Wuchereria bancrofti in Kwale District, Coastal Kenya: patterns of focal distribution of infection, clinical manifestations and anti-filarial IgG responsiveness

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SUMMARY

A cross-sectional study of bancroftian filariasis was conducted in 2 adjacent communities, Mvumoni and Kilore in Muhaka, Kwale District. *Wuchereria bancrofti* infection, clinical manifestations and anti-filarial IgG responsiveness were determined before the long rains, a time coinciding with a low transmission season. The prevalence of microfilaraemia increased gradually with age and was significantly higher in Kilore (24 %) than in Mvumoni (6·3 %, P < 0.001). Similarly, the prevalence of antigenaemia increased with age and also was significantly higher in Kilore, 48.9 % than in Mvumoni, 20·5 % (P < 0.001). Hydrocele, funiculitis, lymphangitis and lymphadenitis were also significantly more common in Kilore than in Mvumoni. In comparing the 2 communities, levels of IgG4 responsiveness in antigen-positive persons were higher in Kilore than Mvumoni (P = 0.034), but this was related to higher antigen loads in persons in Kilore than in Mvumoni. In antigen-negative persons, anti-filarial antibodies of 3 IgG isotypes were significantly higher in Kilore than Mvumoni (P < 0.001, for IgG1, IgG2, IgG4). These results emphasize the highly focal nature of bancroftian filariasis in this setting and demonstrate that anti-filarial antibody levels are related to transmission intensity.

Key words: filariasis, microfilaria, antigen, antibody, focal transmission.

INTRODUCTION

Human lymphatic filariasis is caused by infection with lymphatic-dwelling parasitic nematodes which are prevalent in the humid tropical regions of the world. Wuchereria bancrofti is the only known aetiologic agent of lymphatic filariasis in the African Region where an estimated 26 million infections occur (WHO, 1992). In Kenya, filariasis is endemic in coastal areas along the Indian Ocean, and it is estimated that at least 2.5 million people who are living in these endemic areas are at risk of infection. In the 4 districts of the Coast Province, Kenya, microfilariae rates as described by Wijers (1977) ranged from 28.4 to 56 % and sign rates (hydroceles and elephantiasis) ranged from 30.2 to 64 %. In more recent studies, the microfilaria prevalence in Maili Nane, Kinango, Kwale District was 22.4 % (Wamae et al. 1989) and in the most south-eastern region of Kwale District, circulating microfilariae were found in 13.7% of a population of 1129 individuals (Estambale et al. 1994a). These studies confirm that W. bancrofti is endemic along the Kenyan Coast, but do not address the question of how W. bancrofti

prevalence varies from community to community within this region. In this study, we employed an antigen detection assay to characterize age-specific patterns of bancroftian filariasis in 2 contiguous endemic communities in Muhaka, Kwale District, Coastal Kenya. A significant difference in *W. bancrofti* prevalence between these 2 communities provided us with an opportunity to study the relationship between community infection level and anti-filarial IgG responsiveness.

MATERIALS AND METHODS

Description of the study site

The study was conducted in Muhaka Mvumoni and Muhaka Kilore which are 2 of the 13 communities that constitute Muhaka, Kwale District. The District is hot and dry from January to April/May, the average daily temperatures ranging from 25 to $26 \cdot 6 \,^{\circ}$ C. Kwale District experiences 2 rainy seasons. The long rains occur between March/April and June/July and the short rains fall in the months of October and November. Humidity varies between $60 \text{ and } 95 \,^{\circ}_{\circ}$ throughout the year making the ambient conditions optimal for *W. bancrofti* development, survival in the mosquito vectors and transmission to the human host. There are 2 seasonal rivers in the study area, the Chamanzi and the Mtawa. Seasonal

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marshes that provide rain-fed, rice-growing fields ('burani') and favourable breeding sites for *Ano-pheles* mosquitoes during the rainy seasons are also common.

