The effect of pregnancy on *Wuchereria bancrofti* microfilarial load in humans

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SUMMARY

As part of a drug trial against bancroftian filariasis in the East Sepik Province of Papua New Guinea we measured the pre-treatment microfilarial densities of 2219 individuals. Mean levels generally increased with age in both sexes, with a tendency to plateau at the highest ages. However, there was a reduction among women of approximately reproductive age. Allowing for the tendency for aggregation to decrease with age, this reduction was statistically significant. However, a comparison of pregnant women and controls showed no evidence that the reduction is specifically related to pregnancy. Moreover, a simple differential equation model of microfilarial acquisition and loss suggests that age-specific patterns of exposure are also unlikely to be solely responsible. Therefore, we suggest that the observed reduction in microfilarial intensity may result from hormonal changes associated with female reproduction, possibly in combination with other factors.

Key words: *Wuchereria bancrofti*, lymphatic filariasis, age factors, sex factors, infection susceptibility.

INTRODUCTION

The global prevalence of lymphatic filariasis has recently been estimated as approximately 2%, or 120 million cases (Michael, Bundy & Grenfell, 1996), with sex being an important determinant of disease burden (Pani *et al*. 1991). Reviewing more than 40 studies, Brabin (1990) noted that microfilarial prevalence and density frequently dropped among women of reproductive age, which she hypothesized was due to pregnancy. The nature of the review, with data extracted from published reports, meant that analysis was restricted to simple subgroup analyses. Our baseline data from a drug trial against lymphatic filariasis (Bockarie *et al*. 1998) enable us to look at this issue in more detail. The initial aim is to quantify the extent of any drop in microfilarial density among women of reproductive age, and determine whether it could plausibly have occurred by chance. Our demographic surveillance then allows us to look for the hypothesized pregnancy-specific effect. Changing patterns of exposure are another possible cause of age differences in microfilarial density. We have not made direct observations of individuals' exposure, but a simple mathematical model can be used to estimate whether they might be sufficient to cause differences of the observed magnitude.

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MATERIALS AND METHODS

This work is based on a randomized community-based trial of ivermectin and DEC against bancroftian filariasis which is taking place in the East Sepik Province of Papua New Guinea (Bockarie *et al*. 1998). The counts of *Wuchereria bancrofti* microfilariae were done by membrane filtration of 1 ml night blood samples, taken in 1994 as part of a pre-treatment clinical and parasitological survey. Only those aged 5 years or more were included. Oral informed consent was obtained from all adults, and from guardians of children younger than 16 years. The protocol was approved by the Medical Research Advisory Committee of Papua New Guinea.

Statistical methods

The age and sex data on each individual permits more detailed analysis than in Brabin's (1990) review. For example, a simple difference between males and females does not necessarily indicate a pregnancy-specific effect, because the difference could be consistent over reproductive and non-reproductive ages. We modelled the mean microfilarial density, μ, as continuous functions of age and sex using negative binomial distributions fitted by maximum likelihood. The negative binomial distribution function is (McCullagh & Nelder (1989), page 199):

$$P(x) = \frac{\Gamma(k+x)}{\Gamma(k)x!}\frac{\mu^x k^k}{(\mu+k)^{k+x}}. \quad (1)$$

This distribution has been found to be widely

applicable to parasite counts (Grenfell *et al*. 1990; Anderson & May, 1991; Grenfell, Dietz & Roberts, 1995). The k parameter (ν in the notation of McCullagh & Nelder) is an inverse measure of parasite aggregation. Analysis by a standard generalized linear model would involve estimating a single value of k and applying it to all the data. However, in fact, k often varies with age. Such patterns have intrinsic interest because of the hypotheses advanced to explain them (Pacala & Dobson, 1988; Quinnell, Grafen & Woolhouse, 1995). They are also important for statistical estimation and testing, because, if the assumption of constant k is not justified, then the accuracy of the estimates of the mean parasite burden (μ) will be over-estimated at some ages, and under-estimated at others. Similar problems of changing dispersion, or heteroscedasticity, are also found in Gaussian regression. However, tools to accommodate the problem have been less well developed for the negative binomial case. To fit our models, we note that each data point contributes one term such as equation (1) to the overall likelihood. We then write μ and k as functions of age and sex, yielding an expression which can be maximized in terms of those functions' parameters.

