Epidemiology and immunology of *Necator americanus* infection in a community in Papua New Guinea: humoral responses to excretory–secretory and cuticular collagen antigens

D. I. PRITCHARD, R. J. QUINNELL*, A. F. G. SLATER†, P. G. McKEAN, D. D. S. DALE*, A. RAIKO‡ *and* A. E. KEYMER*

Department of Zoology, University Park, Nottingham NG7 2RD

(*Accepted 10 August 1989*)

SUMMARY

Baseline data from an immuno-epidemiological study of hookworm infection in a rural village in Madang Province, Papua New Guinea are reported. *Necator americanus* was found to be the commonest helminth infection, with a prevalence of near 100% and intensity of 40 worms per host in adults. *Enterobius vermicularis*, *Ascaris lumbricoides* and *Trichuris trichiura* were also present, at prevalences of 53, 10 and 3% respectively; *Ancylostoma duodenale* was absent. The frequency distribution of *N. americanus* was highly over-dispersed, and was well described by a negative binomial distribution with aggregation parameter, k, of 0·370. Intensity of infection was significantly related to host age, but did not differ between the sexes. Haemoglobin levels and haematocrit values were indicative of anaemia in the community, but were unrelated to hookworm infection. Levels of antibodies (IgG, IgA and IgM combined) against adult *Necator* cuticular collagen and excretory–secretory (ES) products were determined. Serum concentrations of the two types of antibody were significantly correlated with each other. Significant positive correlations were found between anti-ES antibody levels and hookworm egg production, and between anti-collagen antibody levels and host age. It is suggested that the level of anti-collagen antibodies may reflect cumulative exposure to infection, whereas levels of anti-ES antibodies may be more dependent on current worm burden. No evidence was found to suggest that either antibody response is important in regulating parasite population growth. Similarly, the presence of a positive correlation between eosinophil concentration and infection intensity in adults indicates that eosinophilia reflects, rather than determines, the host's worm burden.

Key words: hookworm, *Necator americanus*, Papua New Guinea, epidemiology, antibody response, excretory–secretory antigens, collagen

INTRODUCTION

Hookworms (*Necator americanus* and *Ancylostoma duodenale*) are among the most prevalent of all human infections. The distribution of these parasites extends throughout the tropics and subtropics, where an estimated 1000 million people are infected (Warren, 1988). A person living in an endemic area is likely to be infected with hookworm during childhood, and to be repeatedly reinfected for the rest of his or her life. The pathology associated with hookworm infection is not simply related to the presence of the parasite, but rather is dependent upon the number of worms harboured. Two factors determine the intensity of infection: the degree of exposure to infective larvae and the survival of the parasites within the host.

* Present address: Department of Zoology, South Parks Road, Oxford OX1 3PS.
† Present address: Laboratory of Medical Biochemistry, The Rockefeller University, 1230 York Avenue, New York 10021–6399
‡ Present address: Institute for Medical Research, PO Box 378, Madang, Papua New Guinea.

Immune responses could act to decrease the survival of hookworm parasites, thereby lowering infection intensity and reducing pathology. The human host is known to mount a variety of immunological responses to hookworm infection. For example, volunteers infected with *N. americanus* produce antibodies against both larval and adult worms (Ball & Bartlett, 1969; Ogilvie *et al.* 1978), and a number of antigens are recognized by a range of sera from infected patients (Carr & Pritchard, 1986, 1987; Pritchard *et al.* 1986). Cellular responses, including a pronounced eosinophilia, are also associated with hookworm infection (Grove, Burston & Forbes, 1974; Taylor & Turton, 1976; White, Maxwell & Gallin, 1986; Maxwell *et al.* 1987).

At present, the importance of acquired immunity in the epidemiology of hookworm infection is unknown (reviewed by Anderson, 1986; Behnke, 1987). Even relatively simple questions, such as the extent to which a person's past exposure to infection or current worm burden correlates with antibody levels, and the changes in the nature of the antibody response with age, remain unanswered.

In this paper we report the study design and

baseline data from an immuno-epidemiological study of hookworm infection in a rural village in Madang Province, Papua New Guinea. This area was chosen because of the extensive data on the epidemiology of *N. americanus* and other intestinal infections there (Vines, 1970; Kelly, 1974; Hornabrook, Kelly & McMillan, 1975; Jones, 1976; Shield *et al.* 1984). *N. americanus* is reported to exist at a prevalence of 80–100%; other intestinal helminths (*Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis* and *Enterobius vermicularis*) are present, but their distribution is patchy. *Ancylostoma duodenale* is reputedly absent. The antibody responses to two antigen preparations were studied: excretory–secretory antigens and cuticular collagen. Collagen antigens are likely to be exposed during moulting or after worm damage, and the response to such hidden epitopes may be of special interest (Pritchard, McKean & Rogan, 1988*a*,*b*).

MATERIALS AND METHODS

Study design

Fieldwork was carried out in Kebasob village on Karkar Island, Papua New Guinea, with the support of the Madang Provincial Government, Papua New Guinea Institute of Medical Research and the Christensen Research Institute. Karkar (Dampier) Island (146° E, 4° S) lies 50–60 km NW of Madang off the north coast of New Guinea. It is a volcanic island approximately 80 km in circumference, with a maximum elevation of 1849 m. The majority of the population of 16800 (Hornabrook *et al.* 1975) resides on a coastal strip 4–8 km wide. Vegetation in this coastal strip is largely coconut and cocoa plantations and native gardens; the mountain slopes are covered in rain forest. Average temperature is around 28 °C, and rainfall is approximately 355 cm per annum. Kebasob is a village of approximately 750 people, mostly subsistence farmers and plantation workers; some cocoa and copra are grown as cash crops. Water is available from standpipes, piped from a local stream; the village has no electricity. Medical services are provided by the Gaubin Mission Hospital, 3 h walk or 1 h drive away.

