PNG Med J 2000 Sep-Dec;43(3-4):161-165

Prevalence of filarial antigenaemia in Papua New Guinea: results of surveys by the School of Public Health and Tropical Medicine, James Cook University, Townsville, Australia

WAYNE MELROSE[1], PAULINE PISTERS[1], PAUL TURNER[1], ZURE KOMBATI[1], BILLY P. SELVE[2], JEFFREY HII[1,3] AND RICHARD SPEARE[1]

School of Public Health and Tropical Medicine, James Cook University, Townsville, Australia and Misima Mines Ltd, Milne Bay, Papua New Guinea

SUMMARY

During the period from 1991 to 1997 the School of Public Health and Tropical Medicine, James Cook University carried out filariasis surveys in several parts of Papua New Guinea using the newly introduced *Onchocerca gibsoni* monoclonal (Og4C3) and immunochromatographic test (ICT) antibody-based assays for filarial antigen and, in some cases, a Knott's test for microfilariae. The average prevalence of filarial antigenaemia and microfilaraemia was 56% and 35% respectively confirming earlier survey results that filariasis is hyperendemic in many parts of the country. The antigen tests detected 25% more cases than the Knott's test and the simplicity of the ICT and its capacity to produce almost instant results make it an ideal tool for surveys.

Introduction

A number of filariasis prevalence surveys have been done in various areas of Papua New Guinea, such as the Fly River area (1,2), Star Mountains (3,4), the Sepik region (5,6), Schraeder Mountains (7) and Mount Bosavi (8). Traditionally, the diagnosis has depended upon the detection of microfilariae (mf) in blood samples collected during the night. Some surveys used methods such as wet preparations and thick films (2-4), which have been shown to be unreliable in detecting low numbers of mf (9,10). Others utilized the filtration technique (1,4,7). This increased the sensitivity but did not solve the problem of having to take night bloods or detecting amicrofilaraemic cases. Even with these limitations, filariasis was found to be highly endemic in the areas surveyed. If the samples taken by Taukuro et al. at Lake Murray (2) are excluded (because they were taken during the day) the average mf prevalence in these earlier surveys was 51%. The highest recorded prevalence was in a community at North Bosavi where the prevalence rate was 95% (8).

The diagnosis of filariasis has been revolutionized by the introduction of sensitive, specific filarial antigen tests for *Wuchereria bancrofti* such as the Og4C3 ELISA (TropBio, Townsville, Australia) and the immunochromatographic rapid card test (ICT AMRAD, Sydney, Australia). Blood can be taken at any time, day or night, and both microfilaraemic and amicrofilaraemic cases will be detected. Both tests have almost identical sensitivity and are specific for *W. bancrofti*. There is no cross reaction with other filarial species or other helminths (11-13).

The first filarial antigenaemia surveys in Papua New Guinea were done by Turner et al. in the Western Province in 1991 and 1992 using the Og4C3 test (12,13). Since then the School of Public Health and Tropical Medicine, James Cook University has used both tests to conduct a number of surveys in

1 School of Public Health and Tropical Medicine, James Cook University, Townsville, Qld 4811, Australia

2 Misima Mines Ltd, PO Box 38, Misima Island, Milne Bay Province 222, Papua New Guinea

3 Present address: Health Advisor, AusAID, GHEG, GPO Box 887, Canberra, ACT 2601, Australia

other localities in Papua New Guinea on behalf of local health authorities and the private sector.

Methods

The Og4C3 test is a sandwich ELISA based on a monoclonal antibody raised against *Onchocerca gibsoni* antigens (14). The test was carried out according to the manufacturer's instructions. Briefly, blood was collected (at night if mf were also going to be counted) and the serum was separated, diluted 1:3 with the diluent supplied and placed in boiling water for 5 minutes. The boiled samples were centrifuged and the supernatant, which contains the filarial antigen, dispensed into ELISA plates pre-coated with Og4C3 antibody. All subsequent steps were carried out at room temperature. The plates were incubated, washed 3 times and, after rabbit anti-onchocerca antibody had been added, incubated for 1 hour. The plates were washed again, antibody conjugate was added and they were incubated for a further hour. After a final washing 2,2'-azino-di [3-ethylbenzothiazoline sulphonate] (ABTS) chromogen was added; after 1 hour of incubation stopping solution was added and the plates were read at a wavelength of 414 nm. A positive test is denoted by an optical density (OD) >2 SD above the mean OD of the negative control readings.

