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Wuchereria bancrofti in a community with seasonal transmission: stability of microfilaraemia, antigenaemia and filarial-specific antibody concentrations

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The effect of seasonal transmission on microfilaraemia, antigenaemia and filarial-specific antibody levels in individuals infected with *Wuchereria bancrofti* was investigated in a follow-up study in an endemic community in north–eastern Tanzania. The subjects were 37 adult male residents who were found to be positive for circulating filarial antigen (CFA) at the beginning of the study (26 of whom were also found microfilaraemic with *W. bancrofti* at this time). Blood samples were collected from each subject in July 1998, January 1999 and July 1999, during the seasons when transmission intensity was high, low and high, respectively. The mean intensities of microfilaraemia and the mean concentrations of CFA were each slightly higher during the low-transmission season than during the two high-transmission seasons but the differences were not statistically significant ($P > 0.05$). Similarly, the mean levels of filarial-specific IgG_1, IgG_2, IgG_3, IgG_4 or IgE did not differ to a statistically significant degree between the three examination times. Microfilaraemias and the levels of CFA and filarial-specific antibodies all therefore appeared to be remarkably stable and largely unaffected by the seasonal variation in transmission. That no variation in the mean IgG_4/IgE ratio was observed over the study period may indicate that the level of resistance to *W. bancrofti* infection in the study subjects was also unaffected by the transmission season.

Marked seasonal variation in the abundance of its mosquito vectors means that transmission of *Wuchereria bancrofti* is often seasonal, with a peak occurring during and shortly after the rainy season (Wijers and Kiilu, 1977; McMahon *et al.*, 1981). Since the hosts' immune responses are directed against all stages in the parasite's life-cycle, including the infective larval stage (Bailey *et al.*, 1995), it is reasonable to assume that these responses, and perhaps infection intensities, could vary with the seasonal exposure. If this is so, those interpreting cross-sectional data obtained from an endemic area should take careful account of

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the season during which the data were collected.

Bancroftian filariasis is highly endemic in the coastal areas of Tanga region, in north–eastern Tanzania (McMahon *et al.*, 1981; Simonsen *et al.*, 1996). In these areas, most transmission of the causative parasite, *W. bancrofti*, occurs during and shortly after the major rainy season, which runs from April to June (McMahon *et al.*, 1981). There is thus a well defined season when transmission intensity is high (a 'high-transmission' season) and a season when relatively little transmission of *W. bancrofti* occurs (a 'low-transmission' season). An endemic community in Tanga region should therefore provide a good site for determining the possible effect of seasonal variation on infection and filarial-specific antibody responses in the infected population. Adult males from one such community, where detailed monitoring of *W. bancrofti* transmission also took place, were therefore selected for the present study. They were examined three times over a 1-year period (during one high-transmission season, the following low-transmission season and the next high-transmission season), for microfilaraemia, circulating filarial antigenaemia and filarial-specific IgG_1, IgG_2, IgG_3, IgG_4 and IgE antibodies. The levels of these parameters during the different transmission seasons were analysed and compared.

SUBJECTS AND METHODS

Subjects

The study was carried out in the village of Masaika, in a part of the Tanzanian region of Tanga which is highly endemic for *W. bancrofti* infection (Simonsen *et al.*, 1996). At the start of the study, in July 1998, two blood samples, one of finger-prick blood and a larger one of venous blood, were collected from each member of this community during a cross-sectional survey (see below). Microfilariae were found in the finger-prick samples from 24.9% of the residents and specific circulating filarial antigen (CFA) was found in 52.2% of the samples of venous blood (unpubl. obs.). Thirty-seven of the adult males (aged 20–40 years) who had been found positive for CFA (26 of whom had also been found to be carrying *W. bancrofti* microfilariae) were recruited for follow-up. Each of these 37 subjects of the present study gave his informed verbal consent at the start of the study and each was treated with diethylcarbamazine at the end of the investigation.

Blood Samples, Parasitology and Serology

Two samples, one of finger-prick blood (100 μl) immediately followed by one of venous blood (5 ml), were collected from each subject, between 21.00 and 24.00 hours, on three occasions: during the cross-sectional survey at the beginning of the study (July 1998); 6 months later (January 1999); and 12 months later (July 1999). The exact time of each sampling of finger-prick blood was noted.

