New Tools for Diagnosis and Monitoring of Bancroftian Filariasis Parasitism: The Polynesian Experience

L. Nicolas

Bancroftian filariasis is endemic in French Polynesia and control programs with diethylcarbamazine, started in the 1950s, led to a sharp reduction of the microfilaria prevalence. Consequently, the control program was interrupted in 1982. Ten years later, however, the incidence of the parasitism again reached pre-control levels (20–30% microfilaremia in some islands), indicating that the adult worms (for which no diagnostic tool was available) had persisted. Apart from research on chemotherapy strategies, the Institut Malardé has been actively involved in developing and evaluating more-powerful diagnostic tools than the unique detection of microfilariae by blood smear examination. These include: (1) the detection of adult worm circulating antigens in humans, and (2) the detection of Wuchereria bancrofti *larvae in mosquitoes, using DNA probes. In this paper, Luc Nicolas reviews the available diagnostic tools to detect* W. bancrofti *and their implementation in epidemiological areas, based on the Polynesian experience.*

At least 120 million people, in 73 countries of the tropical world, are estimated to harbor agents of lymphatic filariasis, 90% of which are *Wuchereria bancrofti* and 10% *Brugia malayi*[1]. Severe clinical syndromes, such as hydroceles, elephantiasis or lymphedema with irregular fever episodes, occur in 44 million people, while another 76 million harbor circulating microfilariae (Mf) in their blood without any external clinical symptoms[1]. It is now recognized, however, that most of these 'asymptomatic' microfilaremic individuals suffer lymphatic and renal damage[2,3]. Brugian filariasis is restricted to Asia, but bancroftian filariasis persists as a major cause of morbidity and social handicap in many countries in Asia, East Africa, Latin America, the Middle East, the Pacific Rim and the Pacific Islands. The levels of parasite-harboring patients, based on records of Mf carriers and individuals with external symptoms are, therefore, underestimated because they do not include 'cryptic' parasitemias. Furthermore, epidemiological data are not available from many African countries, and recent findings of *W. bancrofti* foci with severe symptoms in Ghana[4] indicated that *W. bancrofti* could be widespread on this continent as well.

New strategies for efficient control have recently emerged, and elimination of lymphatic filariasis is now considered a reasonably achievable goal by the World Health Organization[1]. Control of lymphatic filariasis still relies on uptake of diethylcarbamazine (DEC), an antifilarial drug discovered 50 years ago. In addition, after its success in *Onchocerca volvulus* control, ivermectin was shown to be lethal against *W. bancrofti* Mf as well. Simplified and cost-effective strategies are now based on single yearly or half-yearly distribution of DEC and ivermectin[5–8] to the entire population. Another strategy, based on daily consumption of DEC-medicated salt as an additive to table/cooking salt, has been tried with success[9]. In addition, vector control plays a significant role in interruption of transmission, and should be implemented as a complementary tool to drug administration-based programs whenever feasible[1].

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The need for diagnostic tools for filariasis

Transmission of *W. bancrofti* is due to the presence of Mf in human blood; these are taken by mosquito females during their bloodmeal and develop into infective larvae (L3) within the mosquitoes before transmission to another individual. In humans, L3 larvae mature into adult worms (commonly named 'macrofilariae') which can persist for more than ten years in the lymphatic system[10], mate and produce Mf. Interruption of transmission can be achieved with microfilaricidal drugs, but elimination of the parasitism requires the clearance of adult worms with macrofilaricidal drugs as well. Most microfilaremic individuals remain asymptomatic but some develop well-known filarial symptoms such as hydroceles, lymphangitis or elephantiasis.

Microfilaria detection

Detection of Mf in blood remains the only diagnostic test available for filariasis in most endemic areas, apart from clinical examination. However, in most endemic areas, blood needs to be sampled at night when Mf are present in the peripheral vascular system (nocturnal periodicity). This problem is not encountered in Polynesia, where *W. bancrofti* is aperiodic. Furthermore, in several vector–parasite pairs (eg. *Aedes polynesiensis–W. bancrofti* in Polynesia) the relationship is such that proportionally more infective larvae develop in the insect as Mf uptake decreases[11]. Therefore, very sensitive Mf detection methods are needed.

