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Immuno–parasitological assessment of bancroftian filariasis in a highly endemic area along the River Sabaki, in Malindi district, Kenya

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Lymphatic filariasis is endemic in the coastal areas of Kenya, with four major foci identified in the early 1970s. The prevalence and intensity of *Wuchereria bancrofti* infection, together with antifilarial antibody responses, were assessed in a historically highly endemic focus along the River Sabaki, in Malindi district. The prevalences of microfilaraemia and antigenaemia (detected by Og4C3 ELISA) were >20% and >40%, respectively, and both increased steadily with age. The high prevalences of antifilarial IgG_1 (86%) and IgG_4 (91%) responses indicate that most people living in this setting are exposed to *W. bancrofti* infection. The children investigated had higher levels of antifilarial IgG_1 than the adults.

The results of this study, based on a battery of currently available parasitological and immunological methods, provide an epidemiological update on lymphatic filariasis on the northern Kenyan coast. They show that the River Sabaki area is still an important focus for bancroftian filariasis and highlight the importance of implementing an elimination programme, to interrupt the transmission of *W. bancrofti* in all areas of endemicity in Kenya. The detailed baseline data collected in the River Sabaki area make the communities studied ideal as sentinel sites for epidemiological monitoring and the evaluation of the impact of mass drug administrations to eliminate lymphatic filariasis.

Lymphatic filariasis (LF), one of the most prevalent tropical diseases, is caused by mosquito-transmitted nematodes that are estimated to be present in >120 million people world-wide (Michael *et al.*, 1996; Zagaria and Savioli, 2002). More than 40 million of those infected are suffering from one or more of the overt clinical manifestations caused by the infection (Ottesen *et al.*, 1997; Molyneux and Zagaria, 2002), and more than 1000 million people live in endemic areas, where they risk infection by virtue of their continuous exposure to the mosquito vectors (WHO, 2002).

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Recent research advances in the diagnosis and treatment of LF have led to optimism that the disease could be eliminated as a public-health problem (Ottesen *et al.*, 1997). The introduction of effective treatment regimens to control transmission, by reducing microfilaraemias, has been primarily responsible for the identification of LF as an eradicable, or potentially eradicable, infectious disease, by an International Task Force for Disease Eradication (Anon., 1993). In 2000, 3 years after a World Health Assembly resolution to eliminate LF as a public-health problem (WHO, 1997), the World Health Organization (WHO) initiated the Global Programme to Eliminate Lymphatic Filariasis (GPELF; Molyneux and Zagaria, 2002). The principal goals of the GPELF are to interrupt