Each sample of finger-prick blood was collected in a heparinized capillary tube, transferred to a specimen tube with 1 ml 3% acetic acid, and later examined for microfilariae (mff) under a light microscope, using the counting-chamber method (McMahon *et al.*, 1979).

The samples of venous blood were collected in plain Vacutainer tubes (Becton Dickinson), allowed to clot overnight in a refrigerator and then centrifuged so that the sera could be removed. The sera, with sodium azide added as a preservative (15 mM), were initially stored at $-20°C$ in the field and then at $-80°C$ in the main laboratory until use. Before further handling and testing of sera, lipid-coated viruses were eliminated by addition of 3 μl tri-*N*-butyl phosphate and 10 μl Tween-80/ml to each sample (Poulsen and Sørensen, 1993).

Each serum specimen was checked for CFA using a commercial ELISA (TropBio, Townsville, Australia; Simonsen and Dunyo, 1999). Serum specimens with ≥ 32 antigen units (i.e. at least titre-group 3) were considered positive for CFA, and specimens with a response of at least standard 7 (i.e. titre-group 8) were assigned a fixed value of 32 000 CFA units. Sera were also examined for filarial-specific antibodies (IgG_1, IgG_2, IgG_3, IgG_4 and IgE), using ELISA based on the antigens in a *Brugia pahangi* adult-worm ho-

mogenate (Estambale *et al.*, 1994; Simonsen and Meyrowitsch, 1998). Prior to measurement of IgE, sera were absorbed with a Protein-A–agarose bead suspension (Kem-En-Tec, Copenhagen) at a ratio of 50:140, to remove any IgG_4-blocking antibodies (Jaoko *et al.*, 2001). To avoid problems with inter-plate variations in the ELISA for CFA and the five types of filarial-specific antibodies, serum samples taken at the three different time-points from each individual were examined on the same ELISA plate.

Transmission Studies

Longitudinal entomological studies were also conducted in Masaika during the 1-year study period. These will be described and their results analysed in detail in a separate article. Briefly, 50 households with two or more inhabitants each were randomly selected, and mosquitoes were caught during the night once each week, in a light trap placed in each house beside an unimpregnated bed net. Vector mosquitoes caught were identified to species, and the live ones dissected for filarial larvae. The total number of mosquitoes trapped during the 12 month-period were, in order of their abundance, 12 827 *Anopheles gambiae*, 7904 *Culex quinquefasciatus* and 5313 *An. funestus*. The infectivity of each mosquito species was calculated for each study month, as the number of mosquitoes positive for infective larvae, divided by the number of mosquitoes dissected. The monthly infective biting rate (i.e. the number of infective bites per person per month) was calculated for each species as: [(no. mosquitoes caught during the month) × (infectivity rate of the species) × (no. of days in the month) × (conversion factor for light traps)]/[(no. catching days in the month) × (no. light traps/day)]. The conversion factor for light traps was as described by Lines *et al.* (1991). The combined monthly infective biting rate for the three species of vector mosquitoes was obtained by adding the monthly infective biting rates of each of the species.

Data Analysis

The intensities of the microfilaraemia detected (mff/ml) were adjusted for sampling time by multiplying the counts with a time-specific factor, as described by Simonsen *et al.* (1997). Geometric mean 'intensities' (GMI) of microfilaraemia, antigenaemia and filarial-specific antibody were calculated as the antilog of $\{[\Sigma \log(x+1)]/n\} - 1$, where x is the number of mff/ml, the number of CFA units or the optical-density (OD) value, respectively, and n is the number of subjects examined. Upper and lower values for S.E. were calculated as the antilog of [(mean of log-transformed values) ± (S.E. on log-transformed values)]. IgG_4/IgE ratios were first calculated for individual sera, and mean IgG_4/IgE ratios and S.E. were thereafter calculated as described above. GMI or mean ratios for the three time-points were compared statistically by one-way analysis of variance on the log-transformed values. P-values of <0.05 were considered statistically significant.

RESULTS

Seasonality of Transmission

The combined infective biting rates for each month during the study year are shown in Figure 1. Transmission intensity was high in July 1998 (during the first blood sampling), low from October–December 1998 (prior to the second blood sampling, in January 1999), and high again from March–June 1999 (prior to the last blood sampling, in July 1999).