Two methods, based on microscopic examination of blood samples, are routinely used: (1) the sampling of 20–60 µl of capillary blood by finger-prick, followed by smearing onto a glass slide (thick-film or blood smear method); and (2) the sampling of venous blood, followed by filtration of 1 ml of blood using a Nucleopore membrane and microscopic counting (membrane filtration method). Often, Mf prevalence surveys are conducted using the finger-prick method, which is less invasive than venous blood sampling. However, accurate determination of Mf prevalence in an endemic area requires comparison of the sensitivity of the two methods, and determination of the

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Table 1. Sensitivity and specificity of circulating filarial antigen ELISAs

CFA ELISA	Country	Mf prevalence[a] (%)	CFA prevalence (%)	CFA prevalence/ Mf prevalence	% CFA⁺/Mf⁺ (n)	% CFA⁺/Mf⁻ (n)	Ref.
Og4C3	French Polynesia (Raiatea)	27 [vb]	49	1.8	94 (274)	32 (751)	19
Og4C3	French Polynesia (Tahaa)	22 [vb]	46	2.1	99 (187)	31 (354)	22
Og4C3	French Polynesia (Marquesas)	11 [vb]	27	2.4	100 (29)	18 (230)	b
Og4C3	Haiti	29 [fp]	49	1.7	100 (121)	30 (287)	21
Og4C3	Papua New Guinea	26 [vb]	57	2.2	100 (175)	42 (498)	20
Og4C3	Brazil	nd [fp]	nd	–	100 (25)	40 (25)	c
AD12	India	30 [fp]	40	1.3	98 (52)	16 (70)	15
AD12	Egypt	64 [vb]	88	1.4	97 (122)	16 (305)	44
		50 [fp]	88	1.8	97 (95)	23 (332)	44

[a] Abbreviations: Mf, microfilariae; [vb], membrane filtration of 1 ml of venous blood; [fp], finger-prick of 20–60 µl of capillary blood; CFA, circulating filarial antigen; nd, not done.
[b] L. Nicolas *et al.*, unpublished.
[c] L. Nicolas and A.F. Furtado, unpublished.

periodicity of Mf in the vascular system, in order to optimize the results. In Polynesia, Mf periodicity was determined in 12 Mf carriers over a 48 h period (every 4 h)[12]. No nychthemeral variations of Mf counts were observed by membrane filtration; conversely, the blood film (20 µl finger-prick) showed an up to five times higher microfilaremia during the day than during the night. Another study involved 782 individuals who had venous and finger-prick blood samples taken simultaneously during the day[13]; the Mf prevalence was 1.8 times higher by the filtration method than by blood film. An accurate comparison of the periodicity and intravascular distribution of *W. bancrofti* Mf, assessed by membrane filtration and capillary blood film (60 µl), was also carried out in Recife, Brazil[14]. The shape of periodicity curves was found to be fairly uniform between the two techniques in 18 individuals, with a nocturnal peak between 11.00 pm and 1.00 am. However, the filtration method gave more reproducible results than did finger-prick tests, and proved to be more sensitive as well, especially for low-Mf carriers, because of the higher volume of blood sample used.

Monitoring adult worm populations

A major technical breakthrough in the monitoring of the presence of adult worms was the development of two monoclonal antibodies (mAbs), AD12 (Ref. 15) and Og4C3 (Ref. 16), which bind to circulating *W. bancrofti* filarial antigen(s) (CFA) in enzyme-linked immunosorbent assays (ELISAs). AD12 recognizes a 200 kDa antigen in sera of filarial patients[17]. Infestation of monkeys with *W. bancrofti* larvae has recently permitted elucidation that the antigen originates from the adult worm[18]. Indirect evidence suggests that the antigen recognized by Og4C3 is also of adult worm origin[19]. Both mAbs appear to be specific for *W. bancrofti* infection when they are used to detect antigen in plasma or serum from people naturally exposed to a range of parasitic infections[15,16]. The Og4C3 antigen assay has been commercially available for a few years now as an ELISA kit (Trop-Ag *W. bancrofti* ELISA kit, JCU Tropical Biotechnology Pty Ltd, Queensland, Australia) that is sufficiently simple to be used in most endemic countries. The AD12 antigen assay is still under development. In addition, individual immunochromatographic cards (ICT Diagnostics, Balgowlah, Australia), coated with AD12 or Og4C3 mAbs, have been developed recently, to detect *W. bancrofti* CFA, and are under evaluation. These could be convenient tools for direct assessment of parasitological burden.