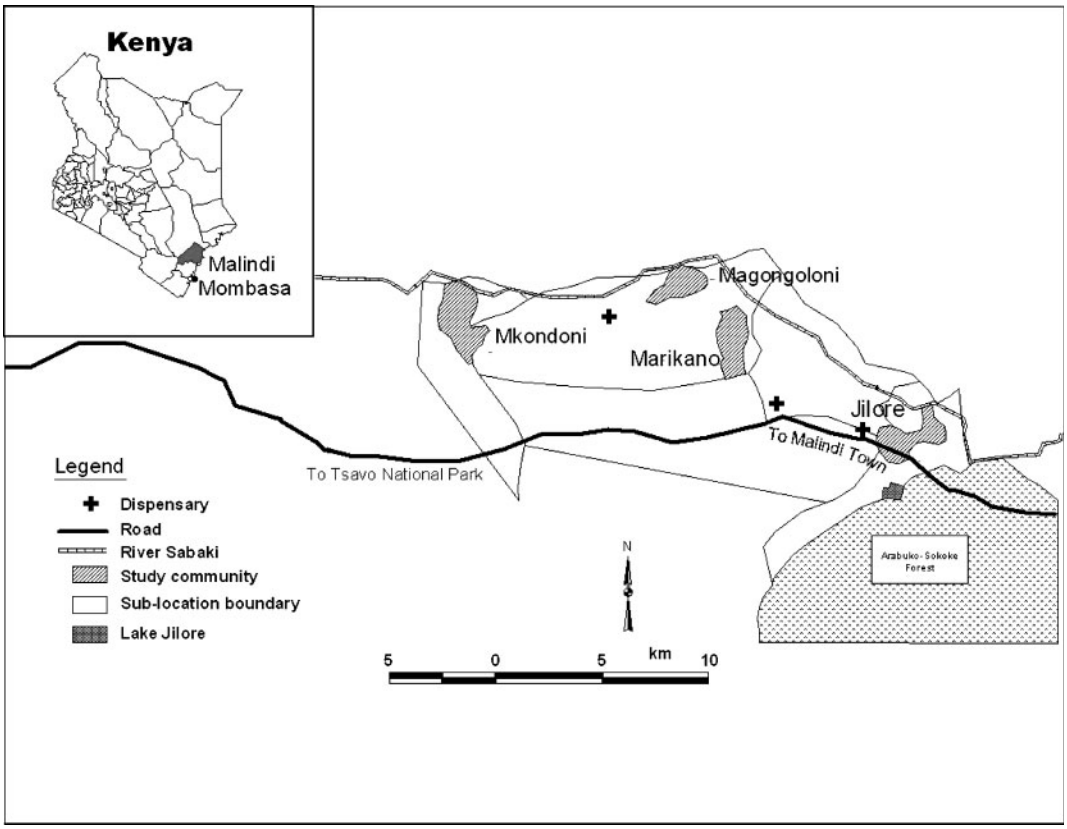

FIG. 1. Map showing the locations of the four study communities.

transmission of the parasites that cause LF and to alleviate and prevent the suffering and disability caused by the disease.

Although studies conducted in the 1960s and 1970s revealed that one form of LF, bancroftian filariasis, was highly endemic in coastal Kenya (Nelson *et al.*, 1962; Wijers, 1977), few relevant epidemiological data were subsequently collected in this region until the 1990s. Then, several research groups began to collect new information on bancroftian filariasis in one area of coastal Kenya: Kwale district in the south (Estambale *et al.*, 1994; Wamae *et al.*, 1998; Njenga *et al.*, 2000; Mukoko *et al.*, 2004).

Between 1971 and 1973, the area along the River Sabaki, close to Kenya's northern coast, was shown to be one of four major foci for bancroftian filariasis in the coastal areas of Kenya (Wijers, 1977). In 2001, this area was selected for a pilot LF-elimination programme, based on annual, single-dose, mass treatments with a combination of diethylcarbamazine (DEC) and albendazole. The objective of the present study was to assess the immuno–parasitological indicators of *Wuchereria bancrofti* infection in communities living along the River Sabaki, in an effort to provide baseline data to be used in the epidemiological monitoring of the elimination programme. This is the first published epidemiological study of bancroftian filariasis in northern coastal Kenya for more than 30 years.

MATERIALS AND METHODS

Study Area

The study area (Fig. 1) is located in Malindi district, which is one of the seven administrative districts forming the Coast province

of Kenya. Malindi town, the administrative headquarters of the district, is located on the shores of the Indian Ocean, 120 km north of Mombasa Island. The study was conducted in 2002 in four communities (Jilore, Marikano, Magongoloni and Mkondoni) which all lie close to the River Sabaki (also known as the River Galana). The area has a hot and dry climate, with two moderate rainy seasons (in March–June and October–November). The Giriama, a major subgroup of the Miji Kenda (who occupy most of Coast province), are the predominant ethnic group in Malindi district. The study communities are rural, with homesteads and households dispersed in the countryside. Most of the houses are made of mud walls, with roofs, of coconut leaves or grass, that have open eaves that allow entry of mosquitoes. The inhabitants of the area practise subsistence agriculture, growing mainly maize and cassava and keeping chickens, ducks, goats and cattle. The languages predominantly spoken in the study area are Giriama and Kiswahili.

Study Population

The purpose of the present study was explained to each study community, during public meetings conducted in Kiswahili and Giriama. During subsequent house-to-house visits, baseline demographic information (name, gender, year of birth, ethnicity and duration of residency in the area) was collected on all members of each household in the four communities.

In each community, approximately 200 individuals were recruited for blood sampling. The household occupied by each villager providing a blood sample was mapped using a global positioning system (Garmin® eTrex™; Garmin International, Olathe, KS).

The study protocol was approved by the Scientific Steering and Ethical Review Committees of the Kenya Medical Research Institute, Kenya, and the Research Ethics Committee of Liverpool School of Tropical Medicine, U.K.

