Analysis of the prevalence of infection and associations between human gastrointestinal nematodes among different age classes living in the urban and suburban communities of Port Harcourt, Nigeria

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Abstract

Fresh stool samples from 300 subjects presenting at out-patient clinics in two major hospitals in Port Harcourt were analysed for the prevalence and intensity of gastrointestinal (GI) nematode infections and 85.3% were found to carry at least one of the four species of GI nematodes recognized during the study. The most common species was *Ascaris lumbricoides* (54%), followed by *Trichuris trichiura* (43.7%) and *Necator americanus* (42.7%) and lastly *Strongyloides stercoralis* (33%). Peak prevalence for *A. lumbricoides* was among the ≤9 years age cohorts, whilst those for the remaining species all coincided among the 15-19 years age cohort. The frequencies of single, double and triple-species infection categories, across the species and within most of the specific combinations were lower than the expected frequencies calculated from overall prevalence data for each species. Observed cases of non-infected individuals and those carrying all four species were higher than expected, suggesting that a greater than expected subset of the population was free from infection, particularly among subjects ≥30 years old and another subset more prone to infection by all four species, predominantly among the age cohorts ≤29 years. *N. americanus* and *T. trichiura* were more commonly encountered among multiple species infection combinations than expected, but the intensity of the former declined as the number of other concurrently infecting species increased. In contrast, the intensity of infection with *S. stercoralis* increased as the number of other species increased. Positive associations between *A. lumbricoides* and *T. trichiura*, *N. americanus* and *S. stercoralis*, and *T. trichiura* and *N. americanus* were identified in respect of greater than expected co-occurrence of these combinations and significant positive correlations between their respective intensities of infection. These were related to specific age cohorts which were identified as particularly prone to multiple infections.

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Introduction

Intestinal helminthiases are widespread in rural communities in most third world countries. Among these helminths, the triad of *Ascaris lumbricoides*–hookworm–*Trichuris trichiura* have been identified as the commonest gastrointestinal (GI) nematodes which, by WHO statistics, infect a significant proportion of the world population. It is estimated that approximately one billion (10^9), 900 million, and 500 million persons are infected by *A. lumbricoides*, hookworm, and *T. trichiura* respectively (Bundy *et al.*, 1991). In many endemic areas of the tropics, environmental contamination interacts with human behaviour and low socio-economic living standards to provide situations which favour the transmission of these parasites. In consequence, a complex picture is generated in which combinations of these species affect sectors of the community prone to particular activities or characterized by specific socio-economic standards (Holland & Asaolu, 1990; Killewo *et al.*, 1991). Although there are data to indicate the existence of predisposition to multiple GI nematode infections (Haswell-Elkins *et al.*, 1987) the factors involved are not clearly understood. As Bundy *et al.* (1991) observed, the proportion of concurrent multiple infections exhibits considerable geographical variation, but is usually highest in areas where the prevalence of GI helminths is also high.

Numerous studies have documented the high prevalence of GI nematode infections in Nigeria (Holland & Asaolu, 1990; Onubogu, 1978; Oyerinde, 1978; Nwosu & Anya, 1980; Udonsi, 1983, 1984, 1985; Udonsi & Amabibi, 1992). In a study evaluating the benefits of Primary Health Care intervention in controlling GI nematodes, Udonsi & Ogan (1993) reported high prevalence for *A. lumbricoides*, *N. americanus* and *T. trichiura* among communities in the vicinity of Port Harcourt and drew attention to the large numbers of individuals carrying concurrent infections with two or more GI nematode species. This observation motivated further studies to elucidate the underlying causes of the concurrent infections in the Port Harcourt metropolis where high transmission rates are usual. New data gathered from the same communities are analysed in the present report with three principal objectives: firstly to establish whether the frequencies of single and multiple infection cases are consistent with those that would be predicted from prevalence data, secondly to establish whether associations between GI nematodes exist in this region and thirdly to determine whether any specific age classes were particularly prone to multiple infections.

Materials and methods

Study site and population

The study was carried out in Port Harcourt metropolis, an industrial Atlantic coastal city in the mangrove forest zone of the Niger Delta of Nigeria. As a state capital city, Port Harcourt comprises a conurbation of numerous suburban villages and communities including Diobu, Rumuolumeni, Nkpolu and Rumuola. The inhabitants of these villages and communities are subsistence farmers, fishermen, artisans, and local craftsmen. Although the standard of sanitation in the city is fairly high, that of the suburbs is of an inferior standard in which open-air defaecation still occurs indiscriminately. Environmental contamination with the infective stages of GI nematodes is therefore high in these suburban districts. Only two government owned hospitals serve the city and the surrounding communities: the University Teaching Hospital, and the Braithwaite Memorial Hospital. Crowded out-patient departments are common in both hospitals. It is these out-patients that formed the study population.

Collection of stool samples

Stool samples were collected from 300 outpatients (152 males and 148 females and covering an age range from <1 to >60 years) attending morning clinics in both hospitals between August 1991 and June 1992. Each hospital was visited weekly and stool samples collected from the subjects after consultation with the medical directors. On each sampling morning, at least 20 wide-mouth screw top plastic containers were given to randomly selected out-patients of both sexes and different ages to produce stools in nearby hospital toilets after registering their cards. Each stool sample was duly identified by name, sex, age, occupation and locality of donor, and was taken to the parasitology laboratory of the University of Port Harcourt, a distance of 20 km from the city, for immediate examination.

