

RESEARCH NOTE

FIELD EVALUATION OF TWO DIAGNOSTIC ANTIGEN TESTS FOR *WUCHERERIA BANCROFTI* INFECTION AMONG INDIAN EXPATRIATES IN SAUDI ARABIA

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Abstract. Two commercially available diagnostic tools (Tro-Bio ELISA and ICT card test) were used to detect circulating filarial antigen of *Wuchereria bancrofti* infections among Indian expatriate workers in Saudi Arabia. Daytime serum samples collected from 302 individuals (210 men and 92 women) were tested. Night blood surveys for microfilaraemia were restricted to those individuals who became positive for the trop-Bio assay test. The overall prevalence of filarial antigenaemia was 10.6% (32 individuals). Of these 32 antigen positive cases, microfilariae were found in 10 men (31.3%), with a mean microfilarial count of 105 mff/ml. No positive antigen results were found in control sera from 200 native healthy Saudis or from patients with helminthic infections (schistosomiasis, echinococcosis, hookworm, ascariasis and trichuriasis). All 32 positive sera with the Trop-Bio ELISA showed a positive ICT card reaction (specificity and sensitivity 100%). It is concluded that, in Saudi Arabia and other Gulf states, where a continuous flow of south- and southeastern workers coming from areas endemic for bancroftian filariasis, the ICT card test may be useful in monitoring the potential risk of introducing bancroftian filariasis to the host countries.

Lymphatic filariasis caused by the filarial parasite *Wuchereria bancrofti* is an important public health and socioeconomic problem for nearly 120 million people worldwide. Thanks to current breakthroughs in the development of new tools and control strategies made available to the affected communities, lymphatic filariasis is nearing the stage of being one of the six "potentially eradicable" infectious diseases (Ottesen, 1997). The development of new immunological tests for *W. bancrofti*, that fulfill all diagnostic requirements under field conditions, may be an important asset for future community-based control strategy of the disease.

In the present study, we have evaluated the performance of the ICT Filariasis test (Balgowlah, NSW, Australia) against the commercial TropBio *W. bancrofti* ELISA assay (JCU Tropical Biotechnology, Townsville, Australia) for the detection of *W. bancrofti* circulating antigen (CFA) in a field trial of an endemic population of expatriate workers from India residing in Abha city, the capital

of Asir Province, southwestern Saudi Arabia. Firstly, antigen detection was done by the capture sandwich ELISA, according to the manufacturer's recommendations, to determine the antigenic status among the study population. Only *W. bancrofti* antigen-positive patients were reexamined for the presence of microfilariae in the blood by conventional methods. Secondly, the results of the ELISA assay and blood examination were checked against those of the ICT filariasis card test in terms of sensitivity, specificity and practical utility of the latter test as a screening tool for *W. bancrofti* infections in primary filariasis field surveillance.

The study was conducted with sera separated from daytime venous blood collected from 302 Indian expatriates working for 4 service companies located in the Abha area. All workers 21 years of age and older, who were living in aggregations in company housing and consented to participate, were included in the study. The mean length of residence of the participants in the host country at the time of the investigation was 2.4 years (range one month to 14 years). The individuals surveyed came from various endemic states in India: Kerala, Tamil Nadu, Andhra Pradesh, Uttar Pradesh, Maharashtra. For comparison, serum samples were collected from 200 healthy native Saudi blood donors aged 18-

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60 years (mean 27 years), who were living in non-endemic filariasis areas in and around Abha. In addition, 60 sera from patients with other serologically and/or parasitologically confirmed helminthic infections (31 schistosomiasis; 28 echinococcosis; 1 mixed infection with hookworm, ascariasis and trichuriasis) were immunologically assayed. Serum was separated from the blood cells by centrifugation and stored at -45°C for later antigenic assays. The specific CFA was detected using the Og4C3 capture sandwich ELISA (More and Copeman, 1990) and was performed on 50- μ l serum samples at 1:4 dilution. Sixty sera were pretreated with acid and boiling before being tested in parallel. All antigen-positive sera were re-tested with the same TropBio ELISA for confirmation. Detection of microfilaria was restricted to those individuals who turned out to be positive for the antigen test results. To determine the prevalence rate and densities of microfilaria among the test persons, night blood samples were obtained between 9:00 pm and midnight. Parasitemia was expressed as the number of microfilariae per ml of venous blood (mff/ml) and was determined by using the modified Knott test (Manson-Bahr and Bell, 1987). In addition, 3 Giemsa-stained 20 μ l blood smears obtained at the same time by finger prick were examined for the detection of microfilariae.

Eighty of the 302 expatriate sera including 32 antigen-positives and 48 randomly selected antigen negative sera (TropBio ELISA) were blindly tested

with the ICT Filariasis card test (ICT Diagnostics). The latter test is an *in vitro* immunodiagnostic test for the detection of *W. bancrofti* antigen in serum or plasma. The results were read after 2 minutes and at 15 minutes for maximum sensitivity and rechecked at 30 minutes before being recorded as negative. For confirmatory purposes, questionable samples were re-tested with both the ICT Filariasis card and TropBio ELISA tests.