The ICT test consists of a card which is folded into two halves. On one side of the card there is a filter paper pad impregnated with a *W. bancrofti*-specific polyclonal antibody attached to colloidal gold. The other side of the card has a strip of membrane with a line of immobilized monoclonal antibody. Blood is collected by finger prick and 100 µl of whole blood is placed on the lower part of the filter paper pad. Red cells are retained on this portion of the pad and plasma flows upwards; any filarial antigen present will bind with the gold-labelled polyclonal antibody. When the card is closed the sample and the labelled antibody will come in contact with the strip of membrane and migrate upwards to cross the monoclonal antibody line. In a positive sample the gold-labelled antibody-antigen complex will be captured by the monoclonal antibody and a pink line will result. Results are available within a few minutes and the pink line remains visible for many months, which facilitates the checking of results and record keeping. In our surveys, cards were reread after 1 hour, and the results checked by a second person.

Microfilariae were counted using an improved Knott's technique (15). 1 ml of blood was mixed with 0.9 ml of 2% Triton X-100 in water rather than the usual 1% formalin. This results in a reduction in the amount of proteinaceous sludge and makes the subsequent counting of the microfilariae easier. The mixture was left to stand at room temperature for 30 minutes and centrifuged at 120 g for 5 minutes. The supernatant was removed and the deposit suspended in 100 µl of 1% formalin in water. A drop of 1% methylene blue was added and the deposit examined for microfilariae.

Results

Over a period of 6 years (1991-1997) a total of 5 surveys were carried out in the Western Province, 6 in Milne Bay Province, 2 in New Ireland Province and 1 each in Madang, Gulf, Southern Highlands, Western Highlands and West New Britain Provinces. The average prevalence of antigenaemia in the filaria-endemic areas surveyed was 56% with the highest prevalence at Nomad in the Western Province, where it was 88%. The average prevalence of microfilaraemia was 35%, 10% higher than the previous estimate by the World Health Organization (16). The results are summarized in Table 1.

Discussion

The results confirm earlier observations that filariasis is highly endemic in lowland areas with pockets of endemicity extending to highland fringe and some highland areas. They also demonstrate that the prevalence of filariasis can be underestimated by around 25% if reliance is placed solely upon the detection of mf, even if concentration methods are used. The discrepancy between microfilaraemia and antigenaemia has also been observed in other parts of the world and can be due to infection by a single sex parasite, failure of the female worm to produce mf, or the presence of ultra-

TABLE 1

THE PREVALENCE OF MICROFILARAEMIA AND ANTIGENAEMIA FROM VARIOUS FILARIA-ENDEMIC AREAS IN PAPUA NEW GUINEA

Year	Location	Number tested	Prevalence of mf	Prevalence of antigen
	Western Province			
1991	Upper Fly	600	54%	76% (Og4C3)
1991	Mogulu	300	51%	82% (Og4C3)
1993	Waiwoi Falls	485	52%	76% (Og4C3)
1999	Lake Murray	500	-	70% (Og4C3)
2000	Nomad	262	-	88% (Og4C3)
	Madang Province			
1996	Karkar Island	133	-	10% (Og4C3)
	Gulf Province			
1994	Near Kerema	222	35%	65% (Og4C3)
	Southern Highlands Province			
1994	Moro	181	37%	52% (Og4C3)
	Western Highlands Province			
1998	Mount Hagen	200	-	0% (ICT)
	East New Britain Province			
1994	Witu Island	69	10%	38% (Og4C3)
	New Ireland Province			
1998	South Coast	140	21%	32% (Og4C3)
				32% (ICT)
1993	Lihir Island	575	20%	55% (Og4C3)
	Milne Bay Province			
1996	Alotau	212	23%	52% (Og4C3)
				53% (ICT)
				53% (Og4C3)
1997	Misima Island	144	-	56% (ICT)
1997	Panapompom Island			18% (ICT)
1996	Kimuta Island	50	-	39% (ICT)
1996	Rossel Island	121	-	67%
1997	Paneati Island	97	-	0%

low mf densities which cannot be detected even by concentration methods (16,17). Ultrasound examination has shown that many individuals who have antigenaemia without microfilaraemia have adult worms in situ (17-19). The simplicity of the ICT means that it can be accurately carried out after minimal amount of training and is ideal for field surveys, but its cost of around $US1.90 per test is a major obstacle for widespread use in developing countries unless financial support can be obtained. The Og4C3 test requires laboratory facilities, but it gives a quantitative result, and is very useful for research purposes, or when large numbers of serum samples have to be analyzed.

Although these surveys have gone some way towards mapping the prevalence of filariasis in Papua New Guinea there remains a lot more work to do. Many surveys excluded children below the age of 10 years. In hindsight this was a mistake as it is now believed that filarial infection occurs early in life (19) and questions are being raised about the epidemiology of filariasis in children, the possible effects of filaria-induced immunosuppression on the efficacy of vaccination and the contribution of childhood filarial infection to renal disease. Further surveys therefore should also include young children.

ACKNOWLEDGEMENTS

We thank the many people who have made this study possible, in particular the health workers and village people of Papua New Guinea and other colleagues at James Cook University. We gratefully acknowledge the generous logistic support we received from private companies, missions and churches, Missionary Aviation Fellowship, Royal Australian Air Force and the Australian Army.