Fieldwork was carried out in July–August 1988 and was organized as follows. After an introductory meeting with the village elders, the purpose of the study was explained in Pidgin to a general gathering of the villagers, when questions about the study were invited. Having obtained the consent of the villagers, 200 people occupying five distinct areas of the village were selected to take part. Each area was assigned a day for 'laboratory' visits and, on the day preceding the visit, the village councillor visited the head of each household to encourage cooperation. All members of each household, excepting children under 3 years old, were included in the study. The age and sex distribution of the study group is shown in Fig. 1. Percentage weight for height ratios, assessed according to the methods of Jelliffe (1966), indicated that the population was generally well nourished; 5 out of 172 people (2·9%) had a weight for height ratio less than 80% of standard, a level which indicates marked undernutrition (Waterlow, 1976).

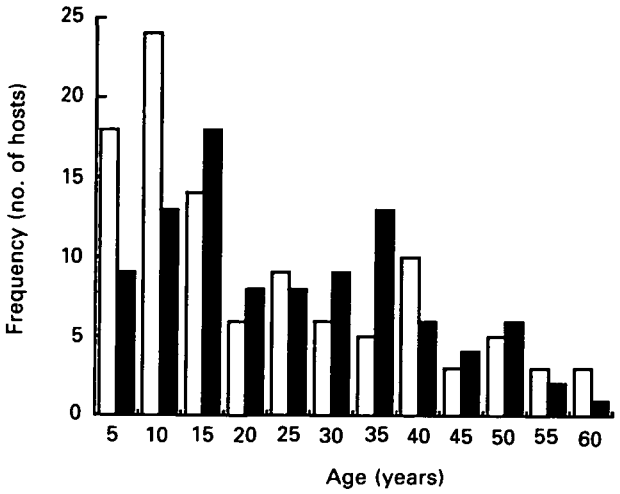

Fig. 1. The age and sex distribution of the study population (n = 206). □, Males: ■, females.

At 6 pm, when families had returned from their work, each subject was given a 1 l sealable plastic bucket, clearly labelled with their name and house number. Subjects were asked to provide a small faecal sample, and to attend at a temporary field laboratory the following morning. The next morning faecal samples were collected and preserved in 10% formalin, and a fingerprick blood sample was taken from each subject.

Having established the prevalence of infection by faecal analysis, each subject was treated with the anthelminthic pyrantel pamoate ('Combantrin', Pfizer Ltd, Sandwich, Kent), administered as a single dose of 10 mg/kg. No side-effects were reported. Subjects were then asked to collect all faeces for 48 h post-treatment. Faecal collections were obtained for 72 h from 19 individuals. A venous blood sample (4 ml) was taken during this period. Of the 202 people who began participation, 123 provided full post-treatment faecal samples. Between 6 and 12 days after chemotherapy, a further faecal sample was obtained from 25 individuals to assess treatment efficacy. Finally, the anthelminthic treatment was offered to all villagers who had not been included in the study group.

Parasitology and haematology

Pre- and post-treatment faecal samples of known weight (1–2 g) were preserved in 10% (v/v) formalin. Samples were processed according to a modified formalin-ether concentration technique (Hall, 1981). One drop of sediment was examined and eggs per

gram (epg) values calculated; if no eggs were found in the first drop, a further two drops were examined.

After anthelminthic treatment, faeces were washed through two grades of sieve (1 mm and 300 μm) with unlimited water and the sediment preserved in 10% formalin. Worms were recovered from the sediment after careful searching by eye, sexed, and preserved in 10% formalin. Hookworms were identified to species by microscopic examination after clearing overnight in lactophenol.

Haemoglobin concentration (fingerprick samples) was assessed using a portable haemoglobinometer, which was recharged and calibrated each day prior to use. Haematocrit was measured by a micro-haematocrit method, using heparinized capillary tubes. Thin smears were prepared and immediately fixed in methanol for differential white cell counts.

At the time of anthelminthic treatment, 4 ml of venous blood were collected into EDTA-coated tubes. A small aliquot was transferred into a heparinized tube for determination of total leucocyte count. The remainder of the sample was centrifuged and the plasma recovered. These procedures were carried out in the field immediately after blood collection, and plasma was frozen at the Gaubin Mission Hospital within 2 h of recovery. Samples were then air-freighted on dry ice to the UK; they were still frozen on arrival at Nottingham.

Immunology

Adult excretory–secretory (ES) products were prepared according the methods of Carr & Pritchard (1987). The method for cuticular collagen preparation was that used by Pritchard *et al.* (1988*b*). Briefly, cuticular collagen was prepared by extracting whole worms in a sequence of steps involving firstly sodium dodecyl sulphate (SDS) and then 2-β mercaptoethanol (Pritchard *et al.* 1988*b*). The resultant collagen preparation resolved with protein bands of M_r 55000 and 110000 kDa on SDS–polyacrylamide gel electrophoresis. Both bands were completely susceptible to digestion by bacterial collagenase. A 50 μl quantity of adult ES products or cuticular collagen preparation (each diluted to a concentration of 2·5 μg/ml with 0·01 M carbonate–bicarbonate buffer, pH 9·6) was added to each well of a 96-well ELISA plate and incubated at 4 °C overnight. After washing 5 times with 0·05% PBS Tween, 100 μl of a 5% skimmed milk solution were added to each well and incubated for 1 h at room temperature. The skimmed milk was removed and the plate washed 3 times with PBS Tween before 50 μl of the test serum were added to an individual well. Triplicates of 1:250 serum dilutions were used with the ES products; a single 1:3200 dilution with the collagen preparation. The concentration of antigen and dilutions of sera used had been determined by checkerboard titrations, on a subset of sera, to give greatest contrast between control and test sera. The solubility of the collagen preparation in the assay buffer was confirmed using a rabbit antiserum to *N. americanus* cuticular collagen (McKean, 1989).