The study population included 302 Indian expatriate workers aged 21-55 years (mean 32.9 years), who were living in Abha during the time of the survey. This group of individuals comprised 92 women and 210 men. The overall prevalence of filarial antigenemia in that group was 10.6% (32 individuals). Among the men 28 (87.5%) had positive CFA results, compared to 4 (12.5%) among the women. Microfilaria was present in 10 men (31.3%) out of 32 subjects with positive antigen results who were recalled for night blood examination. The mean microfilarial counts in these microfilaria-positive subjects was 105 mff/ml (range 10-1148 microfilariae). Individuals with microfilarial counts lower than 20 in the modified Knott test were usually microfilaria negative in the thick smears. Filarial antigenemia was not detected in sera from non-endemic Saudis or in sera from patients with other helminthic infections (Table 1).

All 32 sera of the antigen-positive individuals detected by the ELISA assay had positive results with the ICT Filariasis antigen test. All 10 indi-

Table 1
Circulating *Wuchereria bancrofti* antigen detected by TropBio ELISA assay and ICT filariasis rapid card test in a population of expatriate workers from India residing in Abha, southwestern Saudi Arabia.

Group tested	No. Positive/No. tested	% Positive
A. Expatriate endemic sera		
TropBio ELISA	32 ^a /302	10.6
ICT Filariasis Card Test		
Antigen-positive sera	32/32 ^b	100
Antigen-positive sera (Mf+)	10/10 ^c	100
Antigen-negative sera	0/48	0
B. Non-endemic control sera		
TropBio ELISA		
Saudis (blood bank)	0/200	0
Other helminthic infections	0/60	0

^aIncludes 10 mff-positive patients.

^bSix samples were classified as weakly positive after confirming testing.

^cMean microfilarial density 105 mff/ml blood (range 10-1,148).

viduals with microfilaremia had positive results (100%) determined by both ELISA and ICT card tests. In the latter test, a visible pink line could be identified as early as 2 minutes with microfilaria-positive sera which was confirmed at subsequent 15 and 30 minute-readings. Six of the 32 positive-antigen samples were first classified as questionable in the capture ELISA due to low antigen level test results. These samples gave weak positive readings after 15-30 minutes in the ICT Filariasis card test. All 6 samples were reclassified as antigen positive after in parallel tests of acid pretreatment and boiling sera (n=2) and confirmatory testing (n=4). One of the questionable sera came from patient who was microfilaria positive in an earlier survey and became microfilaria negative after DEC-treatment.

Previous baseline information on the prevalence of bancroftian filariasis among Indian expatriate workers in Saudi Arabia was based primarily on the detection of microfilariae in thick films of night blood surveys (Omar, 1996). In that study, a prevalence microfilarial rate of 3.5% was found among 259 Indian expatriates. This percentage compares well with the present microfilarial findings of 3.3% and the overall microfilarial infection rate of 3.4% in India (WHO,1994). It can be seen from Table 1 that the prevalence rate of CFA among the study sample of Indian expatriates was 10.6% as measured by the TropBio ELISA assay. No false positive antigen results were found in control sera from either native Saudis or from patients with other helminthic infections. This finding was confirmed by the ICT Filariasis test which detected virtually all of the antigen-positive individuals and proved to be as sensitive and specific for *W. bancrofti* infection as the TropBio ELISA assay. Both tests were capable of detecting CFA in microfilaremic and amicrofilaremic individuals. As reported in other studies (Ramzy *et al*,1991; Simonsen *et al*, 1996; Simonsen and Dunyo, 1999), the prevalence of antigenemia in the present sample was higher (3 times) than the prevalence of microfilaremia. Since amicrofilaremic antigen-positive endemic normals presumably harbor *W. bancrofti* adult worms (Weil *et al*,1996), a higher number in our study sample who would have appeared falsely negative by microfilaria detection alone may have in reality active infection.

The present ICT Filariasis test performance agrees fully with the results of other studies performed with banked or field sera from various *W. bancrofti* endemic areas (Weil *et al*,1997; Zheng

et al, 1998; Simonsen and Dunyo, 1999). In these studies, the ICT Filariasis card test gave the highest specificity and sensitivity (96-100%) with microfilaria positive sera when compared to other filarial antigen tests. Similarly, clinical trials have shown that the ICT Filariasis card test has a sensitivity, specificity and efficiency of 93.3% and 98.5% respectively when compared to the TropBio ELISA test (Mearns,1997). In conclusion, the use of the ICT Filariasis test for field surveys proved to be a reliable technique, since it is simpler to perform than the TropBio ELISA method and requires no laboratory or technical equipment. In both cases, however, examination of night blood samples for microfilaria can be confined to antigen-positive individuals only. Saudi Arabia and other Gulf states of the Arabian peninsula, where a continuous influx of mainly south and southeast Asian migrant workers from countries endemic for *W. bancrofti* reside for long periods, the ICT Filariasis card test could be of practical use in investigating the potential risk of introduced bancroftian filariasis in the host countries.

ACKNOWLEDGEMENTS

We would like to thank the JCU Tropical Biotechnology Pty Ltd (Townsville, Queensland, Australia) for providing the TropBio ELISA kits for detecting *Wuchereria bancrofti* antigen, and Dr G Burgess for valuable information. We are also indebted to the staff members of the Diagnostic Center of the Swiss Tropical Institute, Basel, Switzerland, where the rapid Filariasis card test (ICT Diagnostics) was performed. The willing co-operation of the General Directorate of Health Affairs in Asir, Saudi Arabia as well as the sponsors, supervisors and expatriate workers of the companies surveyed is greatly appreciated.

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