Microfilaraemia

Among the 26 individuals who were microfilaraemic at the beginning of the study, the GMI for microfilaraemia in July 1998, January 1999 and July 1999 were 909, 1175 and 843 mff/ml, respectively (see Table and Figure 2). The difference in these GMI between the three time-points was not statistically significant ($P>0.05$). At the individual level, microfilarial intensities also remained quite stable over the observation period; for 21 (81%) of the 26 microfilaraemics, the log-transformed intensities always lay within a narrow range defined by the mean value for the subject ± 20%. All 26 subjects who were

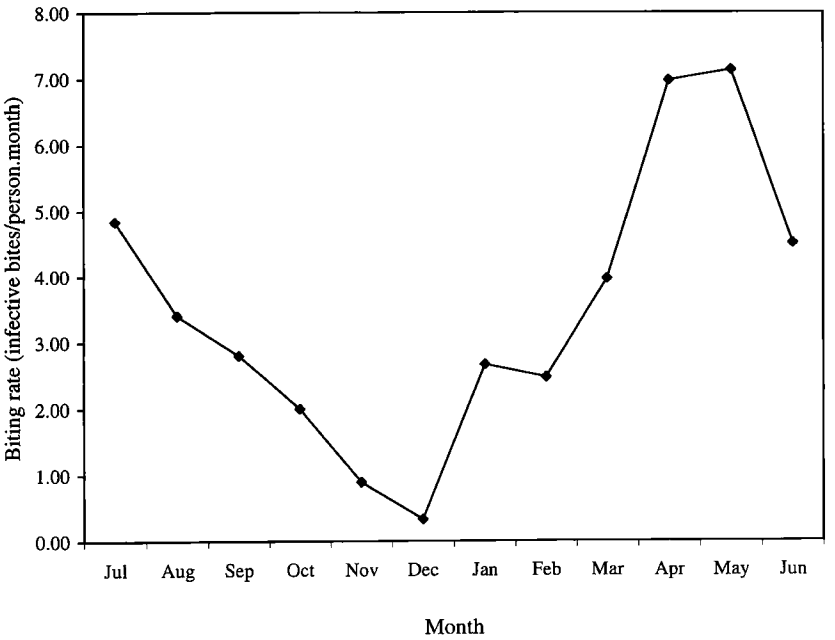

Fig. 1. Changes in monthly infective biting rate (combined for all vector species) over the 1-year study. The rate shown represents the mean number of bites by mosquitoes carrying the human-infective larvae of *Wuchereria bancrofti* experienced by each resident of Masaika in each month.

microfilaraemic in July 1998 were also microfilaraemic 6 months later but one had apparently become amicrofilaraemic by the end of the study.

Among the 11 subjects who appeared to be amicrofilaraemic at the beginning of the study, three were microfilaraemic 6 months later, and four (including two of the three who were microfilaraemic at 6 months) were microfilaraemic at the end of the study. The intensities of microfilaraemia seen in these individuals were always low, however (Table).

Antigenaemia

The geometric mean CFA concentrations among the subjects are shown in the Table and Figure 2, split by microfilarial status at the beginning of the study (i.e. amicrofilaraemic or microfilaraemic) and by time-point. These CFA were higher in the low-transmission season than in either of the two high-transmission seasons, but the differences between the three time-points were not statistically significant for those subjects who appeared amicrofilaraemic in July 1998, for those who were microfilaraemic in July 1998, or for all subjects combined ($P > 0.05$ for each). At the individual level, CFA concentrations also remained quite stable over the observation period; for 31 (84%) of the subjects they were always within a narrow range defined by the mean titre-group for the subject ± one titre-group. None of the subjects became CFA-negative during the study period.