Study design

Community meetings were held to discuss the purpose of the study. After registration of all members of each household, age 1 year and above, a simple random selection of 100 households from each community was done. Subsequently, 200 participants were randomly selected from each community (n = 400).

Blood collection and examination

Beginning at 22.00 h, venous blood samples (2–4 ml) were collected from consenting persons using vacutainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) and by finger-prick (20 μ l) from children aged less than 4 years. Using a micropipette, a second blood sample (20 μ l) collected from children less than 4 years was spotted onto 2 filter paper discs, 10 µl each, for elution of antigen (JCU Tropical Biotechnology, Townsville, Queensland). For venous samples, 1 ml was filtered by polycarbonate nuclepore filters, $25 \text{ mm} \times 5.0 \mu \text{m}$ (Nuclepore Corporation, Cambridge, MA) and examined for the presence of W. bancrofti microfilariae after staining with Difil-test stain (Evsco Pharmaceuticals, Buena, NJ). Children's samples were similarly examined after preparation of Giemsa-stained, thick smears. The microfilariae count was expressed as mf/ml. The remaining volume of blood was centrifuged for plasma separation. Separated plasma samples were frozen for later immunological analyses.

Antigen-detection assay

An IgM monoclonal antibody (Mab), designated Og4C3 (More & Copeman, 1990) was used for the antigen-detection ELISA as per manufacturer's instructions (JCU Tropical Biotechnology, Townsville, Queensland). Briefly, $50 \,\mu l$ of each serum sample was added to 50 μ l of solution A preparation buffer and after 2 min 50 μ l of solution B preparation buffer was added. Immediately after mixing, 50 µl of the resulting solution was added into each of 2 microtitre wells pre-coated with the Og4C3 Mab. In the case of the children's samples, elution of antigen from the filter discs containing samples from children was done according to the package insert instructions (JCU Tropical Biotechnology, Townsville, Queensland). Briefly, after boiling the discs in 200 μ l of elution diluent, 50 μ l aliquots of supernatant containing the stable antigen were added to each of 2 microtitre wells pre-coated with the Og4C3 Mab and thereafter processed in the same manner as

those from older persons. After 2 h incubation, the plates were washed 3 times and 50 μ l of rabbit anti-Onchocerca antibody were added to each well and incubated for 1 h. After washing 3 times, 50 μ l of anti-rabbit conjugate (JCU, Tropical Biotechnology) were added to each well and incubated for a further 1 h. Following this incubation, the plates were washed and 100 μ l of chromogen added to each well and incubated for 1 h. The results of the reaction were read at 405 nm wavelength by an ELISA reader using Softmax program (Molecular Devices, Palo Alto, CA). An optical density value of ≥ 60 antigen units was considered positive.

Clinical examination

The physical examination was restricted to the inguinal lymph nodes, the male genitals, the legs and arms. Acute manifestations including fever, adeno-lymphangitis (excluding inguinal nodes), funiculitis and orchitis were given special attention. For the purpose of this study, fever was defined as chills and fever similar to those experienced during a malaria infection ('homa ya malaria') and was self-reported. Signs and symptoms of chronic filarial involvement were recorded according to a modified grading key as described by Wijers (1977).

Antigen preparation for the antibody-detection assays

A soluble antigenic extract of *Brugia pahangi* adult was prepared from adult worms of *B. pahangi* maintained in jirds (*Meriones unguiculatus*). Parasites were provided by supply contract AI-02642 of the NIAID US–Japan Cooperative Medical Sciences Program. Soluble *B. pahangi* antigens were prepared as previously described (Lammie *et al.* 1988). The protein concentration of the supernatant was determined by the BCA protein assay reagent (Pierce, Rockford, IL).

