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Screening of army soldiers for *Wuchereria bancrofti* infection in the metropolitan Recife region, Brazil: implications for epidemiological surveillance

Zulma Medeiros[1], Judilson Gomes[2], Fátima Béliz[1], Amaury Coutinho[1], Patricia Dreyer[3] and Gerusa Dreyer[1]

1 *Departamento de Parasitologia do Centro de Pesquisas Aggeu Magalhães – Fundação Oswaldo Cruz, Recife, Brazil*
2 *Seção do Serviço de Saaúde da 7ª Região Militar e 7ª Divisão de Exército, Recife, Brazil*
3 *Universidade Federal de Pernambuco, Brazil.*

Summary Between 1989 and 1995, blood surveys were performed for *Wuchereria bancrofti* infection in several barracks of the Brazilian army in the metropolitan Recife region. For initial screening, 60 μl of capillary blood were examined for microfilaria. All men who tested positive had microfilaria quantified by filtration of venous blood through a polycarbonate membrane. Of 23773 men screened, 585 (2.5%) had microfilaria (mf). Microfilarial density ranged from < 1–8706 mf/ml of blood. Thirteen individuals had ultra-low microfilarial densities (1 mf/11 ml of blood). Characterization of 174 autochthonous cases made it possible to map 8 new districts in 4 cities within metropolitan Recife region where transmission of *W. bancrofti* was previously unknown. Routine screening of soldiers in the military may provide important surveillance data for national programmes to eliminate transmission of *W. bancrofti*.

keywords *Wuchereria bancrofti*; *Mansonella ozzardi*; public health; epidemiology; surveillance.

correspondence Dr Gerusa Dreyer, Departamento de Parasitologia – Centro de Pesquisas Aggeu Magalhães – FIOCRUZ, Av. Moraes Rego, s/No., Câmpus da UFPE, Cidade Universitária, Recife PE, CEP 50.670–420, Brazil.

Introduction

Although it has long been a neglected disease of the tropics, lymphatic filariasis has received increased attention in recent years with the development of improved diagnostic tools and the recognition that periodic single-dose mass treatment can profoundly suppress levels of microfilaria in the blood (Kessel *et al*. 1970; Mahoney & Kessel 1971; Eberhard *et al*. 1991; Andrade *et al*. 1995; Dreyer *et al*. 1995), thereby leading to interruption of transmission of the parasite (Panicker *et al*. 1991; Eberhard *et al*. 1992; Ismail *et al*. 1996; Kimura & Mataika 1996; Kazura *et al*. 1997; Bockarie *et al*. 1998). In May 1997 the World Health Assembly passed a resolution calling for the global elimination of lymphatic filariasis as a public health problem, and by November of that year, 13 countries had developed national filariasis elimination plans (WHO 1996; Ottesen *et al*. 1997).

One of the first major challenges facing these countries will be to determine the geographical distribution of filarial infection and to decide where filariasis elimination activities will be implemented. Surveillance for *Wuchereria bancrofti* infection presents several potential difficulties. Unlike diseases for which elimination campaigns have been successful, such as smallpox, dracunculiasis and polio, most patent *W. bancrofti* infections are asymptomatic and many years may elapse between infection and development of obvious clinical sequelae such as hydrocele and lymphoedema. Thus, although rapid assessment techniques based on clinical disease have been proposed for lymphatic filariasis (Pani *et al*. 1995, 1997; Gyapong *et al*. 1996a, b, c, 1998), the sensitivity of these methods, if used alone, may be inadequate to detect infection in areas of low-level or recent transmission. Further, these techniques probably require training of many community-level health workers or deployment of mobile assessment teams. Particularly to identify foci of transmission in areas where lymphatic filariasis is not suspected to occur (making these areas likely to be overlooked or of low priority for rapid assessment), and for the purposes of certifying that *W. bancrofti* has been eliminated in a country, a routine, ongoing, 'passive' system is needed for detection of current *W. bancrofti* infection in a nationally representative sentinel population. Our experience in Recife, Brazil, where bancroft-

ian filariasis is endemic (Dreyer 1987; Medeiros *et al*. 1992; Maciel *et al*. 1994, 1996), suggests that filarial screening of soldiers living or working in the military barracks may provide such a system.

