Seroconversion to Filarial Antigens in Australian Defence Force Personnel in Timor-Leste


Abstract. To investigate whether Australian soldiers were exposed to filarial parasites that cause lymphatic filariasis during a 6-month deployment to Timor-Leste, antifilarial antibody levels were measured in 907 soldiers using an enzyme linked immunosorbent assay (ELISA). Initial testing using *Dirofilaria immitis* antigen demonstrated that 49 of 907 (5.4%) soldiers developed antifilarial antibodies of the IgG1 subclass after deployment, whereas 1 of 944 (0.1%) seroconverted to the IgG4 subclass. When a sub sample of 88 *D. immitis*-reactive sera was subject to testing with an antifilarial antibody test using *Brugia malayi* antigen, 46 had elevated IgG antibodies, whereas 5 had elevated antibodies of the IgG4 subclass. A total of 24 soldiers seroconverted to *B. malayi*, as measured by parasite-specific IgG, whereas 1 seroconverted to IgG4. The relatively low number of seroconversions indicates a low but measurable risk of exposure to human filarial parasites among Australian soldiers deployed to Timor-Leste. However, to reduce the risk of exposure to these parasites, soldiers deploying to endemic areas should practice strict adherence to personal protective measures against mosquito bites.

INTRODUCTION

Lymphatic filariasis (LF) is caused by infection with the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, and is transmitted by mosquitoes. These parasites are endemic in many countries in the tropics and subtropics, where they cause significant morbidity.1

Lymphatic filariasis has been a significant concern for military personnel operating in filaria-endemic areas. During World War II, Australian and American military personnel serving in the South Pacific region were exposed to parasites causing LF, with an estimated 38,300 United States military personnel exposed, among whom 10,421 were diagnosed with active lymphatic filarial infections.2 In a second study, a total of 2,124 US soldiers were reported to have developed symptomatic filarial infections; however, as is generally observed in individuals from non-endemic areas who contract filarial infection, only 16 had patent infections with microfilariae detected in their blood.3 Only 24 cases of LF were recorded among Australian Defense personnel during World War II.4

Records indicate that US soldiers serving in Vietnam in the 1960s were also exposed to parasites causing LF. In one unit, 11% of 406 soldiers screened were found to have been exposed, with a single serviceman being identified as having microfilaraemia.5 In contrast, no lymphatic filariasis was detected among 4,575 US Special Forces soldiers living in 28 camps in South Vietnam.6

Lymphatic filariasis has also been observed among missionaries, expatriates working in endemic areas and travelers, and among transmigrants in Indonesia.8 In people from non-endemic areas infection is characterized by a brisk immune response and a good prognosis, even untreated, as long as exposure ceases.9

In 1965, the prevalence of LF in the East Timorese population was reported to be between 3.5 and 9.3%.10 Further work suggested that this infection was due to a distinct species of filaria, which was named *Brugia timori*.11 In a recent survey among indigenous Timorese, the prevalence of microfilaraemia was measured at 12.7%, with most of these infections being due to *B. timori* (11.6%), whereas a smaller number were due to *W. bancrofti* (1.1%).12

In September 1999, Australian soldiers were deployed to Timor-Leste for peacekeeping duties. The aim of the current study was to determine if soldiers deployed to Timor-Leste were exposed to filarial parasites. As microfilaraemia is a rare finding in expatriates, the study was undertaken by serologic testing, initially with an antibody test using antigen prepared from adult stage *Dirofilaria immitis* filarial worms, the only test available in Australia. Positive samples were then retested using antigen from *B. malayi*, a filarial parasite of humans most closely related to the two species of filarial parasite endemic in Timor-Leste. Because detection of antifilarial IgG4 antibody has been shown to enhance diagnostic specificity for filarial infections by eliminating some of the non-specific cross reactions,13 and IgG1 an indication of filarial infectivity for filarial infections by eliminating some of the non-specific cross reactions,13 and IgG1 an indication of filarial

MATERIALS AND METHODS

Study population. Australian soldiers who spent approximately 6 months in the country participated in the study. Soldiers were deployed to Bobonaro District, Timor-Leste, close to the western border with West Timor province, Indonesia. The first group of 447 soldiers was deployed for 6 months between April 2000 and October 2000, whereas the second group of 497 was deployed for 6 months between October 2000 and April 2001.

Enzyme-linked immunosorbent assay (ELISA) for detection of antifilarial antibodies using *D. immitis* adult worm antigen. Serum samples collected prior to deployment and 6 months later prior to return to Australia were stored at −70°C.
until assayed. A screening antifilarial ELISA was undertaken as described previously. The antigen used was a commercial preparation of adult dog heartworm (D. immitis) (Agen, Brisbane, Queensland, Australia). A pool of 100 sera obtained from Papua New Guinean subjects infected with W. bancrofti was used on each test plate in serial dilution as a positive control. Negative control sera were obtained from 27 anonymous individuals (males 18–40 years old); all patients attended the Royal Brisbane Hospital.