Serum samples were incubated on the plate for 2 h at room temperature before being removed and the plate washed 5 times with PBS Tween. Fifty microlitres of alkaline phosphatase-conjugated goat anti-human polyvalent immunoglobulin (α, γ and μ-chain specific) serum (1:350) were added to each well. After 2 h at room temperature, the plate was washed 5 times with PBS Tween and 100 μl of substrate solution (*p*-nitrophenyl phosphate) added to each well. Absorbance at 410 nm was determined using an automated ELISA plate reader after 15, 30 and 60 min. Results are expressed as optical density (O.D.).

Statistical analysis

Data on parasite intensity, antibody levels, eosinophil counts and age showed highly skewed distributions, so were logarithmically transformed before analysis. Sex was coded as 1-male, 2-female. Age-corrected data were calculated as the residual from the linear regression of the variable on age. Two variables, anti-ES antibody level and pinworm burden, had a convex relationship with age; in these cases age-corrected values were taken from regression lines fitted separately to those ages before the peak and those after the peak.

RESULTS

Parasitology

The prevalence of gastro-intestinal helminth infection, as assessed by egg counts and worm recovery, is shown in Table 1. The prevalence of hookworm infection was similar in villagers of both sexes, 91% in males and 87% in females. Prevalence increased rapidly with age, reaching 100% in the 16- to 20-year old age class (Fig. 2). This pattern was similar in both sexes, although prevalence in the 3 to 6-year-olds was twice as high in males (41%) as females (20%).

A total of 3116 hookworms were recovered from 123 hosts, a mean worm burden of 25·3 worms/host. All the worms identified (34 individuals from a number of hosts) were *N. americanus*. Hookworm burden increased significantly with host age ($r = 0{\cdot}514$, $n = 121$, $P < 0{\cdot}001$; Fig. 3), reaching a plateau of around 40 worms/host in adults. Mean intensities were slightly, but not significantly, lower in females than males (ANOVA, $F = 0{\cdot}181$, D.F. = 1,121, N.S.).

The sex ratio of the hookworms was not significantly different from unity, with totals of 1551 male and 1565 female worms recovered ($\chi^2 = 0{\cdot}063$,

Table 1. Prevalence of gastro-intestinal helminth infection in Kebasob. Prevalence was assessed either by worm recovery after treatment or by faecal egg count

	Prevalence (%) of infection as assessed by	
	Worm recovery	Egg production
Necator americanus	81	90
Enterobius vermicularis	53	—
Ascaris lumbricoides	10	8
Trichuris trichiura	1	3

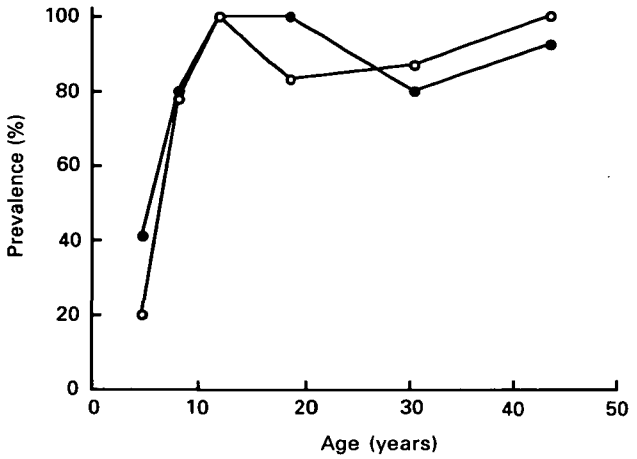

Fig. 2. The relationship between hookworm prevalence, assessed by faecal egg count, and host age. ●, Males; ○, females.

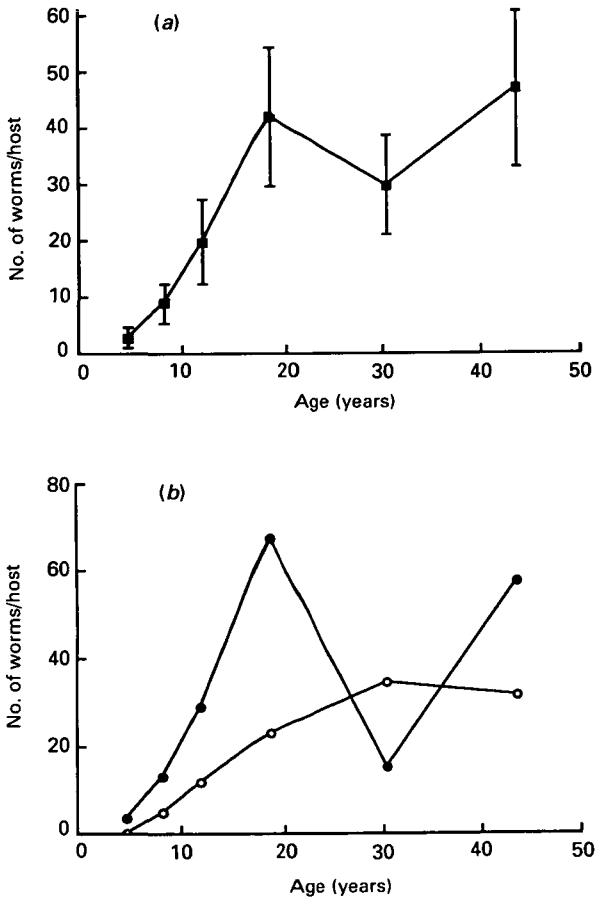

Fig. 3. The relationship between hookworm burden and host age. (a) All hosts (mean±S.E.); (*b*) stratified by host sex (●, males; ○, females).

