

The epidemiology of human hookworm infections in the southern region of Mali

J. M. Behnke¹, D. De Clercq², M. Sacko³, F. S. Gilbert¹, D. B. Ouattara⁴ and J. Vercruyse²

¹ School of Biological Sciences, University of Nottingham, UK

² Department of Parasitology, Faculty of Veterinary Medicine, University of Gent, Belgium

³ Institut National de Recherche en Santé Publique, Service de Parasitologie, Bamako-Coura, Mali

⁴ Medecin-Chef Adjoint des Services Socio-Sanitaires du Cercle de Bougouni, Mali

Summary

Two surveys of hookworm (*Necator americanus*) infections, conducted three years apart (December 1994 and January 1998) in a village in the Sikasso region of Mali, revealed that overall prevalence of infection was 68.7% and 53%, respectively. In both years there was a highly significant difference between the sexes in the prevalence and abundance of infection, with male subjects carrying heavier infections than females. Both prevalence and abundance of infection increased with age, although in 1998 there was a strong interaction between sex and age, arising from the declining egg counts among 16–20-year-old females and the continuing increase among males, reinforced by the subsequent reduction among the older males (≥ 61 years) and concomitant increase among females. After controlling for the effects of age, sex and their interaction, a highly significant positive relationship was detected between faecal egg counts of individuals who were examined in both 1994 and 1998 ($n = 134$), indicating predisposition to infection. This relationship remained significant in each of 4 age classes spanning 7–79 years. The members of some family compounds were shown to carry heavier infections than expected whilst others were less infected, suggesting compound-related clustering of hookworm infections. The use of footwear increased with age but there was no significant relationship between the extent of use of footwear and the abundance of hookworm infection. Eyesight deteriorated with age and impaired vision was particularly prominent among the older sectors of the community, a legacy from the time when onchocerciasis was widely prevalent in the region. Although men with partially damaged eyes carried lower infections than expected for their age, no overall significant relationship was found between quality of vision and hookworm infections. These results are discussed in relation to hookworm epidemiology in general and in Mali in particular.

keywords *Necator americanus*, hookworms, familial clustering, predisposition, footwear, eyesight

correspondence Dr. J.M.Behnke, School of Biological Sciences University Park, University of Nottingham, Nottingham NG7 2RD, UK. E-mail: jerzy.behnke@pln1.life.nottingham.ac.uk

Introduction

Human hookworm infections are widespread in the tropics and collectively the two anthrophilic species of hookworms are second only to *Ascaris lumbricoides* in respect of the number of people infected throughout the world (Bundy 1997; Chan 1997). The dominant species in Africa is *Necator americanus*, a species whose epidemiology has been investigated in many countries in Africa (Bradley *et al.* 1992; Palmer & Bundy 1995; Brooker *et al.* 1999) and elsewhere (Miller 1979; Schad & Warren 1990; Ye *et al.* 1994; Humphries *et al.* 1997), but for which there is little comparable data in

respect of communities living in the Sahel region of sub-Saharan West Africa (Kilama 1990).

Although *N. americanus* is generally confined to locations where the climate is warm and moist (Udonsi *et al.* 1980; Schad *et al.* 1983), the parasite also occurs locally in more arid regions of Africa. In Mali, this species has been recorded in six of the seven regions into which the country is divided and in the district of Bamako (De Clercq *et al.* 1995). The only region where no hookworms were found was in the extremely arid, northern 6th region of Tombouctou, which reaches deep into the Saharan Desert. As expected, the relationship between the prevalence of hookworms and mean annual rainfall was

positive and highly significant, and the highest prevalence of infection was recorded in the region of Sikasso (Region 4) in the humid south of the country (De Clercq *et al.* 1995).

The prevalence of *N. americanus* in the Sikasso Region has been found to be as high as 70% in some villages (De Clercq *et al.* 1997), but little is known about the factors which influence its transmission locally and how the worms are distributed among host populations. In this paper we present data from two surveys in a village in the Sikasso region of Mali conducted 3 years apart. We provide data on the age-prevalence and age-abundance profiles of *N. americanus* in a community in Mali. We also sought evidence of predisposition to infection as well as familial clustering of infections and we examined relationships between abundance of infection and the extent to which people use footwear. Lastly we examined the idea that deteriorating eyesight might increase the risk of infection because the village was situated in a region where onchocerciasis was once highly endemic.

Materials and methods

Study site and subjects

The study was conducted in a village in the district of Bougouni (South-east Mali) in the Sikasso region (3rd Region). The village comprised 24 family groups and an estimated total population of 352–419 subjects (352 in an official census conducted in 1987 and 419 from our records in 1998). Each family group occupied a family compound, comprising a circle of huts arranged around a communal open central area. Most of these were clustered around the larger space in the middle of the village, although some were located on the periphery of the village and some were more distant. The village elders comprised the heads of each family group. The main activities in the village are cultivation of millet, cotton and some livestock husbandry. The first survey took place in mid-December 1994, when 247 subjects (70.2% of the population) were enrolled for the study but 182 (106 males and 76 females) provided stools for examination. Most of the population were treated with mebendazole after participating in a chemotherapy trial in January 1995. The second survey was conducted in January 1998, when 323 subjects (77.1%) were initially registered and 285 provided stools for analysis (136 males, 149 females).

Ethical approval

The surveys were approved by the Directorate of the National Institute for Research in Public Health (INRSP), in Bamako, Mali. The background and the purpose of the work were explained at a meeting with village elders and heads of families and their approval was obtained. Participation in the surveys was subject to informed, voluntary consent of each

individual or parents and/or head of family in the case of children. Free medical examinations were available to all villagers at both surveys.

Quantification of hookworm infections

Fresh overnight stools were obtained from individuals who participated in the study and two slides were prepared from each specimen and examined on the same day and within 50 min of preparation by the Kato-Katz technique (Katz *et al.* 1972). Hookworm eggs were identified, the intensity of infection was calculated as eggs per gram of faeces (EPG) and then adjusted for stool consistency and host age using WHO (1963) guidelines. The same procedure was used in both surveys. Faecal cultures revealed only larvae of *N. americanus*.

Assessment of eyesight, footwear and other relevant information

In 1998 one of the authors (DBO) examined and interviewed most villagers ($n = 260$), to assess eyesight and the extent to which footwear was worn, and to obtain additional information relevant to transmission of hookworms in the region. Eyesight was assessed on a semiquantitative scale of 1–3, where 1 = eyesight severely compromised; 2 = some evidence of impaired eyesight and 3 = eyesight apparently normal. Each respondent was also asked to state whether they wore footwear always (score = 4), often but not always (3), rarely – sometimes (2) or never (1).

Statistical analysis

Prevalence of infection was analysed by maximum likelihood techniques based on log linear analysis of contingency tables implemented by the software package Statgraphics V. 7. Beginning with the most complex model involving all possible main effects and interactions, those combinations which did not contribute significantly to explaining variation in the data were eliminated stepwise beginning with the highest-level interaction. A minimum sufficient model was then obtained, for which the likelihood ratio of χ^2 was not significant, indicating that the model was sufficient in explaining the data.