Microfilaria Detection and ICT

Between 20.30 and 24.00 hours — a period of the night when the numbers of microfilariae (mff) in the peripheral blood were found to peak elsewhere in Kenya (Gatika *et al.*, 1994) — two 100-μl samples of fingerprick blood were collected, into heparinized capillary tubes, from each of 748 subjects. At the same time, a 2-ml sample of venous blood was collected into a tube containing EDTA and used as a source of plasma for testing for circulating filarial antigens (CFA) and antifilarial IgG_1 and IgG_4 antibodies, using ELISA (see below).

One of the fingerprick blood samples from each of 740 subjects was transferred into a tube containing 0.9 ml 3% acetic acid solution and mixed gently. The acetic-acid-diluted samples were kept at room temperature until the following day, when any mff in them were counted using the counting-chamber method (McMahon *et al.*, 1979). Eight subjects were not checked for microfilaraemia because insufficient blood was collected from the fingerpricks.

The second fingerprick sample from each subject was tested for CFA using a commercial immunochromatographic-test (ICT) card (ICT Diagnostics/AMRAD ICT, Sydney, Australia).

ELISA

Overall, 463 of the plasma samples were tested for CFA using a commercial ELISA based on the Og4C3 monoclonal antibody (TropBio, Townsville, Queensland, Australia), following the manufacturer's instructions. Each sample was run initially at a dilution of 1:10. If this gave a reading that fell beyond the standard curve, the sample was re-run at a higher dilution, to give a reading within the curve. Each reading was then multiplied with an appropriate factor, based on the dilution used, to give arbitrary 'antigen units'. Specimens with $\geqslant 128$ antigen units were considered positive for CFA.

The plasma specimens were also tested for antifilarial immunoglobulin G_1 (IgG_1)

and IgG_4 (Hitch *et al.*, 1991). Briefly, 96-well polystyrene microtitre plates (Dynex Technologies, Chantily, VA) were coated, at 50 μl/well, with 0.1 M $NaHCO_3$ buffer, pH 9.6, containing 2 μg *Brugia pahangi* antigen/ml. The plates were incubated overnight at 4°C and then blocked for 1 h, at 100 μl/well, with 0.01 M phosphate-buffered saline (PBS), pH 7.2, containing 0.3% (v/v) Tween 20. After a washing step using PBS/0.05% Tween 20, each test plasma, diluted 1:50 in PBS/0.05% Tween 20, was added to two wells, at 50 μl/well. Serial dilutions of a standard serum sample with known amounts of antifilarial IgG_1 and IgG_4 were included in each plate, to generate a standard curve. The plates were incubated at room temperature for 2 h and, after another washing step, biotin-conjugated mouse anti-human-IgG_1 monoclonal antibody (Zymed Laboratories, South San Francisco, CA) or a similar conjugate of mouse anti-human-IgG_4 monoclonal antibody (Zymed Laboratories) was added, at 50 μl/well. The plates were incubated at room temperature for 1 h and, after washing, streptavidin conjugated to alkaline phosphatase was added. The plates were developed by addition to each well of 50 μl of a solution (1 mg/ml) of p-nitrophenyl phosphate (Sigma) in 10% diethanolamine, pH 9.8, containing 3 mM $MgCl_2$. The absorbance of the contents of each well was read at 405 nm, in a UVmax plate reader (Molecular Devices, Sunnyvale, CA).

Plasma specimens from individuals in two of the study communities (Magongoloni and Marikano) were also used to determine IgG_4 antibody responses to the recombinant antigen Bm14. Magongoloni and Marikano were selected for this study to represent areas of high and moderate levels of *W. bancrofti* transmission (based on intensities of CFA and microfilaraemia), respectively. The anti-Bm14 IgG_4 assays were conducted like the assays of antifilarial IgG_1 and IgG_4 except that plates were coated with the Bm14 recombinant antigen and the plasma specimens were diluted 1:250.

A plasma sample giving an absorbance that exceeded a cut-off threshold, set as the corresponding mean value plus two S.D. for 20 negative-control plasma (all collected in an area of Kenya where filariasis is non-endemic), was considered positive.

The immunological assays were all conducted at the laboratories of the U.S. Centers for Disease Control and Prevention (Atlanta, GA).