D.F. = 1, N.S.). However, when only lightly infected hosts (1–3 worms) were considered, there was a significant excess of females, 40 females and only 15 males being recovered ($\chi^2 = 11{\cdot}4$, D.F. = 1, $P < 0{\cdot}001$).

Hookworm burdens varied from 0 to 263 worms/host. The frequency distribution of hookworm infection showed the expected over-dispersed pattern (Fig. 4), with the most heavily infected 10% of the host population holding 52% of the parasite population. The frequency distribution was well described by a negative binomial distribution with aggregation parameter, k, of 0·370 (Table 2). Analysis within age classes showed that the lowest value of k, i.e. the greatest over-dispersion, was recorded in the 3 to 6-years-olds; in the older age classes k values were variable but higher. Male hosts showed a slightly more over-dispersed distribution than females (Table 2).

The mean egg count was 300 epg (range 0–3918). There was a highly significant correlation between epg and worm burden ($r = 0{\cdot}649$, $n = 122$, $P < 0{\cdot}001$). *Per capita* egg production showed a significant negative correlation with female worm burden ($r = -0{\cdot}208$, $n = 96$, $P = 0{\cdot}042$), while the correlation with total worm burden was almost significant ($r = -0{\cdot}198$, $n = 96$, $P = 0{\cdot}053$). When female-only infections were excluded both correlations were significant (females: $r = -0{\cdot}239$, $n = 83$, $P = 0{\cdot}030$; total worms: $r = -0{\cdot}235$, $n = 83$, $P = 0{\cdot}033$). There were no significant correlations between fecundity and either host age or sex (age: $r = 0{\cdot}0034$, $n = 94$, N.S.; sex: $r = -0{\cdot}14$, $n = 95$, N.S.).

Post-treatment egg counts were carried out on a subgroup of 25 individuals, of whom 20 were initially egg-positive, 6–12 days after treatment. Eight people

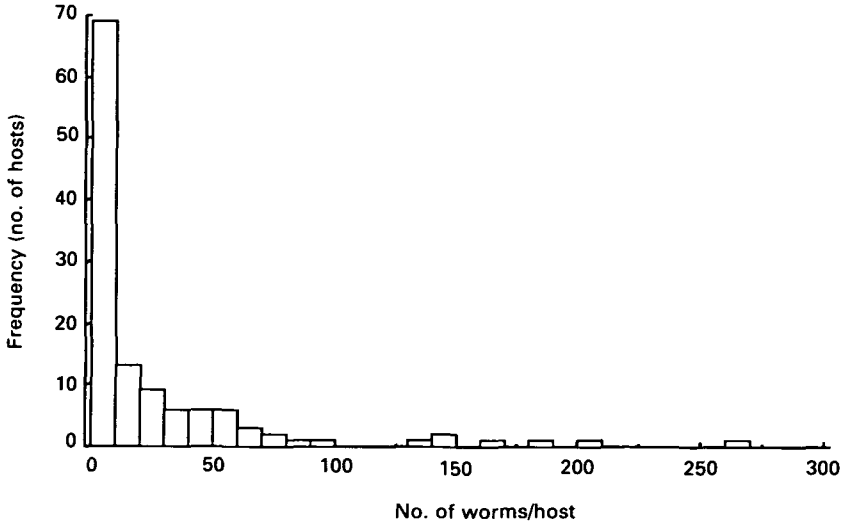

Fig. 4. The frequency distribution of hookworm burdens within the study population ($n = 123$).

Table 2. Frequency distribution of hookworm numbers per person: the aggregation parameter (k) and the goodness of fit of the negative binomial distribution (χ^2) within age groups and sexes

Age class (year)	n	k value	χ^2
3–6	22	0·147	—*
7–9	19	0·452	—
10–13	17	0·889	—
14–23	21	0·542	—
24–35	20	0·429	—
36+	22	0·680	—
Total	123	0·370	7·71†
Males	63	0·332	1.38†
Females	59	0·425	8·91†

* Insufficient degrees of freedom for χ^2 test.
† $P > 0·05$ (i.e. no significant departure from negative binomial distribution).

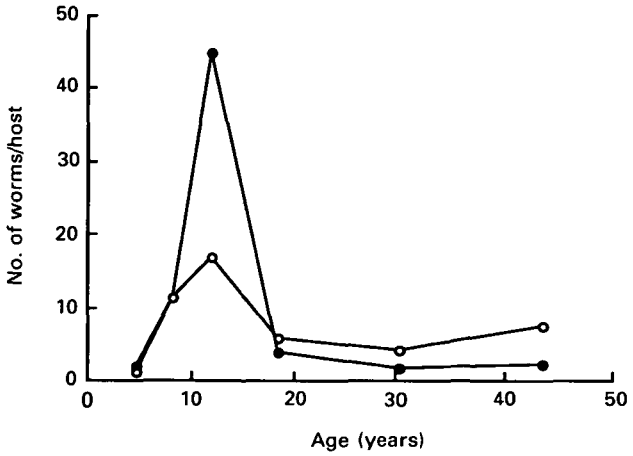

Fig. 5. The relationship between pinworm burden and host age. ●, Males; ○, females.

were still excreting hookworm eggs, giving a cure rate of 60%. The mean (±S.E.) egg count was reduced from 449±156 to 26±12 epg, a reduction of 94%. Faecal collections were made for 72 h from 19 individuals, 10 of whom proved to be worm-positive. These 10 people expelled a mean of 82% of their worms during the first 48 h.