Materials and methods

The project was approved by the Ethical Committee of Hospital das Clínicas – Universidade Federal de Pernambuco. Soldiers from 85 barracks in the metropolitan Recife region were targeted for screening. Before they were invited to have their blood examined for filarial infection, the soldiers attended an educational seminar that provided information on filarial infection, its diagnosis and prevention and signs and symptoms of acute and chronic filarial disease. After each soldier gave verbal consent to participate, information was collected on place of origin, current and past residential address and duration of residence in each location. Infections were considered authochthonous if they occurred in lifelong residents of metropolitan Recife region who had not travelled outside the area.

Fingerprick blood specimens were collected between 2300h and 0100h, the peak period of microfilaraemia in Metropolitan Recife Region (Dreyer *et al*. 1996a). For each soldier, three drops of capillary blood (approximately 60 μl) were placed on a glass slide, dried, stained with Carrazi haematoxilin-eosin, and examined under the microscope. To more accurately estimate microfilarial density in men who tested positive on the thick smear, one additional ml of peripheral blood was collected at night, filtered through a 3 μm polycarbonate membrane, and examined microscopically (Dennis & Kaen 1971). If microfilaria (mf) were not detected in 1 ml of filtered blood, this test was repeated by filtering 5 ml of blood, and if necessary, an additional 5 ml the following week. Every patient who tested positive for *W. bancrofti* mf was treated with diethylcarbamazine (DEC) or ivermectin by physicians working at the Military Hospital or the Filariasis Clinic of Centro de Pesquisas Aggeu Magalhães (CPqAM/FIOCRUZ), both in Recife.

Results

Between 1989 and 1995, 23773 men from 85 barracks in metropolitan Recife region (the cities of Recife, Jaboatão dos Guararapes and Olinda) were screened for microfilaraemia. None of the soldiers who attended the introductory lecture refused to undergo a blood examination. Mean age was 22 years (range: 18–58). 585 men (2.5%) were mf-positive (Table 1). Of these, 582 were infected by *W. bancrofti* while 3 individuals, aged 36, 48, and 39 years, were infected with *Mansonella ozzardi* (Manson 1897) between 1989 and 1991. These three men were lifelong residents of areas with known *M. ozzardi* transmission in Brazil (Rachou 1956). The annual filarial infection prevalence ranged from 1.3% to 4.1% (Table 1). The median interval between joining the army and screening was 6 months.

Table 1 Prevalence of *W. bancrofti* and *M. ozzardi* infection among Army's military units in Metropolitan Recife Region, by year 1989–95

Year	N° of barracks	No. men examined	No. (%) with microfilaraemia†
1989	13	3594	147 (4.1)*
1990	07	2072	68 (3.3)
1991	16	3434	111 (3.2)**
1992	10	2346	60 (2.6)
1993	10	2664	57 (2.1)
1994	13	4489	74 (1.6)
1995	16	5174	68 (1.3)
Total	85	23773	585 (2.5)

† All infections are *W. bancrofti* except 3 cases represented by: *2 men infected by *M. ozzardi*; **1 man infected by *M. ozzardi*.

In 1989–91, the prevalence of *W. bancrofti* infection was significantly higher among soldiers 18–30-year-old (3.8%) than among those who were older (RR = 2.6, CI 1.5–4.6, $P = 0.00028$); 96% of infections were detected in soldiers aged 18–30 years (310/323). After the first year therefore screening was focused on military personnel < 30, with the exception of those who had been transferred from barracks elsewhere. Of 601 men who had been transferred from elsewhere and were examined between 1990 and 1991, 5 (0.8%), aged 31–58, had *W. bancrofti* microfilaraemia, 1 case being detected in 1990 and 4 cases in 1991 (Table 2).

