Associations among multiple geohelminth species infections in schoolchildren from Pemba Island

M. BOOTH†[1]*, D. A. P. BUNDY†[2], M. ALBONICO†[3], H. M. CHWAYA†[4], K. S. ALAWI†[5] *and* L. SAVIOLI†[3]

Department of Biology, Imperial College of Science Technology and Medicine, London SW7 2BB, UK

(*Received 15 November 1996; revised 13 March and 3 July 1997; accepted 3 July 1997*)

SUMMARY

In order to estimate the potential benefits of interventions against multiple geohelminth species in endemic areas, an improved understanding of the population biology of multiple infections is required. This paper presents a detailed analysis of the associations among *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections in 1539 schoolchildren on Pemba Island, Tanzania, where 58% of the sampled children carried infections of all 3 parasites at the time of the study. Infection intensities of different species were positively correlated, and individuals with single-species infections had generally lower species-specific egg counts than individuals with multiple-species infections. There was no age- or sex-related clustering of infections. A weak clustering of intense infections among individuals with multiple-species infections was observed, which became more pronounced as the threshold defining an intense infection increased for each species. The results suggest that individuals with multiple species infections are likely to be at highest risk of geohelminth-related morbidity, not only because of the number of infections they harbour, but also because they generally carry heavier infections of each species.

Key words: *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms, multiple-species infections, infection clustering.

INTRODUCTION

In any community where multiple geohelminth infections are endemic, a proportion of the population may be infected by 2 or more species. This proportion is determined by the overall prevalences of the different infections, and the degree of infection clustering (Booth & Bundy, 1992, 1995). A large degree of clustering will obviously reduce the overall proportion of a population that are infected, but at the same time will increase the proportion of the population with multiple-species infections.

Among infected individuals, the risk of morbidity associated with any one species is related to infection intensity (Roche & Layrisse, 1966; Biagi, Lopez & Viso, 1975; Cooper *et al.* 1992; Ramdath *et al.* 1995). There is also evidence that the risk of morbidity is related to the number of species harboured by an individual (Buck, Anderson & Macrae, 1978; Robertson *et al.* 1992; Stephenson *et al.* 1993*a*, *b*). Thus, the risk of an individual suffering geohelminth-related morbidity may be a joint function of the number of infections they carry and/or the intensity of each infection.

As a consequence of some of the above observations, theoretical studies have used a range of threshold intensities to define an infection intense enough to cause morbidity (Guyatt & Bundy, 1991; Lwambo, Medley & Bundy, 1992, Booth, 1994). In this paper, we extend the concept to cover the situation where multiple species are endemic in the same community. We also extend the concept of infection clustering to cover clustering of intense infections, since it is this latter measure which will determine the overall morbidity distribution.

Through complementary analyses of 1 data set, aspects of the combined population biology of *A. lumbricoides*, *T. trichiura* and hookworm infections are examined within a population of school children, the age group at highest risk of geohelminth-related morbidity (Bundy *et al.* 1990; Hall, 1993). We ascertain the extent of infection clustering, and determine whether the infection intensity of individual species was related to the presence of concurrent infections. Finally, we estimate the prevalence of multiple-intense infections, using a

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† Present addresses: †[1] Department of Public Health and Epidemiology, Swiss Tropical Institute, CH 4002, Basel, Switzerland.
†[2] Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.
†[3] World Health Organization, Geneva, Switzerland.
†[4] Ministry of Health, P.O. Box 236, Zanzibar Town, Zanzibar.
†[5] Helminth Control Programme, P.O. Box 122, Chake-Chake, Pemba.
* Corresponding author: Department of Public Health and Epidemiology, Swiss Tropical Institute, CH 4002, Basel, Switzerland. Tel: +41 61 2848109. Fax: +41 61 271 7951. E-mail: booth@ubaclu.unibas.ch.

Parasitology (1998), **116**, 85–93. Printed in the United Kingdom

range of different thresholds to define an infection intense enough to cause morbidity. The results are used to infer the overall likely morbidity distribution within the infected population.

