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Reinfection with hookworm after chemotherapy in Papua New Guinea

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SUMMARY

Reinfection with hookworm (*Necator americanus*) following chemotherapy was studied over 2 years in a rural village in Madang Province, Papua New Guinea. The prevalence of hookworm infection had returned to pre-treatment levels after 2 years, and the geometric mean hookworm burden had returned to 58% of the pre-treatment value. The rate of acquisition of adult worms was independent of host age, and was estimated as a geometric mean of 2.9–3.3 worms/host/year (arithmetic mean 7.9–8.9 worms/host/year). There was significant predisposition to hookworm infection; the strength of this predisposition did not vary significantly between age or sex classes.

Key words: hookworm, *Necator americanus*, reinfection, predisposition, chemotherapy, Papua New Guinea.

INTRODUCTION

Hookworms (*Necator americanus* and *Ancylostoma duodenale*) are among the most prevalent of all human infections. The distribution of these parasites extends throughout the wet tropics and subtropics, and an estimated 1150 million people are infected (Bundy, 1990). A large number of studies of hookworm epidemiology have been carried out, especially in the 1920s and 1930s, when the Rockefeller Foundation funded studies and control programmes in many countries (Ettling, 1990). However, since this period the study of hookworm has been neglected in comparison to that of other human helminths. In particular, there have been very few studies involving the use of anthelmintic-induced worm expulsion to accurately determine worm burdens (Bundy, 1990). Worm expulsion has been used in recent studies in India and Zimbabwe (Anderson & Schad, 1985; Schad & Anderson, 1985; Bradley *et al.* 1992), but to date there has been only one community-based reinfection study with intensity assessed by worm counts, that of Haswell-Elkins *et al.* (1988).

Studies on hookworm in Papua New Guinea have concentrated on assessing its distribution and prevalence, and its relationship to nutritional status, particularly anaemia (Zigas, 1973; Shield *et al.*

1981). Hookworm is the commonest helminth infection in Papua New Guinea: in 1919–1923 the Australian Hookworm Campaign recorded a prevalence of 68% from a countrywide survey of more than 46000 people (Sweet, 1924), while more recent estimates give a prevalence of 74–90% (Vines, 1970). Despite its commonness little is known about the local population dynamics of the parasite, and estimates of intensity have relied on the use of faecal egg counts. In the only published reinfection study from Papua New Guinea, Ashford & Barnish (1989) found no predisposition to high or low hookworm egg count in children from a highland village.

The present study was carried out in a lowland village on Karkar Island, Madang Province, Papua New Guinea. This study is part of a wider investigation of the epidemiology and immunology of *N. americanus*, the only species of hookworm found in the area; the study design and pre-treatment results have been previously published (Pritchard *et al.* 1990). The present paper describes the extent and pattern of reinfection 1 and 2 years after treatment.

MATERIALS AND METHODS

Fieldwork was carried out in the village of Kebasob, Karkar Island, Papua New Guinea. Details of the study area, study design and methods used have already been described (Pritchard *et al.* 1990). Briefly, an initial survey was carried out in July–August 1988. Faecal samples were obtained from 202 people, who were then treated with the anthelmintic pyrantel pamoate (Combantrin, Pfizer

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Ltd) at a dose of 10 mg/kg. All faecal output was collected for 48 h and expelled worms counted. Later, anthelmintic treatment was offered to all villagers who had not been included in the study group: in total, an estimated 60% of the village population received treatment.

In August 1989 faecal samples were collected from as many as possible of the original sample. Five people were found to be infected with *Ascaris lumbricoides* and were treated with Combantrin; the rest of the population was not treated. In July–August 1990 faecal samples were collected again, all people treated and counts of expelled worms performed as in 1988.

Only data from people who provided samples in all years are analysed: 140 people provided faecal samples in all 3 years, and 75 people provided full post-treatment faecal output in both 1988 and 1990.

The efficacy of anthelmintic treatment was assessed in a subsample of 25 people in 1988: the cure rate was 60% and the reduction in arithmetic mean faecal egg count 94% (Pritchard *et al.* 1990). Seventy-two h faecal collections were made from a total of 20 worm-positive individuals in 1988 and 1990. These people expelled a mean of 88% of their worms during the first 48 h.