Quantitative faecal egg counts were expressed as geometric means \pm 95% confidence limits (CL) of the adjusted EPGs because the data were highly overdispersed (Elliott 1977; Dash *et al.* 1988). In some cases arithmetic mean and standard errors of the means are also provided. These means reflect the abundance of infection as defined by Margolis *et al.* (1982) and include all subjects within the specified group, infected and not infected, for whom relevant data were available. The degree of aggregation in the data was calculated by the Index of Discrepancy (D) as described by Poulin (1993) (a value of 0

indicates an even distribution of counts across all hosts and a value of 1 indicates all parasites aggregated in a single host). Distributions were tested for goodness of fit to the negative binomial distribution through χ^2 and the negative binomial exponent k is given as appropriate (Elliott 1977).

The quantitative faecal egg count (FEC) data from each survey were analysed by GLIM (A statistical system for generalized linear interactive modelling; GLIM 4, PC version, Royal Statistical Society 1993) as described previously, using models with negative binomial or normal errors as appropriate (Crawley 1993; De Clercq *et al.* 1997). Host sex (2 levels) was entered as a factor. Age was also entered as a factor (In 1994 at 7 levels; age classes corresponding to 3–5, 6–10, 11–20, 21–30, 31–40, 41–60 and ≥ 61 years. In 1998, the 11–20 years class was divided into two classes of 11–15 and 16–20 years age groups). For models employing negative binomial errors the change in deviance is divided by the scale parameter and the resulting scaled deviance is distributed as χ^2 . For models with normal errors the change in deviance is divided by the scale parameter and the result divided by the change in degrees of freedom (dof) following each deletion, to give a variance ratio, F .

For analysis of correlation in EPGs between 1994 and 1998, we initially examined the relationship between EPGs in both years by Spearman's Rank-order correlation test with and without subjects who did not pass hookworm eggs. In a second approach, we first used 2-way ANOVA (Type III sums of squares in Statgraphics Version 7) with sex and age as factors on $\log_{10}(x + 1)$ transformed EPGs to remove variation arising from these factors and then correlated the residuals from each ANOVA against each other.

Differences in infection intensities between the members of family compounds in the village (familial predisposition to infection) were illustrated with data after standardization which took into account sex and age effects (The arithmetic mean EPG of each single-sex and age class was subtracted from the EPG of each individual in that class and the difference was divided by the standard deviation for that class (SD)). The mean departure from zero in SD units of each family compound was then calculated, where values above zero indicated higher than expected EPGs and negative values corresponded to lower ones). A similar approach was used to illustrate the effect of vision score on EPGs. However, in both cases statistical analyses were based on full ANOVAs in Glim with relevant factors (age and sex, and family or eye score, respectively) taken into account as described above.

Results

1994 Survey

In December 1994, 182 subjects (106 males, 76 females) provided stools for examination. The age structure of this subset of the population is illustrated in Figure 1a, which

shows that there were relatively few 16–20 years-olds of both sexes and females aged 60+. For this reason subsequent analysis of the data by age class was based on the age categories illustrated in the figure with the exception of the 11–15 and 16–20 age groups, which were collapsed into a single class. For the purpose of illustration of the data, but not data analysis, the 3 females aged > 60 were combined with the 41–60 year female class.

The frequency distribution of the faecal egg counts is shown in Figure 2a. Fifty-seven individuals did not pass hookworm eggs, and the remaining 125 had EPGs in the range 12–4656. However, most infections were low-intensity with EPGs < 1000 (116 subjects) and only 9 subjects had EPGs > 1000 . The data were aggregated with $D = 0.572$ but the tail of heavily infected individuals and the lower than expected number showing EPGs in the 1–30 and 151–180

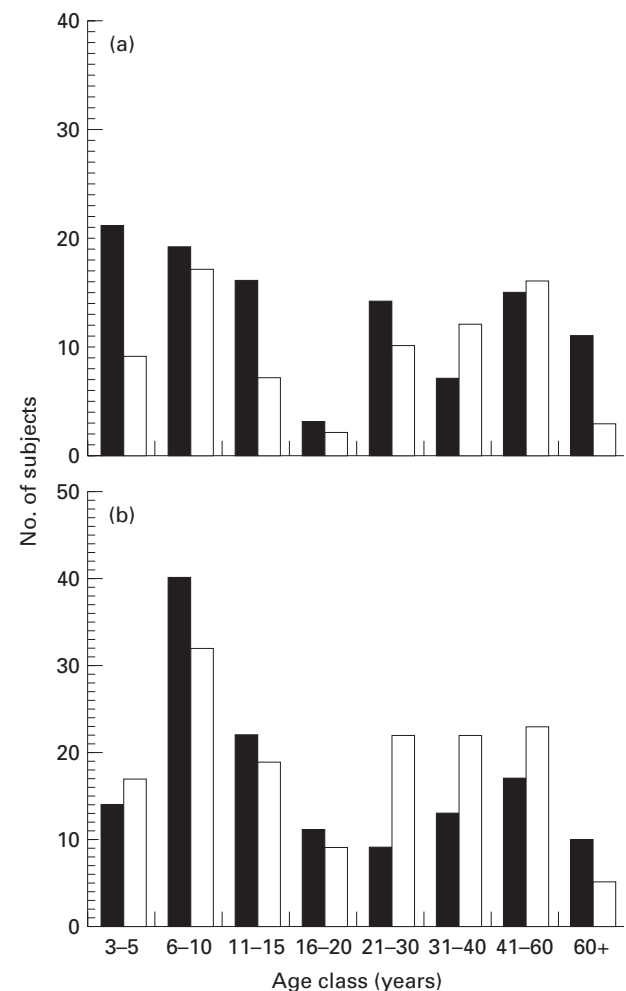


Figure 1 Age structure of the population sampled for hookworm infections in (a) 1994 and (b) 1998. ■ Males; □ Females.

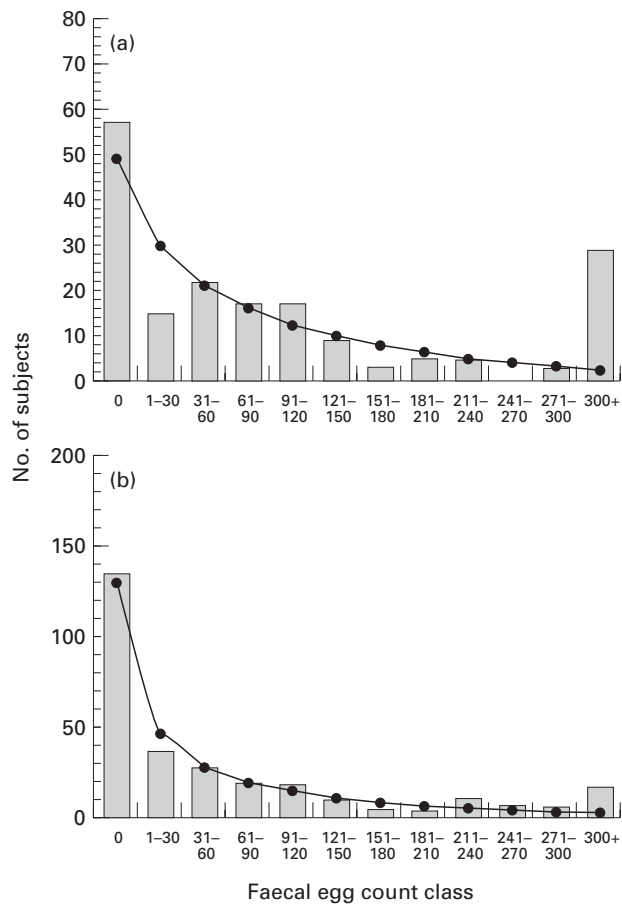


Figure 2 Frequency distribution of subjects by faecal egg count class, in (a) 1994 and (b) 1998. Columns show the observed frequencies and the line graph expected frequencies from the fitted negative binomial distribution.