Mean pinworm burden was 8·4 worms/host. Mean burden was slightly higher in males than females (9·1 versus 7·7 worms/host), but this difference was not significant (ANOVA, $F = 0·009$, D.F. = 1,121, N.S.). The age–intensity profile for pinworm shows a marked peak in the 10 to 13-year-olds, especially in males (Fig. 5). Pinworms were highly aggregated in the host population ($k = 0·173$), although the frequency distribution differed significantly from a negative binomial distribution ($\chi^2 = 32·8$, D.F. = 8, $P < 0·001$). Analysis of the relationship between hookworm burden and pinworm burden (both age-corrected) revealed a significant positive correlation ($r = 0·32$, $n = 122$, $P < 0·001$).

Mean *Ascaris* burden was 0·12 worms/host, with a maximum burden of 2 worms. *Ascaris* was found only in the younger age classes, the oldest infected host being 26 years old. There was no correlation between *Ascaris* burden and sex of host, or between *Ascaris* burden and hookworm burden.

Serology

Levels of antibodies against adult ES products and collagen in the different host age classes are shown in Fig. 6. Levels of both antibodies increased with age in children, and anti-collagen antibody levels continued to increase in adults. Age-corrected levels of the two antibodies were significantly correlated ($r = 0·26$, $P < 0·01$).

Correlations between antibody levels and hook-

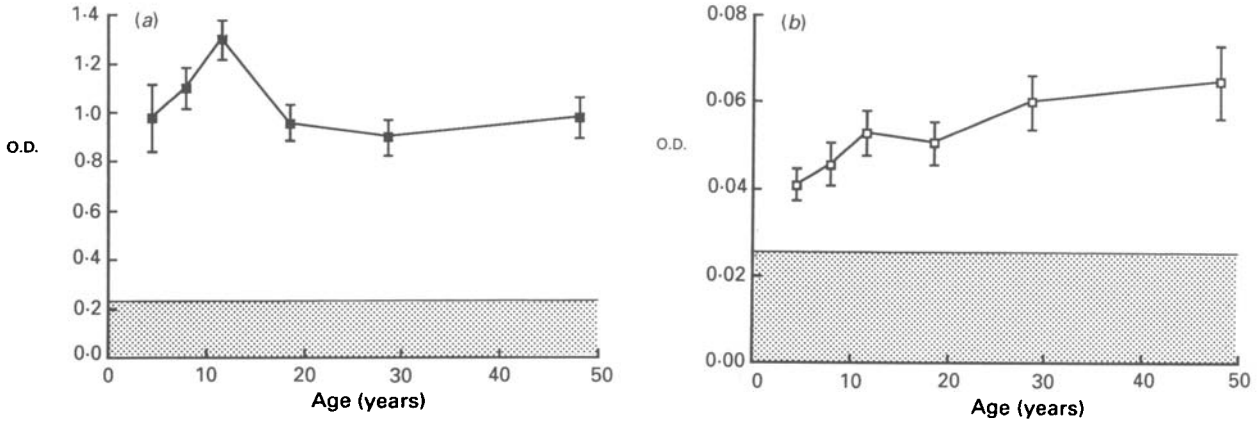

Fig. 6. Anti-hookworm antibody levels in different host age classes (mean ± S.E.). (*a*) Anti-ES antibodies; (*b*) anti-collagen antibodies. The shaded area indicates background (normal human serum) absorbance.

Table 3. Correlation coefficients between anti-hookworm antibody levels and hookworm intensity or host age

(Correlations between antibody level and worm burden or faecal egg count (epg) were calculated between age-corrected data. Correlations between antibody level and host age were performed after correction for epg count.)

Antibody	Age class	Hookworm burden	Epg	Age
Anti-ES	All	0·17	0·32**	−0·18
Anti-collagen	All	0·02	0·13	0·18*
Anti-ES	< 20 years	0·13	0·32*	0·10
Anti-collagen	< 20 years	0·23	0·27**	0·05
Anti-ES	> 20 years	0·20	0·30*	0·04
Anti-collagen	> 20 years	−0·13	−0·01	0·16

* $P < 0·05$; ** $P < 0·01$.

worm intensity (both age-corrected) and between antibody levels and host age (both corrected for epg count) are given in Table 3. The level of anti-ES antibodies showed a significant positive correlation with worm egg production; the correlation with worm burden was also positive, but was weaker and not quite significant ($P < 0·1$). In contrast, anti-collagen antibody levels were significantly positively correlated with host age, but not with infection intensity.

Examination of the hookworm age-intensity profile (Fig. 3) shows that intensity increased with age in hosts up to 20 years old and then reached a plateau. The correlation analysis was thus repeated for these two age classes. The results show that, in the under 20-year-old age class, levels of both antibodies were positively correlated with worm egg production (Table 3); the rise in antibody levels in children is due to increasing infection intensity, rather than being a purely age-related phenomenon. In adults, levels of anti-ES antibodies were still positively correlated with egg production, while levels of anti-collagen antibodies were unrelated to either measure of infection intensity.