Additional venous blood was collected from all 582 men with *W. bancrofti* detected in fingerprick samples and filtered for quantification of microfilarial density. All had *W. bancrofti* mf. The geometric mean microfilarial density was 239/ml (range: 1 mf/11 ml to 8,700/ml). 163 (28.0%) men had low-density microfilaraemia (1–100 per ml) and 13 (2.2%) ultra-low microfilarial densities (< 1 mf/ml). Geometric mean microfilarial density did not vary significantly between 1989 and 1995.

Of 582 men infected by *W. bancrofti*, only 174 (29.9%) had infections that were considered authochthonous to metropolitan Recife region (Figure 1). Other cases probably resulted from transmission there, but because the soldiers had lived in several areas within the metropolitan region, the precise location could not be determined. 165 soldiers with authochthonous infections resided in areas previously recognized to be endemic for filariasis (Jaboatão dos Guararapes, Olinda and Recife) and 9 lived in areas not previously recognized as having *W. bancrofti* transmission – Abreu e Lima (1

Table 2 Distribution of filarial infection among soldiers at military bases, by age

	mf + /all (%)							
Age	1989	1990	1991	1992	1993	1994	1995	Total
18–20	104/2175 (4.8)	59/1304 (4.5)	89/2047 (4.3)	53/1600 (3.3)	34/1450 (2.3)	45/2435 (1.8)	53/2664 (2.0)	437/13675 (3.2)
21–30	33/1112 (3.0)	8/618 (1.3)	17/936 (1.8)	7/611 (1.1)	23/1006 (2.3)	29/1635 (1.8)	15/2068 (0.7)	132/7986 (1.6)
31–40	5/197 (2.5)*	0/98	4/258 (1.5)*	0/91	0/121	0/253	0/277	9/1295 (0.7)
41–50	5/103 (4.8)*	1/46 (2.2)	1/174 (0.6)	0/40	0/82	0/149	0/137	7/731 (1.0)
>50	0/7	0/6	0/19	0/4	0/5	0/17	0/28	0/86
Total	147/3594 (4.1)	68/2072 (3.3)	111/3434 (3.2)	60/2346 (2.6)	57/2664 (2.1)	74/4489 (1.6)	68/5174 (1.3)	585/23773 (2.5)

* Including cases of *M. ozzardi*.

case from Caetes district), Cabo de Santo Agostinho (1 case from Ponte dos Carvalhos), Camaragibe (2 cases from Fabrica district, 1 case from Bairro dos Estados district and 1 case from Bairro Novo district), and Paulista (1 case from Maranguape district, 1 case from Janga district and 1 case from Paratibe district). Subsequent follow-up of these cases and their family members documented transmission in these areas (Zulma Medeiros, unpublished data).