ranges generated sufficient discrepancy for the distribution to differ significantly from the negative binomial ($k = 0.73 \pm 0.11$, $\chi^2 = 59.53$, $\text{dof} = 9$, $P < 0.0001$).

The overall prevalence of infection was 68.7% (males, 76.4%; females, 57.9%) and the age prevalence profile, illustrated in Figure 3a, shows that whilst prevalence among young males increased with age to peak in the 31–40 age class, prevalence in females peaked in the 11–20 age class and declined only slightly in successive older age groups. Log-linear analysis yielded a model with the following term: sex \times age, sex \times infection and age \times infection (likelihood ratio of $\chi^2 = 8.63$ d.f. = 6, $P = 0.195$), confirming that there was no interaction between age and sex in determining prevalence, but that there were significant independent effects of both age and sex on the prevalence of infection.

The mean abundance of infection by age is illustrated in Figure 4a and the statistical analysis is given in Table 1. The

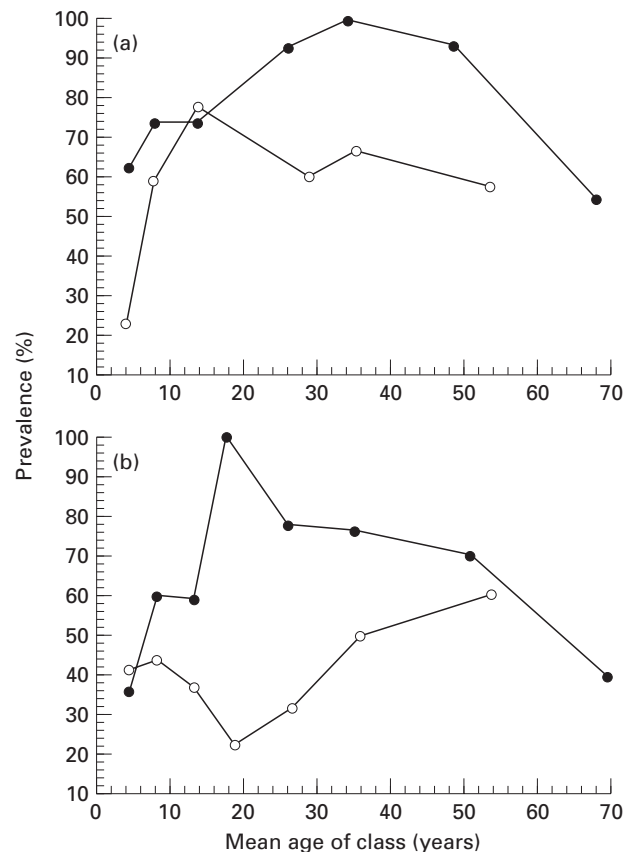


Figure 3 Age-prevalence profiles in (a) 1994 and (b) 1998. ● Males; ○ Females.

overall geometric mean EPG was 26.8 (95% CL of 18.3–38.9 and an arithmetic mean EPG of 220.3 ± 38.9). There was no significant interaction between sex and age (Table 1), but there was a significant effect of age and a much stronger effect of sex (Geometric mean EPGs in males = 44.3 (95% CL 27.5–71.1), females = 13.1 (95% CL 7.0–23.7)).

1998 Survey

In January 1998, 285 subjects (136 males, 149 females) provided stools for examination. The age structure of this subset is illustrated in Figure 1b and again shows relatively few subjects in the 16–20 age group, but they were sufficient this time to merit statistical analysis by 8 age classes. However, for the purpose of illustration, and for comparison with the 1994 data, the 5 females aged > 60 were combined with the 41–60 age range class.

The frequency distribution of the faecal egg counts is illustrated in Figure 2b. 134 individuals did not pass hookworm eggs, and the EPGs ranged from 6 to 2340 among

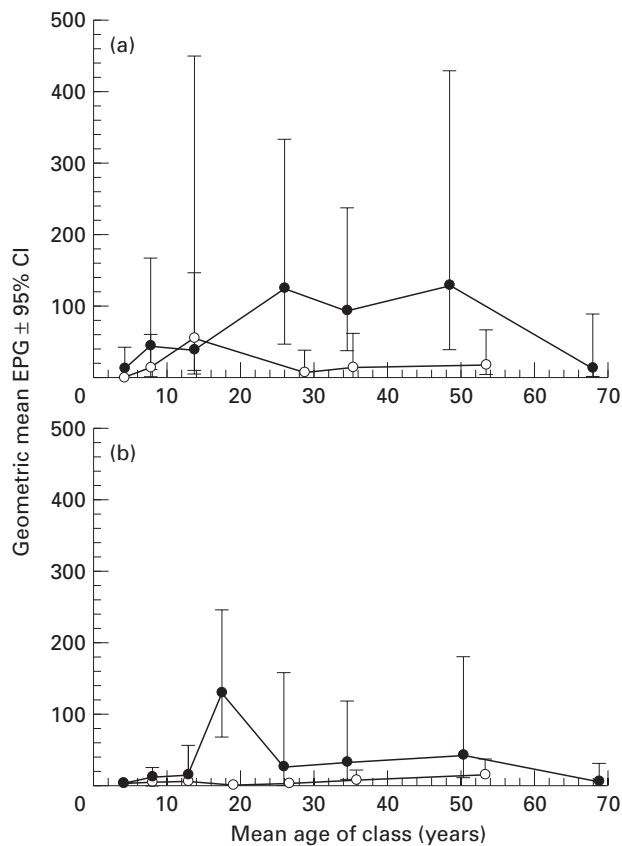


Figure 4 Age – abundance profiles in (a) 1994 and (b) 1998. ● Males; ○ Females.

infected subjects. Again, most infections were low-intensity with EPGs < 1000 and only 4 subjects yielding EPGs > 1000. The data showed a greater aggregation than in 1994 with $D = 0.691$, but again the tail of high EPGs generated sufficient discrepancy for the distribution to differ

Table 1 Statistical analysis of factors (sex × age) affecting intensity of infection (log EPG [x + 1] transformed data) with *N.americanaus* in 1994 through a 2-way ANOVA with normal errors

Source of variation	Change in deviance*	Degrees of freedom	Scale parameter	Scaled deviance†	P
Sex	80.41	1	6.075	13.236	< 0.001
Age	84.18	6	5.928	2.367	0.05 > P > 0.025
Age × sex	43.75	6	5.648	1.291	NS

The full model deviance was 939.03 with a scale parameter of 5.589. NS, Not significant. * Change in deviance following removal of combination specified in 'source of variation' column from the full factorial model. We begin by removing the age by sex interaction from the model. We then progressively remove the combinations in order from the base of the table towards the top. The main effect, of age, was removed to assess the change in deviance but then replaced before removing the effect of sex. † Scaled deviance = measure of contribution of factor specified under column labelled 'source of variation' to explaining variation in the data, calculated by fitting ANOVA with normal errors through GLIM. It is distributed as F.

significantly from the negative binomial ($k = 0.422 + 0.052$, $\chi^2 = 44.41$, dof = 8, $P < 0.0001$).