We have used the Og4C3 antigen ELISA test in French Polynesia to evaluate the parasitic status of Polynesian asymptomatic individuals and the macrofilaricidal effect of chemotherapy strategies, using CFA reduction as a readout assay. This ELISA has been evaluated as a diagnostic tool also in Papua New Guinea[20], Haiti[21] and Brazil (L. Nicolas and A.F. Furtado, unpublished) and results obtained in those different groups are fairly consistent (Table 1). Importantly, the level of CFA remains constant in infected individuals throughout the 24 h of the day[12,21], in contrast to microfilaremia which is periodically nocturnal in most endemic areas. Therefore, CFA detection avoids the necessity to take blood samples at night. The Og4C3 antigen assay detects almost all Mf carriers (Table 1), even those with low Mf density. Out of 53 sera from Polynesians harboring <50 Mf ml^{-1} (by membrane filtration), 51 (96%) were CFA positive, and the two CFA-negative sera had only 1 and 4 Mf ml^{-1} (Ref. 22). CFA are found in all ages, even young children (<5 years old)[20,21,23]. With regard to epidemiological assessment, the highlight is that a large proportion (18–40%) of Mf-negative individuals are CFA positive and, therefore, suspected of harboring adult worms. This means that the CFA prevalence in populations where *W. bancrofti* is circulating is approximately twice the Mf prevalence (Table 1). Although it is not known why a large proportion of Mf-negative individuals harbor adult worms or whether or not these individuals are always amicrofilaremic, they are potential parasite reservoirs, and thus need the clearance of their adult worms with DEC[22].

The Og4C3 antigen ELISA is a powerful method for evaluating the efficiency of chemotherapy (Fig. 1). Annual single doses of DEC at 6 mg kg^{-1}, either with or without ivermectin at 400 µg kg^{-1}, reduced CFA levels by 40–50% after one year and by 60–70% after two years[22,24] in Mf-positive individuals. In contrast, reduction of CFA levels following treatment with

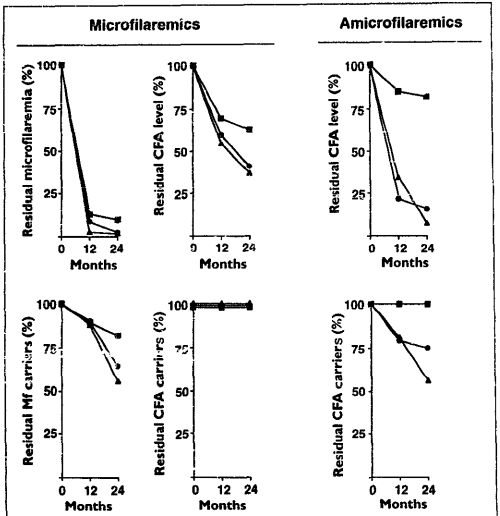

Fig. 1. Effect of annual single-dose treatments with filaricidal drugs on microfilariae and adult worms of *Wuchereria bancrofti* in endemic adult Polynesian individuals (data from Ref. 21, with permission). Adult individuals (>20 years old) living in Tahaa Island, Society Archipelago, were treated at month 0 and month 12 with a single dose of either DEC at 6 mg kg^{-1} (closed circle) or ivermectin at 400 μg kg^{-1} (closed square), or a combination of both drugs (closed triangle). For each treatment, two groups were considered according to their microfilaremia status prior to the treatments ('microfilaremics' or 'amicrofilaremics'). Venous blood samples were collected prior to treatment (month 0), immediately before the second treatment (month 12) and one year after the second treatment (month 24). Microfilaremia was determined by membrane filtration (1 ml) and CFA by Og4C3 antigen ELISA. All individuals considered were CFA positive prior to treatment. Microfilaremics included 46 to 47 individuals per treatment group, with pretreatment microfilarial intensity ranging from 2 to 6816 Mf ml^{-1} (geometric mean 552) and CFA level ranging from 244 to 20416 antigen units (geometric mean 3952). Amicrofilaremic (but CFA-positive) individuals included 27 to 28 individuals per treatment group, with pretreatment CFA level ranging from 123 to 3912 antigen units (geometric mean 613).

ivermectin alone was poor. Reduction of CFA levels followed the same pattern in Mf-negative and Mf-positive individuals. Nevertheless, CFA clearance with DEC was only observed in Mf-negative individuals, even after two annual treatments, probably because the pretreatment antigen level was 4–6 times lower in Mf-negative than Mf-positive individuals[22]. These data, obtained in an area where concurrent re-infection occurs, show that the eradication of *W. bancrofti* parasitism (Mf and adult worms) with annual doses of DEC, either alone or combined with ivermectin, is likely to be achieved within a few years, but might take longer in those individuals harboring a high number of parasites.