Filarial-specific Antibodies

The GMI (OD) of the filarial-specific antibodies are shown in the Table and Figure 2, split by microfilarial status at the beginning of the study (i.e. amicrofilaraemic or microfilaraemic) and by time-point. Concentrations of filarial-specific IgG_1, IgG_2, IgG_3 and IgE were generally higher in the subjects who

TABLE

Geometric mean 'intensities' (and ranges) of microfilaraemia, antigenaemia and filarial-specific IgG_1, IgG_2, IgG_3, IgG_4 and IgE, and IgG_4/IgE ratios among the 37 subjects, in relation to their microfilarial status at the start of the study

	26 subjects who were microfilaraemic in July 1998			*11 subjects who were amicrofilaraemic in July 1998*		
Parameter	*July 1998*	*January 1999*	*July 1999*	*July 1998*	*January 1999*	*July 1999*
Microfilaraemia (microfilariae/ml)	919 (60–12 050)	1187 (60–8310)	927 (0–6390)	0 (–)	2.2 (0–130)	3.6 (0–370)
Antigenaemia (CFA units)	5473 (608–32 000)	9165 (893–32 000)	5475 (536–32 000)	1385 (158–32 000)	3271 (185–32 000)	2707 (607–32 000)
FILARIAL-SPECIFIC ANTIBODY CONCENTRATION (ELISA optical density)						
IgG_1	0.627 (0.168–1.830)	0.541 (0.083–1.734)	0.547 (0.052–1.557)	1.117 (0.166–3.126)	0.931 (0.209–3.038)	0.811 (0.202–2.234)
IgG_2	0.23 (0.041–0.725)	0.197 (0.039–0.557)	0.178 (0.056–0.353)	0.655 (0.097–2.096)	0.555 (0.045–1.378)	0.524 (0.078–1.529)
IgG_3	0.108 (0.029–0.225)	0.09 (0.022–0.249)	0.09 (0.025–0.243)	0.235 (0.018–1.029)	0.168 (0.016–0.696)	0.2 (0.014–0.676)
IgG_4	0.828 (0.016–2.684)	0.839 (0.016–2.572)	0.773 (0.021–2.362)	0.714 (0.040–1.629)	0.693 (0.032–1.732)	0.696 (0.079–1.554)
IgE	0.205 (0.035–1.061)	0.206 (0.032–0.988)	0.181 (0.036–0.809)	0.273 (0.023–0.873)	0.235 (0.014–0.702)	0.25 (0.020–0.677)
IgG_4/IgE ratio	4.42 (0.10–30.16)	4.48 (0.04–29.52)	4.74 (0.09–34.74)	3.62 (0.54–17.90)	4.1 (0.64–31.49)	3.88 (0.47–42.05)

CFA, Circulating filarial antigen (all subjects were CFA-positive whenever checked).

