

USE OF BRUGIA RAPID DIPSTICK AND ICT TEST TO MAP DISTRIBUTION OF LYMPHATIC FILARIASIS IN THE DEMOCRATIC REPUBLIC OF TIMOR-LESTE

W Melrose¹ and N Rahmah²

¹Lymphatic Filariasis Support Center, School of Public Health and Tropical Medicine, James Cook University, Townsville, Australia; ²Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Penang, Malaysia

Abstract. The newly-introduced Brugia Rapid dipstick for filarial antibodies and ICT filarial antigen card test were used to confirm historical data on the distribution of lymphatic filariasis in the Republic of Timor-Leste. Twelve out of thirteen districts were confirmed as being endemic. Brugian filariasis predominates, with an average prevalence of 11.6%. The average prevalence of Bancroftian filariasis was 1.1%. The study demonstrated that the Brugia Rapid test can provide useful information about the distribution of Brugian filariasis in circumstances where it is difficult or impossible to obtain night blood samples for microfilariae.

INTRODUCTION

Traditionally, the diagnosis of both Bancroftian and Brugian filariasis was done by the detection of microfilariae (mf). In areas where mf show nocturnal periodicity, blood must be collected at night, which is inconvenient for both patient and health worker, and difficult, if terrain or security concerns make traveling at night, or remaining overnight at field sites, inadvisable. The diagnosis of Bancroftian filariasis was revolutionized in the 1990s by the introduction of tests that detect circulating filarial antigen, because day blood can be used, and both microfilaremic and amicrofilaremic cases are detected (More and Copeman 1990; Turner *et al*, 1993). The ICT[®] rapid card filarial antigen test (now marketed as the Binax NOW filariasis test[®]) has become the tool of choice for mapping the distribution and prevalence of Bancroftian filariasis (Weil *et al*, 1997; Simonsen and Dunyo, 1999).

No antigen test is currently available for Brugian filariasis, but the newly-introduced Brugia Rapid[®] dipstick antibody test shows considerable promise as a mapping tool for *Brugia malayi* and *Brugia timori* (Supali *et al*, 2004). In

multi-center trials, it has shown 95% and 100% sensitivities compared with microscopy for *B. malayi* mf, but because it is an antibody test, those who are amicrofilaremic, or who have cleared their infection may also test positive. The test is not specific for *Brugia*, as it reacts with most, but interestingly not all, *Wuchereria bancrofti*-positive samples. There is no cross-reaction with other helminths (Rahmah *et al*, 2001, 2003; Melrose *et al*, 2004; Supali *et al*, 2004). It is a two stage test which requires the dipstick to be moved into a reagent well after blood is applied. This makes it more suitable for laboratory rather than field use, but a single-stage cassette variation suitable for field use is under development.

The Republic of Timor-Leste (formally known as East Timor) is situated at the southeastern end of the Indonesian archipelago and occupies the eastern end of the island of Timor. It has thirteen districts. Historically, *B. timori* has been the most prevalent species, accounting for 95% of the cases. The other 5% are made up mostly of *B. malayi* with occasional cases of *W. bancrofti*. Microfilariae exhibit a nocturnally periodic or nocturnally sub-periodic pattern. In 1965, David and Edeson, who first described *Brugia timori*, carried out a survey on 982 military recruits and found that 3.5% were positive. A village-based survey was then carried out in

Correspondence: Dr Wayne Melrose, SPHTM, James Cook University, Townsville, Australia 4811.
Tel: +61 7 47 8175
E-mail wayne.melrose@jcu.edu.au

ten areas of the country. *B. timori* was found at all sites and the average prevalence was 5.9%. The highest prevalence was 15.3% in Manatuto, followed by 11.3% at Colomera, a suburb of Dili. *W. bancrofti* was found in 50% of the sites with an average prevalence of 4.9%. The highest prevalence was recorded at Batugade on the West Timor border, where it was 18.7%, much higher than the *B. timori* prevalence of 3.2%. The next highest prevalence was 2.1% in the Dili suburb of Bidau. No *B. malayi* was detected during that survey (David and Edeson, 1964, 1965).