The Og4C3 antigen assay is useful for diagnosis, as well as for following chemotherapy of clinically symptomatic individuals, who are often amicrofilaremic. In French Polynesia, external filarial symptoms have been recorded in 2% of the adult inhabitants of Tahaa Island (Society Archipelago) where a large-scale chemotherapy trial was initiated in 1994 (Ref. 24). Among 36 symptomatic people studied, 33% were CFA positive while only 22% were Mf positive (all Mf-positive individuals were CFA positive) (Fig. 2). This confirms previous data from other endemic areas[19,21,25] showing a higher CFA prevalence than Mf prevalence in clinical cases. Of practical interest for therapy, DEC reduced CFA levels in symptomatic individuals of Tahaa Island to levels found in asymptomatic individuals (L. Nicolas *et al.*, unpublished).

Studies using AD12 instead of Og4C3 for monitoring CFA levels led to similar conclusions on the parasitism status of endemic populations (Table 1) and in evaluation of chemotherapy to reduce the adult worm burden[6,15,26–29]. Paired samples of sera from Mf-positive Polynesians, collected before and after chemotherapy, were analyzed in our laboratory with the Og4C3 antigen assay and in Washington University of St Louis (G.J. Weil) with the AD12 assay[7]. Reduction of antigen was higher with the AD12 assay than with the Og4C3 assay, but it is unknown whether this reflects the different sensitivities of the individual ELISAs or whether the two mAbs recognize different *W. bancrofti* antigen(s). Larger formal comparisons of these mAbs are needed, evaluating CFA reduction in the same cohort of patients, in order to establish when parasitism is eliminated from individuals.

Considering the availability of sensitive CFA detection, the detection of antifilarial antibodies[30] is now of less interest in monitoring programs.

Polymerase chain reaction (PCR) poolscreening

Monitoring the parasite presence among mosquitoes is necessary to evaluate the transmission of the parasite and the efficacy of filariasis control programs. Such a goal requires the dissection and individual microscopic observation of several hundreds of mosquitoes, especially in endemic areas where parasitism rates are low. Although dissection provides detailed information on the numbers of the different stages and their distribution within the mosquito body, and allows determination of entomological indexes such as the infection rate (percentage of mosquitos infected by any stage of the parasite) and the infectivity rate (percentage of mosquito carriers of infective larvae L3), it remains tedious work. In addition, there is also the risk of neglecting the presence of filarial species that may 'co-infect' a mosquito population.

We have, therefore, developed a PCR strategy to evaluate the infection rate of a mosquito population by screening pools of vectors with a *W. bancrofti* molecular probe (Fig. 3). A PCR assay that amplifies a DNA repeat sequence, specific to *W. bancrofti* parasites but not stage specific (the *Ssp*I DNA repeat), was first established[31,32] and then improved[33]. Using artificially bloodfed *A. polynesiensis* mosquitoes, we have shown that a mosquito harboring as few as one or two Mf, the smallest stage of the parasite, can be detected among 50 parasite-free mosquitoes[33]. Thus, PCR can be performed, with an excellent sensitivity, on pools of mosquitoes instead of individuals. The percentage of positive individuals (infection rate) can be deduced from the percentage of pools that are PCR positive, using a computer program (Poolscreen Program™) designed by Katholi *et al.*[34] Also, an internal PCR standard plasmid has been constructed which enables the validation of PCR reactions to be achieved, thereby avoiding false-negative results[35]. This plasmid contains a copy of the *Ssp*I DNA repeat which has been mutagenized by inserting 34 additional base pairs. When added to DNA extracted from biological samples (mosquitoes or blood) containing *W. bancrofti* larvae in a single PCR reaction using common primers, two PCR products of 225 bp and ~188 bp are obtained, from the standard plasmid and from the target *W. bancrofti* DNA, respectively. PCR products can be visualized either on an agarose gel or by a DNA-ELISA[36], using two specific oligonucleotide probes. This should also help in standardizing the assay among different laboratories. Infection rates of large batches of field-captured *A. polynesiensis* were higher in all batches by PCR poolscreening than by individual dissection[33]. Two hypotheses might be considered. First of all, the dissection may miss some small larvae, due to the difficulty of dilacerating tissues, while the PCR assay can detect any parasite-carrying mosquito. Second, the DNA from the *W. bancrofti* Mf that do not complete their development within the mosquito (and are not detected by dissection) can still be amplified by PCR, leading to some false positives. However, this minor overestimation of parasite carriage is likely to be fairly constant in a given endemic situation where the vectorial competence of a mosquito species is known. Although the *Ssp*I PCR assay is not stage specific, it is very useful for monitoring infection rates, especially following chemotherapy campaigns.