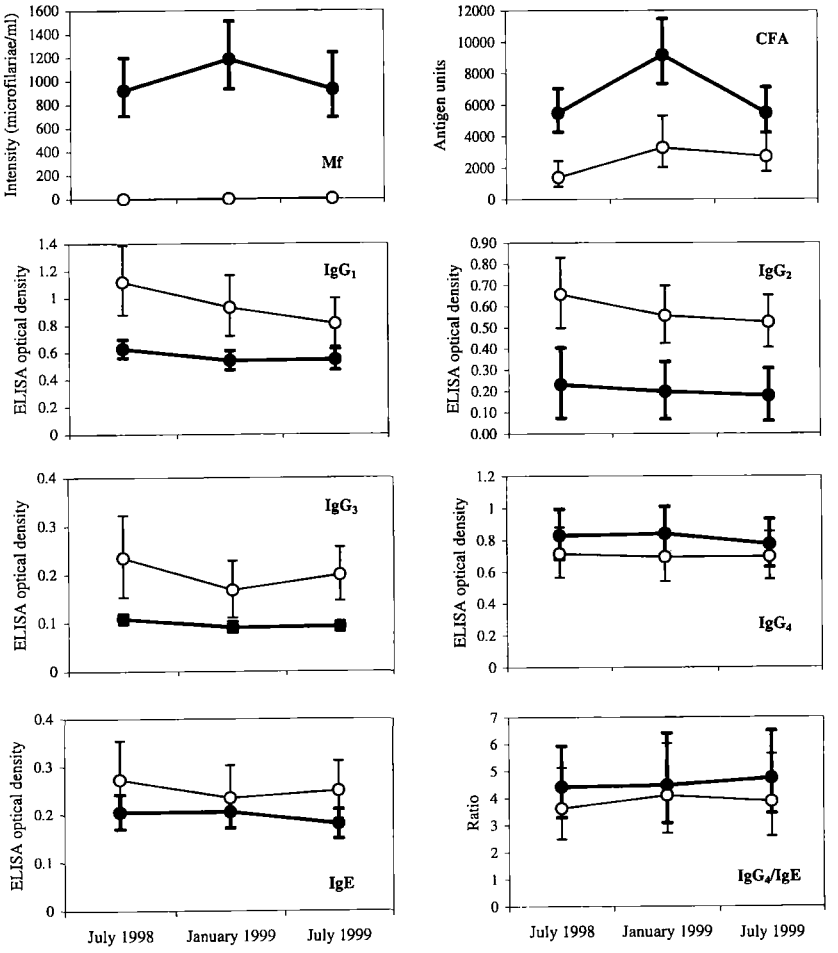

Fig. 2. Geometric mean 'intensities' of microfilaraemia (Mf), circulating filarial antigenaemia (CFA), and filarial-specific IgG_1, IgG_2, IgG_3, IgG_4 and IgE antibodies, and the corresponding IgG_4/IgE ratios for the subjects who appeared microfilaraemic (•) or amicrofilaraemic (○) at the beginning of the study. Vertical lines indicate S.E.

appeared amicrofilaraemic at the beginning of the study than in those who were then found to be microfilaraemic, whereas IgG_4 showed the opposite trend. None of the observed seasonal differences in the geometric mean concentrations of any of the antibodies—for those who appeared amicrofilaraemic in July 1998, those who were microfilaraemic at that time, or for all subjects combined—was statistically significant ($P > 0.05$ for each). At an individual level, most of the subjects showed little seasonal variation in their concentrations of filarial-specific antibodies.

IgG_4/IgE Ratio

The mean IgG_4/IgE ratios are also shown in the Table and Figure 2. At each time-point, the ratio for those who were microfilaraemic at the beginning of the study was slightly higher than that for the subjects who appeared ami-

crofilaraemic at the start. Again, however, none of the seasonal differences observed in these ratios—whether for those who appeared amicrofilaraemic in July 1998, those who were microfilaraemic at that time, or for all subjects combined—was statistically significant ($P > 0.05$ for each). Similarly, IgG_4/IgE ratios at the individual level were quite stable in each of the subjects.

DISCUSSION

Although transmission of *W. bancrofti* occurred all year round in the study community, there was considerable seasonal variation. Highest transmission intensity was seen during and immediately after the rainy season (March–June), when mosquitoes were most abundant. The lowest intensity of transmission coincided with the dry season, when mosquito numbers were low. The combined monthly infective biting rates indicate that an individual in the study community was exposed, on average, to 11.4 times as many bites by infective mosquitoes during the months of April–May 1999 than during the months of November–December 1998.

As the microfilariae of *W. bancrofti* have nocturnal periodicity in the present study area, it was essential to consider sampling time when comparing the intensities of microfilaraemia in the repeated samples. This was facilitated by collecting all the samples of finger-prick blood in the evening (between 21.00 and 24.00 hours), noting the exact sampling time, and multiplying each count of mff with a time-specific adjustment factor (Simonsen *et al.*, 1997). The samples used to estimate the concentrations of CFA or filarial-specific antibodies were all collected immediately after the finger-prick blood samples, and concentrations of CFA and specific antibodies are not known to show circadian or other short-term variation in the host's blood.

Despite the seasonal variation in intensity of transmission, no statistically significant variation was observed in the mean microfilaraemias or CFA concentrations between the three examination times. Variations in individual microfilaraemias and CFA levels were also limited. Although not statistically significant in the present study, the tendency for microfilaraemias and CFA concentrations to be slightly higher in the low-transmission season (January 1999) than in either high-transmission season is interesting. It may indicate that the relatively low level of exposure in the low-transmission season results in diminished host response against the parasite. As no individuals became CFA-negative during the study period it is unlikely that any lost their infection with *W. bancrofti*. Although some subjects appeared to become amicrofilaraemic during the study, such a change in microfilarial status may be ascribed to the relatively low sensitivity of the microscopical method used to detect microfilaraemia. Microfilaraemias and CFA concentrations appeared to be remarkably stable in the present subjects, and largely independent of the variation in transmission intensity. A high degree of stability in microfilaraemia was similarly observed in longitudinal studies on *Loa loa* infections in Cameroon (Garcia *et al.*, 1995).