We used splines to model μ and k as functions of age. Splines are regression polynomials which are fitted piecewise over intervals of the data range, in such a way that the fits are continuous at interval boundaries (Hastie & Tibshirani, 1990). When the polynomials have degree 1, the model is piecewise linear. In that case, the fits are continuous but the slopes are not, because adjacent line segments generally meet at an angle. Higher order polynomial splines allow the slopes to be continuous as well. We used both linear (degree 1) and cubic (degree 3) splines. In each case, one function was estimated independently for each sex. This technique is a generalization of that used by Pacala & Dobson (1988). Modern software allows more general functions to be fitted without resort to low-level programming languages; in particular, we used the 'nlminb' function of S-PLUS. The piecewise linear is a more crude model but permits direct estimation of the rate of change of microfilarial density within any of the age intervals. Hypothesis tests and confidence intervals were constructed using the profile likelihood (McCullagh & Nelder (1989) page 254).

A possible pregnancy-specific effect was investigated using the birth records which are maintained as part of the drug trial's demographic surveillance. Pregnancy status at the time of the microfilarial sample was estimated according to whether or not a live birth occurred in the following nine months. Each pregnant woman was matched to a control, chosen as the non-pregnant woman closest to her in age. This procedure was executed in order of increasing age, with no-one permitted to be a control more than once. If multiple potential controls had the same age, then the tie was broken by choosing one at random.

Mathematical models

The effect of possible differences in exposure were investigated by a simple immigration–death model expressed by the following differential equation:

$$\frac{d\mu(t)}{dt} = \Lambda(t) - c\mu(t). \tag{2}$$

Here, $\Lambda(t)$ is the net acquisition rate, and c is the *per capita* loss rate, which is assumed to be constant. This means that the balance between loss and gain, but not the *per capita* loss rate, varies over time. For consistency with equation (1), our notation differs from the more usual one, in which M stands for our μ, and μ for our c (Anderson & May, 1991). If the loss rate is allowed to vary ($c = c(t)$), then equation (2) yields solutions which are not of closed form, but which are convex functions of age, not qualitatively different from those obtained below. We also assume that the process is in equilibrium with respect to time, so that age can be used as a proxy for calendar time. That is not unreasonable, given the apparent lack of factors which might have significantly changed transmission over the life-time of the present inhabitants. Most are still engaged in shifting subsistence agriculture, and virtually no houses are made of modern materials. Moreover, although there were attempts at malaria eradication by insecticide spraying in East Sepik Province, they did not reach as far west as the current area (Kwan-Lim, Forsyth & Maizels, 1990). We use the following simple form of net acquisition rate:

$$\Lambda(t) = a\,e^{-bt}. \tag{3}$$

This can represent constant ($b = 0$) or decaying ($b > 0$) acquisition, which may result from such factors as a reduction in vector contact with age, or acquired immunity (Anderson & May, 1991). A more general type of acquisition function with a turning point was also fitted ($\Lambda(t) = a\,e^{-bt} - \gamma\,e^{-\delta t}$) with similar results (not shown). Multiplying through equation (3) by an integrating factor of e^{ct} yields the solution as a difference of exponentials:

$$\mu(t) = \frac{a}{c-b}(e^{-bt} - e^{-ct}). \tag{4}$$

This was fitted to μ by maximum likelihood as before, with a fresh spline model for k being fitted simultaneously. Equation (4) has at most one turning point. To simulate a more general pattern resulting from age-dependent changes in exposure, we modified the acquisition function $\Lambda(t)$, and used the

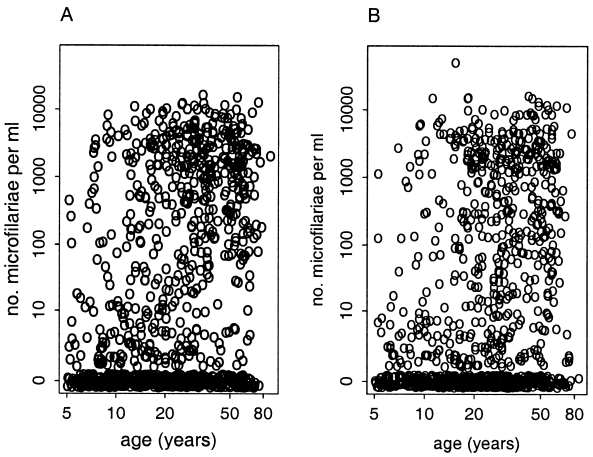

Fig. 1. Plots of the microfilarial counts per ml against age in years, for (A) males ($n = 1075$), and (B) females ($n = 1144$). The data have been 'jittered' by adding a small amount of random noise to separate identical points. The scale of the horizontal axis is logarithmic in age, and the scale of the vertical axis is logarithmic in the microfilarial count plus 1. (Adding 1 to the microfilarial counts was done only for the plot, not for the negative binomial analyses.) Maximum likelihood estimates and confidence intervals for the mean and k are shown for age and sex classes in Fig. 3.