Haematology

Correlations between eosinophil levels (no. of eosinophils/μl blood), hookworm intensity, age and antibody levels are shown in Table 4. In adults there was a significant positive correlation between eosinophil levels and hookworm intensity, although there was no correlation in children. Eosinophil levels were generally not related to antibody levels, although there was a significant positive correlation with anti-ES antibody levels in children (Table 4).

Mean haemoglobin and haematocrit levels, grouped according to age and sex, are given in Table 5. Using the WHO (1975) standards for anaemia, the percentage of the population classified as anaemic was 19%; this proportion was similar in males and females. The relationships between haemoglobin levels, hookworm burden and host sex (all age-corrected) were analysed by the calculation of partial correlation coefficients; only the correlation with host sex was significant (hookworm burden: $r = 0·11$, N.S.: host sex: $r = -0·22$, $P < 0·05$; $n = 121$). Comparison of worm burdens in anaemic and normal hosts revealed no significant differences.

Table 4. Correlation coefficients between eosinophil levels and hookworm intensity, host age or anti-hookworm antibody levels

Age class	Hookworm burden	Epg	Age	Antibody level: Anti-ES	Antibody level: Anti-collagen
All	0·16	0·13	0·01	0·09	−0·07
< 20 years	−0·06	0·04	−0·09	0·27*	−0·07
> 20 years	0·33*	0·21*	0·07	−0·07	−0·07

* $P < 0·05$.

Table 5. Haemoglobin levels and haematocrits within age and sex classes

Sex	Age class (year)	*n*	Haemoglobin (g/dl) Mean ± S.E.	Haemoglobin (g/dl) Range	Haematocrit (%) Mean ± S.E.	Haematocrit (%) Range
Male	3–6	26	12·5 ± 0·36	8·2–16·0	40 ± 1·0	25–47
Male	7–14	29	13·0 ± 0·29	10·0–16·4	42 ± 1·1	32–52
Male	15+	51	14·5 ± 0·21	9·8–17·7	47 ± 0·8	28–65
Female	3–6	8	12·7 ± 0·59	10·4–14·8	41 ± 1·6	34–46
Female	7–14	30	13·4 ± 0·23	11·0–15·7	43 ± 0·8	34–50
Female	15+	57	13·2 ± 0·21	10·0–17·6	42 ± 0·7	28–53

DISCUSSION

Hookworms are undoubtedly immunogenic, as illustrated by the observed production of antibodies in response to both natural and artificial infection with *N. americanus* (Ball & Bartlett, 1969; Ogilvie *et al.* 1978; Carr & Pritchard, 1986; Pritchard *et al.* 1986). The role of acquired immunity in the epidemiology of hookworm infection, however, remains a matter for conjecture (reviewed by Behnke, 1987) in the absence of data from integrated immuno-epidemiological studies. Such studies have been carried out on schistosomes (reviewed by Hagan, 1987) and malaria (Marsh *et al.* 1989); the present paper describes initial results from the first study of this kind on nematode infection. The data derive from a horizontal survey and allow relationships between various epidemiological, haematological and immunological parameters to be assessed in a community in which hookworm infection is endemic. Future longitudinal studies will assess the relationship between the immune response and reinfection.

The population dynamics of hookworm in Kebasob appear typical of those reported for hookworm infections elsewhere (Anderson, 1986). However, very few studies of hookworm have counted expelled worms (Haswell-Elkins *et al.* 1988), and modelling of hookworm epidemiology has by necessity relied on inferences about worm burdens from egg counts (Anderson, 1980). In view of the known variability of egg counts, and their observed density-dependence (Anderson & Schad, 1985), it is important that models should be based on accurate worm counts. The age-intensity profile of hookworm infection in the study community was not convex, and thus provides no epidemiological evidence for acquired immunity (Anderson, 1986).

Hookworms were acquired relatively slowly by children, and peak intensities were not reached until 20 years of age (Fig. 3). This pattern of infection contrasts with that typically observed for *Ascaris* and *Trichuris*, worm burdens of which usually peak between 10 and 15 years of age (Anderson, 1986). The sigmoid shape of the age–intensity profile indicates that infection rates are higher in adults than young children, and suggests that most infections are acquired away from the village. It is possible that the bare, unshaded soil and large numbers of coprophagic pigs in the village itself are not conducive to larval survival; transmission may be more effective in the well-shaded gardens and copra plantations.

The dynamics of the antibody responses against ES and collagen antigens were very different (Fig. 6, Table 3). The antibody response to ES antigens was correlated with infection intensity, especially egg production; however, the antibody response against collagen was correlated with host age (Table 3). This suggests that the anti-collagen response reflects the cumulative exposure to hookworm, which will increase with age, rather than current worm burden, which reaches a plateau with age in adults. In children, both worm burden and exposure will increase, and worm burden may be a better indicator of exposure than host age; thus both antibodies correlate with hookworm intensity. This difference in the dynamics of the two antibody responses is not

surprising in view of the likely source of the two antigens. Adult ES products are continuously synthesized by adult worms in the small intestine. In contrast, collagen is likely to be exposed only during moulting or as a result of damage to worms in the intestines or tissues. That levels of anti-ES antibodies were significantly correlated with egg production rather than worm burden suggests that some ES antigens may be involved in or released during egg production. Serological studies on stage-specific antigens may cast more light on these observations.