The overall prevalence of infection in the village was 53% but the age-prevalence profile was complex. The log-linear model could not be simplified from the full three way interaction (age × sex × infection). Prevalence was higher among male (63.2%) compared with female (43.6%) subjects but this sex difference was compounded by age. This can be seen in Figure 3b which shows that prevalence of infection rose to peak among the 15–20-year-old males, when the corresponding female age class showed lowest prevalence. Among the older age classes, the trends were again opposite in the two sexes, with prevalence rising in females and declining among males with increasing age.

The overall abundance of infection (Figure 4b) was lower than in 1994 (geometric mean EPG = 9.4, 95% CL 6.9–12.7; arithmetic mean EPG = 93.1 ± 14.0) but again higher among males (16.5, 95% CL 10.6–25.4) than females (6.5, 95% CL 3.5–8.3). Statistical analysis (Table 2) was by ANOVA with negative binomial errors since despite the significant difference between observed and expected distributions, the data were aggregated as reflected in the value of D , other distributions generated even greater discrepancy and the iterations in GLIM with negative binomial errors converged satisfactorily. The analysis revealed that there was a highly significant interaction between age and sex, arising from the declining egg counts among 16–20 age females and the continuing increase among males, reinforced by the subsequent reduction among the older males and concomitant increase among females. There were highly significant main effects of both sex and age.

Comparison of egg counts in 1994 and 1998

134 subjects were examined in both 1994 and 1998, comprising 52 females and 82 males. Of these 27 had no

Source of variation	Change in deviance*	Degrees of freedom	Scale parameter	Scaled deviance†	P
Sex	8250	1	476.8	17.303	< 0.0005
Age	17331	7	498.7	34.752	< 0.0005
Age × sex	25142	7	448.6	56.045	< 0.0005

The full model deviance was 98669 with a scale parameter of 366.8. a. Scaled deviance = measure of contribution of factor specified under column labelled 'source of variation' to explaining variation in the data, calculated by fitting ANOVA with negative binomial errors through GLIM. It is distributed as χ^2 .

detectable eggs in both years (12 males and 15 females). The log₁₀ EPG ($x + 1$) transformed data is illustrated in Figure 5, by sex. There was a highly significant positive relationship between the 1994 and 1998 EPGs when all the subjects common to both surveys were considered ($r_s = 0.483$, $n = 134$, $P < 0.001$), which remained significant when the 27 subjects who had zero egg counts in both years were removed ($r_s = 0.257$, $n = 107$, $P = 0.008$) and also when the analysis

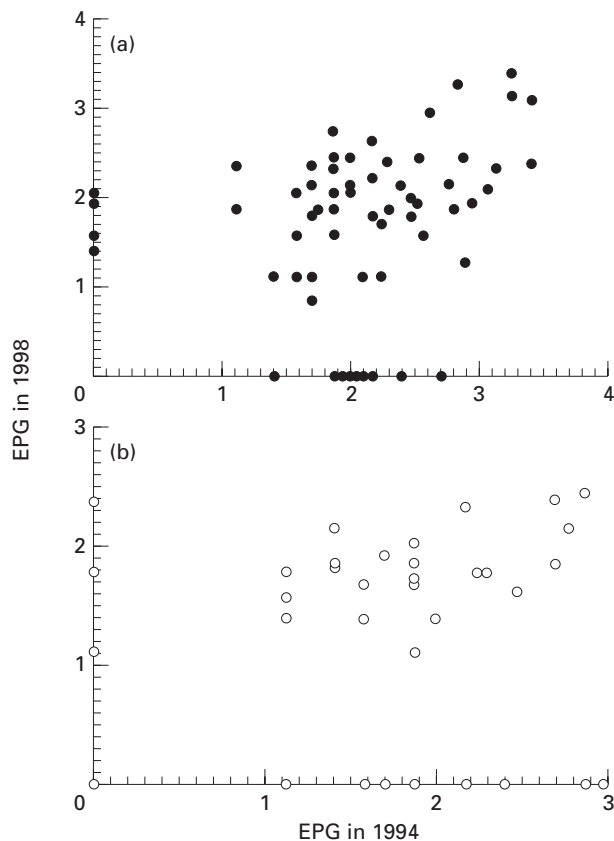


Figure 5 The relationship between EPGs in 1994 and 1998 for (a) male and (b) female subjects. All data are Log₁₀ ($x + 1$) transformed and $n = 134$

Table 2 Statistical analysis of factors (sex × age) affecting intensity of infections with *N. americanus* in 1998 through a 2-way ANOVA with negative binomial errors

was based only on subjects who had positive EPGs in both years ($r_s = 0.445$, $n = 73$, $P < 0.001$).

However, the above analysis did not take into account the sex and age effects described earlier. Therefore, to control for the contribution to variation in EPGs attributable to the effects of age and sex, we first carried out a 2-way ANOVA (Statgraphics version Plus 7) of age and sex on Log₁₀ ($x + 1$) transformed EPGs from both the 1994 and 1998 data-sets. Even on this limited subset of data, in both years there was a clear sex effect (main effect of sex in 1994, $F_{1,126} = 12.036$, $P = 0.0007$; 1998, $F_{1,126} = 4.365$, $P = 0.0387$) but only in 1998 was an age effect detected ($F_{3,126} = 5.046$, $P = 0.0025$). Correlational analysis of the residuals (Figure 6) gave $r = 0.502$ ($n = 134$, $P < 0.0001$), confirming that, with age, sex and possible interactions between these factors taken into account, there was a highly significant relationship between the abundance of infection carried by people in 1994 and 1998. Moreover, when analysis of the residuals was limited to each of the 4 age classes in turn, the correlation coefficients were significant in each case (7–10 years, $r = 0.544$, $n = 34$, $P = 0.0009$; 11–20 years, $r = 0.447$, $n = 31$, $P = 0.012$;

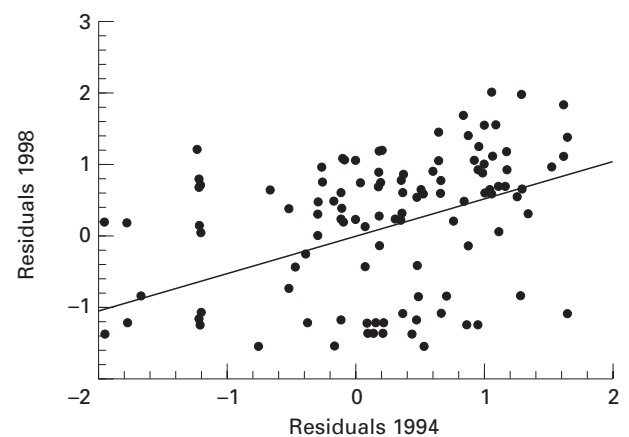


Figure 6 Correlation between the residuals from 2-way ANOVA of sex (2 levels) and age (4 levels) on LOG₁₀ (EPG + 1) in 1994 and 1998. A line of best fit is shown. For statistical analysis see text.