Quantitative analyses of filaria-specific IgG antibody subclasses

Assays to determine serum levels of *B. pahangi*specific IgG antibody isotypes were done as described (Hitch, Lammie & Eberhard, 1989; Lammie *et al.* 1993). Immulon 2 microtitre plates (Dynatech Laboratories, Chantilly, VA) were sensitized with *B. pahangi* adult antigen (2 μ g/ml) in bicarbonate buffer overnight at 4 °C and blocked with PBS–0·3 % Tween 20 (Sigma, St Louis, MO) for 1 h at 4 °C to eliminate non-specific binding. Serum samples were diluted 1:50 in PBS–0·05 % Tween 20, and 25 μ l of the resulting dilution was added to the sensitized plates in triplicate. Biotinylated monoclonal antibody directed against 1 of the human IgG isotypes (Clones: IgG1-HP 6069, IgG2-HP 6002, IgG3-HP 6047 and IgG4-HP 6025; Zymed Laboratories, San

Table 1. Demographic characteristics of the study sample: Muhaka

Community	Mvumoni (%)	Kilore (%)
n	176	152
Participant's age range	2–75 years	2–79 years
Median age	15 years	20 years
Participant's gender	(42·6) male*	(44·7) male*

* There were more females than males in the study sample (P < 0.001).

Francisco, CA) and streptavidin-alkaline phosphatase conjugate (Bethesda Research Laboratories, Gaithersburg, MD), were added in sequence with intermediate washings with PBS-0.05 % Tween 20. After washing 5 times, substrate, p-nitrophenyl phosphate (Sigma) was added. After 10 min development, the reaction was stopped with 0.1 M EDTA. The optical density of the reaction product was read (405 nm) by Softmax program using a u.v. max ELISA plate reader (Molecular Devices, Palo Alto, CA). Anti-filarial antibody levels were standardized with respect to calibrated samples, generously provided by Dr Eric Ottesen (National Institutes of Health, Bethesda, MD). A pool of 8 serum specimens of persons from the study site was used as endemic positive controls and 9 serum samples from Nairobi residents were used as negative controls.

Statistical analysis

Quantitative data were analysed with EpiInfo, version 6 software. Geometric means were calculated from logarithmically transformed data for microfilaraemia, antigenaemia intensities and anti-filarial antibody responsiveness. Levels of microfilaraemia, antigenaemia and anti-filarial antibody responsiveness were compared by Kruskal–Wallis test for 2 groups. Modelling for multiple loglinear regression analysis was performed with SAS software version 6 for determination of Pearson correlation coefficients and linear association of selected variables.

RESULTS

Population demographics

Of the total 328 persons participating in this study, 176 were from Mvumoni and 152 from Kilore (Table 1). The age of participants varied between 2 and 79 years in both communities and there were more females than males in both study populations (P < 0.001). Most study participants were life-long residents of Muhaka (86.9 and 96.7% in Mvumoni and Kilore, respectively). Serum samples for the analyses of anti-filarial responsiveness were available from 276 of the total 328 participants.

Microfilaraemia and antigenaemia

Microfilaria prevalence was 14.5% overall but was significantly higher in Kilore (24%) than in Mvumoni (6.3%; P < 0.001). In both communities, microfilaria prevalence increased gradually with age (Fig. 1A). Overall, microfilaraemia was more common among males (19.5%) than females (9.9%, P = 0.026). However, this was confined to adults of child-bearing age (21-40 years; P = 0.002).

Similarly, the prevalence of antigenaemia was significantly higher in Kilore (48.9%) than in Mvumoni (20.5%) (P < 0.001). In both communities, age-specific prevalence of antigenaemia increased gradually, peaking in the 21-40 years age groups, then declining in Mvumoni and levelling off in Kilore (Fig. 1B). Prevalence of antigenaemia was significantly higher in Kilore than Mvumoni in all age groups except for children less than 10 years of age (P = 0.005, 0.032 and 0.003, for 11-20, 21-40)and > 40 years age groups, respectively). Moreover, the prevalence of antigenaemia was significantly higher than the prevalence of microfilaraemia in every age group in both communities, and more common in males than in females for all age groups in Kilore (P = 0.009), but only in the child-bearing age (P = 0.044) in Mvumoni (data not shown). The qualitative relationship between antigenaemia and microfilaraemia in both communities is shown in Table 2. The Og4C3 assay was positive for 38/39 microfilaraemic persons (97.4%, sensitivity) and 26.1% (60/230) of amicrofilaraemic persons.