Faecal egg counts were performed according to a modified formalin–ether concentration technique (Hall, 1981); results are presented as eggs per gram of faeces (eggs/g). Worms were counted after washing post-treatment faeces through two grades of sieve (1 mm and 300 μ m). The sediment was preserved in 10% formalin and worms were recovered after careful searching by eye, counted and sexed.

During the 3 years village meetings were held when the pathology of hookworm infection and its mode of transmission were explained to interested villagers (in Melanesian pidgin). Leaflets were also distributed advising people on latrine use and the safe disposal of faeces. Ethical clearance for the study was obtained from the Medical Advisory Committee of the Department of Health, Government of Papua New Guinea.

Statistical analysis

The distributions of egg counts, worm burdens and host ages were positively skewed: these data were analysed after logarithmic transformation. Predisposition was analysed by non-parametric methods on untransformed data. Age and sex standardization of data (where indicated) was performed by z -transformation within sex and age classes (3–8 years, 9–13 years, 14–30 years and 31–60 years old in 1988). As there are problems in testing the homogeneity of rank correlation coefficients, the predisposition analysis was also performed parametrically; data were converted to normal scores in order to normalize best the data within age-classes. Homogeneity tests

were carried out according to Sokal & Rohlf (1981). Negative binomial distributions were fitted by an iterative maximum-likelihood method (Elliott, 1977).

RESULTS

Prevalence of hookworm infection

The prevalence of hookworm infection before chemotherapy, assessed by faecal egg counts, was 92.9%, which was reduced to an estimated 37% by chemotherapy. Prevalence had increased to 65.0% and then 80.7% after 1 and 2 years reinfection (Fig. 1). The prevalence assessed by worm recovery was 81.3% in 1988 and 82.7% in 1990.

The age-prevalence profiles of infection in all 3 years are shown in Fig. 2. After reinfection prevalence did not increase with host age, and had returned to pre-treatment levels in the younger age classes in 1990. Prevalence was very similar in male and female hosts in all three years (Fig. 1; $\chi^2 = 1.17$, 0.387 and 0.053 in 1988, 1989 and 1990, D.F. = 1, N.S. in all cases).

Intensity of hookworm infection

After 2 years reinfection the geometric mean (95% c.l.) worm burden was 4.8 (3.3–6.8) worms/host, 58% of the pre-treatment value of 8.3 (5.5–12.3) worms/host. Corresponding arithmetic means were 13.0 worms/host in 1990 and 25.3 worms/host in 1988. Worm burden differed significantly between years, but there was no significant effect of host sex on worm burden. Although the percentage mean reinfection was lower in female than male hosts, 40 compared to 78%, there was no significant effect of host sex on the degree of reinfection (ANOVA: year, $F_{1,73} = 7.12$, $P < 0.01$; host sex, $F_{1,73} = 2.01$, N.S.; interaction, $F_{1,73} = 2.17$, N.S.).

The age-intensity profiles of infection were very different pre-treatment and after reinfection: monotonic pre-treatment and flat after reinfection (Fig. 3 and Pritchard *et al.* 1990). There was a highly significant correlation between worm burden and host age in 1988 ($r = 0.453$, $n = 75$, $P < 0.001$) but not in 1990 ($r = 0.090$, $n = 75$, N.S.); these correlation coefficients differed significantly ($t = 2.39$, $P < 0.05$).

The geometric mean faecal egg counts in 1988, 1989 and 1990 were 93, 23 and 45 eggs/g respectively (Fig. 4). The *per capita* egg production of female worms was similar before treatment and after 2 years reinfection, geometric means (95% c.l.) being 20 (14–30) and 17 (11–27) eggs/g/female respectively (paired t -test: D.F. = 45, $t = 0.61$, N.S.). In 1990, as in 1988, the sex ratio of hookworms was not significantly different from unity, 490 males and 485 females being recovered ($\chi^2 = 0.026$, D.F. = 1, N.S.).

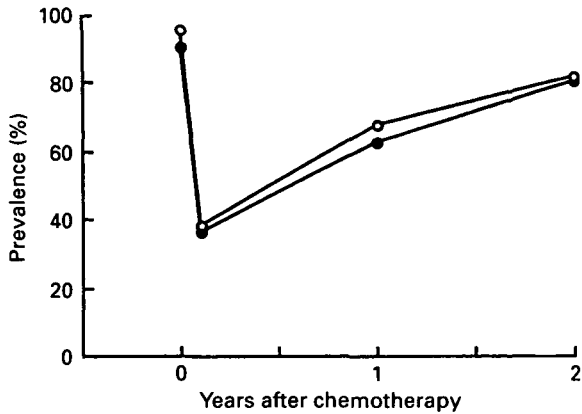


Fig. 1. The prevalence of hookworm infection pre-treatment and at various times post-treatment in Kebasob, Papua New Guinea. Prevalence was calculated from faecal egg counts. (●) Male hosts ($n = 75$); (○) female hosts ($n = 65$).