Data Analysis

All the data were analysed using version 12.0.1 of the SPSS statistical software package (SPSS Inc., Chicago, IL) and Epi Info 2002 (Centers for Disease Control and Prevention, Atlanta, GA). Pearson's χ^2 test was used to compare proportions, in two-by-two contingency tables. Geometric mean intensities (GMI), for microfilaraemia, antigenaemia and levels of antifilarial antibody, were calculated as $\{\text{antilog}[\Sigma\log(x+1)/n]\}$-1, where x is the measure of microfilaraemia, antigenaemia or level of antifilarial antibody, and n is the number of individuals. The Mann–Whitney rank-sum test was used to determine the significance of differences between two independent groups, whereas the Kruskal–Wallis test was employed to explore the significance of differences among multiple groups.

RESULTS

Demography

The total number of people living in the four study communities in 2002 was 2611. There were more females than males (1341 *v.* 1270; $P=0.564$), with the mean age of the females slightly greater than that of the males (20.8 *v.* 19.6 years; $P=0.056$). The overall mean age of inhabitants of the study communities was 20.2 years.

Microfilaraemia

Among the 740 subjects who provided adequate samples of fingerprick blood, the

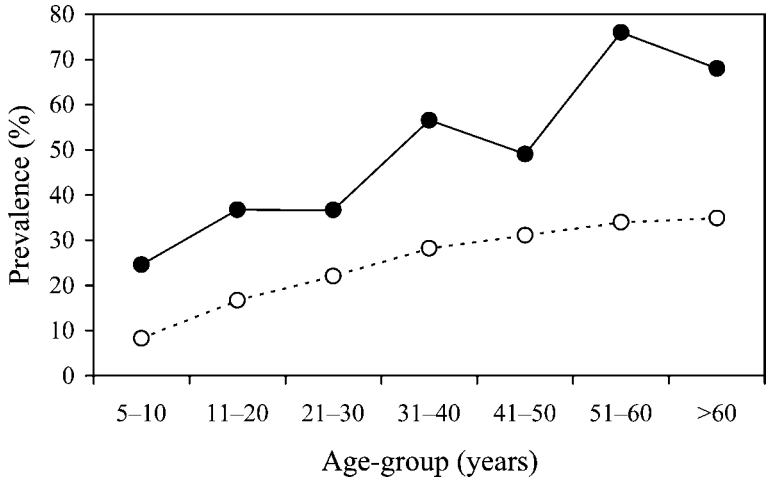

FIG. 2. The age-specific prevalences of microfilaraemia (○) and antigenaemia by Og4C3 ELISA (●) in the subjects from the four study communities along the River Sabaki, Kenya, in 2002.

overall prevalence of microfilaraemia was 20.5%. Table 1 summarizes the prevalences and geometric mean intensities of microfilaraemia observed in the four study communities. The mean age of the villagers checked for microfilaraemia was similar in each study community (24.5–29.1 years; $P=0.056$), as was the mean age of those found microfilaraemic (27.6–38.5 years; $P=0.123$). The overall geometric mean intensity of microfilaraemia, among the microfilaraemics, was 526 mff/ml. The prevalence and GMI of microfilaraemia among the male subjects (20.9% and 543 mff/ml, respectively) were similar to those among the female (20.2% and 512 mff/ml, respectively).

In age-specific analyses, the subjects were categorized as children (<16 years), young adults (16–30 years) and mature adults (>30 years). The prevalence of microfilaraemia in the children (10.8%) was significantly lower than that in the young adults (22.2%; $P<0.001$) or the mature adults (31.3%; $P<0.001$) and the prevalence of microfilaraemia in the mature adults was significantly higher than that in the young adults ($P=0.034$). The prevalence of microfilaraemia rose steadily with age (Fig. 2).