This assay presents several practical advantages over mosquito dissection. It can be performed on mosquitoes stored dried at room temperature (even in tropical climates) up to two years after capture without loss of sensitivity. Thus, mosquitoes can be either sent to a reference laboratory for PCR assay or analyzed locally if proper PCR equipment and training exist. The *Ssp*I PCR assay (and the internal standard plasmid) is ubiquitous for all geographical isolates of *W. bancrofti* so far tested and has been applied successfully to other mosquito species, such as *Culex quinquefasciatus*[37]. Between 1000 and 2000 mosquitoes can be analyzed per (8 h) day by a technician, with a single thermocycler. This has to be compared with the ability of a well-trained technician to dissect and examine ~100–200 mosquitoes per day. DNA extracts from mosquitoes can be counterchecked by PCR in other laboratories. Also, they can be screened for the presence of several parasites using

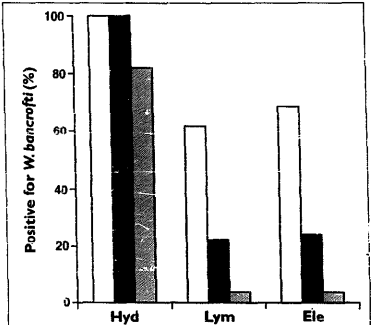

Fig. 2. Parasitological and immunological detection of *Wuchereria bancrofti* in adult Polynesian individuals exhibiting external filarial-like symptoms. Symptoms: Hyd, scrotal hydrocele ($n=7$); Lym, lymphangitis of a leg ($n=33$); Ele, elephantiasis ($n=19$). The following diagnostic tests were performed: immunodetection of antifilarial antibodies (IgG BmA) (open bar), Og4C3 antigen ELISA (CFA) (closed bar) and microfilaremia by membrane filtration (Mf) (shaded bar).

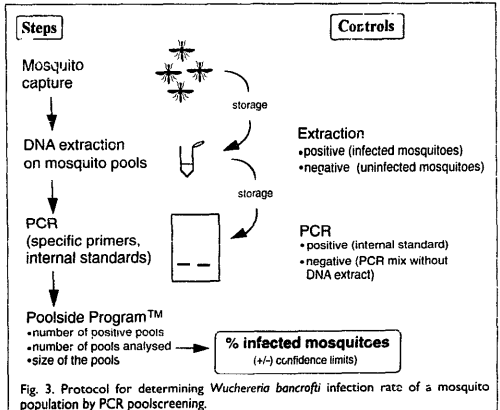

Fig. 3. Protocol for determining *Wuchereria bancrofti* infection rate of a mosquito population by PCR poolscreening.

different molecular probes, for example, probes for detecting *W. bancrofti* and the dog heart worm *Dirofilaria immitis*, both transmitted by *A. polynesiensis* in French Polynesia[38].

New tools in community diagnosis

The history of filariasis epidemiology in French Polynesia has shown that 'control' of bancroftian filariasis requires complete elimination of adult worms in humans, and not simply the reduction of microfilaremia and Mf prevalence.

The availability of sensitive and convenient immunodiagnostic tools for monitoring the adult worm burden opens the way for novel approaches to epidemiological assessment of filariasis and monitoring control programs. With the objective of the elimination of filariasis, parasitological assessment in humans could rely on CFA detection, Mf monitoring being a complementary assessment but no longer the priority, for the following reasons: (1) the CFA assay detects approximately twice as many parasitized individuals as does Mf detection, including all microfilaremics; (2) it can be performed on diurnal blood samples; and (3) the technique is rather simple and can be applied in most endemic areas. Sera can be collected by finger-prick onto filter papers and stored until analysis or separated from venous blood by centrifugation. The Og4C3 antigen ELISA can be performed in poorly equipped laboratories. Because the final step of the reaction is a colorimetric reaction, results can be read visually or, more accurately, quantified with an optical density reader. Reagents cost less than one US$ per sample. Aliquots of sera can be kept frozen for better evaluation of kinetics by analyzing samples collected at different times simultaneously on the same plate. Individual immunochromatographic cards (ICT) to detect *W. bancrofti* CFA could be a convenient tool for direct assessment of infection in the field without equipment. However, as they indicate only whether an individual is CFA positive or not (yes/no), they will only ascertain prevalence and not CFA level. If data on Mf prevalence are needed (for identifying those individuals transmitting the parasite), CFA detection should be complemented by Mf detection, as CFA detection does not indicate whether a CFA-positive individual is microfilaremic.