MATERIALS AND METHODS

Data collection

During October and November 1992, as part of an evaluation of the relative efficacies of albendazole and mebendazole against intestinal nematodes, a parasitological survey was conducted amongst schoolchildren from 10 schools on the island of Pemba, Tanzania. Details of the survey methods have been described by Albonico *et al*. (1994), and the island topology by Savioli *et al*. (1989). Briefly, each child between 7 and 12 years of age (inclusive) donated a single stool sample from which *A. lumbricoides*, *T. trichiura*, and hookworm egg counts were recorded by Kato Katz quantitative cellophane thick smear technique (Katz, Chavez & Pellegrino, 1972). Egg counts were converted to the number of eggs per gramme of faeces (epg). No attempt was made to identify the hookworm species in each infection due to the methodological complexities of larval identification; however, *N. americanus* is known to be the dominant species in the area. Diarrhetic stools were excluded from analysis, due to their dilutive influence on eggs counts. When the concentration of eggs was extremely high ($> 100\,000$ epg), stools were processed by the Stoll dilution method and re-examined, in order to maintain egg-counting accuracy.

Data analysis

Infection prevalences of *A. lumbricoides*, *T. trichiura* and hookworms were estimated, for each age group and sex, from the number of egg positive stools in the sample of children. Numbers of individuals in the following infection categories were estimated: *A. lumbricoides* alone, *T. trichiura* alone, hookworms alone, *A. lumbricoides* + *T. trichiura*, *A. lumbricoides* + hookworms, *T. trichiura* + hookworms, *A. lumbricoides* + *T. trichiura* + hookworms. A simple probabilistic model, described by Booth & Bundy (1995), was used to produce expected numbers of multiple-species infections for comparative purposes. This model is based on the assumption that infections of different species are transmitted independently of each other (i.e. there is no excess infection clustering).

Poisson regression

Poisson regression was applied to the data, in order to test whether the prevalence of each infection varied with age and sex, and also to test whether certain combinations of infections occurred more or less often than expected by chance. This analysis tested for clustering at the level of presence and absence of infections.

Infection intensity correlations

Data from individuals with multiple-species infections were tested for correlations between the infection intensities of different species. For each pair of possible species combinations, the Kendall rank coefficient of correlation (*Tau*) was calculated (Kendall, 1938). Infection-intensity relationships were examined for the pooled data, and also within each age group and sex.

Variation in egg counts with different concurrent infections

Preliminary analysis indicated that egg-count distributions were overdispersed. Non-parametric analysis of variance (Kruskall–Wallis) was therefore used to assess whether egg counts of individual species varied significantly among different infection categories in each age group. Where there was significant variation, the multiple-comparison test of Dunn, as described by Zar (1996), was applied, in order to detect among which categories egg counts differed significantly. In order to examine directly the direction and magnitude of the variation, median and percentile infection intensities of each species in each infection category were estimated.

Clustering of infections at different intensities

For each species, a series of egg-count values were chosen as potential thresholds defining an infection intense enough to cause morbidity (hence called an intense infection). Although the actual values used for the thresholds were arbitrary, they represented the full range of observed egg counts, and reflected differences in the observed magnitude of egg counts for each species. We then determined whether the degree of infection clustering between 2 species was related to these thresholds. Matrices were constructed for each combination of 2 species, with row and column headings containing the thresholds defined for each parasite. Each cell in the matrix contained a ratio value ($R_{j,k}$) derived from the following equation

$$R_{j,k} = N\left(\frac{O_{jk}}{A_j \, . \, B_k}\right), \tag{1}$$

where N is the total sample size, and j and k represent the threshold egg counts for species A and B respectively. O_{jk} represents the number of individual with egg counts of $A >= j$ and $B >= k$, A_j represents the overall number of individuals with $>= j$ eggs in their stool, and B_k represents the same measurement for parasite B, with k eggs. Ratios of value greater than 1 were taken as evidence of infection clustering at the given thresholds. χ^2 analysis was applied to test whether each ratio value differed significantly from 1.

Prevalence of multiple-intense infections at different intensity thresholds

Finally, we examined how changing the definition of an intense infection, for multiple species simultaneously, affected estimates of the proportion of the population carrying 1 or more intense infections. Starting with a threshold of 0 for each species, the number of individuals fulfilling the following criteria was counted for successive increases in the threshold values for each parasite species: intense infection of all 3 species; intense infection of at least 2 species; intense infection of at least 1 species. Thresholds were increased with intervals of 1000 epg for *A. lumbricoides*, and 400 epg for *Trichiura* and hookworms. The highest thresholds were 19000 epg for *A. lumbricoides*, and 7600 for *T. trichiura* and hookworms. Expected numbers (calculated by assuming there was no infection clustering at any level of infection) were also estimated for each set of thresholds, for comparative purposes.