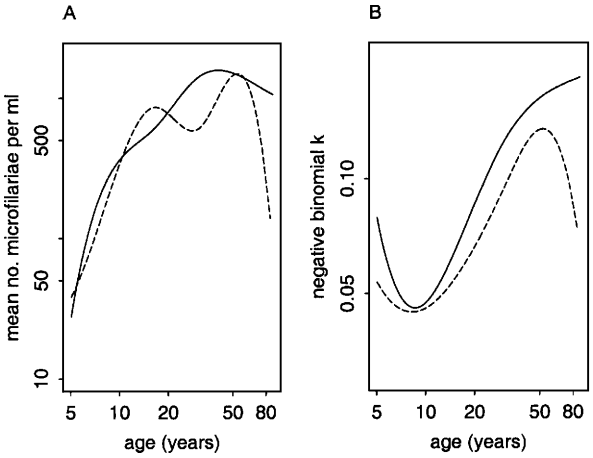

Fig. 2. Negative binomial parameters for the microfilarial counts, fitted as spline functions of age and sex by maximum likelihood: (A) mean number per ml, and (B) k. (——) Males; (– – – –) females.

more general solution to equation (2) (Anderson & May (1991) page 502):

$$\mu(t) = e^{-ct} \int_0^t \Lambda(s)\, e^{cs}\, ds. \qquad (5)$$

The integration was done numerically with S-PLUS' 'integrate' function.

RESULTS

The microfilarial counts analysed are the same 2219 as in Bockarie *et al*. (1998), and are shown in Fig. 1. Cubic spline fits to the data are shown in Fig.2. In males, the mean density increased rapidly through childhood, then less rapidly in mature years, before

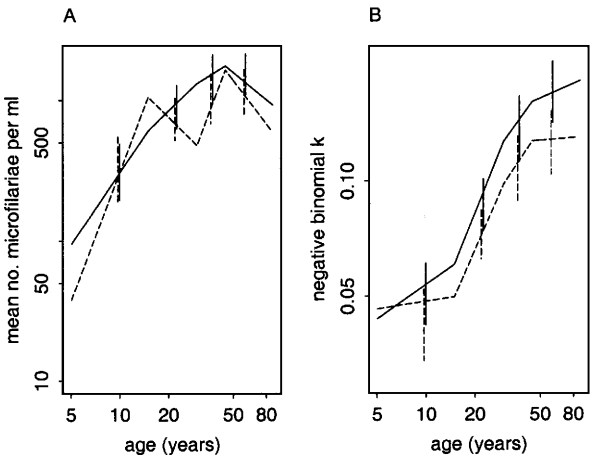

Fig. 3. Negative binomial parameters for the microfilarial counts, fitted as piecewise log-linear functions of age and sex by maximum likelihood: (A) mean number per ml, and (B) k. The vertical lines (——) males; (– – – –) females) are the subgroup 95% confidence intervals for 4 age classes, defined by boundaries at 5, 15, 30 and 45 years. Those whose age lay exactly on a boundary were included in the lower class. The respective numbers of people in the classes were 353, 342, 185 and 195 males; and 295, 435, 210 and 204 females.

dropping off slightly in the highest ages. The pattern was similar in females, except the final reduction was more pronounced, and an earlier reduction occurred among those of approximately reproductive age. In males, k increased monotonically with age, after a reduction in the youngest children. The pattern of k was similar in females, except for a reduction in the highest ages.

Fig. 3 shows the piecewise linear fits, the change points having been chosen at 15, 30 and 45 years of age. The vertical lines in the figure are the 95% confidence intervals for the maximum likelihood estimates of μ and k for each of those 4 subgroups. The results of the piecewise linear fits are generally similar to the spline ones. Except for the reduction in the highest ages, Fig. 3 is similar to Fig. 3 of Brabin (1990), which was redrawn from the results of Knight *et al*. (1979) in a different region of Papua New Guinea. In this case we can do statistical estimation on the rate of change. In those aged between 15 and 30 years, mean microfilarial density decreased in females and increased in males, both effects being statistically significant. In females, microfilarial density changed on average by $-4{\cdot}6\%$ per 10% increase in age, with a 95% confidence interval of $-6{\cdot}0$ to $-3{\cdot}1\%$ ($P < 0{\cdot}001$). In males, the corresponding change was $+4{\cdot}8\%$ (95% confidence interval 3·4 to 6·3%, $P < 0{\cdot}001$). These estimates of the rates of change of μ and k use the exact age data at the individual level, as opposed to the subgroup estimates, which pool all ages within each class.