Monitoring the risk of transmission in mosquitoes is a complementary tool to CFA detection in humans, and control programs could be based on these two techniques (PCR poolscreening and CFA detection) only. The sensitivity of PCR poolscreening and the possibility of using ubiquitous primers and an internal standard should make this technique easy to use in endemic areas. However, precautions should be taken to avoid the risk of contamination in PCR handling. Data generated by (1) dissection and microscopic observation, and (2) PCR, differ. Dissection and microscopic observations of larvae can establish the presence of infective larvae (L3) in vector mosquitoes. PCR poolscreening determines the percentage of mosquitoes infected by any filarial stage, and enables the kinetics of this system to be followed. Moreover, although PCR can detect DNA from dead *W. bancrofti* in mosquitoes, this indicates that mosquitoes have ingested Mf, evidence of parasite circulation in the human population.

Filarial parasitism could be considered to be eliminated from an area when humans are cleared of CFA and PCR on mosquitoes remains negative. Complementary tools might be Mf detection and antifilarial antibody detection in children. Following elimination and the end of control measures, a surveillance program should be implemented on the basis of CFA detection in a sentinel human population and the monitoring of mosquitoes by *W. bancrofti*-based PCR.

Special attention should be paid to areas where multifilarial parasitism occurs, such as Central Africa or India. As specific probes now exist for several filarial species, in particular *W. bancrofti*[32,39], *B. malayi*[40] and *Loa loa*[41], PCR diagnosis on blood could be useful in these areas and is being investigated[36,42,43].

Finally, data on *W. bancrofti* prevalence in unsurveyed areas could be obtained by screening banks of sera collected in the course of other infection studies (and kept frozen) with the CFA assay. This should help identify new filariasis foci with limited costs.

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References

1 World Health Organization (1994) **Lymphatic filariasis infection and disease: control strategies.** TDR/CTD/FIL/PENANG/94.1
2 Dreyer, G. *et al.* (1992) **Renal abnormalities in microfilaremic patients with bancroftian filariasis.** *Am. J. Trop. Med. Hyg.* 46, 745–751
3 Freedman, D.O. *et al.* (1994) **Lymphoscintigraphic analysis of lymphatic abnormalities in symptomatic and asymptomatic human filariasis.** *J. Infect. Dis.* 170, 927–933
4 Gyapong, J.O., Magnussen, P. and Binka, F.N. (1994) **Parasitological and clinical aspects of bancroftian filariasis in Kassena-Nankana district, Upper East Region, Ghana.** *Trans. R. Soc. Trop. Med. Hyg.* 88, 555–557
5 Addiss, D.G. *et al.* (1993) **Comparative efficacy of clearing-dose and single high dose ivermectin and diethylcarbamazine against *Wuchereria bancrofti* microfilaremia.** *Am. J. Trop. Med. Hyg.* 48, 178–185
6 Kazura, J. *et al.* (1993) **Comparison of single-dose diethylcarbamazine and ivermectin for treatment of bancroftian filariasis in Papua New Guinea.** *Am. J. Trop. Med. Hyg.* 49, 804–811
7 Moulia-Pelat, J.P. *et al.* (1995) **Combination ivermectin plus diethylcarbamazine, a new tool for control of lymphatic filariasis.** *Trop. Med. Parasitol.* 46, 9–12
8 Meyrowitsch, D.W., Simonsen, P.E. and Makunde, W.H. (1996) **Mass diethylcarbamazine chemotherapy for control of bancroftian filariasis: comparative efficacy of standard treatment and two semi-annual single-dose treatments.** *Trans. Roy. Soc. Trop. Med. Hyg.* 90, 69–73
9 Gelband, H. (1994) **Diethylcarbamazine salt in control of lymphatic filariasis.** *Am. J. Trop. Med. Hyg.* 50, 655–662
10 World Health Organization (1984) **WHO Expert Committee on Filariasis, Fourth Report.** *WHO Techn. Rep. Ser.* No. 702
11 Southgate, B.A. (1992) **The significance of low density microfilaremia in the transmission of lymphatic filarial parasites.** *J. Trop. Med. Hyg.* 95, 79–86
12 Moulia-Pelat, J.P. *et al.* (1993) **Periodicity of *Wuchereria bancrofti* var. *pacifica* filariasis in French Polynesia.** *Trop. Med. Parasitol.* 44, 83–85
13 Moulia-Pelat, J.P. *et al.* (1992) **A comparative study of detection methods in lymphatic filariasis control programmes.** *Trop. Med. Parasitol.* 43, 146–148
14 Dreyer, G. *et al.* (1996) **Studies on the periodicity and intravascular**