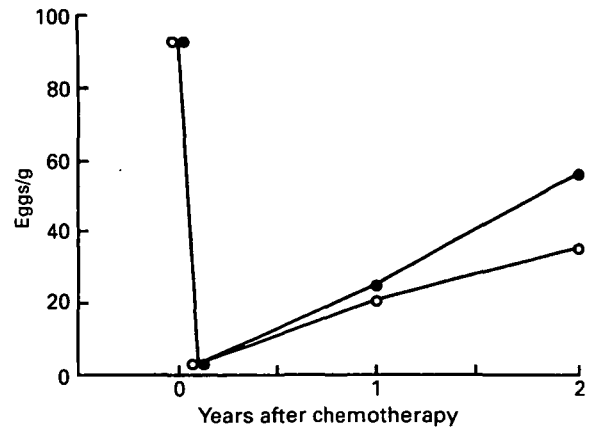


Fig. 4. The intensity of hookworm infection pre-treatment and at various times post-treatment. Intensity was assessed as geometric mean faecal egg production (eggs/g). (●) Male hosts ($n = 75$); (○) female hosts ($n = 65$).

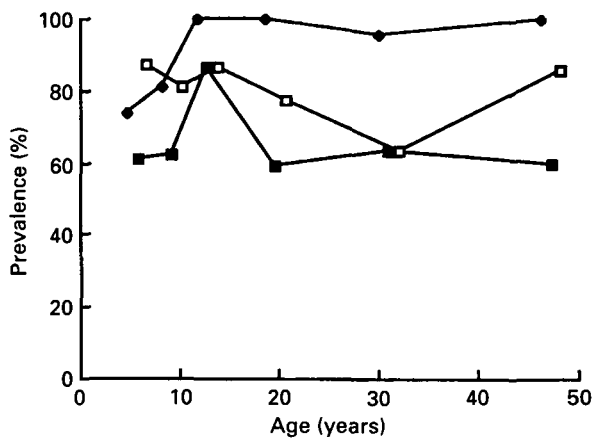


Fig. 2. Age-prevalence profiles of hookworm infection pre-treatment (1988) and 1 and 2 years post-treatment (1989 and 1990). Prevalence was calculated from faecal egg counts. (◆) 1988; (■) 1989; (□) 1990. Each point represents 16-35 hosts.



Fig. 3. Age-intensity profiles of hookworm infection pre-treatment (1988) and 2 years post-treatment (1990). Intensity was assessed as geometric mean (\pm S.D.) worm burden. (◆) 1988; (□) 1990. Each point represents 17-20 hosts.

Table 1. Frequency distribution of hookworm numbers per person in 1990: the aggregation parameter (k) and the goodness of fit to the negative binomial distribution (χ^2 test) within age groups and sexes

Age class (years)	n	k	χ^2
5-10	18	0.328	—
11-15	20	0.549	—
16-32	17	0.481	—
33+	20	0.477	—
Total	75	0.447	4.22†
Males	39	0.451	4.18†
Females	36	0.614	2.24†

—, Insufficient degrees of freedom for χ^2 test.
 † $P > 0.05$ (i.e. no significant departure from negative binomial distribution).

Table 2. Correlation coefficients between initial and reinfection hookworm egg counts

(Kendall's rank correlation coefficient for raw data (τ) and data standardized by age and sex (standardized τ).

	n	τ	Standardized τ
1988 vs 1989	140	0.262***	0.211***
1988 vs 1990	140	0.188***	0.227***
1989 vs 1990	140	0.330***	0.253***

*** $P < 0.001$.

The frequency distribution of hookworm burdens

The frequency distribution of hookworm burdens in 1990 was highly overdispersed, and could be described by a negative binomial distribution with aggregation parameter, k , of 0.447 (Table 1). There was little variation in k with host age or sex (Table 1).

Table 3. Correlation coefficients between initial and reinfection hookworm burdens

(τ , Kendall's rank correlation coefficient; standardized τ , Kendall's rank correlation coefficient on age- and sex-standardized data; r , Pearson's correlation coefficient on data converted to normal scores.)