RESULTS

Prevalence of infection

A total of 1539 stools from children aged 7–12 years inclusive were examined for the presence of *A. lumbricoides*, *T. trichiura* and hookworm eggs. A total of 1032 contained *A. lumbricoides* eggs, 1498 contained *T. trichiura* eggs, and 1355 contained hookworm eggs. Only 3 individuals were counted as negative for all 3 species. For the purpose of analysis, it was assumed that the sensitivity of egg counts was 100%; thus the estimated infection prevalences were 66, 97 and 88%, for *A. lumbricoides*, *T. trichiura* and hookworms respectively. Multiple-species infections were very common (Table 1). In total 93% of the children harboured more than 1 species, and most carried 3 species.

Poisson regression

There was very little variation in either prevalence or mean intensity among different age groups for each species (Table 2). Even the youngest children had high levels of infection, and there was evidence that only the prevalence of *hookworm* infection increased with age (Table 3). In terms of mean infection intensity, there was significant variation only for hookworms (Table 3), but closer inspection of the data revealed that this was not systematic, and consisted mainly of variation in the number of outliers in different age groups. There were no sex differences in infection levels for any species; indeed sex was an insignificant main-effect term in the regression, indicating no stratification or adjustment was necessary in further analysis. Having adjusted for the effects of age on the prevalence of hookworm infections, the results of the Poisson regression also revealed no evidence of excessive clustering at the level of infection presence/absence (Table 3).

Infection-intensity relationships

Despite the lack of association at the level of infection presence/absence, further analysis indicated inter-species associations amongst individuals with multiple-species infections. Relationships between infection intensities of different species were characterized by positive correlations, and large amounts of scatter in the data. Rank correlations were highly significant for each combination (*A. lumbricoides* versus *T. trichiura*: $N = 1011$, $Tau = 0{\cdot}31$, $P < 0{\cdot}0001$; *A. lumbricoides* versus hookworm: $N = 921$, $Tau = 0{\cdot}20$, $P < 0{\cdot}0001$; *T. trichiura* versus hookworm: $N = 132$, $Tau = 0{\cdot}30$, $P < 0{\cdot}0001$). The correlations remained highly significant ($P < 0{\cdot}001$) when data from each age group or sex were analysed separately.

Variation of intensities among different infection categories

The mean ages of infected individuals in each infection category are listed in Table 2. Although there was a trend for the mean age to increase with an increasing number of infections, Kruskall–Wallis analysis revealed that the age distribution did not vary significantly among the infection categories ($H = 12{\cdot}97$, $P = 0{\cdot}0728$). The slight increase in mean age with increasing multiplicity of infection is therefore explained by the slight increase in overall infection prevalences with age.

Box and whisker plots depict the variation in egg counts of each species across different infection categories (Fig. 1). Infection intensities were generally lower in single-species categories, although both hookworm and *A. lumbricoides* egg counts were low in the category consisting of these 2 infections only. Since this category contained only 17 individuals, this observation is likely to be a sampling artefact. Only 4 individuals carried *A. lumbricoides* worms only, and the same reason may therefore explain the low egg counts of this species when it was the only infection.

When Kruskall–Wallis analysis was applied to test the significance of the observed variation, it was found that *T. trichiura* egg counts varied significantly ($H = 31{\cdot}4$, $P < 0{\cdot}001$), as did hookworm egg counts

Table 1. Observed number (*Obs N*) and proportion (*Obs P*) with each combination of infections, for comparison with the expected proportion (*Exp P*) as calculated on the basis of no interaction (Booth & Bundy, 1995)

(The mean ages of infected individuals (standard deviations in parentheses), are also given for each infection combination.)