Flat bottom 96-well plates (Limbro, ICN Biomedicals) were coated with 50 μL/well of D. immitis antigen at a dilution of 1:2,500 for IgG1 assays and with a 1:10,000 dilution of antigen for IgG4 assays, and incubated at 4°C overnight. Plates were washed in a Plate Washer (Wallac, Delfia, ADIL Instruments, France) with Phosphate Buffered Saline (PBS) and 0.05% v/v Tween 20. Test sera were diluted 1:10 in PBS and 50 μL added to wells in the 96-well plates and incubated at 37°C for 1 h. After washing 50 μL of Horse Radish Peroxidase (HRP) conjugated anti human IgG1 (Diluted 1:2,500 in PBS) or IgG4 (Diluted 1:5,000 in PBS) (ICN Biochemicals) was added and incubated at 37°C for 1 h. Plates were then washed and 100 μL per well of tetramethylbenzidine (TMB, Dade Bearing) substrate added before incubation in the dark at room temperature for 15 minutes. The reaction was stopped by adding 100 μL/well of H2SO4 (Dade Bearing). Optical density was read on an ELISA plate reader (Spectrum Max Plus 384, Molecular Devices, Sunnyvale, CA) at a wavelength of 450 nm.

Samples were considered reactive if the mean optical density (OD) was 3 standard deviations above the mean OD for the negative control sera (> 0.13 for IgG1 and > 0.09 for IgG4).

A subsample of 25 reactive sera for IgG1 using D. immitis antigen were also tested using an immunochromatographic test (ICT, Binax™ Now) specific for W. bancrofti.

ELISA using B. malayi antigen. To define further the antibody response among the study group a subset of 88 sera that had initially been tested using D. immitis antigen and found positive were subject to screening with an ELISA using adult worm antigen of the parasite B. malayi. An additional 10 sera found negative using D. immitis antigen was also subjected to the B. malayi ELISA. Testing was performed at the National Institutes of Health as has been described previously.

Consent/ethics. The study was conducted in a military operational setting and was approved by the Director of Health Capability and Development, Australian Defense Force. Sera were obtained from stored specimens from previous studies where informed consent was provided to obtain the samples.

RESULTS

Screening enzyme-linked immunosorbent assay for detection of antifilarial antibodies using D. immitis antigen. The results of the screening serodiagnostic testing of sera are shown in Table 1. Thirty-seven sera could not be tested for the presence of IgG1 antibodies; 109 study subjects showed a positive antibody response to D. immitis, of whom 53 were positive before deployment. Of these, 46 remained positive after deployment. Six study subjects tested positive for D. immitis-specific IgG4 before deployment, with 4 of these 6 remaining IgG4 positive after deployment.

<table>
<thead>
<tr>
<th>Antibody subclass</th>
<th>Response</th>
<th>No. positive/No. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive before</td>
<td>7/907</td>
<td>2/944</td>
</tr>
<tr>
<td>deployment only</td>
<td>(0.8%)</td>
<td>(0.2%)</td>
</tr>
<tr>
<td>Reactive both</td>
<td>46/907</td>
<td>4/944</td>
</tr>
<tr>
<td>before &amp; after</td>
<td>(5.1%)</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>Sero-converted</td>
<td>49/907</td>
<td>1/944</td>
</tr>
<tr>
<td>after deployment</td>
<td>(5.4%)</td>
<td>(0.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IgG4</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive before</td>
<td></td>
<td></td>
</tr>
<tr>
<td>deployment only</td>
<td>(0.8%)</td>
<td>(0.2%)</td>
</tr>
<tr>
<td>Reactive both</td>
<td>(5.1%)</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>before &amp; after</td>
<td>(5.4%)</td>
<td>(0.1%)</td>
</tr>
</tbody>
</table>

* Positive where optical density > 0.13 for IgG1 and > 0.09 for IgG4.

More interesting was the finding that 49 subjects seroconverted to IgG1 after deployment to Timor-Leste (Table 1). A single subject had an IgG4 seroconversion. The rate of seroconversion did not differ between two different battalion groups deployed, with 24 of 460 soldiers in the first group and 25 of 447 soldiers in the second group seroconverting after deployment. None of the 25 sera tested using the Binax ICT was positive.

ELISA using B. malayi antigen. A total of 46 of 87 individuals had raised B. malayi-specific antifilarial antibodies, of which 24 seroconverted after deployment (Table 2). Of these 46, 19 were seropositive both before and after deployment, whereas 3 were positive only before deployment. One person had an elevated B. malayi-specific IgG4 antibody after deployment to Timor-Leste.

