 512 (49.7) | 10-63 | 197 (38.5) | 315 (61.5) |
| Total | | 1,030 (100) | 7-63 | 534 (51.8) | 497 (48.2) |

cessful National Program is contingent on monitoring and measuring yearly trends in parasite transmission and infectivity as a result of anti-parasite interventions (Michael *et al*, 2007). In Thailand, all LF endemic areas, except Narathiwart Province bordering Malaysia in the South, are moving to this phase.

The purpose of the present study was to assess bancroftian filariasis among Myanmar migrant communities in Ranong and Bangkok Provinces using 2 parameters: *Wuchereria bancrofti* antigen (ICT) and antifilarial IgG4 antibodies. The prevalence and infectivity rates were compared with previous reports. The data obtained from the present study should provide an epidemiological window on the status of bancroftian filariasis in Myanmar immigrant workers in Thailand.

## MATERIALS AND METHODS

### Study location and study samples

This study was conducted as part of a study protocol to determine the effectiveness of MDA for bancroftian filariasis among Myanmar migratory workers when they enter Thailand seeking employment. The two provinces serving as predominant destinations for Myanmar immigrants to Thailand are Ranong Province, located on the Isthmus of the Malaysian Peninsula, 586 km south of Bangkok, and Bangkok, the capital of Thailand. Surveillance was carried out among 1,031 subjects divided into 3 groups: 512 from Ranong, 438 from Bangkok and 81 Thai residents living adjacent to Myanmar immigrant communities in Bangkok, serving as controls. In Bangkok, spot checks were carried out in four locations; Padipat (*n*=20), On Nut (*n*=133), Asok (87), Nong Khaem (*n*=133) and Bang Khae (*n*=65).

Table 1 presents demographic data for the subjects. There were 534 males and 497 females included in the study. The average ages of male and female subjects were 25.6 years (95% CI 19.1-32.00 and 27.4 years (95% CI 20.2-34.6) (Bangkok), and 26.7 years (95% CI 16.9-36.5) and 24.7 years (95% CI = 15.41-34.0) (Ranong), respectively. The age differences were not significant between males and females.

After informed consent was obtained, finger prick capillary blood samples (120 µl) were collected from each participant, and tested for circulating filarial antigens (CFA) with the rapid-format immunochromatography (ICT) filariasis card test and an antifilarial IgG4 antibody test.

### DEC mass treatment

The policy of the Thai Ministry of Public Health is multiple-dose DEC treatments for all legitimate Myanmar immigrant worker in Thailand. An oral dose of

Table 2
Results of *Wuchereria bancrofti* ICT test antigen and antifilarial IgG4 antibody detection from the serum of 437 Myanmar workers in Bangkok Province.

| Age range (years) | Sex | | Total | Antigen | | Antifilarial IgG4 antibody | |
|---|---|---|---|---|---|---|---|
| | Male | Female | | Positive | Negative | Positive | Negative |
| 1-10 | 2 | 2 | 4 | 0 | 4 | 0 | 4 |
| 11-20 | 61 | 30 | 91 | 1 | 90 | 10 | 81 |
| 21-30 | 119 | 61 | 180 | 4 | 176 | 7 | 173 |
| 31-40 | 46 | 35 | 81 | 0 | 81 | 1 | 80 |
| 41-50 | 9 | 11 | 20 | 0 | 20 | 1 | 19 |
| 51-60 | 1 | 1 | 2 | 0 | 2 | 0 | 2 |
| NA | 52 | 8 | 60 | 0 | 60 | 2 | 58 |
| Total | 290 | 148 | 438 | 5 | 433 | 21 | 417 |

DEC 300 mg was given twice yearly to all registrants, and at the time of the annual health examination a single oral dose of DEC 300 mg was given as a provocation dose, followed 30-minutes later by a blood test for microfilaremia via thick film blood smear or the Knott technique (Knott, 1939). For the present study, those who tested positive were then asked about drug compliance. Asking before testing would have prevented the subject from participating for fear of deportation as an illegal immigrant. All 35 subjects positive for MF admitted to being non-compliant with DEC treatment; 2 who had positive titers of 1:100 said they had received prior DEC treatment.

**Ethics**

This study was approved by the Ethics Committee on Research Involving Human Subjects, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. All subjects, including children with their parent's permission, voluntarily agreed to participate and gave written informed consent after receiving detailed information about the study.