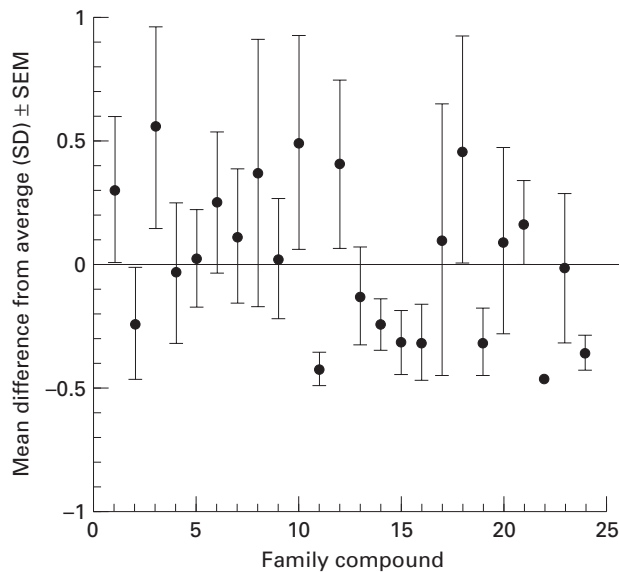


Figure 7 Evidence for clustering of hookworm infection in relation to family compounds. The data for each individual were standardized for host sex and age (i.e. were re-expressed in terms of the deviation in SD units from the mean for the relevant sex and age class). These data were then averaged for the members of each family compound. The horizontal line (zero) represents the average level of infection controlling for the effects of age and sex. Figures below this line represent family groups with lower than expected intensity of infection and those above with greater than expected intensity of infection. The number of subjects (total $n = 285$) in each family compound from 1 to 24 was as follows: 18, 22, 15, 12, 19, 6, 7, 8, 19, 11, 13, 16, 23, 11, 12, 17, 4, 5, 16, 3, 9, 11, 3, 5.

20–40 years, $r = 0.435$, $n = 30$, $P = 0.016$; 41–78 years, $r = 0.547$, $n = 39$, $P = 0.0003$, respectively).

Family compound-related clustering of infections

In 1998 the abundance of infection was examined in relation

to subjects living in the 24 family compounds comprising the village. Since different compounds comprised different numbers of adult males, females and children and since in 1998 there was a highly significant effect of age and sex on abundance of infection, we controlled via standardization for these factors (i.e. by re-expressing the data for each subject in terms of departure from the mean (in SD units) of the respective sex and age class and then averaging the data by family compound). These data are illustrated in Figure 7.

Analysis was by a 3-way ANOVA with normal errors as shown in Table 3. It can be seen that the interaction between sex and age, the main effects of age and sex were all significant as expected. However, taking all these sources of variation into consideration, there was a highly significant effect of family compound. Figure 7 shows that family compounds 11, 15, 16, 19, 22 and 24 had subjects with lower than expected mean EPGs. Moreover, they were clearly less predisposed to infection than those associated with family compounds 3, 10, 12 and 18 which, despite the variation, clearly showed greater predisposition to infection with hookworms. There was no significant relationship between the mean EPG of the subjects in each family compound (after adjustment for age and sex) and the number of individuals registered as living there.

Does the extent to which people rely on footwear affect infection with hookworms?

We obtained information on the extent of use of footwear from 260 subjects in the village and these data are illustrated for each of the age classes and by sex in Figure 8a. As expected, children rarely wore footwear but did so increasingly with age. There was then little change in the year groups covering the range from 20 to 60, but a slight increase followed in both sexes in the older members of the community. This age effect was highly significant, but there was no effect of sex on footwear score (2-way ANOVA in

Table 3 Statistical analysis of family compound-related clustering of infections in 1998 with *N.americanus*

Source of variation	Change in deviance	Degrees of freedom	Scale parameter	Scaled deviance*	<i>P</i>
Family	261.8	23	5.328	02.136	< 0.005
Sex	53.47	01	4.970	10.759	< 0.005 > <i>P</i> > 0.001
Age	68.85	07	4.914	02.002	< 0.05
Age × sex	94.10	07	4.778	02.813	< 0.01 > <i>P</i> > 0.005

Sex and age were fitted together with their interaction and the main effect of family in a three-way ANOVA with normal errors on $\log_{10}(x + 1)$ transformed data. The full model deviance was 1114.7 with a scale parameter of 4.531

*Scaled deviance = measure of contribution of factor specified under column labelled 'source of variation' to explain variation in the data, calculated by fitting ANOVA with normal errors through GLIM. It is distributed as *F*.

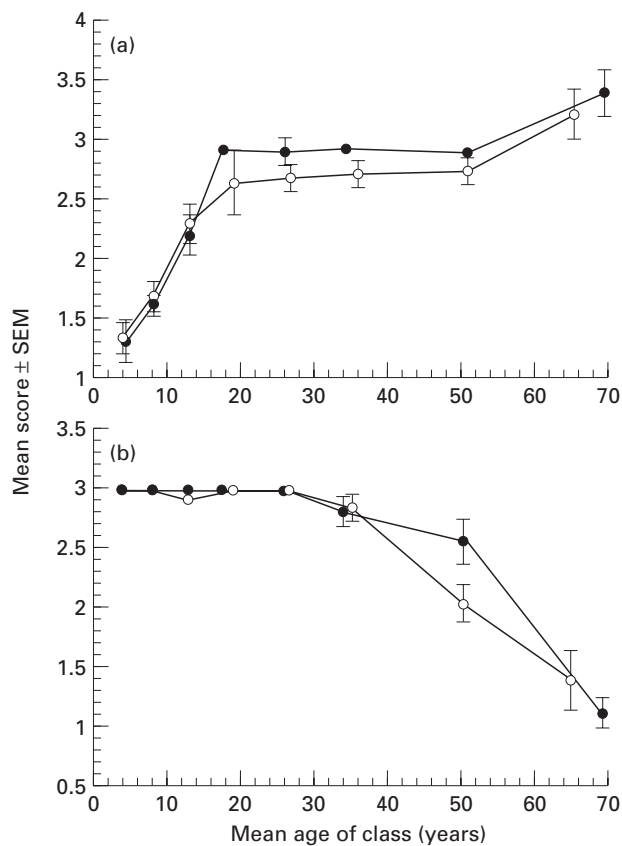


Figure 8 Changes with age in (a) use of footwear and (b) in vision. ● Males; ○ Females.