<table>
<thead>
<tr>
<th>Antibody subclass</th>
<th>Response</th>
<th>No. positive/No. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive before</td>
<td>3/87</td>
<td>2/88</td>
</tr>
<tr>
<td>deployment only</td>
<td>(3.4%)</td>
<td>(2.3%)</td>
</tr>
<tr>
<td>Reactive both</td>
<td>19/87</td>
<td>2/88</td>
</tr>
<tr>
<td>before &amp; after</td>
<td>(21.9%)</td>
<td>(2.1%)</td>
</tr>
<tr>
<td>Sero-converted</td>
<td>24/87</td>
<td>1/88</td>
</tr>
<tr>
<td>after deployment</td>
<td>(27.6%)</td>
<td>(1.1%)</td>
</tr>
</tbody>
</table>

* Positive where > 130 μg/mL of IgG anti B. malayi antigen and 0 ng/mL for IgG4.
Although no parasitologic follow-up was undertaken, no reports of symptoms of lymphatic filariasis have been received from medical services supporting the military.

The use of *D. immitis* antigen as a screen for filarial antibodies has been used in northern Queensland since the assay was developed in the early 1990s. Because dog heartworm is common in parts of Australia the occurrence of a positive reaction in some soldiers prior to deployment in the current study may be due to the use of *D. immitis* antigen (more antigenic cross-reactions). A possible explanation for the seroreversion to *D. immitis* antigen observed in seven individuals in this study is that their antibody response was to a zoonotic filarial parasite. However, sera obtained from residents of the Northern Territory and Queensland where dog heartworm is present has typically been non-reactive (Melrose, personal communication). Moreover, the serologic reactivity in zoonotic filarial infections (including dirofilariasis) is often negative. Nevertheless, the observed frequency of seroresponsiveness to *B. malayi* antigen among those seropositive to *D. immitis* antigen was only 52% (46 of 87), suggesting that this test may be less specific.

Of note, the rate of seroconversion did not differ between two different battalion groups deployed in the current study. This is significant because the servicemen received different antimalarial prophylaxis regimens, the first receiving doxycycline, which may have some antifilarial activity based on its action against the Wolbachia endosymbiont; the latter group received tafenoquine or mefloquine, neither of which has known antifilarial activity.

Personal protective measures such as using mosquito repel-lents, wearing long-sleeved shirts and trousers, and sleeping under a mosquito bed net remain the first line of protection against vector-borne diseases such as malaria, dengue, and filarial infections including LF. The main vector of Brugian filariasis in Timor-Leste is *Anopheles barbirostris*. This species was commonly found biting ADF personnel during the night (biting rate: 6.5 bites/human/night) in the Bobanaro District in surveys conducted in 2001 (Cooper and others, unpublished). After the initial deployment of ADF personnel to Timor-Leste in September 1999, the incidence of malaria was high, indicating significant exposure to mosquitoes and thus vector-borne diseases. In the subsequent deployments there was increased awareness and more vigilant adherence to personal protective measures to minimize mosquito bites among soldiers, including the two groups in this current study.

The current study was conducted on stored sera, and there was no clinical or parasitological data obtained. In addition, because the personnel dispersed after return to Australia follow-up, to date, has not been possible. The traditional diagnostic testing for lymphatic filariasis has relied on the detection of microfilariae in blood samples. In recent years diagnosis has been enhanced by the introduction of filarial antigen tests, which are specific for *W. bancrofti*. Recently a PCR-based method to detect the presence of *B. timori* microfilariae on blood slides has been used successfully. A rapid immunochromatographic dipstick test (Bruga Rapid) has also been tested as a simple serological test for the identification of *B. timori*, and has shown > 97% percent sensitivity and specificity. The current study clearly showed that soldiers in Timor-Leste can be exposed to filarial parasites that cause LF, and reinforces the need to minimize individual exposure to mosquito bites and to consider long-term follow-up to ensure that the pathological consequences of this exposure are minimized.

Received January 18, 2007. Accepted for publication January 18, 2008.

Acknowledgments: The authors thank the Commanding Officers and men of the two battalion groups who participated in this study. The opinions expressed herein are those of the authors and do not necessarily reflect those of the Defence Health Service (Australia) or any extant health policy.

Authors’ addresses: Stephen P. Frances and Lisa M. Baade, Australian Army Malaria Institute, Gallipoli Barracks, Enoggera, Queensland, 4051, Australia. Joseph Kubofcik and Thomas B. Nutman, Helminth Immunology Section, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD 20892-0425. Wayne D. Melrose, Lymphatic Filariasis Support Centre, School of Public Health and Tropical Medicine, James Cook University, Townsville, Queensland, 4811, Australia. James S. McCarthy, Australian Centre for International and Tropical Health, School of Medicine, University of Queensland, Royal Brisbane Hospital, Herston, Brisbane, Queensland, Australia. Michael D. Nissen, University of Queensland, Queensland Institute of Medical Research and Royal Brisbane and Women’s Hospital, Herston, Queensland, 4029, Australia.

REFERENCES