From published information, it appears that the last countrywide survey of microfilaremia was carried out in 58 areas of the country by the Portuguese administration in the 1970s (Janz *et al*, 1977). It showed that filariasis was widely distributed with only 14 areas mostly in the mountains, being filariasis free. At 31 sites *B. timori* was the only species detected. At another 11 sites *B. timori* was still the predominant species but *B. malayi* and *W. bancrofti* were also present. After combining data for all three species the overall country prevalence was 5.2%, but two sites, Atabai in Bobonaro district and Illomar in Lautem district, had prevalences of 21% and 19.6%, respectively. The microfilaremia densities were high, with an average of 548/ml, and the highest being 2,007/ml.

In 2001, it was decided by the WHO that updated information had to be obtained prior to the drawing up of a national filariasis elimination program. Following in the footsteps of David and Edeson, a survey was conducted among military recruits in 2002. This was seen as a convenient way of obtaining a "snapshot" of filariasis distribution, as the recruits represented all districts of the country. Small surveys were also carried out in two communities and a small number of blood samples were obtained from the hospital in Dili, the capital city.

After informed consent was obtained, blood was drawn from 383 male and 32 female military recruits aged between 20 and 42 years as part of a comprehensive health check. Blood was also obtained from 21 males and 26 females at Batugade in Bobonaro district, 26 males and 32 females from Rembor in the Manatutu district

and 68 adult samples, gender unknown, from the outpatient clinic at Dili Hospital. It should be emphasized that the purpose of this study was to gain an overview of filariasis distribution and divide the country into "red" (endemic) and "green" (non-endemic) areas according to the protocols of the Global Filariasis Elimination Program (WHO, 2000) rather than obtain detailed data on prevalence. All samples were tested with the Brugia Rapid and ICT tests, and at Rembor in Manatutu district, the only site where night blood could be obtained, mf were separated and counted by a modified Knott's test (Melrose *et al*, 2000).

RESULTS

The results are shown in Table 1. Samples were considered to be positive for Brugian filariasis if they were positive by Brugia Rapid and Negative with ICT, and Bancroftian if they were positive with both tests. No samples were positive on ICT and negative on Brugia Rapid, and none were positive for mf and negative on Brugia Rapid. No attempt was made to identify the species of *Brugia*. There were no significant gender differences. Microfilaremia prevalence at Rembor was 7% and mf density ranged from 5 to 33 per ml of blood.

DISCUSSION

It is not possible to directly compare this survey, which used an antibody and an antigen test, with earlier ones which used mf, because no previous studies have shown a consistent, direct, correlation between mf and filarial antibody. It can be said from previous studies that a high prevalence of IgG4 antibody is consistent with filarial endemicity, and positive antigen tests are diagnostic for *W. bancrofti* (Ottesen *et al*, 1985; Turner *et al*, 1993; Melrose, 2002, 2004). The results confirm that 12 of the 13 districts are endemic for lymphatic filariasis and that Brugian filariasis predominates. Too few samples were obtained from the Aileu district to obtain a meaningful result and sample collection will need to be repeated. Historically, however, this district is filarial-endemic. The results at Rembor are not unexpected as previous surveys had

Table 1

Summary of Brugia Rapid and ICT test results from 12 districts of the Republic of Timor-Leste.

District	Number of samples	Brugian filariasis prevalence %	Bancroftian filariasis prevalence %	Total filariasis prevalence %
Dili	173	5.1	2.8	7.9
Liquica	29	6.8	0	6.8
Oecusse	59	3.3	0	3.3
Manatutu	77	24.5	1.7	26.2
Bobonaro	82	17.1	0	17.1
Ainaro	39	7.6	0	7.6
Baucau	16	12.5	0	12.5
Lautem	17	5.1	0	5.1
Covalima	24	20.6	3.4	24.0
Emera	30	3.3	2.3	5.6
Viqueque	15	26.6	0	26.6
Manufahi	27	7.4	3.0	10.4
Total for 12 districts	588	11.6	1.1	12.7

found a high prevalence of microfilaremia in Manatutu district, and the site was suggested to us by local health workers who were concerned about the number of people with elephantiasis there. The mf density was lower than those previously reported but samples were collected between 19 00 and 20 00 hours. The mf prevalence and density might have been higher had the samples been taken later in the night. The negative results at Batugade were surprising given that historically it has had one of the highest prevalences in the country. Local health workers have a vague recollection that a control program may have been conducted in the 1970s but no documentary evidence has been found to date. Dili has been identified as a possible site for transmission of *W. bancrofti* during entomological surveys carried out in 1991 and 1999 (Whelan, 1991; Whelan and Habgood, 1999) and these results confirm that there is transmission of both Brugian and Bancroftian filariasis within the city.