distribution of *Wuchereria bancrofti* microfilariae in paired samples of capillary and venous blood from Recife, Brazil. *Trop. Med. Intern. Health* 1, 264–272
15 Weil, G.J. *et al.* (1987) **A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in bancroftian filariasis.** *J. Infect. Dis.* 156, 350–355
16 More, S.J. and Copeman, D.B. (1990) **A highly specific and sensitive monoclonal antibody based ELISA for the detection of circulating antigen in bancroftian filariasis.** *Trop. Med. Parasitol.* 41, 403–406
17 Weil, G.J. and Liftis, F. (1987) **Identification and partial characteriz-ation of a parasite antigen in sera from humans infected with *Wuchereria bancrofti*.** *J. Immunol.* 138, 3035–3041
18 Weil, G.J. *et al.* (1996) **Parasite antigenemia without microfilaremia in bancroftian filariasis.** *Am. J. Trop. Med. Hyg.* 55, 333–337
19 Chanteau, S. *et al.* (1994) **Og4C3 circulating antigen, a marker of infection and adult worm burden in *Wuchereria bancrofti* filariasis.** *J. Infect. Dis.* 170, 247–250
20 Turner, P. *et al.* (1993) **A comparison of the Og4C3 antigen capture ELISA, the Knott test, an IgG4 assay and clinical signs, in the diagnosis of bancroftian filariasis.** *Trop. Med. Parasitol.* 44, 45–48
21 Lammie, P.J., Hightower, A.W. and Eberhard, M.L. (1994) **Age-specific prevalence of antigenemia in a *Wuchereria bancrofti* exposed population.** *Am. J. Trop. Med. Hyg.* 51, 348–355
22 Nicolas, L. *et al.* (1997) **Reduction of *Wuchereria bancrofti* adult worm circulating antigen after annual treatments of diethylcarbamazine and ivermectin in French Polynesia.** *J. Infect. Dis.* 175, 489–492
23 Chanteau, S. *et al.* (1995) ***Wuchereria bancrofti* filariasis in French Polynesia: age-specific patterns of microfilaremia, circulating antigen, and specific IgG and IgG4 responses according to transmission level.** *Int. J. Parasitol.* 25, 81–85
24 Moulia-Pelat, J.P. *et al.* (1995) **Advantages of an annual single dose of the combination ivermectin 400 μg/kg plus diethylcarbamazine for community treatment of bancroftian filariasis.** *Trans. R. Soc. Trop. Med. Hyg.* 89, 682–685
25 Addiss, D.G., Eberhard, M.L. and Lammie, P.J. (1994) **Filarial adenolymphangitis without filarial infection.** *Lancet* 343, 597
26 Weil, G.J. *et al.* (1988) **Persistence of parasite antigenemia following diethylcarbamazine therapy of bancroftian filariasis.** *Am. J. Trop. Med. Hyg.* 38, 589–595
27 Weil, G.J. *et al.* (1991) **Changes in circulating parasite antigen levels after treatment of bancroftian filariasis with diethylcarbamazine and ivermectin.** *J. Infect. Dis.* 164, 814–816
28 Ramzy, R.M.R. *et al.* (1994) **Efficient assessment of filariasis endemicity by screening for filarial antigenaemia in a sentinel population.** *Trans. R. Soc. Trop. Med. Hyg.* 88, 41–44
29 McCarthy, J.S. *et al.* (1995) **Clearance of circulating filarial antigen as a measure of the macrofilaricidal activity of diethylcarbamazine in *Wuchereria bancrofti* infection.** *J. Infect. Dis.* 172, 521–526
30 Lal, R.B. and Ottesen, E.A. (1988) **Enhanced diagnostic specificity in human filariasis by IgG4 antibody assessment.** *J. Infect. Dis.* 158, 1034–1037
31 Chanteau, S. *et al.* (1994) **PCR-based detection of *Wuchereria bancrofti* larvae in pools of mosquitoes.** *Trans. R. Soc. Trop. Med. Hyg.* 88, 665–666
32 Zhong, M. *et al.* (1996) **A PCR assay for the detection of *Wuchereria bancrofti* in blood.** *Am. J. Trop. Med. Hyg.* 54, 357–363
33 Nicolas, L. *et al.* (1996) **Evaluation of a polymerase chain reaction assay to determine infection of *Aedes polynesiensis* by *Wuchereria bancrofti*.** *Trans. R. Soc. Trop. Med. Hyg.* 90, 136–139