Microfilaraemias ranged between 7 and 907 mf/ml (median = 23.01 mf/ml) and 1 and 2468 mf/ml (median = 181.9 mf/ml) in Mvumoni and Kilore, respectively. This difference was not significant (P = 0.228). The intensity of microfilaraemia was low in persons ≤ 20 years and increased sharply in the age group 21–40 years; there was no detectable microfilaraemia in persons > 40 years in Mvumoni (Fig. 2A). In Kilore, age-specific intensity of microfilaraemia peaked in the 21–40 years age group and decreased sharply in persons > 40 years.

Overall, the intensity of antigenaemia both among the antigen-positive persons and in the total population was significantly higher in Kilore than in (P < 0.001), data not shown. In Mvumoni Mvumoni, the median antigenaemia in antigenpositive persons was 109.6 units/ml compared to 763.8 units/ml in Kilore. In both communities, intensity of antigenaemia increased with age in young people and levelled off in adults over 20 years of age (Fig. 2B). Levels of antigenaemia were higher in Kilore than in Mvumoni in all age groups (P = 0.050, 0.017, 0.016 and 0.013, respectively). Moreover, among antigen-positive persons in Kilore, microfilaraemic individuals had significantly higher levels of antigenaemia than microfilaria-negative persons (P = 0.001).



Fig. 1. Age-specific prevalence of *Wuchereria bancrofti* (A) microfilaraemia and (B) antigenaemia in Mvumoni (■) and Kilore ().



Table 2. Qualitative relationship between antigenaemia and microfilaraemia: Muhaka

Fig. 2. Age-specific intensity of *Wuchereria bancrofti* (A) microfilaraemia and (B) antigenaemia in Mvumoni (■) and Kilore (🖾).

In further analyses of microfilaraemic individuals in both communities, microfilaraemia and antigenaemia were poorly correlated ($r^2 = 0.34$). However, using a multiple linear regression model with log-transformed data adjusted for age as a continuous variable and gender as a dichotomous variable, microfilaraemia was associated with antigenaemia (P < 0.001). This association was significantly influenced by community location (P < 0.001), but not age or gender.

Clinical manifestation	Mvumoni No. pos/total (%)	Kilore No. pos/total (%)	<i>P</i> -value (between communities)
Hydrocele*	3/54 (5.6)	18/60 (30)	0.001
Elephantiasis (leg, arm and breast)	2/139 (1.4)	6/130 (4.6)	Not significant
Inguinal lymphadenopathy	5/138 (3.6)	36/129 (27.9)	< 0.001
Other lymphadenopathy	2/138 (1.4)	24/129 (17.3)	< 0.001
Lymphangitis	(0)	16/129 (12.4)	< 0.001
Funiculitis/Orchitis*	3/54 (5.6)	20/60 (33.3)	< 0.001
Fever	6/138 (4.3)	28/129 (21.7)	< 0.001

Table 3. Prevalence of clinical signs and symptoms: Muhaka

* In male participants only.



Fig. 3. (A) The relationship between *Wuchereria bancrofti* anti-filarial antibody levels and microfilaraemic status. (\blacksquare) Mf-positive (n = 9 and 30 in (a) Mvumoni and (b) Kilore, respectively); (\boxdot) mf-negative (n = 122 and 93 in Mvumoni and Kilore, respectively). (B) The relationship between *W. bancrofti* anti-filarial antibody levels and infection status. (\blacksquare) Antigen-positive (n = 28 and 64 in (a) Mvumoni and (b) Kilore, respectively); (\boxdot) antigen-negative (n = 118 and 67 in Mvumoni and Kilore, respectively).



Fig. 4. A comparison in *Wuchereria bancrofti* anti-filarial antibody responsiveness by infection status between Mvumoni (\blacksquare) and Kilore (\boxdot), (A) antigen-positive persons and (B), antigen-negative persons.