Investigating a possible pregnancy-specific effect,

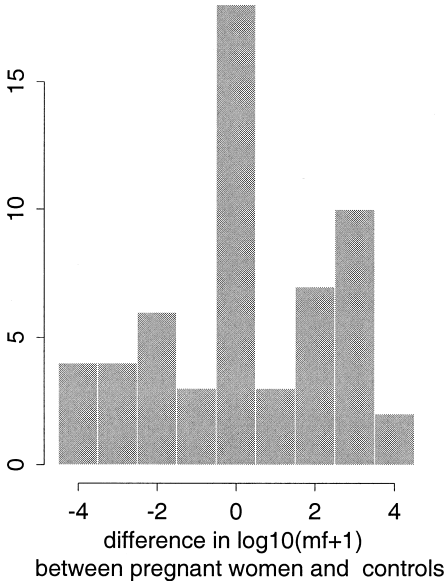

Fig. 4. Histogram of the differences in $\log_{10}$(microfilariae/ml + 1) between pregnant women and matched non-pregnant controls (a positive value indicates the pregnant woman had the higher density).

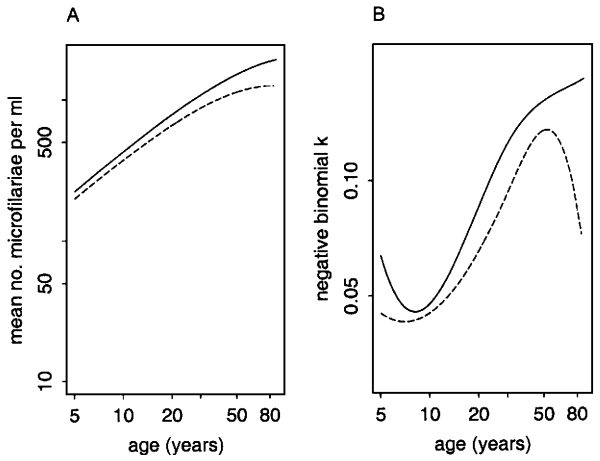

Fig. 5. (A) Mean number of microfilariae per ml, fitted by maximum likelihood from equation (4). (B) k fitted as splines. (——) Males; (– – – –) females.

57 women were found to have given birth within 9 months of participating in the survey, and were therefore presumed to have been pregnant at that time. For each pregnant woman, a non-pregnant control was found whose age matched within 6 months. The differences in microfilarial density are shown in Fig. 4. There is very little difference between the matched microfilarial densities ($Z = 0{\cdot}10$ from the Wilcoxon test, $P = 0{\cdot}92$). Among the pairs which differed in microfilarial density, the pregnant woman had the higher level in exactly half of them: 24/48, with a 95% confidence interval of 35 to 65% (Armitage & Berry, 1994). There is therefore no evidence that pregnancy is causally associated with a reduction in microfilarial density.

The fits to equation (4) are shown in Fig. 5, with k fitted by splines. The general patterns of mean

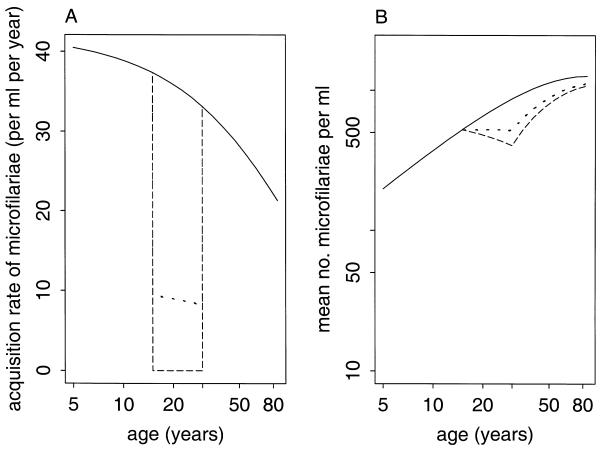

Fig. 6. (A) Acquisition function of microfilariae in females. (——) Exponential decay fitted from equation (3); (. . . . .) as (——) but with 75% reduction in 15 to 30-year-olds; (– – – –) no acquisition in 15 to 30-year-olds. (B) Resulting mean microfilarial densities per ml, according to equation (5). The maximum likelihood estimates (95% confidence intervals) for the parameters of the solid lines are: a = 42 (35–52) microfilariae/ml/year; b = 0·008 (−0·002–0·018)/year; c = 0·009 (−0·001–0·019)/year.