These results suggest that the humoral responses measured here simply reflect the epidemiology of the parasite, and no evidence was found that they are operative in the regulation of parasite population growth or the generation of heterogeneity in individual infection levels. However, the lack of correlation between anti-collagen responses and worm burden in adults suggests that exposure is not correlated with worm burden in adults, and thus that there may be an immune response reducing worm burden in some individuals. It is also possible that differences in the recognition of specific antigens exist between heavily and lightly infected adult, or that there are differences in the classes of antibody produced. These possibilities are currently under investigation.

A further factor of relevance for the interpretation of the results presented here is the specificity of the antibody responses measured, since cross-reactivity between hookworm antigens and antigens of other helminth species is known to occur (Ball, Voller & Taffs, 1971). Cross-reactivity between *N. americanus* and *Ascaris lumbricoides* could be particularly problematic for epidemiological studies using immunological markers in many communities (Neppert & Warns, 1974; Turner, Fisher & McWilliam, 1980). Experiments to analyse the cross-reactivity between the species encountered in the present study have revealed that, although a high degree of cross-reactivity between *Ascaris* and hookworm was seen, extensive absorption of sera with *A. lumbricoides* pseudo-coelomic fluid (sufficient to remove anti-*Ascaris* activity on ELISA) reduced but failed to remove antibodies to *N. americanus* (Pritchard *et al.* 1990). This indicates the apparent presence of a species-specific response, although the role of antibodies to *Enterobius* will also have to be taken into consideration in future.

A significant positive correlation was detected in adults between the number of hookworms recovered and the number of eosinophils in the peripheral blood. Eosinophilia has long been recognized as a characteristic indicator of helminth infection (reviewed by Butterworth, 1984), but this is the first report of a correlation of this type in human intestinal nematode infection. The result suggests that an increase in the number of circulating eosinophils is insufficient to reduce infection with *Necator*, as the highest concentrations of this cell type were found in the blood of the most heavily infected people.

There was no correlation between hookworm burden and haemoglobin and haematocrit values in the present study. Hookworm burdens were, however, relatively low: only 3 individuals had egg counts of over 2000 epg, the level above which significant effects on haemoglobin values are typically recorded (Roche & Layrisse, 1966). The relatively low hookworm burdens recorded in the study area should obviously also be borne in mind in the interpretation of the functional significance of the immunological assays reported.

The results presented here quantify for the first time the relationship between hookworm burden, eosinophilia and the production of antibodies in individuals in a naturally infected human community. Future studies will monitor reinfection of the individuals in the village with hookworm over the next 3 years. Schad & Anderson (1985) observed that some individuals were predisposed to re-acquire high or low hookworm burdens following chemotherapy; the identification of differences in the immune response of such individuals would have important consequences for the design of control programmes based on either chemotherapy or vaccination (Anderson & May, 1985; Anderson & Medley, 1985). Long-term immuno-epidemiological studies of this type are essential if the role played by acquired immunity in the population biology of hookworm infection is to be understood.

We are pleased to acknowledge the support and assistance of the Institute of Papua New Guinea Studies and the Madang Provincial Government. Financial support was provided by the Wellcome Trust, Christensen Research Institute (D.I.P), SERC (R.J.Q. and P.M.), Pfizer Ltd, Janssen Pharmaceutical Ltd and Smith, Kline and French Ltd. Pyrantel pamoate ('Combantrin') was donated by Pfizer Ltd. Imferon was donated by Fisons plc. We would like to thank Dr M. Alpers (Institute of Medical Research, Goroka), Dr K. Trenholme (IMR, Madang), Dr M. Jebb and T. Reardon (Christensen Research Institute, Madang) and E. Tscharke (Gaubin Mission Hospital, Karkar) for help and advice throughout the project. IMR provided our field assistant, A. Raiko, as well as field equipment and reagents. Finally, the project would have been impossible without the cooperation and hospitality of the villagers of Kebasob.

This is Publication no. 9 from the Christensen Research Institute, PO Box 305, Madang.

REFERENCES

ANDERSON, R. M. (1980). The dynamics and control of direct life cycle helminth parasites. *Lecture Notes in Biomathematics* **39**, 278–322.

ANDERSON, R. M. (1986). The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 686–96.

ANDERSON, R. M. & MAY, R. M. (1985). Herd immunity to

helminth infection and implications for parasite control. *Nature, London* **315**, 493–6.

ANDERSON, R. M. & MEDLEY, G. F. (1985). Community control of helminth infections of man by mass and selective chemotherapy. *Parasitology* **90**, 629–60.

ANDERSON, R. M. & SCHAD, G. A. (1985). Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 812–25.

BALL, P. A. J. & BARTLETT, A. (1969). Serological reactions to infection with *Necator americanus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 362–9.

BALL, P. A. J., VOLLER, A. & TAFFS, L. F. (1971). Hypersensitivity to some nematode antigens. *British Medical Journal* 23 Jan. 1971, 210–11.

BEHNKE, J. M. (1987). Do hookworms elicit protective immunity in man? *Parasitology Today* **3**, 200–6.

BUTTERWORTH, A. E. (1984). Cell-mediated damage to helminths. *Advances in Parasitology* **23**, 143–235.

CARR, A. & PRITCHARD, D. I. (1986). Identification of hookworm (*Necator americanus*) antigens and their translation *in vitro*. *Molecular and Biochemical Parasitology* **19**, 251–8.

CARR, A. & PRITCHARD, D. I. (1987). Antigen expression during development of the human hookworm *Necator americanus* (Nematoda). *Parasite Immunology* **9**, 219–34.

GROVE, D. I., BURSTON, T. O. & FORBES, I. J. (1974). Immunoglobulin E and eosinophil levels in atopic and non-atopic populations infested with hookworm. *Clinical Allergy* **4**, 295–300.