Infection combination	*Obs N*	*Obs P*	*Exp P*	Mean age in years
A. lumbricoides only	4	0·003	0·002	9·00 (1·15)
T. trichiura only	70	0·046	0·038	9·44 (1·36)
Hookworms only	17	0·011	0·008	9·29 (1·53)
A. lumbricoides + *T. trichiura*	107	0·070	0·078	9·86 (1·14)
A. lumbricoides + hookworms only	17	0·011	0·016	9·47 (1·33)
T. trichiura + hookworms only	417	0·271	0·283	9·89 (1·40)
A. lumbricoides + *T. trichiura* + hookworms	904	0·589	0·576	9·92 (1·39)

Table 2. The infection prevalence, expressed as the percentage infected (*Prev*), and arithmetic mean intensity (*Int*) of each infection by age group

		A. lumbricoides		*T. trichiura*		Hookworms	
Age	*N*	*Prev.*	*Int.*	*Prev.*	*Int.*	*Prev.*	*Int.*
7	58	63·8	10773	94·8	1467	86·2	1638
8	204	62·7	9012	94·1	1468	90·2	1492
9	332	66·0	9580	98·9	1663	85·9	1703
10	505	70·3	9102	97·4	1580	85·7	1635
11	158	62·7	8710	97·4	1696	89·9	1701
12	281	69·0	9314	98·5	1363	92·3	1926

Table 3. Results of Poisson regression

(Tests for variation in infection prevalence by age and sex, and also for whether certain combinations of infections occurred more often than expected by chance. Each listed interaction represents 1 term in the regression model. Degrees of freedom of each term (D.F.) are also given. The χ^2 and associated *P* values are calculated by comparing a model containing all possible interactions of the same and lower orders, with a model missing the interaction of interest.)

Interaction	D.F.	χ^2	*P*
Age.Asc	5	5·56	0·3513
Age.Tri	5	9·97	0·0762
Age.Hkw	5	11·50	0·0423
Asc.Tri	1	3·67	0·0553
Asc.Hkw	1	3·61	0·0576
Tri.Hkw	1	0·735	0·3912
Age.Asc.Tri	5	3·25	0·6614
Age.Asc.Hkw	5	7·97	0·1579
Age.Tri.Hkw	5	4·78	0·4430

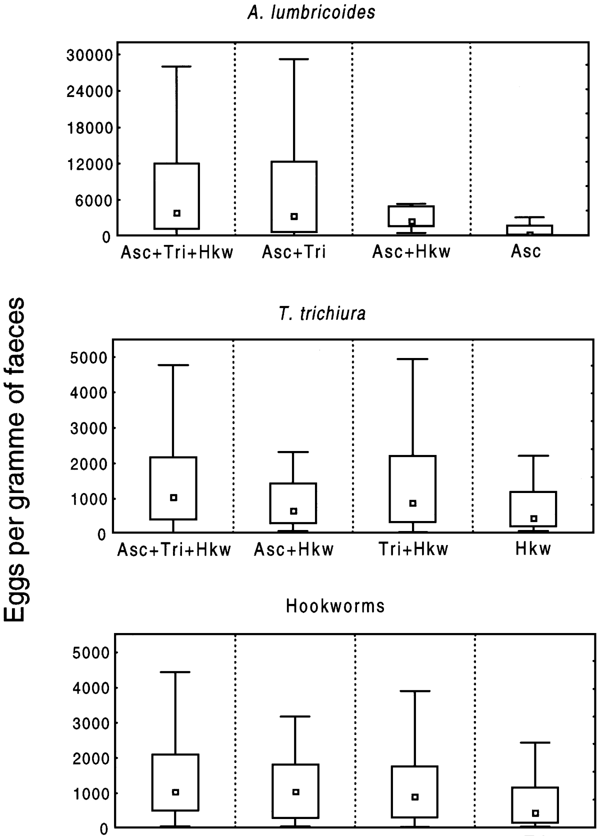

Fig. 1. Box and whisker plots depicting the distribution of egg counts for each species according to different infection categories tested (Asc, *Ascaris lumbricoides*; Tri, *Trichuris trichiura*; Hkw, hookworm). Median egg counts are shown as small squares inside rectangles which form the 25- and 75-percentiles. Whiskers represent the extent of data outside the percentiles, but within a range of 1·5* height of the rectangle.

($H = 11{\cdot}5$, $P = 0{\cdot}0094$). *A. lumbricoides* egg counts did not vary significantly ($H = 7{\cdot}3$, $P = 0{\cdot}0628$), and multiple-comparison tests were therefore conducted for *T. trichiura* and hookworms only. In a total of 12 tests, there was no significant effect of age on egg-count variation for any species in any infection category ($0{\cdot}06 < P < 0{\cdot}60$).