GLIM with normal errors, sex and age as factors on footwear score $F_{7,258} = 20.13$, $P < 0.001$). However, with both sex and age taken into account, there was no significant effect of footwear score on EPG (3-way ANOVA in GLIM with sex \times age + footwear score as factors on $\text{Log}_{10} [\text{EPG} + 1]$).

Does the quality of vision affect infection with hookworms?

Vision scores were uniformly 3 in all subjects (except 1 female in age class 3) until 30 years in both sexes (Figure 8b) and then deteriorated with further age. There was no main effect of sex on eyesight score, but there was a significant interaction between age and sex (2-way ANOVA with normal errors in GLIM with sex \times age on vision score, $F_{7,251} = 2.89$, $P < 0.01$) arising from the differences in vision score between the sexes in the age classes > 31 years, and a highly significant main effect of age ($F_{7,258} = 22.84$, $P < 0.001$).

The data in Figure 9(a) suggest that males with normal sight (Vision category 3) had higher EPGs than females, which is consistent with the general finding that males carried

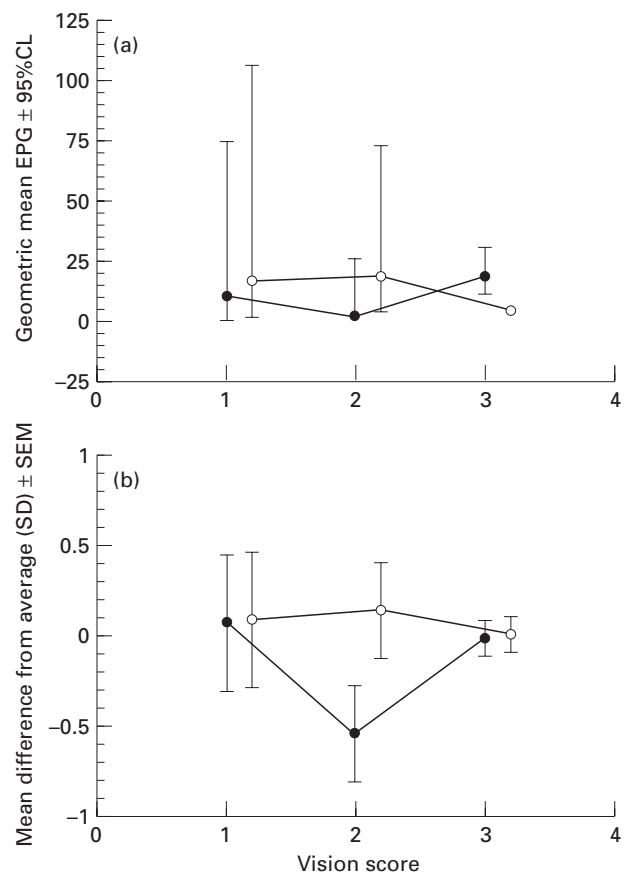


Figure 9 Relationship between faecal egg counts and vision. A, Data expressed as the geometric mean faecal egg counts. B, Data expressed as the mean difference, in SD units, from sex and age class average. See text for full details. ● Males; ○ Females. The number of male and female subjects in each vision score category (from 1 to 3) were as follows: 9, 6, 112, 9, 15, 109, respectively.

heavier infections than females. However, when the data for each vision score category were recalculated after standardization for age and sex effects (Figure 9b), it is evident that only males with vision score category 2 had lower than expected EPGs. Analysis by 3-way ANOVA (GLIM with normal errors and age \times sex \times eyesight score as factors, on $\text{Log}_{10} [x + 1]$ transformed EPG) confirmed that, as expected, the main effect of sex was significant ($F_{1,250} = 8.672$, $P < 0.005$) but there was also a significant interaction between sex and vision score ($F_{2,245} = 4.568$, $P < 0.025$) although no main effect of vision score on EPG.

Discussion

The most consistent finding from the data presented in this paper was the strong sex difference in prevalence and

abundance of infection. More male subjects were infected compared with females and overall they passed higher egg counts than females. This sex bias was observed in both surveys 3 years apart and was particularly prominent in age classes > 21 years. Our observations confirm and extend an earlier report of a pronounced sex bias in hookworm infections in Mali (Ecole Nationale de Médecine et de Pharmacie du Mali 1980). They are consistent with reports from other African (Bradley *et al.* 1992; Brooker *et al.* 1999) and Asian (Nawalinski *et al.* 1978) countries where males have been shown to carry heavier infections with hookworms than females but contrast with surveys where no sex-bias has been detected (Higgins *et al.* 1984; Bradley & Chandiwana 1990; Pritchard *et al.* 1990; Palmer & Bundy 1995) or where females were found to carry heavier infections (Haswell-Elkins *et al.* 1988; Needham *et al.* 1998). The possible mechanisms underlying such differences in the pattern of parasitic infections carried by the sexes have been reviewed in the past (Alexander & Stimson 1988; Bundy 1988; Brabin & Brabin 1992; Roberts *et al.* 1996), and although innate and immunological factors may contribute, the most important factor in the case of human GI nematodes is believed to be human behaviour and associated sex-dependent differences in exposure to infection. Clearly it is important to understand the basis of the sex bias because control strategies aimed at males may have beneficial consequences for the whole community if the bulk of the egg laying hookworm population resides in male hosts.

In villages in southern Mali, human faeces is occasionally combined with animal faeces in fertilizing agricultural plots, a practice which has been shown to be associated with higher abundance of hookworm infection in those who use fresh, as distinct from treated, human faeces or non faecal fertilisers in Vietnam (Humphries *et al.* 1997). There were some indications of indiscriminate deposition of faeces in humid places on the village periphery, which people frequently cross barefoot. However, significantly, human faecal material is also often incorporated into materials used to strengthen dwellings and it is predominantly men who are involved in these activities. Consistent with this, the younger age classes (< 15 years) showed little sex bias in FEC, but the difference arose in the next age groups with a pronounced rise in EPGs among males in the 16–20 years age group in 1998 and the 21–30 years class in 1994. However, these age classes were least well represented in our surveys as can be seen from the age profiles in Figure 1. Young adults and late adolescents often move away from their home villages in search of jobs in towns, or are the least compliant in participating in surveys because they are highly mobile and stay away from the village for long periods of time. Nevertheless, those who return to their village become increasingly involved in agricultural pursuits to support their new families. In consequence the sex

difference in hookworm infections is likely to have arisen primarily through greater exposure of males to hookworm larvae through activities after the rainy season. The rainy season ends in October in this part of Africa and both of our surveys were conducted soon afterwards (December 1994 and January 1998).