**Detection of *W. bancrofti* circulating antigens using the ICT card test**

Binax ICT filariasis cards (Binax, Scarborough, ME) were used for the detection of *Wuchereria bancrofti* circulating antigens in the study subjects. The test employs both a polyclonal antibody (PAb) and a monoclonal antibody specific for *W. bancrofti*. The PAb is conjugated to colloidal gold and impregnated in a specific area of the pad. The monoclonal antibody (MAb) is immobilized in a line across the membrane (test area). When whole blood is added, blood containing the antigen will flow into the area of the pad and bind with the colloidal gold-labeled PAb, thereby forming a complex. Upon closing the card, the antigen-PAb conjugate complex migrates across the membrane into the immobilized MAb and is captured by the MAb. A pink line appears if the test is positive and no line of the test is negative.

**Detection of antifilarial IgG4 antibodies**

Antifilarial IgG4 was detected using an ELISA test kit developed in Thailand. Briefly, antifilarial IgG4 in the serum of the studied subject reacts with *B. malayi*

antigen coated in microtiter wells, which is then incubated for one hour. After washing, the conjugated solution provided with the test kit is added to each well and incubated for another hour. The substrate solution is then added, then ten minutes later a color reaction is looked for in the test wells compared to the control.

**Data analysis**

Patients were grouped by age, sex, test results and location. Means and standard deviations were then calculated. Positive scores with the IgG4 ELISA test were compared using the non-parametric Wilcoxon-Mann-Whitney test. The number positive scores from both provinces with the ICT and IgG4 tests was evaluated with the chi-square test while the results were compared with the Wilcoxon-Mann-Whitney test. Significance was set at $p<0.05$.

## RESULTS

The results of the screening test by age group and sex for Bangkok Province are found in Table 2 and for Ranong Province are found in Table 3. In Bangkok Province, antifilarial IgG4 antibodies were detected in 16 males and 5 females. The antibody titers among positive subjects were 1:100 ($n$=9, all from On Nut checkpoint), 1:200 ($n$=3), 1:400 ($n$=2), 1:800 ($n$=6) and 1:1,600 ($n$=1). Five of 21 subjects with IgG4 antibodies were positive on the ICT test for CFA (all males). The age group with the greatest number of positive IgG4 results was the 11-30 year old group, comprising 80.9% (17/21) of the positive results (12 males, 5 females).

In Ranong Province (Table 3), antifilarial IgG4 antibodies were detected in 6 males and 8 females while the ICT test was positive in only 1 female subject, who also had an IgG4 titer of 1:800. The antibody titers among positive subjects were 1:100 ($n$=6), 1:200 ($n$=5), 1:400 ($n$=1) and 1:800 ($n$=2). Similar to Bangkok Province, the age group with the greatest number of positive antifilarial IgG4 results was 11-30 year old group, comprising 85.7% (12/14) of the postitive results (5 males, 7 females).

Table 4 shows the lab results by location and group (Thai and Maynmar) among all subjects ($n$=1,031) in the study. Six subjects (0.58%) had positive results with the ICT test and 35 (3.39%) had positive test results with IgG4 test. The difference in test results by test type was significant ($p \leq 0.0005$). There was no significant difference in positive results between males and females ($p = 0.22$).

## DISCUSSION

The length of the border separating Myanmar and Thailand is more than 1,000 km long and while there are a number of legitimate crossing sites (bridges and roads), there are many other unmonitored crossing-points. It has been estimated that hundreds of thousands of potential Myanmar workers enter Thailand legally or illegally yearly seeking employment, usually in agriculture, domestic work, construction, manufacturing, hotels, restaurants or fisheries. They are willing to work for lower wages. Some of them carry *Wuchereria bancrofti*. This cross-border migration of Myanmar migrant workers may lead to recrudescence or reemergence of bancroftian filariasis among Thai citizens living in close proximity to Myanmar communities in Thailand.

Previous surveys of infection among Myanmar immigrants to Thailand between 1999 and 2005 are shown in Table 5 (Triteeraprapab *et al*, 1999, 2001; Keeratihuttayakorn, 2002; Koyadun *et al*, 2003, 2005; Nuchprayoon *et al*, 2003a; Bhumiratana *et al*, 2004, 2005). The most fre-

Table 3
Results of *Wuchereria bancrofti* ICT test antigen and antifilarial IgG4 antibody detected from the serum of 512 Myanmar workers in Ranong Province.

| Age range (years) | Sex | | Total | Antigen | | Antifilarial IgG4 antibody | |
|---|---|---|---|---|---|---|---|
| | Male | Female | | Positive | Negative | Positive | Negative |
| 1-10 | 7 | 8 | 15 | 0 | 15 | 0 | 15 |
| 11-20 | 70 | 136 | 206 | 1 | 205 | 9 | 197 |
| 21-30 | 52 | 85 | 137 | 0 | 137 | 3 | 134 |
| 31-40 | 38 | 55 | 93 | 0 | 93 | 1 | 92 |
| 41-50 | 20 | 23 | 43 | 0 | 43 | 0 | 43 |
| 51-60 | 9 | 7 | 16 | 0 | 16 | 0 | 16 |
| 61-70 | 1 | 1 | 2 | 0 | 2 | 1 | 1 |
| Total | 197 | 315 | 512 | 1 | 511 | 14 | 498 |