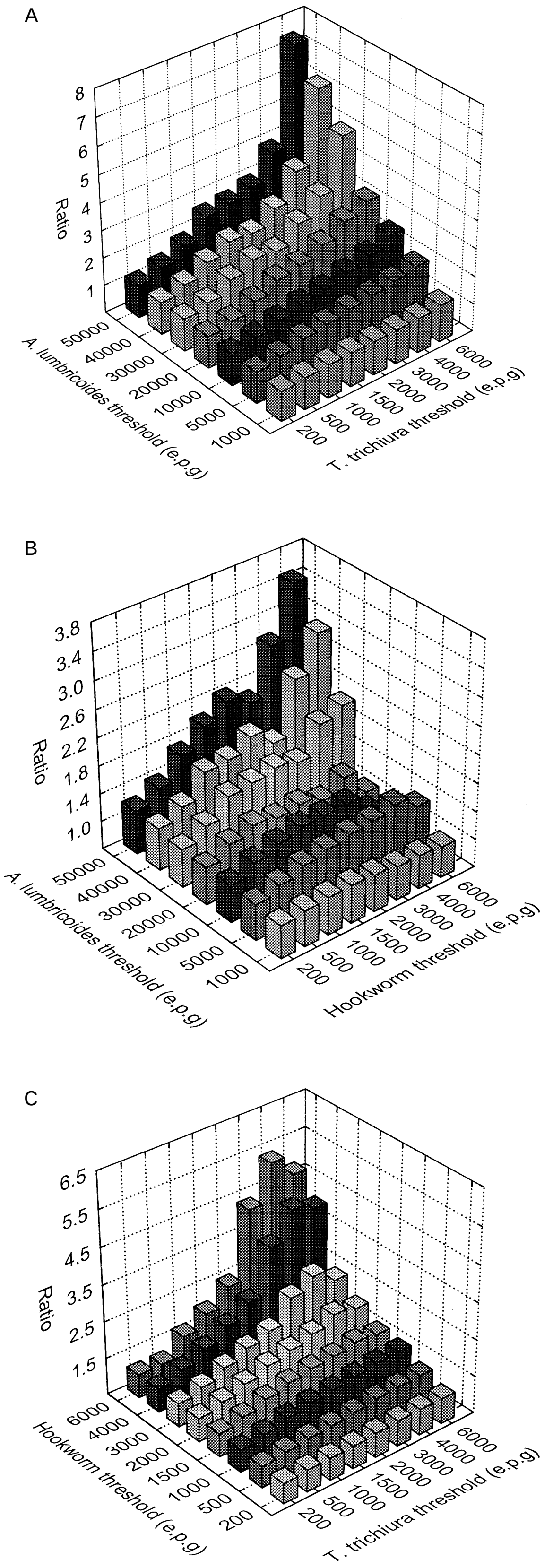

Fig. 2. Results of ratio analysis for *Ascaris lumbricoides* and *Trichuris trichiura* infections (A), *A. lumbricoides* and hookworm infections (B), and *T. trichiura* and hookworm infections (C). Each plot depicts the ratio of observed to expected proportions of the population who had infection intensities of both species which were higher than the thresholds defined in the x and y axes.

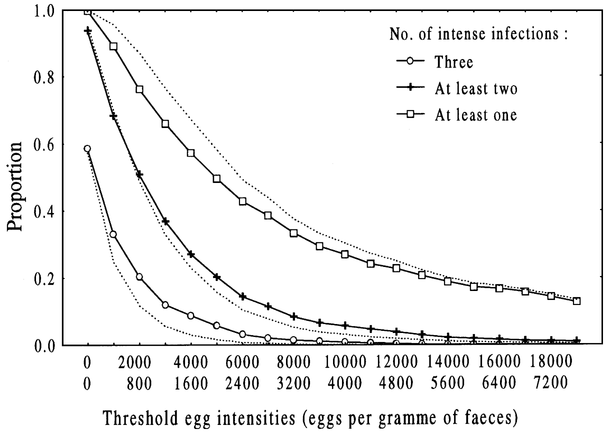

Fig. 3. Results of varying the threshold intensity for each parasite species on the prevalence of intense infections. The top row of threshold values on the x axis correspond to *Ascaris lumbricoides* infection, and the bottom row correspond to *Trichuris trichiura* and hookworm infections. The solid curves describe the proportion carrying at least 1, 2 or 3 intense infections. Dotted curves nearest to each solid curve are the values for each statistic that would be expected if each infection were transmitted independently.