In contrast to the present results, Ravindranathan *et al.* (1980) reported that *W. bancrofti* microfilaraemias in Kerala, India, were, on average, more intense in the post-rainy season than in the dry season, although no indication of the statistical significance of this difference was given. In a more recent study on brugian filariasis in Indonesia, Sartono *et al.* (1999) observed a significant decrease in mean microfilarial intensity between the end of the rainy season and the middle of the dry season. However, as no entomological data appear to have been collected, it is not certain whether this fluctuation was associated with variation in transmission intensity. The hypothesis advanced by Sartono *et al.* (1999), that the parasites regulate production of mff to coincide with high levels of biting activity of mosquitoes, so that the most intense microfilaraemias occur during the high-transmission season, is not supported by the present results.

The humoral immune responses of populations living in areas where bancroftian filariasis is endemic have been investigated several

times (Estambale *et al.*, 1994; Simonsen *et al.*, 1996; Simonsen and Meyrowitsch, 1998; Wamae *et al.*, 1998). Observations made in these studies, like the present, have shown that microfilaraemics tend to have lower levels of filarial-specific IgG_1, IgG_2, IgG_3 and IgE and higher levels of specific IgG_4 than amicrofilaraemics. However, although marked (and statistically significant) seasonal variations in immune-response patterns have been observed in several vector-borne parasitic infections, including malaria (Hviid and Theander, 1993), dirofilariasis (Espinoza *et al.*, 1993) and dracunculiasis (Bloch and Simonsen, 1998), there appears to be no clear-cut evidence of such variation in bancroftian filariasis. In the present study, no statistically significant seasonal variation was observed in any filarial-specific antibody, whatever the microfilarial status of the subject. It has been suggested that IgG_4/IgE ratios might be associated with immunological resistance to infection in lymphatic filariasis (Kurniawan *et al.*, 1993). If this hypothesis holds true, the lack of seasonal variation in the mean IgG_4/IgE ratio in the present study would appear to indicate that host resistance to *W. bancrofti* infection is also independent of the transmission season.

It should be noted that the absence of any clear seasonal variation in antibody concentrations in the present study does not exclude the possibility that other immune responses than those measured (e.g. of antibodies directed specifically towards the infective larval stages, or even cellular responses) may vary with the season. It is also possible that a greater variation in antibody responses may occur in uninfected individuals from the same community, or in individuals who are younger or older than the present subjects. Overall, the present results indicate that the levels of microfilaraemia, CFA and filarial-specific antibodies investigated were largely unaffected by the marked seasonal variation in transmission intensity (and therefore, implicitly, by short-term variation in exposure intensity). This remarkable stability probably relates to the fact that bancroftian filariasis is a highly chronic infection adapted for long-term, stable persistence in the human host.

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REFERENCES

BAILEY, J. H., HIGHTOWER, A. W., EBERHARD, M. L. & LAMMIE, P. J. (1995). Acquisition and expression of humoral reactivity to antigens of infective stages of filarial larvae. *Parasite Immunology*, **17**, 617–623.

BLOCH, P. & SIMONSEN, P. E. (1998). Immunoepidemiology of *Dracunculus medinensis* infections. II. Variation in antibody responses in relation to transmission season and patency. *American Journal of Tropical Medicine and Hygiene*, **59**, 985–990.

ESPINOZA, E., CORDERO, M., MURO, A., LORENTE, F. & SIMON, F. (1993). Anti-*Dirofilaria immitis* IgE: seroepidemiology and seasonal variation in an exposed human population. *Tropical Medicine and Parasitology*, **44**, 172–176.

ESTAMBALE, B. B. A., SIMONSEN, P. E., VENNERVALD, B. J., KNIGHT, R. & BWAYO, J. J. (1994). Bancroftian filariasis in Kwale district of Kenya. II. Humoral immune responses to filarial antigens in selected individuals from an endemic community. *Annals of Tropical Medicine and Parasitology*, **88**, 153–161.

GARCIA, A., ABEL, L., COT, M., RANQUE, S., RICHARD, P., BOUSSINESQ, M. & CHIPPAUX, J. P. (1995). Longitudinal survey of *Loa loa* filariasis in southern Cameroon: long-term stability and factors influencing individual microfilarial status. *American Journal of Tropical Medicine and Hygiene*, **52**, 370–375.