Table 4
The prevalences of antifilarial IgG4 antibodies and ICT antigens for *Wuchereria bancrofti* in Thai residents and Myanmar workers in Thailand.

| Province | Study population | Prevalence | |
|---|---|---|---|
| | | Antigen (ICT) | Antifilarial IgG4 |
| Bangkok | Myanmar immigrants | 1.14% (5/438) | 4.79% (21/438) |
| | Thais | 0 (0/81) | 0 (0/81) |
| Ranong | Myanmar immigrants | 0.2% (1/512) | 2.73% (14/512) |
| Total | | 0.58% (6/1,031) | 3.39% (35/1,031) |

quently used technique to diagnose bancroftion filariasis from previous studies is a thick film blood smear from finger prick blood. The DEC provocation test was used by Bhumiratana *et al* (2005) for all their subjects except 2. Using meta-analysis, the total prevalence of filariasis among the 7 studies listed in 5 was 2.49% (89/3,574). The prevalences ranged from 0.0 (Bhumiratana *et al*, 2004) to 7.8% (Triteeraprapab *et al*, 2001). Koyadun *et al* (2005) found MF prevalences ranged from 4.5% in 1996 to 0.4% in 2000 for Ranong and 9.2% in 1997 to 0.1% in 2000 for Phang-Nga. Four studies used Og4C3 to give a prevalence of 12.68% (141/1,112). Four studies used the ICT test and found a prevalence of 7.78% (85/1,092). Two studies used the IgG4 test and found a prevalence of 32.66% (210/643). This cumulative data suggests the tests may be ranked from the least to the most sensitive as follows: thick blood film for microfilaria, ICT card test, Og4C3 antigen test, and IgG4 antibody tests.

The results of the present survey are in contrast to previous studies as indicated by the lower prevalence of antigens (0.63%) and antifilarial antibodies (6.68%) among Myanmar immigrants in Bangkok and Ranong Provinces. This

Table 5
Surveillance studies of imported bancroftian filariasis among Myanmar immigrants 1999 to 2005.

| Study | Province | District | MF (%) | Og4C3 (%) | IgG4 (%) | ICT (%) |
|---|---|---|---|---|---|---|
| Triteeraprapab and Songtrus (1999) | Tak | Mae Sot | 29/654 (4.4) | | | |
| Triteeraprapab *et al* (2001) | Tak | Mae Sot | 29/371 (7.8) | 38/371 (10.2) | 157/371 (42.3) | |
| Keeratihuttayakorn (2002) | Ranong | Kanti Island | 1/227 (0.44) | | | 9/227 (3.96) |
| Koyadun *et al* (2003) | Phang-Nga | Takua Pa<br>Takua Thung<br>Thai Mueang | | | | 34/357 (9.52) |
| Nuchprayoon *et al* (2003a) | Tak | Mae Sot | 11/337 (3.26) | 28/220 (12.7) | 53/272 (19.5) | |
| Bhumiratana *et al* (2004) | Phang-Nga | | 1/435 (0.23) | 9/100 (9.0) | | 7/100 (7.0) |
| | Suphan Buri | | 2/338 (0.59) | 4/100 (4.0) | | 3/100 (3.0) |
| | Tak | | 0/87 (0.0) | 9/100 (9.0) | | 2/87 (2.3) |
| Koyadun and Bhumiratana (2005) | Ranong | Mueang Ranong<br>Kra Buri | 4/485 (0.82)<br>5/419 (1.19) | | | |
| Bhumiratana *et al* (2005) | Not specified part of Thailand | | 7/221[a] (3.17) | 53/221 (24.0) | | 30/221 (13.6) |

[a]These 7 were from 54 antigenemic Myanmar workers detected from the original 221 workers.

lower incidence may reflect: (a) the initiation of MDA in Myanmar which began in 2001 with monitoring by the year 2007, indicating a significant reduction in microfilaremia, both in frequency and in density; (b) the launching, during the past 10 years, of concurrent strategies by Thailand to control the spread of LF among Myanmar immigrant workers. All district hospitals have conducted and are continuing to implement a screening program for bancroftian filariasis among all legally registered Myanmar immigrant workers at the time of the annual health examination. If required, single dose DEC treatment may be given. The Bureau of Vector-Borne Disease, Ministry of Public Health oversees a program for spot checking the effectiveness of the control program among legal workers, and for monitoring disease status among illegal Myanmar workers. The Royal Thai Government has also established a registration process for immigrant workers from Myanmar, Lao PDR, and Cambodia. According to this policy, registered Myanmar immigrant workers receive health insurance, and thus are eligible to receive DEC bi-annually (Amnesty International Thailand, 2005).