Clinical signs and symptoms

The prevalence of clinical manifestations was significantly higher in Kilore than in Mvumoni for all manifestations except elephantiasis (Table 3). The overall prevalence of hydrocele and elephantiasis (leg, breast and arm) was 18.4 and 3 %, respectively. The prevalence of these conditions and of funiculitus or orchitis (20.2 %) increased with age. The prevalence of lymphangitis, adenopathy (excluding inguinal lymphadenopathy) and fever was 5.6, 15.7 and 12.7 %, respectively. Of the 21 male participants with hydrocele, 7 were also microfilaraemic (35 %) while only 1 of 8 participants with elephantiasis had microfilaraemia (data not shown).

Quantitative analyses of filaria-specific IgG antibody subclasses

To compare the differences in anti-filarial responsiveness among microfilaraemic and amicrofilaraemic persons in each community, anti-filarial IgG responsiveness was determined by ELISA (Fig. 3A). Levels of IgG4 were significantly higher in microfilaria-positive than in microfilaria-negative persons (P = 0.002 and 0.008 in Mvumoni and in Kilore, respectively). However, levels of IgG2 in Kilore were significantly lower in microfilariapositive than in microfilaria-negative persons (P =0.003). In Mvumoni, anti-filarial IgG3 levels were lower in microfilaria-positive persons, but this difference was of borderline significance (P = 0.053).

Although differences in anti-filarial antibody responsiveness were associated with microfilaraemia status, this association may have been confounded by the presence of adult worms in some amicrofilaraemic persons since circulating filarial antigen is also contributed by stages of the worm other than microfilariae. To understand the influence of antigenaemia on anti-filarial IgG production, the association between antigen status and antibody reactivity was examined (Fig. 3B). As was observed when groups were defined by microfilaraemic status, levels of IgG4 among antigen-positive persons were significantly higher than in antigen-negative persons (P < 0.001) in both Mvumoni and Kilore. Similarly, the levels of IgG2 in antigen-positive persons in Kilore were also significantly lower than in antigennegative persons (P = 0.014).

Because we had observed that the prevalence of filarial infection as determined by antigenaemia was significantly higher in Kilore than in Mvumoni (48.9 and 20.5 %, respectively), anti-filarial responsiveness in the 2 communities was compared (Fig. 4A). Levels of anti-filarial IgG4 responsiveness among antigen-positive persons were higher in Kilore than in Mvumoni (P = 0.034). To investigate further the relationship between IgG4 and infection status, linear regression analyses using the IgG4 antibody as the dependent variable were carried out. Controlling for differences in microfilaraemia levels, there was no signifcant difference in anti-filarial IgG4 responses between Kilore and Mvumoni (P = 0.0713). Similarly, after controlling for antigenaemia level, there was no difference in the anti-filarial IgG levels between antigen-positive persons residing in the 2 communities.

Among antigen-negative persons, all 4 IgG antibody responses were significantly higher in Kilore than Mvumoni (Fig. 4B, P < 0.001 for IgG1, IgG2



Fig. 5. Age-specific prevalence of *Wuchereria bancrofti* anti-filarial antibody levels by infection status in Kilore. (\blacksquare) Antigen-positive (n = 64); (\boxdot) antigen-negative (n = 67).

and IgG4), marginally so in the case of IgG3 (P = 0.055). Further regression analyses showed that in contrast to the observation made in antigenpositive persons, the anti-filarial IgG responses were significantly influenced by community location among antigen-negative persons for all 4 isotypes (P < 0.001, = 0.002, = 0.013 and = 0.044, for IgG1, IgG2, IgG3 and IgG4, respectively).