Discussion

Surveillance systems for lymphatic filariasis are urgently

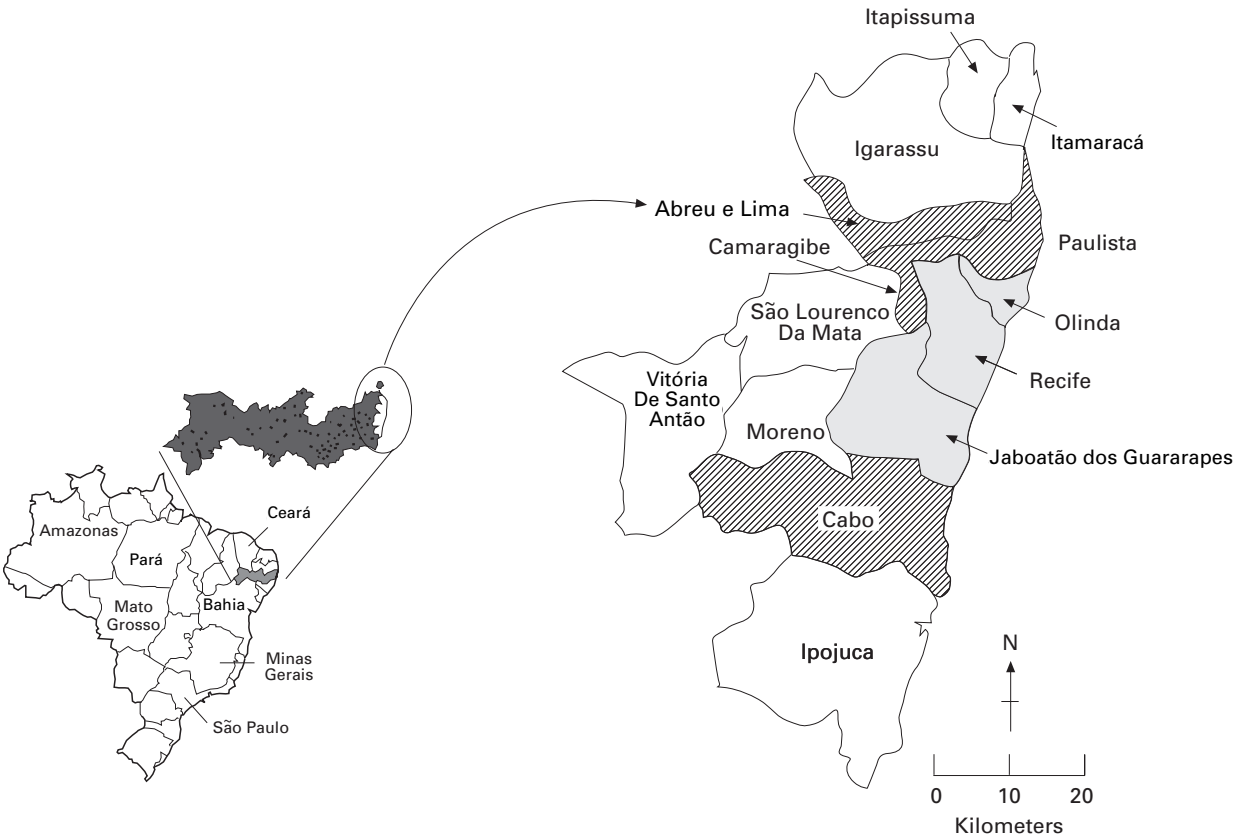

Figure 1 Distribution of autochthonous cases of lymphatic filariasis in the region of metropolitan Recife, northeastern Brazil. ▢ Previously recognised foci; ▨ Newly discovered foci.

needed to support and guide the global elimination programme recently announced by the World Health Organization (Ottesen & Ramachandran 1995; WHO 1995; Ottesen *et al*. 1997). It is likely that no single type of surveillance will meet the needs of all filariasis-endemic countries or of filariasis elimination programmes in all phases of their evolution (e.g. initial geographical assessment, programme monitoring, and certification of elimination). Recent work to develop techniques for initial filariasis assessment has focused on the use of clinical signs, particularly hydrocele, as indirect indicators of transmission (Pani *et al*. 1995, 1997; Gyapong *et al*. 1996a, b, c, 1998), or on collection and testing of fingerprick blood specimens for *W. bancrofti* infection by mobile survey teams. Our work with soldiers in the military as part of the lymphatic filariasis research and treatment programme at CPqAM in Recife, Brazil suggests that routine annual screening of military recruits is feasible and may complement these other surveillance methods. This type of ongoing routine surveillance may play a useful role in identifying new areas of transmission and in providing a back-up system that can enhance the level of certainty among public health officials when it appears that filariasis is being eliminated.