In addition to the ICT filariasis antigen test which detects AD12 antigen (Weil and Liftis, 1987), the present immunoepidermiological survey also utilized the antifilarial IgG4 antibody test. There are two advantages to using assays for antifilarial antibodies: (1) the time needed to detect an infection is much less than using the thick film blood smear to detect microfilaremia or of the test to detect antigenemia; (2) parasitological evaluations are time-point estimates, while measuring antibody levels gives a cumulative history of the infection (Lammie *et al*, 2004) providing information about lifetime exposure to bancroftian filariasis (Weil and Ramzy, 2007).

A downside of the ICT card test is the sensitivity. Pani *et al* (2000) found 71.9% of microfilaremics detected with the membrane filtration technique were ICT positive. The absence of infection in subjects less than 20 years old may be due to the inability to detect low infection levels (Liang *et al,* 2008). This is a major reason suggesting the use of more than one assay for surveillance monitoring in endemic areas, especially using a test with greater sensitivity, such as the antifilarial IgG4 antibody test. This assay may be a more sensitive marker of filarial infection, detecting infection earlier than those detecting antigenemia or microfilaremia (Lammie *et al*, 2004). It appears, the antifilarial IgG4 antibody test is more sensitive than the MF thick blood film test and in certain circumstance may outperform the ICT card test (Jiraamonnimit *et al*, 2009). Noordin *et al* (2004) found that using native filarial antigen as opposed to recombinant antigen, the antifilarial IgG4 test detected infection 2-4 months post-exposure in a population of immigrants. Using soluble worm antigen for antibody testing, post-GPLF surveillance may benefit by earlier detection of re-emergence of the disease.

The results of the present study suggest the antifilarial IgG4 assay is 5.84 times more sensitive in detecting active bancroftian infections than the ICT card test. All but two antibody positive subjects indicated they had not received prior DEC treatment; the two that did receive treatment previously had low antibody titers. Preliminary parasitological (unpublished) data from ten children < 10 years old residing in a Ranong Myanmar community reveal 5 of them were infected with gastrointestinal parasites, including

*Ascaris lumbricoides*, *Trichuris trichiura* and Hookworm, but were without bancroftian infection as measured by antifilarial IgG4 antibodies. This suggests the positive IgG4 antibodies detected in Myanmar workers > 15 years old in the present study cannot be construed as false positives (Rodriguez-Perez *et al*, 1999; Weil, 2005).

A previous study of cross-reactivity showed no effect on the outcome of the antifilarial antibody assays (Wongkamchai *et al*, 2006). Another study of cross-reactivity (Wongkamchai, 2010 unpublished) showed low cross-rectivity (3.77%) with the antifilarial IgG4 test and parasites detected in the following 106 subjects in Bangkok, Ammart Charoen and Phuket Provinces: *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, Hookworm, *Angiostrongylus cantonensis*, *Capillaria philippinensis*, *Ophistorchis viverrini*, *Cysticercus*, *Echinostoma* spp, *Giardia lambria*, *Blastocystis hominis*, *Entamoeba coli*, *E. histolytica*, *Isospora belii*, *Trichomonas hominis* and mixed infection with *A. lumbricoides*, *T. trichiura*, Hookworm and/or *G. lamblia*. The only cross-reactivity was found with *Taenia* spp, *Capillaria philippinensis,* and *Strongyloides stercoralis.*

There is evidence to suggest antifilarial antibody tests may detect LF infection prior to the appearance of microfilaremia or antigenemia (Lammie *et al*, 2004). The present survey showed in both provinces no children <10 years old tested positive for filarial antigens or antifilarial IgG4 antibodies. Children born after filariasis control measures have not been exposed to the parasite. Those aged >30 years had decreasing levels of infection (Gao *et al*, 1994). The observation that no Thai citizens residing near Myanmar immigrant communities in Bangkok, where the vector *Culex* spp abounds, displayed signs of antigenemia or antibodies is strong evidence that recrudescence has not occurred. This, validates the effectiveness of MDA as a public health strategy.

We concluded that: 1) the prevalence of *Wuchereria bancrofti* infection has been significantly reduced in Myanmar immigrants in Bangkok and Ranong Provinces; 2) the Thai MDA program to eliminate LF among Myanmar immigrants nationals living and working in Thailand is a successful public health strategy; 3) from personal interviews it was determined the positive subjects in the present study were workers newly immigrated from Myanmar, suggesting the necessity for continuing surveillance; 4) the antifilarial IgG4 antibody test is a more sensitive measure of disease progression than the IGT card test; and 5) the results of this study provide an epidemiological update on lymphatic filariasis among Myanmar immigrants working in Thailand.

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