The age prevalence and age abundance profiles in our study were compiled using geometric means because these reflect more accurately the central tendency of values when overdispersed data are involved (Dash *et al.* 1988). Overall, but with the exception of the oldest age classes, the profiles were consistent with a monotonic or asymptotic age-abundance pattern for both males and females, as in many other studies with human hookworms (Behnke 1987). In the oldest male age class the age abundance curve dropped in both 1994 and 1998. This subset of the male population generally represented the village elders and those who were severely affected by river blindness, subjects who were for the most part village bound. Many had to be led around the village in the traditional manner with sticks held by younger members of the family. Thus we are confident that this reduction in the prevalence and intensity of infection in the oldest age class among the male population was primarily attributable to less exposure to hookworm larvae through reduced involvement in agricultural and house building pursuits.

Consistent with earlier findings (Schad & Anderson 1985; Haswell-Elkins *et al.* 1987, 1988; Bradley & Chandiwana 1990), our data provide strong support for the existence of predisposition to infection in the village; this was evident in both male and female subjects, but most convincing when age and sex effects were controlled for in the analysis. There appeared to be a subset of the population which remained relatively free of infection and another which carried infection and became more readily re-infected after chemotherapy. Moreover, this relationship held significance among each of the four age-restricted subsets which were examined in turn, although the highest correlation coefficients were obtained in the oldest and the youngest age classes.

Members of particular family compounds showed higher than average infections, as was evident in our analysis of family compound-related clustering of infections, in agreement with Forrester *et al.* (1988) and Chan *et al.* (1994), who reported familial clustering of *Ascaris lumbricoides* and *Trichuris trichiura* in Mexican and Malaysian households, respectively, but contrasting with Killewo *et al.* (1991) who failed to find evidence to support familial clustering of hookworm infections in Tanzania. The inhabitants of villages in this region of West Africa live in family groups with huts of all members grouped together in a family compound such that entrances lead into an open, mostly circular arena in

which cooking and other family based activities take place. Although we did not investigate these effects further, we believe that as with the sex difference, the clustering of infections in family compounds can be accounted for by behavioural and exposure-related explanations. There was evidence of specialization in the village with some families being more involved in building and others in agricultural activities, although we did not relate these quantitatively to any of the families involved.

One factor which has been known for many years to have a major influence on transmission of hookworms is the extent to which people use footwear (Smillie & Augustine 1925), and a recent study in Thailand has drawn attention to the use of footwear as the dominant factor in protection against hookworm infection (Chongsuvivatwong *et al.* 1996). Hookworms are acquired by infective third stage larvae which penetrate skin predominantly on the feet and often between the toes. We quantified use of footwear by asking directly those who were prepared to give us information about their use of footwear and grading this on a simple subjective scale of 1–4. Analysis of these data revealed, much as expected, that the use of footwear increased with age but our attempt to link predisposition to use of footwear was not successful. From interviews it was apparent that footwear was considered to be a valuable, prized asset and that people working in the fields removed their sandals. In the wet season soil sticks to sandals, making them uncomfortable and frustrating to wear when tilling soil, and risking damage. As a result of this practice, those who often wore shoes still became infected through bare skin. An approach based on quantifying shoe wear in relation to village activities by direct observation, as has been done so successfully in relation to water-contact and schistosomiasis (Bundy & Blumenthal 1990), may have yielded more relevant data, but was not feasible for us because of time constraints.

Onchocerciasis was endemic in the region until the recent Onchocerciasis Control Programme and the inhabitants of the study village were treated with ivermectin in the past. However, at the doses recommended for human use, ivermectin is without effect on *N. americanus*, and the hookworm burdens would not have been affected (Behnke *et al.* 1993; Richards *et al.* 1995). Nevertheless, there has been a great improvement in the prevalence of onchocerciasis and during our visits only the older members of the community still bore its legacy through persisting damage to their eyes. We examined the relationship between vision and hookworm infection because it has been suggested in the past that deteriorating eyesight among older inhabitants of villages, and hence reduced capacity to detect disturbed soil at defecation sites, may contribute to their heavier hookworm infections (Schad *et al.* 1983). In our study the age-abundance profiles revealed a drop in older males, but river

blindness among the middle-aged villagers may have contributed to their higher infections. Our assessment of vision was based on interview and a brief medical inspection. Analysis of these data revealed that vision was mostly satisfactory in subjects < 30 years of age, but deteriorated severely in older individuals. However, when age and sex differences were taken into account, the only significant effect on parasite burdens (as reflected in EPGs) came from an interaction with sex. The explanation for this lay in the lower than expected levels of infection in male subjects showing partial damage to their eyes and partial lack of vision. The individuals in this group ranged from 34 to 71 years of age, and it is likely that such persons would have been less active in agricultural pursuits and consequently less exposed to infection than others in the same age range. Those with severely handicapped vision (Vision score 1) ranged from 55 to 78 years in the case of men and 38–70 years in the case of women, but these individuals would have had vision scores typical for their age and sex, because impaired vision was very common in the older classes.

These two surveys provide the first quantitative, comprehensively analysed data on the distribution of hookworm infections among the inhabitants of a community in the Sahel. Although some of the patterns of infection which we detected were similar to those reported elsewhere, there were nevertheless some unique features which are worthy of further investigation.

Acknowledgements

We thank Prof. S. Bayo, Director of the INRSP in Bamako, for making this study possible. The contributions of the technicians of the Laboratory of Parasitology, INRSP (Bamako-Coura), the medical staff and the technical staff of the National Schistosomiasis Control Programme is acknowledged. We are grateful to the Medical Health Officer of Bougouni and the villagers for their help and hospitality. This study was supported by the V.I.I.R. (Flemish Inter University Council), Belgium and a travel grant to JMB from the Wellcome Trust.

References

- Alexander J & Stimson WH (1988) Sex hormones and the course of parasitic infections. *Parasitology Today* **4**, 189–193.
- Behnke JM (1987) Do hookworms elicit protective immunity in man? *Parasitology Today* **3**, 200–206.
- Behnke JM, Rose R & Garside P (1993) Sensitivity to ivermectin and pyrantel of *Ancylostoma ceylanicum* and *Necator americanus*. *International Journal for Parasitology* **23**, 945–952.
- Brabin L & Brabin BJ (1992) Parasitic infections in women and their consequences. *Advances in Parasitology* **31**, 1–81.