REFERENCES

1 **Knight R, McAdam KPWJ, Matola YG, Kirkham V.** Bancroftian filariasis and other parasitic infections in the Middle Fly River region of western Papua New Guinea. I. Clinical, parasitological and serological studies. *Ann Trop Med Parasitol* 1979;73:563-576.

2 **Taukuro BD, Antoni S, Ashford RW, Downes TJ, Ellis B, Flemming F, Gibson FD, Han C, Hogan P, Hutchinson P, Matheson FA, Molineaux L, Narara A, Nurse GT, Otto P, Pilecki B, Ponta S, Rashid K, Schuurkamp G, Taufa T, Vaterlaws AL, Welsch RL.** The World Health Organization North Fly clinico-epidemiological pilot study. *PNG Med J* 1980;23:80-86.

3 **Cattani J, Taufa T, Anderson W, Lourie J.** Malaria and filariasis in the Ok Tedi region of the Star Mountains, Papua New Guinea. *PNG Med J* 1983;26:122-126.

4 **Schuurkamp GJT, Kereu RK, Bulungol PK, Kawereng A, Popon WH, Crane GG, Greenidge J, Spicer PE.** Diethylcarbamazine in the control of splenomegaly associated with bancroftian filariasis in the Ok Tedi area of Papua New Guinea. *Trans R Soc Trop Med Hyg* 1992;86:531-536.

5 **Kazura JW, Spark R, Forsyth K, Brown G, Heywood P, Alpers M.** Parasitologic and clinical features of bancroftian filariasis in a community in East Sepik Province, Papua New Guinea. *Am J Trop Med Hyg* 1984;33:1119-1123.

6 **Bryan JH, Dagoro H, Southgate BA.** Filarial vector studies in a diethylcarbamazine-treated and in untreated villages in Papua New Guinea. *J Trop Med Hyg* 1995;98:445-451.

7 **Desowitz RS, Jenkins C, Anian G.** Bancroftian filariasis in an isolated hunter-gatherer shifting horticulturist group in Papua New Guinea. *Bull World Health Organ* 1993;71:55-58.

8 **Prybylski D, Alto WA, Mengeap S, Odaibaiyue S.** Introduction of an integrated community-based bancroftian filariasis control program into the Mount Bosavi region of the Southern Highlands of Papua New Guinea. *PNG Med J* 1994;37:82-89.

9 **Southgate BA.** Studies on filariasis in the Pacific. 1. A field trial of a counting-chamber technique for the determination of microfilarial rates and densities. *Southeast Asian J Trop Med Public Health* 1973;4:172-178.

10 **Panicker KN, Krishnamoorthy K, Sabesan S, Prathiba J, Abidha K.** Comparison of effects of mass annual and biannual single dose therapy with diethylcarbamazine for the control of Malayan filariasis. *Southeast Asian J Trop Med Public Health* 1991;22:402-411.

11 **Simonsen PE, Dunyo SK.** Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific circulating antigens. *Trans R Soc Trop Med Hyg* 1999;93:278-282.

12 **Turner PF.** Filariasis in the Western Province of Papua New Guinea. PhD Thesis. James Cook University of North Queensland, Townsville, Australia, 1993.

13 **Turner PF, Copeman B, Gerisi D, Speare R.** A comparison of the Og4C3 antigen capture ELISA, the Knott test, and IgG4 assay and clinical signs in the diagnosis of bancroftian filariasis. *Trop Med Parasitol* 1993;44:45-48.

14 **More SJ, Copeman DB.** A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. *Trop Med Parasitol* 1990;41:403-406.

15 **Melrose WD, Turner PF, Pisters P, Turner B.** An improved Knott's concentration test for the

detection of microfilariae. *Trans R Soc Trop Med Hyg* 2000;94:176.

16 **Weil GJ, Ramzy RM, Chandrashekar R, Gad AM, Lowrie RC Jr, Faris R.** Parasite antigenaemia without microfilaraemia in bancroftian filariasis. *Am J Trop Med Hyg* 1996; 55:333-337.

17 **Dreyer G, Santos A, Noroes J, Rocha A, Addiss D**. Amicrofilaraemic carriers of adult *Wuchereria bancrofti*. *Trans R Soc Trop Med Hyg* 1996;90:288-289.

18 **Faris R, Hussain O, El Setouhy M, Ramzy RM, Weil GJ.** Bancroftian filariasis in Egypt: visualisation of adult worms and subclinical lymphatic pathology by scrotal ultrasound. *Am J Trop Med Hyg* 1998;59:864-867.

19 **Dreyer G, Noroes J, Addiss D, Santos A, Medeiros Z, Figueredo-Silva J.** Bancroftian filariasis in a paediatric population: an ultrasonographic study. *Trans R Soc Trop Med Hyg* 1999;93:633-636.