To determine whether anti-filarial antibody responsiveness was age-dependent, mean antibody responsiveness was analysed as a function of age and antigenaemia status. There were no significant agedependent differences in IgG antibody responsiveness in Mvumoni as a function of antigen status (data not shown). In Kilore, although there were no obvious patterns of change in age-specific antibody levels, the levels of anti-filarial IgG2 and IgG3 in antigen-positive persons did differ significantly by age (P = 0.040 and 0.034, respectively) (Fig. 5). Furthermore, among antigen-negative persons, levels of IgG4 responsiveness were also significantly different between age groups (P = 0.015). In analyses using antibody as the dependent variable and 1-10 years age group as the baseline, all 4 isotypes in all age groups were significantly higher in persons living in Kilore than in Mvumoni (P < 0.001) = 0.002, = 0.041 and = 0.031 for IgG1, IgG2, IgG3 and IgG4, respectively) among antigen-negative persons (data not shown).

Due to the low prevalence of clinical manifestations in Mvumoni, analyses of the relationship between anti-filarial antibody responsiveness and *W*. *bancrofti* clinical manifestations were carried out for Kilore only. Participants were categorized by microfilaraemia, antigenaemia and the presence of signs or symptoms of filariasis. Controlling for antigen status, there were no differences in the levels of anti-filarial responsiveness between those with and those without clinical evidence of filariasis (data not shown).

DISCUSSION

Lymphatic filariasis has long been known to be endemic in East Africa (reviewed by Hawking, 1943; Jordan, 1995; Nelson, Heisch & Furlong, 1962). The intensity and severity of the disease varies by setting (Wijers, 1977; Wijers & Kinyanjui, 1977; Wegesa et al. 1979; McMahon et al. 1981; Maxwell et al. 1990; Estambale et al. 1994a). Although Mvumoni and Kilore are adjacent and similar in demographic and geographical characteristics, differing only administratively in that each community is headed by a different chairman, dramatic differences in W. bancrofti infection prevalence were observed. Significant differences in microfilaraemia, antigenaemia and clinical manifestations were found between these 2 communities, indicating differences in the factors that influence distribution of filariasis. Observation of distinct levels of infection in areas within close geographical proximity emphasizes the highly focal nature of transmission and underscores the importance of seasonal vector breeding sites in maintaining mosquito populations and the occurrence of filariasis in a community.

The Og4C3 antigen-detection ELISA detected infection in 38/39 (97.4%) of the microfilaraemic persons and 26.1% (60/230) of persons without microfilaraemia. Therefore, the Og4C3 antigen assay

detected a higher prevalence $(33\cdot3\%)$ of *W. bancrofti* infection than parasitological diagnosis $(14\cdot1\%)$, suggesting that the prevalence of *W. bancrofti* in the coastal regions of Kenya may be higher than previously reported. The sensitivity of the Og4C3 assay $(97\cdot4\%)$ for detection of microfilaraemia and its added ability to detect infected but amicrofilaraemic persons are consistent with results reported by other workers (Turner *et al.* 1993; Lammie, Hightower & Eberhard, 1994; Chanteau *et al.* 1995). These observations raise questions about the underestimation of the prevalence of bancroftian filariasis in both epidemiological studies and control programmes, if detection of microfilariae is the diagnostic method employed.

Compared to parasitological diagnosis, infection as diagnosed by antigenaemia was detected earlier in the first decade of life and later in older persons. The absence of microfilaraemia in some antigen-positive persons at both ends of the age range and in other intermediate age groups is consistent with the conclusion that the adult filarial worm contributes to antigenaemia. This suggestion is further supported by the observation that, the correlation between antigenaemia and microfilaraemia among microfilaraemic persons was relatively poor ($r^2 = 0.34$). Thus, the presence and intensity of antigenaemia are not exclusively related to intensity of microfilaraemia.