34 Katholi, C.R. *et al.* (1995) **Determining the prevalence of *Onchocerca volvulus* infection in vector populations by polymerase chain reaction screening of pools of black flies.** *J. Infect. Dis.* 172, 1414–1417
35 Nicolas, L. and Plichart, C. **A universally applicable internal standard for PCR detection of *Wuchereria bancrofti* in biological samples.** *Parasite* (in press)
36 Williams, S.A. *et al.* (1996) **Use of a polymerase chain reaction assay for the detection of *Wuchereria bancrofti* in blood samples from Tahiti.** *Trans. R. Soc. Trop. Med. Hyg.* 90, 384–387
37 Furtado, A. *et al.* (1997) **Improvement and application of a polymerase chain reaction system for detection of *Wuchereria bancrofti* in *Culex quinquefasciatus* and human blood samples.** *Mem. Inst. Oswaldo Cruz* 92, 85–86
38 Nicolas, L. and Scoles, G.A. **Multiplex polymerase chain reaction for detection of *Dirofilaria immitis* (Filarioidea: Onchocercidae) and *Wuchereria bancrofti* (Filarioidea: Dipetalonematidae) in their common mosquito vector *Aedes polynesiensis* (Diptera: Culicidae).** *J. Med. Entomol.* (in press)
39 Siridewa, K. *et al.* (1994) **Cloning and characterization of a repetitive DNA sequence specific for *Wuchereria bancrofti*.** *Am. J. Trop. Med. Hyg.* 51, 495–500
40 Williams, S.A., DeSimone, S.M. and McReynolds, L.A. (1988) **Species-specific oligonucleotide probes for the identification of human filarial parasites.** *Mol. Biochem. Parasitol.* 28, 163–170
41 Toure, S.F. *et al.* **Detection of *Loa loa* specific DNA in the blood from occult infected individuals.** *Exp. Parasitol.* (in press)
42 Lizotte, M.R. *et al.* (1994) **A polymerase chain reaction assay for the detection of *Brugia malayi* in blood.** *Am. J. Trop. Med. Hyg.* 51, 314–321
43 McCarthy, J.S. *et al.* (1996) **Evaluation of a polymerase chain reaction based assay for diagnosis of *Wuchereria bancrofti* infection.** *J. Infect. Dis.* 173, 1510–1514
44 Faris, R. *et al.* (1993) **Community diagnosis of bancroftian filariasis.** *Trans. R. Soc. Trop. Med. Hyg.* 87, 659–661

Sex, Strains and Virulence

I.M. Hastings and B. Wedgwood-Oppenheim

When are populations of infectious agents likely to evolve into distinct strains? Are they likely to differ in their virulence? Will genetically distinct strains or clones remain stable long enough to be useful as epidemiological markers? Sexual recombination can break down the genetic associations that define a strain structure, but if sex is rare or inbreeding is common, can strains persist? In this paper Ian Hastings and Bruce Wedgwood-Oppenheim show how some simple population genetic theory may provide a basis for addressing these questions.

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The past seven years have seen a long-running debate as to the extent to which populations of pathogens can be split into a series of genetically distinct strains or clones (see, for example, Refs 1–11 and references therein)*. A clonal structure has implications for epidemiology because it may allow the history of infections to be traced, and may form the basis for clinical decisions. If genetically stable strains do exist, then genes (including those affecting virulence) will segregate within this strain background and the strains may be associated with clinical or epidemiological differences. Unfortunately, and despite its obvious importance, many points related to this debate remain unresolved. The purpose of this brief discussion is not

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