The corresponding multiple-comparison test results for *T. trichiura* and hookworm infections are given in Table 4. It is clear from information contained in Fig. 1 that individuals with *T. trichiura* infection alone had lower infection intensities of this parasite than individuals with multi-species infections. Individuals infected by *T. trichiura* and hookworms had significantly lower infection intensities of *T. trichiura* than individuals infected by all 3 species. Hookworm egg counts were lower in single-species infections than in infections involving all 3 infections or infections involving hookworms and *T. trichiura* only.

Ratio analysis

Figure 2 displays the results of the ratio analyses of 3-dimensional plots. Each plot is characterized by 2 features. First, each ratio value is higher than 1, indicating that more individuals had intense infections of both species than would be expected by chance, irrespective of the definition of an intense infection. Second, the ratios increase non-linearly as the threshold definitions of intense infections increase for each species.

Chi-square analysis confirmed that most ratios were significantly higher than 1. For *A. lumbricoides* and *T. trichiura* infections, the only non-significant results (a total of 4) were obtained when low threshold values for 1 species were tested against high threshold values for the other species. Only 3 tests were non-significant for *T. trichiura* and hookworm infections, obtained when testing high hookworm thresholds against low *T. trichiura* thresholds. Ten tests were non-significant between

Table 4. Results of multiple comparison analysis for *Trichuris trichiura* and hookworm infections

(Each cell of the table contains a test statistic: details of their calculation are given by Zar (1996). Row and column headings denote infection combinations which were tested (Asc, *Ascaris lumbricoides*; Tri, *T. trichiura*; Hkw, hookworm). The number of individuals with each combination of infections is given in parentheses. Significant test values are indicated by asterisks. The magnitude of each significant result can be gauged from Fig. 1.)

T. trichiura	Asc + Tri + Hkw (904)	Tri + Hkw (417)	Tri + Asc (107)
Tri alone (70)	4·621***	3·736**	2·608†
Tri + Asc (107)	1·687	0·449	
Tri + Hkw (417)	2·727*		
Hookworm	Asc + Tri + Hkw (904)	Hkw + Tri (417)	Hkw + Asc (17)
Hkw alone (17)	2·855*	2·825*	0·972
Hkw + Asc (17)	1·493	1·070	
Hkw + Tri (417)	1·701		

† Borderline significance (critical Q value = 2·639).

A. lumbricoides and hookworm infections. These occurred when medium *A. lumbricoides* thresholds were tested against high hookworm thresholds. No correction for age was necessary, since age was not related to the frequency distribution of infection intensities for any species.

Prevalence of multiple-intense infections

The results of varying intense-infection thresholds on the prevalence of multiple-intense infections are shown in Fig. 3. The expected proportion closely follows the observed proportion for each statistic. However, due to the weak clustering effect described above, the overall proportion with at least 1 intense infection is always lower than expected, whereas the proportion with 2 or 3 intense infections is always greater than expected. At high thresholds, the proportion carrying at least 1 infection remains elevated, yet it is clear that if high thresholds are used for each species to define an intense infection, most individuals carry an intense infection of only 1 species. Note that despite the increase in the degree of clustering at high thresholds (Fig. 2), the observed and expected curves almost converge for each statistic in Fig. 3. This is to be expected since the number of people with very intense infections was relatively low. Again, no correction for age effects was necessary, for the reason given above.

DISCUSSION

The overall objective of this analysis was to estimate the extent to which infections of *A. lumbricoides*, *T. trichiura* and hookworm infections were associated in a population of school children from Pemba Island, Tanzania. Each component of the analysis is discussed below, and inferences are made on the likely distribution of geohelminth-related morbidity in the community at the time of the study.

Poisson regression indicated that certain combinations of infections did not occur more or less often than expected by chance. This is not surprising, given the very high prevalence of both hookworms and *T. trichiura* infections. Thus, the freedom to test for associations at this level was limited. At lower infection prevalences, *A. lumbricoides* and *T. trichiura* are often found together more often than expected by chance (Ashford, Craig & Oppenheimer, 1992; Kightlinger, Seed & Kightlinger, 1995), although the degree of clustering is generally small (Booth & Bundy, 1995). Our subsequent observation of intense-infection clustering suggests that, even if infection probabilities appear to be unrelated, there may still be inter-species associations in hosts with multiple infections.