HVIID, L. & THEANDER, T. G. (1993). Seasonal changes in human immune responses to malaria. *Parasitology Today*, **9**, 26–27.

JAOKO, W. G., LUND, M., MICHAEL, E. & SIMONSEN, P. E. (2001). A simple and quick method for enhanced detection of specific IgE in serum from lymphatic filariasis patients. *Acta Tropica*, in press.

KURNIAWAN, A., YAZDANBAKHSH, M., VAN REE, R., AALBERSE, R., SELKIRK, M. E., PARTONO, F. & MAIZELS, R. M. (1993). Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. *Journal of Immunology*, **150**, 3941–3950.

LINES, J. D., CURTIS, C. F., WILKES, T. J. & NJUNWA, K. J. (1991). Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. *Bulletin of Entomological Research*, **81**, 77–84.

MCMAHON, J. E., MARSHALL, T. F. DE C., VAUGHAN, J. P. & ABARU, D. E. (1979). Bancroftian filariasis: a comparison of microfilariae counting techniques using counting chamber, standard slide and membrane (nuclepore) filtration. *Annals of Tropical Medicine and Parasitology*, **73**, 457–464.

MCMAHON, J. E., MAGAYUKA, S. A., KOLSTRUP, N., MOSHA, F. W., BUSHROD, F. M., ABARU, D. E. & BRYAN, J. H. (1981). Studies on the transmission and prevalence of bancroftian filariasis in four coastal villages of Tanzania. *Annals of Tropical Medicine and Parasitology*, **75**, 415–431.

POULSEN, L. K. & SØRENSEN, T. B. (1993). Elimination of viral infection risk from blood samples for allergy testing. *Allergy*, **48**, 207–208.

RAVINDRANATHAN, T. C., SETHUMADHAVAN, K. V. P., ROYCHOWDHURY, S. P., BABU, C. S., KANOUJIA, K. H., NARASIMHAM, M. V. V. L. & RAO, C. K. (1980). Seasonal variation in microfilaraemia (bancrofti) density and filarial disease. *Journal of Communicable Diseases*, **12**, 73–77.

SARTONO, E., LOPRIORE, C., KRUIZE, Y. C. M., KURNIAWAN-ATMADJA, A., MAIZELS, R. M. & YAZDANBAKHSH, M. (1999). Reversal in microfilarial density and T cell responses in human lymphatic filariasis. *Parasite Immunology*, **21**, 565–571.

SIMONSEN, P. E. & DUNYO, S. K. (1999). Comparative evaluation of three new tools for diagnosis of bancroftian filariasis based on detection of specific circulating antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, 278–282.

SIMONSEN, P. E. & MEYROWITSCH, D. W. (1998). Bancroftian filariasis in Tanzania: specific antibody responses in relation to long-term observations on microfilaraemia. *American Journal of Tropical Medicine and Hygiene*, **59**, 667–672.

SIMONSEN, P. E., LEMNGE, M. A., MSANGENI, H. A., JAKOBSEN, P. H. & BYGBJERG, I. C. (1996). Bancroftian filariasis: the patterns of filarial-specific immunoglobulins IgG1, IgG4 and circulating antigens in an endemic community of northeastern Tanzania. *American Journal of Tropical Medicine and Hygiene*, **55**, 59–75.

SIMONSEN, P. E., NIEMANN, L. & MEYROWITSCH, D. W. (1997). *Wuchereria bancrofti* in Tanzania: microfilarial periodicity and effect of blood sampling time on microfilarial intensities. *Tropical Medicine and International Health*, **2**, 153–158.

WAMAE, C. N., GATIKA, S. M., ROBERTS, J. M. & LAMMIE, P. J. (1998). *Wuchereria bancrofti* in Kwale district, coastal Kenya: patterns of focal distribution of infection, clinical manifestations and anti-filarial IgG responsiveness. *Parasitology*, **116**, 173–182.

WIJERS, D. J. B. & KIILU, G. (1977). Bancroftian filariasis in Kenya. III. Entomological investigations in Mambrui, a small coastal town, and Jaribuni, a rural area more inland (Coast Province). *Annals of Tropical Medicine and Parasitology*, **71**, 347–359.