HAGAN, P. (1987). Human immune response. In *The Biology of Schistosomes* (ed. Rollinson, D. & Simpson, A. J. G.), pp. 295–320. London: Academic Press.

HALL, A. (1981). Quantitative variability of nematode egg counts in faeces: a study among rural Kenyans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 682–7.

HASWELL-ELKINS, M. R., ELKINS, D. B., MANJULA, K., MICHAEL, E. & ANDERSON, R. M. (1988). An investigation of hookworm infection and reinfection following mass anthelminthic treatment in the South Indian fishing community of Vairavankuppam. *Parasitology* **96**, 565–77.

HORNABROOK, R. W., KELLY, A. & MCMILLAN, B. (1975). Parasitic infection of man on Kar Kar Island, New Guinea. *American Journal of Tropical Medicine and Hygiene* **24**, 590–5.

JELLIFFE, D. B. (1966). *The Assessment of the Nutritional Status of the Community*. Geneva: World Health Organization Monograph Series, 53.

JONES, H. I. (1976). A study of human helminthiasis on Karkar Island, Madang Province. *Papua New Guinea Medical Journal* **19**, 165–72.

KELLY, A. (1974). *Alimentary Parasites of Man in Papua New Guinea*. Papua New Guinea: Institute of Medical Research.

MARSH, K., OTOO, L., HAYES, R. J., CARSON, D. C. & GREENWOOD, B. M. (1989). Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relation to protection against infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 293–303.

MAXWELL, C., HUSSAIN, R., NUTMAN, T. B., POINDEXTER, R. W., LITTLE, M. D., SCHAD, G. A. & OTTESEN, E. A. (1987). The clinical and immunological responses of normal human volunteers to low dose hookworm (*Necator americanus*) infection. *American Journal of Tropical Medicine and Hygiene* **37**, 126–34.

MCKEAN, P. G. (1989). Analysis of the antigens of the human hookworm *Necator americanus*. Unpublished Ph.D. thesis, University of Nottingham.

NEPPERT, J. & WARNS, C-M. (1974). Mit Ascaris, Hakenwurm und Onchocerca – Antigenen kreuzreaginerende Seren aus Liberia, Westafrika. *Tropenmedizin und Parasitologie* **25**, 492–7.

OGILVIE, B. M., BARTLETT, A., GODFREY, F. C., TURTON, J. A., WORMS, M. J. & YEATES, R. A. (1978). Antibody responses in self infections with *Necator americanus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **72**, 66–71.

PRITCHARD, D. I., BEHNKE, J. M., CARR. A. & WELLS. C. (1986). The recognition of antigens on the surface of adult and L4 *Necator americanus* by human and hamster post-infection sera. *Parasite Immunology* **8**, 359–67.

PRITCHARD, D. I., MCKEAN, P. G. & ROGAN, M. T. (1988*a*). Cuticular collagens–a concealed target for immune attack in hookworms. *Parasitology Today* **4**, 239–41.

PRITCHARD, D. I., MCKEAN, P. G. & ROGAN, M. T. (1988*b*). Cuticle preparations from *Necator americanus* and their immunogenicity in the infected host. *Molecular and Biochemical Parasitology* **28**, 275–84.

PRITCHARD, D. I., MCKEAN, P. G., LEGGETT, K. V., QUINNELL, R. J., SLATER, A. F. G. & KEYMER, A. E. (1990). The molecular basis of antigenic cross-reactivity between *Necator americanus* and *Ascaris lumbricoides*. *Parasite Immunology* (in the Press).

ROCHE, M. & LAYRISSE, M. (1966). The nature and causes of 'hookworm anaemia'. *American Journal of Tropical Medicine and Hygiene* **15**, 1031–102.

SCHAD, G. A. & ANDERSON, R. M. (1985). Predisposition to hookworm infection in humans. *Science* **228**, 1537–40.

SHIELD, J., ANIAN, G., OSTWALD, R. & RAINER, A. (1984). Reinfection with intestinal helminths after treatment with mebendazole. *Papua New Guinea Medical Journal* **27**, 41–4.

TAYLOR, M. M. & TURTON, J. A. (1976). Antigen-induced lymphocyte blastogenesis in a hookworm (*Necator americanus*) infection in man. *Tropenmedizin und Parasitologie* **27**, 89–92.

TURNER, K. J., FISHER, E. H. & MCWILLIAM, A. S. (1980). Homology between roundworm (*Ascaris*) and hookworm (*Necator americanus*) antigens detected by human IgE antibodies. *Australian Journal of Experimental Biology and Medicine* **58**, 249–57.

VINES, A. P. (1970). *The Epidemiological Sample Survey of the Highlands, Mainland and Island Regions of Papua New Guinea*. Department of Public Health, Port Moresby, 621 pp.

WARREN, K. S. (1988). Hookworm control. *Lancet*, **15**, October 1988, 897.

WATERLOW, J. C. (1976). Classification and definition of protein energy nutrition. In *Nutrition in Preventive Medicine* (ed. Beaton, G. H. & Bengoa, J. M.). Geneva: WHO.

WHITE, C. J., MAXWELL, C. J. & GALLIN, J. I. (1986). Changes in the structural and functional properties of human eosinophils during experimental infection. *Journal of Infectious Diseases* **154**, 78–83.

WORLD HEALTH ORGANIZATION (1975). Control of nutritional anaemia with special reference to iron deficiency. Geneva: *WHO Technical Report Series*, No. 580.