Overall, 314 individuals aged 16–45 years (113 males and 201 females) were tested for microfilaraemia. Within this age-group, 29.2% of the males and 22.4% of the

TABLE 1. *Prevalences and intensities of microfilaraemia in the four study communities along the River Sabaki, Malindi district, Kenya, in 2002*

	No. found microfilaraemic/ no. examined, and (% microfilaraemic):			Intensity of microfilaraemia (microfilariae/ml):*		
Community	Males	Females	All	Males	Females	All
Jilore	17/81 (21.0)	15/92 (16.3)	32/173 (18.5)	516	675	585
Marikano	15/81 (18.5)	29/111 (26.1)	44/192 (22.9)	277	273	274
Magongoloni	19/80 (23.8)	25/114 (21.9)	44/194 (22.7)	655	788	728
Mkondoni	17/83 (20.5)	15/98 (15.3)	32/181 (17.7)	842	637	739
All four	68/325 (20.9)	84/415 (20.2)	152/740 (20.5)	543	512	526

*Geometric mean intensity in the microfilaraemic subjects.

females were found microfilaraemic (P=0.180), and the GMI microfilaraemia was higher among the males (560 mff/ml) than among the females (526 mff/ml) but the difference was not statistically significant (P=0.430).

Antigenaemia

ICT

Of the 748 subjects tested for antigenaemia using ICT cards, 35.4% (27.5%–42.4% of those from each study community) were found ICT-positive.

OG4C3 ELISA

Of the 463 subjects tested for CFA by Og4C3 ELISA, however, 189 (40.8%) were found positive (Table 2). The ELISA results indicated significant inter-community differences in the intensities of antigenaemia (P=0.001), with the subjects from Magongoloni having significantly higher intensities of antigenaemia than those from Jilore or Marikano (P=0.006). Almost all (90.4%) of the 104 microfilaraemic subjects who were tested for CFA in the Og4C3 ELISA were found to be antigenaemic. The 10 microfilaraemic subjects found antigen-negative had significantly less intense microfilaraemias (median=three mff/100 μl; range=two to 97 mff/100 μl) than the 94 microfilaraemics who were found antigen-positive (median=69 mff/100 μl; range=two to 2471 mff/100 μl). Eighty-nine (25.8%) of the 345 apparently amicrofilaraemic subjects investigated in the Og4C3 ELISA were, however, also found antigen-positive.

Both the prevalence of CFA (43.0% *v.* 39.1%; P=0.392) and the mean intensity of the antigenaemia (24,895.2 *v.* 17,960.8 antigen units; P=0.335) were higher in males than in females but the differences were not statistically significant.

The prevalence of CFA tended to increase with age and was, in general, about twice that of microfilaraemia in each age-group (Fig. 2). The prevalence of CFA was similar in the children and young adults (28.6% *v.* 40.0%; P=0.100) but significantly higher in the mature adults (56.0%) than in the children (P<0.001) or young adults (P=0.037). The intensities of antigenaemia were, however, similar, for the children, young adults and mature adults.

Antifilarial Antibody Responses

The overall prevalences of antifilarial IgG_1 and IgG_4 responses to *B. pahangi* antigen recorded in the study area were 86.2% and 90.9%, respectively. The Marikano study population had a significantly lower prevalence of antifilarial IgG_4 than the Magongoloni (84.1% *v.* 97.1%; P<0.01; Table 3).

The intensity of the antifilarial IgG_1 response peaked in the subjects aged 11–20 years and thereafter decreased steadily

TABLE 2. *Prevalences and intensities of circulating filarial antigen (detected in Og4C3 ELISA) in the four study communities along the River Sabaki, Malindi district, Kenya, in 2002*

	Subjects tested:		Subjects found antigenaemic:			
Community	No.	Mean (S.D.) age (years)	No.	Mean (S.D.) age (years)	Prevalence (%)	Intensity* (antigen units)
Jilore	104	25.2 (19.4)	40	34.9 (20.7)	38.5	11,150.2
Marikano	107	27.5 (17.8)	46	35.4 (17.6)	43.0	11,025.6
Magongoloni	138	22.7 (17.8)	62	26.8 (18.7)	44.9	52,917.9
Mkondoni	114	23.8 (16.6)	41	26.9 (18.4)	36.0	19,598.9
All four	463	33.5 (18.7)	189	30.6 (19.2)	40.8	20,945.7
P-value		0.194		0.096	0.471	0.001

*Geometric mean intensity in antigenaemic subjects.