The military offers a convenient and fairly representative population for surveillance and assessment of a variety of diseases (Ali *et al*. 1985; Gill & Bailey 1989; Sharp *et al*. 1995; Drabick *et al*. 1997; Eitzen & Sawyer 1997). For example, serosurveys of military populations in the United States have been used to determine exposure (seropositivity) to *Toxoplasma gondii* (Smith *et al*. 1996). Several features of military bases also make them attractive for the purposes of screening and epidemiological surveillance for *W. bancrofti* infection. First, young adult males represent an ideal screening population because of their elevated risk for *W. bancrofti* infection and microfilaraemia. In most populations where lymphatic filariasis is endemic, the prevalence of microfilaraemia tends to increase with age to about 20 years, after which it remains consistently high (Brabin 1990; Lammie *et al*. 1994; Albuquerque *et al*. 1995; Chanteau *et al*. 1995). In addition, during the reproductive years, the prevalence of microfilaraemia tends to be higher in males than in females (Brabin 1990; Pani *et al*. 1991; Albuquerque *et al*. 1995). The prevalence of chronic filarial disease, such as hydrocele, tends to increase with age; thus most infected young males are asymptomatic (Ottesen 1993; Norões *et al*. 1996). Unless screening is offered to these men, their infections go undetected and they remain untreated. Without effective treatment, they serve as reservoirs for further transmission and their lymphatic damage progresses, thereby increasing their risk of clinical disease.

Secondly, since lymphatic filariasis causes hydrocele, it is a disease of considerable concern to the military authorities and to the soldiers themselves. In Brazil and in other countries, hydrocele is one of several criteria for exclusion from military service. Soldiers often expressed keen interest in lymphatic filariasis; after they were tested at the military base, for example, many of them brought their entire families to the Filariasis Clinic at CPqAM/FIOCRUZ to be screened for *W. bancrofti* infection.

Third, screening military recruits is convenient, safe, and logistically uncomplicated. In Brazil, prospective soldiers are required to undergo a physical examination and screening for certain diseases before being allowed to enter the army. Currently, no testing for microfilaraemia is required, but it would be a simple matter to collect fingerprick blood for detection of mf. In our surveys, soldiers were highly cooperative with this procedure, and medical staff at the base were eager to learn how to diagnose lymphatic filariasis. In fact, in 1992, army personnel began enrolling in courses at CPqAM/FIOCRUZ, which qualified them to diagnose and treat *W. bancrofti* microfilaraemia cases. This was the first time a programme for diagnosis and treatment of an endemic disease was adopted by the Army in Brazil. Nighttime collection of blood on the military base was easy to arrange, and, unlike nighttime blood collection in some peri-urban slums, where lymphatic filariasis is emerging as an important public health problem, it was also safe for the blood collection team. Further, collecting blood at the peak time of microfilarial density, between 2300h and 0100h, was much more feasible on the army base than it is in most communities. Thus, the sensitivity of screening was enhanced.

Finally, at least in Brazil, military recruits are considered a representative cross-section of the young male population in the region where the base is located. Soldiers tend to be stationed at bases located near their town of residence. Screening of army soldiers has already been used elsewhere in Brazil to successfully identify foci of transmission of *W. bancrofti* in areas that were not considered to be endemic. For example, in 1990, two autochthonous *W. bancrofti* infections were detected when solders were screened at the 59º Batalhão de Infantaria Motorizada in Maceió-Alagoas-Brazil (Dreyer *et al*. 1991). At the time, Maceió was considered by the Ministry of Health not to be endemic for *W. bancrofti* (Ministério da Saúde Brasil 1986). As a result of these findings, however, an epidemiological investigation was begun, active transmission was confirmed, and the Health Ministry recognized that filariasis was indeed a public health problem in Maceió. Filariasis research and control programmes have already begun in this area (Fontes *et al*. 1994, 1998).