	<i>n</i>	τ	Standardized τ	r
All hosts	75	0.311***	0.301***	—
Males	39	0.261*	0.277*	0.346*
Females	36	0.422***	0.354**	0.533***
3–8 years†	18	0.180	0.233	0.232
9–13 years	20	0.409*	0.191	0.603**
14–30 years	17	0.195	0.205	0.292
31+ years	20	0.370*	0.389*	0.486*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Age in 1988.

Predisposition to hookworm infection

There was a highly significant correlation between pre-treatment egg counts and those after 1 or 2 years reinfection (Table 2). Standardization of the data by host age and sex (see Materials and Methods section) did not affect the significance of this correlation.

The correlation between pre- and post-treatment worm burdens was also highly significant (Table 3). When hosts were divided into age and sex classes the correlation was significant only in 9–13 year olds and over 31-year-olds, and was stronger in female than in male hosts. However, homogeneity tests on Pearson's correlation coefficients between normal scores showed no significant differences between age classes ($\chi^2 = 2.15$, D.F. = 3, N.S.), and no difference between male and female hosts ($t = 0.967$, N.S.). Similar results were obtained with data analysed after logarithmic transformation.

Other geohelminth infections

A. lumbricoides and *Trichuris trichiura* were present at low prevalences in all 3 years. The prevalence of *A. lumbricoides* was 7, 0 and 3% in 1988, 1989 and 1990; all people infected with *A. lumbricoides* in 1989 were treated and are excluded from these figures. The prevalence of *T. trichiura* was 1, 1 and 3% respectively. All prevalences were calculated from faecal egg counts. *Enterobius vermicularis* was also present at a prevalence (from worm counts) of 53% in 1988 and 7% in 1990.

DISCUSSION

The prevalence of hookworm infection in Kebasob is typical of that in lowland Papua New Guinea, with around 80–90% of the population infected (Vines &

Kelly, 1966; Vines, 1970). The prevalence of hookworm infection in other villages on Karkar I. was reported as 55–78% in the 1970s (Hornabrook, Kelly & McMillan, 1975; Jones, 1976). Few population-based surveys of hookworm intensity have been carried out in Papua New Guinea; reported arithmetic mean intensities are typically < 1000 eggs/g (Ashford, Hall & Babona, 1981; Shield *et al.* 1981; Barnish & Ashford, 1989*a, b*), though sometimes up to 3000 eggs/g (Bearup & Lawrence, 1940). *N. americanus* is much the commonest species of hookworm in Papua New Guinea, although *A. duodenale* has been reported in very small numbers from a few coastal areas (Bearup & Lawrence, 1950).

A rapid recovery of prevalence and intensity after chemotherapy is typical of helminth infections (Anderson & May, 1985; Anderson & Medley, 1985). The percentage reinfection in the present study was relatively high compared to that reported for hookworm infection in other areas (Table 4). The extent of reinfection is expected to be influenced by a number of factors, including the proportion of the population treated, the effectiveness of the anthelmintic used and the basic reproductive rate of the parasite (Anderson & Medley, 1985). The relatively high rate of reinfection recorded in Kebasob may be partly due to the relatively low proportion of the village which was treated, around 60%.

In Kebasob people acquired a geometric mean of 4.8 adult worms after 2 years. The annual rate of acquisition of adult worms can be calculated at 2.9–3.3 worms/host/year from a simple immigration–death model of infection (Anderson, 1986), assuming a constant mortality rate of 0.2–0.33/worm/year (Hoagland & Schad, 1978). Such an annual rate would lead to an expected worm burden of 10–15 worms/host in adults. Similar calculations using arithmetic means give an acquisition rate of 7.9–8.9 worms/host/year, and an expected adult worm burden of 27–40 worms/host. These burdens are similar to, and well within the confidence limits of, the observed mean adult burdens pre-treatment (Fig. 4 and Pritchard *et al.* 1990). This suggests that infection rates after chemotherapy were similar to those pre-treatment.