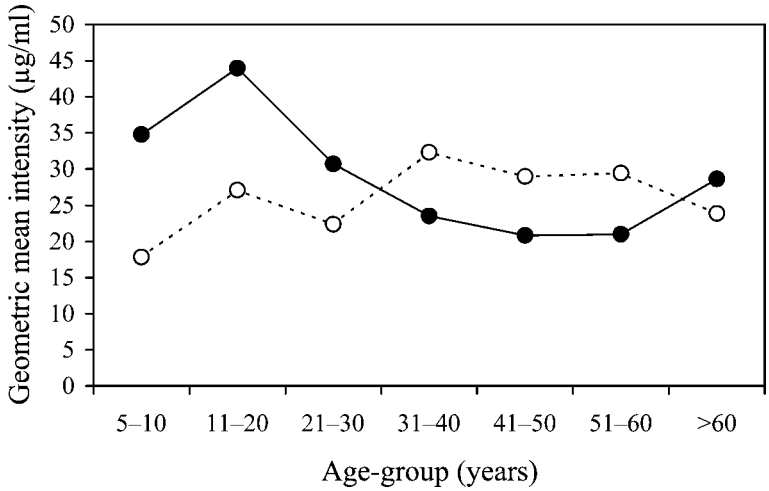

FIG. 3. Age-specific geometric mean intensities of the antifilarial IgG_1 (●) and IgG_4 (○) responses in the subjects from the four study communities along the River Sabaki, Kenya, in 2002.

(Fig. 3). The intensity of the antifilarial IgG_4 response showed less age-related change but was slightly lower in the subjects aged <11 years than in the older subjects, peaked in the subjects aged 31–40 years, and then remained fairly stable (Fig. 3). The peak intensity of antifilarial IgG_4 increased gradually with age until it levelled off at an age of 31–40 years.

The overall prevalence of anti-Bm14 IgG_4, among the 245 subjects from Marikano and Magongoloni who were tested, was 58.8%. The geometric mean intensity in the 144 individuals who showed an antibody response to Bm14 was 957 arbitrary units (AU). Both the prevalence and intensity of the anti-Bm14 IgG_4 responses were higher in Magongoloni (62% and 1027 AU, respectively) than in Marikano (55% and 866 AU, respectively) but these differences were not statistically significant ($P>0.05$). The intensity of the anti-Bm14 IgG_4 response was significantly correlated with that of filarial antigenaemia ($r=0.484$; $P<0.01$). In addition, the GMI of the anti-Bm14 IgG_4 response was more than 16-fold higher in the antigen-positive subjects than in the antigen-negative (748.4 *v.* 46.4 AU). Although only 93 of the 144 samples that were positive for anti-Bm14 IgG_4 came from antigen-positive subjects, the GMI of the anti-Bm14 IgG_4 responses

TABLE 3. *Prevalences of antifilarial IgG_1 and IgG_4 responses in the four study communities along the River Sabaki, Malindi district, Kenya, in 2002*

	Subjects tested:		No. and (%) of subjects positive for:	
Community	No.	Mean (S.D.) age: (years)	Antifilarial IgG_1	Antifilarial IgG_4
Jilore	104	25.2 (19.4)	95 (91.3)	92 (88.5)
Marikano	107	27.5 (17.8)	88 (82.2)	90 (84.1)
Magongoloni	138	22.7 (17.8)	119 (86.2)	134 (97.1)
Mkondoni	114	23.8 (16.6)	97 (85.1)	105 (92.1)
All four	463	24.7 (17.9)	399 (86.2)	421 (90.9)

was significantly higher in these 93 antigen-positive subjects than in the 51 antigen-negative (1442 *v.* 454 AU; $P<0.001$).

DISCUSSION

The major strategy employed by the GPELF to interrupt the transmission of *W. bancrofti* is the mass administration of antifilarial drugs to reduce the reservoir of mff available to the mosquitoes that act as vectors. Collection of baseline data is usually the first step in the implementation of any disease-control programme. Such data provide epidemiological information that is essential in understanding the distribution, prevalence and intensity of the problem targeted for control or for elimination as a public-health problem. After the implementation of a control programme, such data also allow the impact of the programme to be assessed and monitored.