Five years ago, *W. bancrofti* was also considered to be under control in Recife (Ministério da Saúde Brasil 1986). Work by Dreyer and colleagues subsequently documented transmission in 1985 (Dreyer 1987; Medeiros *et al*. 1992; Dreyer *et al*. 1995; 1996). Lymphatic filariasis is now considered an important public health problem of metropolitan

Recife region, with an overall prevalence of ~1%, but with prevalences of up to 15% in some districts (Maciel *et al.* 1996). Because active case detection and surveillance for *W. bancrofti* infection have only begun in recent years, it is difficult to say with absolute certainty whether transmission of this parasite is increasing in intensity and spreading to new areas, but this appears to be the case.

Our screening of military recruits identified 8 districts within four new cities in metropolitan Recife with active transmission of *W. bancrofti*. Autochthonous cases of *W. bancrofti* infection were identified in lifelong residents of a total of 7 cities in metropolitan Recife region; these infected persons resided in 79 districts of the metropolitan area (Figure 1).

Recently, considerable attention has focused on new circulating antigen-detection assays for diagnosis of *W. bancrofti* infection (More & Copeman 1990; Rocha *et al.* 1996; Freedman *et al.* 1997; Nicolas 1997; Weil *et al.* 1997). These assays have a high sensitivity and specificity for bancroftian filariasis infection. However, because the antigen is primarily adult worm-related, caution is warranted in using them for monitoring control programmes where the objective is to reduce or interrupt transmission. Transmission is directly associated with microfilaraemia, and for the first few years after treatment with antifilarial drugs, marked reductions in microfilaraemia are observed, but the prevalence of antigenemia remains constant (Weil *et al.* 1988). Therefore, monitoring mf levels is likely the best option for assessing the effectiveness of treatment in reducing transmission, particularly in the short term. Another drawback of the antigen assays is the fact that they are relatively expensive and their purchase on the international market requires 'hard currency'.

Although antigen-and even PCR-based assays have many advantages and are likely to be used more frequently in the diagnosis of lymphatic filariasis, detection of microfilaraemia using fingerprick blood will probably continue to play an important role in epidemiological surveillance and in monitoring the effectiveness of interventions to interrupt transmission (Nicolas 1997; Weil *et al.* 1997). Although we were unable to measure the sensitivity of the thick smear in this screening programme, it appeared to be relatively high, in view of the fact that 13 of men who had microfilaraemia detected in fingerprick samples had ultra-low microfilarial densities (< 1/ml venous blood). In part, this may have been because we examined 3 drops (60μl), rather than a single drop (20 μl) of capillary blood. Previous work showed that, among persons with < 100 mf/ml, the sensitivity of thick smear increases from 68% to 94% as the volume of examined blood increases from 20 μl to 60 μl (Dreyer *et al.* 1996). Therefore we recommend, not only for the military but also for the national filariasis elimination programme of Health Ministry (Fundação Nacional de Saúde –FNS), that 3 drops (60 μl) of capillary blood be collected by fingerprick for examination of microfilaraemia.

In summary, through routine screening during a 7-year period, we provided health education to thousands of soldiers, identified and treated 582 men 'silently' infected with *W. bancrofti*, and detected several new areas of transmission in metropolitan Recife. Our experience with the army suggests that the military services, including the Navy, Air Force and Military Police, may provide an excellent opportunity for ongoing routine surveillance of *W. bancrofti* infection at the national level. Such a surveillance system can be used by national filariasis elimination programmes to identify new areas of transmission and to monitor trends in prevalence over time in areas where transmission is known to occur. To be useful, military-based surveillance for *W. bancrofti* infection should be linked to thorough and timely case follow-up, particularly when infections are detected in residents of areas where transmission is currently not known to occur. This kind of surveillance should not replace community- or district-level initial assessment and monitoring of interventions to interrupt transmission. However, military-based surveillance can provide an adjunct surveillance system at the national level, particularly for the purpose of certifying countries as free of *W. bancrofti* transmission.

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