microfilarial density are similar to those in Figs 2 and 3, except for the drop in women of reproductive age. As noted above, the model cannot represent this additional turning point. A less important feature which this simple model fails to capture is the steepness of the increase from the age of 5 to 10–15 years, which may result from a delay in the onset of acquisition of infection by the population investigated.

To see whether the reduction in females of reproductive age might plausibly result from changes in exposure, the age-specific acquisition function was modified. Starting from the fitted function of the form of equation (3), the acquisition was set to zero from age 15 to 30 years, corresponding roughly to the extent of the reduction in Fig. 2. The resulting microfilarial density was determined by equation (5). The same was done for a 75% reduction in acquisition in the same age range. The results are shown in Fig. 6. Even when acquisition was totally halted in 15 to 30-year-olds, the resulting drop in microfilarial density is not as large as that seen in Figs 2 and 3. This suggests that, even though we cannot quantify age- and sex-related changes in exposure, they seem unlikely to fully explain as large a reduction as that actually observed.

DISCUSSION

We have used individual-level data to confirm in more detail the finding of Brabin (1990) that mean microfilarial density is reduced in women of reproductive age. However, we have not been able to confirm her hypothesis that this reduction is causally

related to pregnancy. The mean microfilarial density in males is generally consistent with a simple acquisition function, either constant or decreasing slowly with age. Females show a more complex pattern, with a reduction in density starting at the beginning of reproductive age, and a subsequent reduction at higher ages which is more pronounced than in males.

The negative binomial k parameter generally increases with age in both males and females. The model of Pacala & Dobson (1985), implemented for *Wuchereria bancrofti* by Das *et al*. (1990), suggests that such patterns may result from density dependence. However, Quinnell *et al*. (1995) have shown that a very plausible relaxation of the model assumptions – allowing parasite acquisition to be aggregated – can lead to non-constant k even in the absence of density dependence. From these models it is hard to say whether the increase is caused by density dependence, heterogeneities in acquisition, or other factors. Guyatt *et al*. (1990) reached similar conclusions for *Ascaris lumbricoides*, as did Woolhouse, Ndamba & Bradley (1994) for *Schistosoma haematobium*.

The model of Quinnell *et al*. (1995) can yield patterns in k similar to those observed here. However, the assumed form of acquisition function cannot accommodate a turning point in the middle of the age range, a pattern which probably does in fact occur (Vanamail *et al*. 1989). Therefore, a model which could hope to explain detailed patterns in k might well require yet another generalization. This might be possible using the methods used above, although such models should probably not be extended much farther without incorporating stochasticity. One way to do so would be to use moment closure equations to approximate the higher moments of the parasite counts in terms of their mean and variance (Whittle, 1957; Grenfell *et al*. 1995). This would be difficult for human filariasis, because of the lack of information on adult worm numbers and host immunocompetence, although Michael *et al*. (1998) have done so for an animal model.

In any case, more complicated models might be difficult to justify without data on exposure, because otherwise its effect is difficult to distinguish from those of internal host factors. Nevertheless, simple models can be used more heuristically to explore prominent empirical features, such as the observed drop in microfilarial density in females of reproducing age. This indicates that even total interruption of exposure may not by itself cause a drop of the observed magnitude, so it seems likely that physiological factors decrease microfilarial recruitment, either by increasing the death rate of adult worms or by reducing their fecundity. Human hormonal changes are the obvious potential mechanism, although pregnancy does not seem a sufficient cause, so the effect may be related to the increase in oestrogens from puberty (Ganong, 1985). A hormonal explanation has been proposed by Fulford *et al*. (1998) for the observations that women often have less heavy schistosomiasis infections than men, despite having more water contact (Butterworth *et al*. 1984), and that the susceptibility of both sexes decreases during puberty (Butterworth *et al*. 1996). To conclude, puberty-associated hormonal changes merit further study in relation to parasitic infections, although their detailed effects may be hard to separate from other factors such as exposure.

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