The results of earlier surveys showed that bancroftian filariasis was highly endemic in the coastal areas of East Africa (Heisch *et al.*, 1959; Nelson *et al.*, 1962; Wijers, 1977; Abaru *et al.*, 1980; McMahon *et al.*, 1981). Two major surveys, which covered the entire Kenyan coast from Vanga on the southern coast, near the border with Tanzania, to Pate Island in the far north, revealed that the prevalence and intensity of *W. bancrofti* microfilaraemia varied from place to place but were generally higher in the northern coastal areas than further south (Nelson *et al.*, 1962; Wijers, 1977). During the later of these surveys, four major foci of bancroftian filariasis were identified in the Coast province: the area bordering Tanzania, an area west of Mombasa town, an area just inland from Kilifi town, and land along the River Sabaki, in Malindi district (Wijers, 1977). The epidemiological data on bancroftian filariasis on the northern coast of Kenya (Wijers, 1977; Wijers and Kiilu, 1977; Wijers and Kinyanjui, 1977) have not, however, been updated in the last 30 years. The present study was conducted along the River Sabaki, in both the old focus identified in the 1970s and in communities further inland, to provide an epidemiological update that would be useful to those managing and monitoring the current mass-treatment campaigns being run in Kenya, under the GPELF. The overall prevalence and mean intensity of microfilaraemia observed in the present study (20.5% and 526 mff/ml, respectively) are higher than those reported in most areas in Kwale district, on the southern coast (Estambale *et al.*, 1994; Wamae *et al.*, 1998; Njenga *et al.*, 2000; Mukoko *et al.*, 2004). Interestingly, the prevalence of microfilaraemia recorded in the present study was very similar to that reported for Malindi district more than 30 years ago (Wijers and Kinyanjui, 1977). The present results show that the area around the River Sabaki is still an important focus for bancroftian filariasis in Kenya, and corroborate observations, made during long-term follow-up studies in Tanzania, that indicate extraordinary static patterns of infection and disease in communities with endemic bancroftian filariasis (Meyrowitsch *et al.*, 1995, 2004).

It is now clear that, for detecting human infections with *W. bancrofti* in endemic areas, assays based on the demonstration of CFA are superior to those based on the demonstration of microfilariae (Lammie *et al.*, 1994; Wamae *et al.*, 1998). In general, the prevalence of detectable CFA is approximately twice the prevalence of detectable microfilaraemia (Lammie *et al.*, 1994; Wamae *et al.*, 1998; present study). Since filarial antigenaemia is a good indicator of active infection, the apparently amicrofilaraemic individuals who are found to be antigen-positive probably either harbour adult or immature worms that are not releasing microfilariae or have microfilaraemias that are not sufficiently intense to be detected by the tests employed. The reasons why many individuals apparently harbour adult worms but have few if any microfilariae in their peripheral blood are unknown. Some may have worms of only one sex.

Other possible reasons include lack of fecundity of the adult worms, or the ability of some humans to launch an efficient immune response against the mff (Nicolas *et al.*, 1999).

Given that 10 subjects known to be microfilaraemic were found negative in this assay, the sensitivity of the Og4C3 ELISA used in the present study was lower than the sensitivities reported in many previous studies (Chanteau *et al.*, 1994; Lammie *et al.*, 1994; Wamae *et al.*, 1998; Simonsen and Dunyo, 1999). The sensitivity of the Og4C3 ELISA does appear to be relatively poor, however, among individuals with low-level infections, and may be as low as 72%–75% (Chanteau *et al.*, 1994; Rocha *et al.*, 1996). In the present study, the median count of microfilariae in the microfilaraemic individuals who were found antigen-negative using the Og4C3 ELISA was significantly lower than that of the microfilaraemic individuals who were found antigen-positive. It is thus possible that the microfilaraemic individuals who are antigen-negative by the Og4C3 ELISA harbour very low numbers of adult worms. This, however, does not explain why >25% of the apparently amicrofilaraemic subjects were found antigen-positive in this assay. These results underscore the need to include other, more sensitive methods, in addition to microfilaria detection, when carrying out epidemiological assessments, in sentinel sites, to monitor the impacts of mass drug administrations.

In the current study, the prevalence of antigenaemia increased steadily with age and was, in general, two-fold greater than the corresponding prevalence of (detectable) microfilaraemia. The dynamics of the development of a *W. bancrofti* microfilaraemia in humans are not well understood but are probably related to the intensity and duration of exposure (Chanteau *et al.*, 1995). The results of a longitudinal study in a cohort of children in Haiti indicated that, although filarial infections are acquired early in life, the initial intensity of infection is low and requires some time to build up to detectable levels (Lammie *et al.*, 1998). In the present study, the intensity of antigenaemia in children aged >5 years was similar to that in adults. This unexpected observation may indicate that, in high-transmission foci such as the River Sabaki area, *W. bancrofti* infection builds up early in life.