The nature and degree of immune responses made by the host may be a critical determinant of whether microfilaraemia and/or immunopathology develops. To assess immune responses to filarial antigens in these communities and to analyse their relationship to infection and disease status, anti-filarial antibody levels were analysed by ELISA. In both communities, microfilaria-positive persons had significantly higher levels of B. pahangi-specific IgG4 than microfilaria-negative persons. These observations lend support to previous reports that elevated IgG4 is a diagnostic marker for infection (Kwan-Lim, Forsyth & Maizels, 1990). Polyclonal and antigen-specific IgG4 immunoglobulin is characteristically increased in persons with patent filarial infection (King & Nutman, 1992). Several studies, for example, in Papua New Guinea (Kwan-Lim et al. 1990), Haiti (Hitch et al. 1991), Indonesia (Kurniawan et al. 1993), and Kenya (Estambale et al. 1994b; Wamae, Lammie & Muttunga, 1995) have shown that sera from microfilaraemic persons have higher levels of *Brugia*-specific IgG4 than sera from amicrofilaraemic persons.

Similarly, the observation that antigen-positive persons had significantly higher levels of IgG4 than antigen-negative persons is also consistent with previous findings. The association between IgG4 levels and antigenaemia status in antigen-positive persons (in contrast to microfilaraemia) was not influenced by community location. Controlling for antigenaemia, IgG4 responses in infected people were comparable for Mvumoni and Kilore. Thus, intensity of infection (antigen level) is the major determinant of IgG4 levels and the communitydependent difference in IgG4 among microfilaraemia-positive persons reflected the influence of higher antigen loads in Kilore. In infected persons, therefore, a relationship between community infection prevalence and the magnitude of IgG4 response could not be demonstrated.

Among the antigen-negative persons, levels of anti-filarial IgG1, IgG2, IgG3 and IgG4 were higher in Kilore than Mvumoni. In regression analyses, these observations were significantly associated with community location and levels of all 4 isotypes were higher in Kilore than Mvumoni. Mvumoni and Kilore did not differ in geographical or demographic attributes and since there was no record of any previous chemotherapy or vector control measures in either of the 2 communities, the observed elevated levels of anti-filarial responses in antigen-negative persons in Kilore are probably related to the higher transmission levels in Kilore. The intensity of exposure to transmission may be a key factor in the enhancement of anti-filarial IgG production (Bailey et al. 1995).

The IgG1, IgG2 and IgG3 isotypes present a sharp contrast to the pattern of expression seen for IgG4 (Maizels et al. 1995). These 3 isotypes of filaria-specific antibody are minor components of the anti-filarial response in microfilaraemic persons, but are major in elephantiasis patients. It has been suggested that these isotypes may contribute to antibody-dependent pathology through cellmediated cytotoxicity (ADCC) or immune complex formation (van de Winkel & Capel, 1993; Kurniawan et al. 1993; Maizels et al. 1995). However, in the present study, there was no clear relationship between these 3 isotypes and clinical status.

In summary, on the basis of clinical and parasitological observations, we concluded that the distribution of filarial infection in Muhaka was focal. Observations of anti-filarial responsiveness have shown differences between the 2 communities, thus confirming the focal nature of filarial infection in this setting. Four additional observations have emerged. Firstly, the presence of microfilaraemia stimulates enhanced production of anti-filarial IgG4 as previously reported (Kwan-Lim et al. 1990). Secondly, since antigenaemia also stimulates IgG4 production, this isotype may be a diagnostic marker of exposure as well as an indicator of worm burden (Chanteau et al. 1994). Thus, for IgG4 serology to qualify as a useful diagnostic tool in field studies, the choice of the cut-off OD value for a positive response would have to be re-evaluated since all persons in the endemic area were considered to be positive on the basis of IgG4 responsiveness. Thirdly, in an endemic

Focal distribution of Wuchereria bancrofti in Kenya

area, among antigen-negative persons, intensity of exposure may be one of the most important factors influencing anti-filarial antibody levels (Mahanty *et al.* 1994; Bailey *et al.* 1995). Finally, the length of the period of exposure to infective larvae is important since IgG responsiveness changed with age. In order to understand how transmission may influence the differences in anti-filarial responsiveness in this setting, changes in anti-filarial antibody responsiveness following a transmission season were evaluated and the results are reported elsewhere.

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