Age was found not to be a significant confounding factor in any respect of the analysis. Although the data in the present study came from a narrow age window, this observation concords with a previous analysis (Booth & Bundy, 1995). In most endemic areas, younger children have lower levels of infection, and hookworm infections in particular are not often highly prevalent in the age classes represented in the present study (Bundy, 1990). In this respect the endemic situation on Pemba is unusual, but there is no *a-priori* reason why this should lead to confounding. Geohelminth life-span is typically much shorter than human longevity, and even a lengthy duration in an endemic area does not necessarily lead to an accumulation of multiple species within a host.

Additional analysis focused on associations among parasite species within the infected host population. At the simplest level, we correlated egg counts of different species. Correlation coefficients were typically low, and similar to those estimated in other studies (Holland *et al*. 1987, 1989; Robertson *et al*. 1989; Kightlinger *et al*. 1995). A number of factors, including non-homogenous mixing of eggs in the stool, the age of the parasites (Sinniah & Subramaniam, 1991), and daily fluctuations in egg output (Sinniah, 1982; Hall, 1981) may have contributed to the observed scatter in the data. Nonetheless, the results are strongly indicative of a positive correlation between worm burdens.

We also observed that individuals with single-species infections had generally lower infection intensities than individuals with multiple-species infections. Since the analysis ruled out the potentially confounding effect of age, this observation could reflect the result of recent treatment for geohelminth infections, low level exposure, or reduced risk of infection upon exposure, in some individuals. The first explanation is the least likely, since treatment for geohelminth infections was not widely available at the time of the study. The second explanation could arise from a number of behavioural factors, including geophagia (Wong, Bundy & Golden, 1991). The third explanation would imply heterogeneity in immune responses to infection.

The latter 2 explanations are potential mechanisms for generating long-term predisposition to 1 or more infections. Our observation that intense infections of different species were clustered supports evidence of predisposition to multiple species, as demonstrated in a small number of studies (Haswell-Elkins, Elkins & Anderson, 1987; Chan, Kan & Bundy, 1992; Chan *et al*. 1994). However, analysis of data from a follow up study (Albonico *et al*. 1995) would be required for confirmation.

Our observation that the degree of clustering increased with infection intensity implies that the more serious cases of each infection occurred in a very small proportion of the population. Despite this, we can conclude that most individuals at risk of morbidity were only at risk from 1 species, since the overall level of clustering was low. As the threshold defining an intense infection increased for each species, the proportion of the population carrying multiple intense infections rapidly declined, whereas the proportion carrying only 1 intense infection declined much more slowly. This pattern can be expected in any area where the level of infection clustering is low, and suggests that the overall morbidity burden in a community where multiple geohelminth species are endemic will be widely distributed through the infected population.

We can also infer the likely distribution of geohelminth-related morbidity in relation to the different infection categories. Individuals with only 1 species had generally lower infection intensities, and are therefore likely to have made up the group at lowest risk of morbidity. Individuals with double- and triple-species infections had similar infection intensities of each species, and therefore the effect on the hosts' health is likely to have been a function of the total number of infections. Whether harbouring 3 infections increased the risk of suffering a defined symptom, or whether it increased the range of symptoms, cannot be determined from these data. In situations where clinical and parasitological data are available, methods of risk-analysis, such as applied to schistosome infections (Booth *et al*. 1996; Guyatt *et al*. 1995), may be useful.

Overall, our results suggest that individuals with multiple-species geohelminth infections were at highest risk of morbidity, although only a small proportion of that group were at risk of morbidity associated with each species they carried. Since there were relatively few individuals with single-species infections, it remains to be seen whether this conclusion is valid in areas where the different infection categories are more evenly represented. Once this is ascertained, it will improve prospects for evaluating the benefits of intervention programmes which are targeted at multiple geohelminth infections simultaneously.

M.B. was in receipt of a SERC CASE award at the time of this study, with additional support from SmithKline & Beecham. Some analysis was undertaken while in receipt of a Wellcome Trust Travelling Fellowship. Thanks go to the Pemba Helminth Control Team for the parasitology, and to Heide Stirnadel for the SAS programming. D.A.P.B. acknowledges the support of the Wellcome Trust.

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