Reinfection with hookworm, measured by prevalence or intensity, was independent of host age. A constant rate of acquisition of adult worms is expected to result in a monotonic age–intensity profile, as was observed. Such monotonic profiles appear typical of hookworm infection both in Papua New Guinea (Barnish & Ashford, 1989*a, b*) and elsewhere, although the number of such profiles based on worm counts is very small, particularly from high-intensity areas. In contrast, reinfection with *A. lumbricoides* and *T. trichiura* is typically most rapid in children, and age–intensity profiles are convex (Bundy, 1990). These patterns suggest that

Table 4. The relative intensity of reinfection (expressed as the percentage of pre-treatment intensity) with hookworm following treatment in different studies

(The measure of intensity used was arithmetic mean egg count in all studies apart from that of Haswell-Elkins *et al.* (1988), who used arithmetic mean worm burden. Hookworm infections in Sri Lanka, Puerto Rico and Papua New Guinea were all *N. americanus*; those in India and Zimbabwe were a mixture of *N. americanus* and *A. duodenale*. In all studies anthelmintic efficacy was > 90%.)

Study	Country	Reinfection (%)	Time (months)
Sweet (1925)	Sri Lanka	29	12
Hill (1926)	Puerto Rico	10	12
Schad & Anderson (1985)	India	30	21
Haswell-Elkins <i>et al.</i> (1988)	India	35	11
Bradley & Chandiwana (1990)	Zimbabwe	42	9
This study	Papua New Guinea	43	12
This study	Papua New Guinea	54	24

exposure to the infective stages of hookworm does not fall with increasing host age. Most hookworm transmission, particularly of *N. americanus*, is thought to occur around defaecation sites, probably during the process of defaecation (Chandler, 1929; Schad, Nawalinski & Kochar, 1983). Infection by this route may be less age-dependent than oral infection, which depends on the ingestion of contaminated material such as food or soil. Practices such as geophagia are far more widespread in children than adults (Bundy, 1988).

The lack of variation in the rate of acquisition of adult worms with age does not allow inferences to be made about any possible role of acquired immunity. Such a pattern may reflect either age-independent exposure to infective stages or an increase in both exposure and immunity with age. Monotonic age-intensity profiles of helminth infection have been found even when there is significant resistance to reinfection in adults (Quinnell, 1992).

The frequency distribution of hookworm burdens was, as expected, highly overdispersed, with an aggregation parameter, k , of 0.447. This was similar to that reported at the initial survey (0.370) and to other reported values of 0.24 in southern India (Haswell-Elkins *et al.* 1988), 0.346 in Zimbabwe (Bradley *et al.* 1992) and 0.63 in Bengal (Schad & Anderson, 1985).

Predisposition to hookworm infection has been reported previously by Schad & Anderson (1985), Haswell-Elkins *et al.* (1988) and Bradley & Chandiwana (1990). In the latter two studies the strength of predisposition varied with both host age and sex, but the significance of this variation was not reported. Predisposition to hookworm infection may be associated with variation in human behaviour, especially choice of defaecation site, or variation in the strength of the immune response (Schad & Anderson, 1985); both these factors may vary with host age and

sex. However, in the present study, although predisposition was detectable in only 2 of the 4 host age classes, there was no significant heterogeneity in correlation coefficients between age or sex classes. Sample sizes were, however, small: if heterogeneity does exist, larger samples may be needed to detect it. In contrast to the above studies, Ashford & Barnish (1989) found no predisposition to hookworm infection in a highland village in Papua New Guinea. However, this study was restricted to children, in whom predisposition was not detected in the present study nor those of Haswell-Elkins *et al.* (1988) and Bradley & Chandiwana (1990). This lack of detectable predisposition may have a statistical rather than biological explanation, as the lower intensity of infection in children will make predisposition harder to detect (Keymer & Pagel, 1990).

A. lumbricoides and *T. trichiura* infections

A. lumbricoides and *T. trichiura* are typically much less common than hookworm in Papua New Guinea; Sweet (1924) recorded prevalences of 12 and 16% respectively. The factors governing the distribution and abundance of *A. lumbricoides* and *T. trichiura* in Papua New Guinea are largely unknown, although both are more prevalent in the highlands (Vines, 1970).

In other villages on Karkar I. the prevalence of *A. lumbricoides* has been reported as 21–57%, and that of *T. trichiura* as 17–42% (Hornabrook *et al.* 1975; Jones, 1976). The prevalence of both infections reported in the present study was much lower, which may be due to better hygiene and a generally higher standard of living in Kebasob than in neighbouring villages. It seems unlikely that either parasite, especially *T. trichiura*, has a self-sustaining population in Kebasob, and it is possible that most cases result from infection outside the village.

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