J. M. Behnke *et al.* **Epidemiology of hookworms in Mali**

- Bradley M & Chandiwana SK (1990) Age-dependency in predisposition to hookworm infection in Burma valley area of Zimbabwe. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 826–828.
- Bradley M, Chandiwana SK, Bundy DAP & Medley GF (1992) The epidemiology and population biology of *Necator americanus* infection in a rural community in Zimbabwe. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 73–76.
- Brooker S, Peshu N, Warn PA *et al.* (1999) The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 240–246.
- Bundy DAP (1988) Gender-dependent patterns of infection and disease. *Parasitology Today* **4**, 186–189.
- Bundy DAP & Blumenthal UJ (1990) Human behaviour and the epidemiology of helminth infections: the role of behaviour in exposure to infection. In: *Parasitism and Host Behaviour* (eds CJBarnard & JMBehnke), Taylor & Francis, London.
- Bundy DAP (1997) This wormy world – then and now. *Parasitology Today* **13**, 407–408.
- Chan MS (1997) The global burden of intestinal nematode infections – fifty years on. *Parasitology Today* **12**, 438–443.
- Chan L, Bundy DAP & Kan SP (1994) Aggregation and predisposition to *Ascaris lumbricoides* and *Trichuris trichiura* at the familial level. *Transactions of the Royal Society for Tropical Medicine and Hygiene* **88**, 46–48.
- Chongsuvivatwong V, Pas-Ong S, McNeil D, Geater A & Duerawee M (1996) Predictors for the risk of hookworm infection: experience from endemic villages in southern Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 630–633.
- Crawley MT (1993) *GLIM for Ecologists*. Blackwell Scientific Press, Oxford.
- Dash KM, Hall E & Barger IA (1988) The role of arithmetic and geometric mean worm egg counts in faecal egg count reduction tests and in monitoring strategic drenching programs in sheep. *Australian Veterinary Journal* **65**, 66–68.
- De Clercq D, Sacko M, Behnke JM, Traore M & Vercruysse J (1995) *Schistosoma* spp. and geohelminths in Mali, West Africa. *Annales de la Societe Belge de Medecine Tropicale* **75**, 191–199.
- De Clercq D, Sacko M, Behnke J, Gilbert F, Dorny P & Vercruysse J (1997) Failure of mebendazole in treatment of human hookworm infections in the Southern Region of Mali. *American Journal of Tropical Medicine and Hygiene* **57**, 25–30.
- Ecole Nationale de Médecine et de Pharmacie du Mali (1980) *Etat de santé des populations riveraines avant la mise en eau du Barrage de Selingue*. UNDP Programme (Project MLI 77/006) United Nations, New York, Vol. 1, Chapter 5.
- Elliott JM (1977) *Some methods for the statistical analysis of samples of benthic invertebrates*. Freshwater Biological Association, Cumbria, UK.
- Forrester JE, Scott ME, Bundy DA & Golden MHN (1988) Clustering of *Ascaris lumbricoides* and *Trichuris trichiura* infections within households. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 282–288.
- Haswell-Elkins MR, Elkins DB & Anderson RM (1987) Evidence for predisposition in humans to infection with *Ascaris*, hookworm, *Enterobius* and *Trichuris* in a South Indian fishing community. *Parasitology* **95**, 323–337.
- Haswell-Elkins MR, Elkins DB, Manjula K, Michael E & Anderson RM (1988) An investigation of hookworm infection and reinfection following mass anthelmintic treatment in the South Indian fishing community of Vairavankuppam. *Parasitology* **96**, 565–577.
- Higgins DA, Jenkins DJ, Kurniawan L, Purnomo, Harun S & Sundari Juwono S (1984) Human intestinal parasitism in three areas of Indonesia: a survey. *Annals of Tropical Medicine and Parasitology* **78**, 637–648.
- Humphries DL, Stephenson LS, Pearce EJ, The PH, Dan HT & Khanh LT (1997) The use of human faeces for fertilizer is associated with increased intensity of hookworm infection in Vietnamese women. *Transactions of the Royal Society for Tropical Medicine and Hygiene* **91**, 518–520.
- Katz N, Chaves A & Pellegrino J (1972) A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Revista Do Instituto de Medicina Tropical de Sao Paulo* **14**, 397–400.
- Kilama WL (1990) Hookworm infection and disease in Africa and Middle East. In: *Hookworm Disease. Current Status and New Directions* (eds GA Schad & KS Warren), Taylor & Francis, London, pp. 17–32.
- Killewo JZJ, Cairncross S, Smet JEM, Maikwano LF & van Asten H (1991) Patterns of hookworm and *Ascaris* infection in Dar es Salaam. *Acta Tropica* **48**, 247–249.
- Margolis L, Esch GW, Holmes JC, Kuris AM & Schad GA (1982) The use of ecological terms in parasitology (report of an *ad hoc* committee of The American Society of Parasitologists). *Journal of Parasitology* **68**, 131–133.
- Miller TA (1979) Hookworm infection in man. *Advances in Parasitology* **17**, 315–384.
- Nawalinski T, Schad GA & Chowdhury AB (1978) Population biology of hookworms in children in rural West Bengal. I. General parasitological observations. *American Journal of Tropical Medicine and Hygiene* **27**, 1152–1161.
- Needham C, Thi Kim H, Viet Hoa N *et al.* (1998) Epidemiology of soil-transmitted nematode infections in Ha Nam Province, Vietnam. *Tropical Medicine and International Health* **3**, 904–912.
- Palmer DR & Bundy DAP (1995) Epidemiology of human hookworm and *Ascaris lumbricoides* infestations in rural Gambia. *East Africa Medical Journal* **72**, 527–294.
- Poulin R (1993) The disparity between observed and uniform distributions: a new look at parasite aggregation. *International Journal for Parasitology* **23**, 937–944.
- Pritchard DI, Quinnell RJ, Slater AFG *et al.* (1990) The epidemiology and immunology of *Necator americanus* infection in a community in Papua New Guinea: humoral responses to parasite excretory-secretory and cuticular collagen antigens. *Parasitology* **100**, 317–326.
- Richards JC, Behnke JM & Duce IR (1995) *In vitro* studies on the relative sensitivity to ivermectin of *Necator americanus* and *Ancylostoma ceylanicum*. *International Journal for Parasitology* **25**, 1185–1191.
- Roberts CW, Satoskar A & Alexander J (1996) Sex steroids, pregnancy-associated hormones and immunity to parasitic infection. *Parasitology Today* **12**, 382–388.

J. M. Behnke *et al.* **Epidemiology of hookworms in Mali**

- Schad GA & Anderson RM (1985) Predisposition to hookworm infection in humans. *Nature* **228**, 1537–1539.
- Schad GA, Nawalinski TA & Kochar V (1983) Human Ecology and the distribution and abundance of hookworm populations. In: *Human Ecology and Infectious Diseases* (eds. N Croll & J Cross), Academic Press, London, pp. 187–273.
- Schad GA & Warren KS, eds. (1990) *Hookworm Disease. Current Status and New Directions*. Taylor & Francis, London.
- Smillie WG & Augustine DL (1925) Intensity of hookworm infestation in Alabama. *Journal of the American Medical Association* **85**, 1958–1963.
- Udonsi JK, Nwosu ABC & Anya AO (1980) *Necator americanus*: population structure, distribution, and fluctuations in population densities of infective larvae in contaminated farmlands. *Zeitschrift für Parasitenkunde* **63**, 251–259.
- WHO (1963) *CCTA/WHO African conference on ancylostomiasis, Brazzaville 22–29 August 1961. Technical Report Series 225*.
- Ye XP, Wu ZX & Sun FH (1994) The population biology and control of *Necator americanus* in a village community in south-eastern China. *Annals of Tropical Medicine and Parasitology* **88**, 635–643.