In conclusion, this is a preliminary study. The community surveys were small and only convenience sampling was used. It confirms filariasis endemicity in all but one of the districts of the Republic of Timor-Leste and supports the historical data. It shows that the Brugia Rapid test can provide useful information about the distri-

bution of Brugian filariasis in circumstances where it is difficult or impossible to obtain night blood samples for mf.

ACKNOWLEDGEMENTS

The authors thank Dr Alex Andjaparidze, Mr Jose Barreto and other colleagues from the WHO office in Dili and are grateful for the support received from the local communities, the Timor-Leste Ministry of Health, district health professionals, Australian Defence Forces and Curtain Brothers, Ltd. The study was funded in part by European Commission grant number ICA4-CT-2001-10081 and the WHO. We also acknowledge the operational funding supplied to the Lymphatic Filariasis Support Center by Glaxo Smith Kline, Ltd.

REFERENCES

- David H, Edeson J. Microfilariae from man in Portuguese Timor. *Trans R Soc Trop Med Hyg* 1964; 58: 6-11.
- David H, Edeson J. Filariasis in Portuguese Timor with observations of a new microfilariae found in man. *Ann Trop Med Parasitol* 1965; 59: 193-204.
- Janz J, Chung Koo Sito E. [Filariase Linfatica em Timor Leste]. *Ann Instit Hig Med Trop* 1977; 5: 257-80.
- Melrose W, Turner P, Pisters P, Turner B. An improved

- Knott's concentration test for the detection of microfilariae. *Trans R Soc Trop Med Hyg* 2000; 94: 176.
- Melrose W. Tropical Public Health Studies in Papua New Guinea with emphasis on filariasis and other parasitic diseases, Townsville: James Cook University. 2002. Thesis.
- Melrose W, Durrheim D, Burgess G. Updates on immunological tests for lymphatic filariasis. *Trends Parasitol* 2004; 20: 255-7.
- More S, Copeman B. A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in Bancroftian filariasis. *Trop Med Parasitol* 1990; 41: 403-6.
- Ottesen E, Skvaril F, Tripathy S, Poindexter R, Hussain R. Prominence of IgG4 in the antibody response to human filariasis. *J Immunol* 1985; 134: 2707-12.
- Rahmah N, Taniawati S, Shenoy R, *et al.* Specificity and sensitivity of a rapid dipstick test (Brugia Rapid) in the detection of *Brugia malayi* infection. *Trans R Soc Trop Med Hyg* 2001; 95: 601-4.
- Rahmah N, Shenoy RK, Nutman T, *et al.* Multicenter laboratory evaluation of Brugia Rapid dipstick test for detection of brugian filariasis. *Trop Med Int Health* 2003; 8: 895-900.
- Supali T, Rahmah N, Djuardi Y, Sartono E, Ruckert P, Fischer P. Detection of filaria-specific IgG4 antibodies using Brugia Rapid test in individuals from an area highly endemic for *Brugia timori*. *Acta Trop* 2004; 90: 55-61.
- Simonsen P, Dunyo S. Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific antigens. *Trans R Soc Trop Med Hyg* 1999; 93: 278-82.
- Turner P, Copeman B, Gerisi D, Speare R. A comparison of the Og4C3 antigen capture ELISA, the Knott test, and IgG4 test and clinical signs in the diagnosis of bancroftian filariasis. *Trop Med Parasitol* 1993; 44: 45-8.
- Weil G, Lammie P, Weiss N. The ICT filariasis test: a rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitol Today* 1997; 13: 400-4.
- Whelan P. Mosquito survey of Dili, East Timor, Indonesia. Report from Northern Territory Health Services, Darwin, Australia, 1991.
- Whelan P, Habgood G. Mosquito vector survey, Dili, East Timor. Report from Northern Territory Health Services, Darwin, Australia, 1999.
- WHO. Preparing and implementing a national plan to eliminate lymphatic filariasis. Geneva: World Health Organization. 2000.