Although no such trend was observed in the present study, the prevalence of *W. bancrofti* infection is generally higher in males than females. This trend was observed, for example, by Wamae *et al.* (1998), in their study in Kwale district on the southern coast of Kenya. Brabin (1990) suggested that this gender-specific difference may be at least partially hormone-related, since females of reproductive age often have particularly low levels of infection. In the present study, the female subjects of reproductive age had slightly lower prevalences and intensities of microfilaraemia and antigenaemia than their age-matched male counterparts, although the differences were not statistically significant. In areas where *W. bancrofti* is transmitted by day-biting mosquitoes, such as *Aedes* species, it is the male workers engaged in outdoor activities who are probably most likely to be bitten by infected vectors (Chanteau *et al.*, 1995).

The high prevalences of antifilarial IgG_1 (86%) and IgG_4 (91%) responses observed in the present study indicate that most people living in the River Sabaki area, and probably in many other endemic areas, are exposed to infection. Given that, compared with the adults, the children had relatively high levels of antifilarial IgG_1 and relatively low levels of antifilarial IgG_4, it seems likely that initial exposure to the human-infective third-stage larvae of *W. bancrofti* results in a heightened antifilarial IgG_1 response, with a subsequent shift in the ratio of antifilarial IgG_1 to IgG_4 (perhaps reflecting changes in infection status or some form of immune regulation).

Antibody assays based on crude extracts of *Brugia* have been shown to give

false-positive results, because of cross-reactivity, with sera from cases of most other filarial infections, such as bancroftian filariasis, loiasis and onchocerciasis (Maizels *et al.*, 1985). An assay based on detection of IgG_4 antibody responses to a *B. malayi* recombinant antigen (Bm14) was developed to reduce cross-reactivity with intestinal helminths (Lammie *et al.*, 2004). Usefully, at least in areas with endemic *W. bancrofti* but no other human filariasis [the assay acts as a 'pan-filaria' assay, having cross-reactivity with *W. bancrofti, B. malayi*, *Loa loa* and *Onchocerca volvulus*], this anti-Bm14 IgG_4 ELISA provides a sensitive method of detecting *W. bancrofti* infection (Lammie *et al.*, 2004). In the present study, encouragingly, the levels of the IgG_4 response measured in this ELISA were significantly correlated with those of filarial antigenaemia. With detectable microfilaraemia used as the 'gold standard', the sensitivity of the anti-Bm14 IgG_4 ELISA in the present study (79%) was lower than that reported in the assay's validation study (91% for *W. bancrofti*; Lammie *et al.*, 2004). The observation that the prevalence of IgG_4 responses to Bm14 (59%) was higher than the prevalence of antigenaemia detected using the Og4C3 ELISA (41%) indicates that the prevalence of active infection could even be higher than estimated using the Og4C3 ELISA. An alternative explanation for these results is that some individuals living in endemic areas become infected with third-stage larvae of *W. bancrofti* (that elicit a strong antibody response) but are able to clear their infections spontaneously, before the parasites reach adulthood. Since the Bm14 antigen does not cross-react with antibodies to non-filarial helminths, the anti-Bm14 IgG_4 ELISA has been proposed as a potential tool for epidemiological monitoring of filariasis-elimination programmes. In a study involving 57 microfilaraemic individuals in Egypt, the titres of anti-Bm14 IgG_4 antibodies were generally found to decrease after repeated treatment with diethylcarbamazine plus albendazole, with 52.5% of the treated individuals found antibody-negative when tested 48 months post-treatment (Helmy *et al.*, 2006). Further studies are needed to evaluate the usefulness of the Bm14 recombinant antigen in monitoring large-scale filariasis-elimination programmes in different endemic areas.

The present results provide an update on the prevalence of *W. bancrofti* infection in the River Sabaki focus in the Kenyan northern coastal area. The prevalence of infection in this focus is still higher than that reported in the southern coastal areas. The high prevalence of antifilarial antibody indicates that most of the inhabitants of this area have been exposed to infection and, therefore, that the level of transmission is high. The data provide a useful platform for the longitudinal monitoring, using various epidemiological tools, of local programmes to eliminate LF.

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