Analysis of stool samples

Stool samples were examined by two routine methods, the thin smear method with normal saline, and the MFC (merthiolate-formaldehyde-concentration) technique of Blagg *et al.*, (1955). Each stool sample was first examined by a quick saline smear method which detected nematode eggs in heavier infections. When no eggs were detected at first examination, the more sensitive MFC technique was applied as this has been shown to give a higher egg recovery than the saline smear method (Udonsi, 1984). Prevalence data is based on the examination of three slides from each stool sample using each method. Semi-quantitative data are based on the total number of eggs/larvae of each species detected by the saline smear method after the detailed examination of approximately uniform volumes of faecal material corresponding by weight to 0.02 g from each specimen. Since the amount of faecal material sampled was approximately equivalent (as adjudged by eye by experienced microscopists), the data for faecal egg/larval counts were analysed as raw values or after adjustment for faecal consistency. Stool samples were categorized into three types and adjusted as follows: formed × 1, soft formed × 1.5, diarrhoeic × 2. The eggs of the three nematodes - *N. americanus*, *A. lumbricoides*, and *T. trichiura* were identified by their individual characteristic features. The immediate examination of the stool samples enabled the detection of the rhabditiform larvae of *S. stercoralis* larvae, which were recognized by their characteristically short buccal cavity. These were the only

nematode larvae observed in fresh stools. All negative samples were examined further before they were discarded.

Statistical analysis

The prevalence data for each species were used to calculate expected frequencies for the co-occurrence of all the combinations of double, triple and quadruple species infections in the study group. Expected frequencies for occurrence of single, double, triple and quadruple infection categories for all combinations of parasites were calculated by a process of step-wise deletion, beginning with the group carrying all four species. Where relevant, frequencies were compared by either 2 × 2 or 1 × n contingency tables, employing the actual numbers of subjects in relevant categories. However, for ease of interpretation, data are mostly presented as percentages. Age-related changes were examined following stratification of the study population into eight age cohorts with age limits and sample sizes as summarized in table 1. Semi-quantitative data were examined using non-parametric statistics. The Spearman rank-order correlation coefficient (r_s) was used to evaluate the strength of associations between two variables and the Kruskal-Wallis (K-W ANOVA, one-way analysis of variance by ranks) was employed to assess the influence of factors (e.g. age-cohorts, other taxa) on specified variables. A P value ≤0.05 was considered to be significant.

Results

Prevalence of the four species of GI nematodes

The overall prevalence of each species in the study group is given in table 2. It is apparent that $A. lumbricoides$ (54.0%) was the most common and $S. stercoralis$ (33%) the least common species in these communities. The age-prevalence profile for each species is illustrated in fig. 1. $A. lumbricoides$ was the most common species among the three age cohorts corresponding to subjects ≤14 years, with a prevalence of 79.3–86.4% among these groups. Prevalence then declined steadily to a minimum of 16.7% among the oldest individuals in the study. The three remaining species showed an increase in prevalence from the youngest children to a peak among the 15–19 years old group, then a decline followed by a plateau among subjects ≥30 years.

The number of GI nematode species harboured by subjects

Of the study group of 300 subjects, 256 (85.3%) were infected with at least one species of GI nematode and the overall mean no. of species was 1.7 ± 0.06 per subject. Fig. 2 shows this broken down by age cohort and analysis revealed that there was a significant age effect (K-W ANOVA, H=56.237, P<0.001). The youngest children (≤5 years) showed a mean of 1.8, rising to a peak of 2.5 in the 15–19 years age cohort before declining to a plateaux just above a mean of 1 in the three cohorts encompassing subjects ≥30 years. Overall, there was a significant decline in the number of species harboured with age (r_s=-0.29, n=300, P<0.00001).

The relative frequency of uninfected subjects compared with those carrying one or multiple GI nematode infections

The number of subjects carrying one to four species, together with uninfected subjects are illustrated in fig. 3 alongside the expected numbers calculated from overall prevalence data. The combined observed prevalence of GI nematodes (85.3%) was significantly lower than expected (90.0%) from calculations based on the observed

Table 1. Distribution of subjects among age cohorts.

Cohort	Limits of age	Actual range of age	No. of subjects	Mean age ±	S.E.M.
1	<5	5 months–4.5	46	2.1	0.184
2	5–9	5–9	22	6.9	0.31
3	10–14	10–14	29	11.9	0.20
4	15–19	15–19	43	17.0	0.21
5	20–29	20–29	45	23.41	0.47
6	30–39	30–39	50	33.6	0.43
7	40–59	41–59	35	50.9	0.9
8	≥60	60–101	30	72.5	2.02

Table 2. Prevalence of GI nematodes and the frequency of occurence of single species infections in the total and among specific infection subsets comprising the study group.

Species	No. of cases observed	Prevalence (% of total study group) (n=300)	% of subjects carrying ≥1 species (n=256)	Expected[1] no. of subjects	Observed no. of subjects	% of all subjects carrying species	% of total single species infections (n=85)
$A. lumbricoides$	162	54.0	63.3	35.0	35	21.6	41.2
$T. trichiura$	131	43.7	51.2	23.1	18	13.7	21.2
$S. stercoralis$	99	33.0	38.7	14.7	17	17.2	20
$N. americanus$	128	42.7	50.0	22.1	15	11.7	17.6

The header "Single species infections" spans the Expected and Observed columns, and "Observed" spans the last four columns.

[1]Expected values were calculated from the observed prevalence data for each species and were then compared with observed values for occurrence of single species infections by a 1 × 4 contingency table and χ^2=3.77, df=3, P=NS.
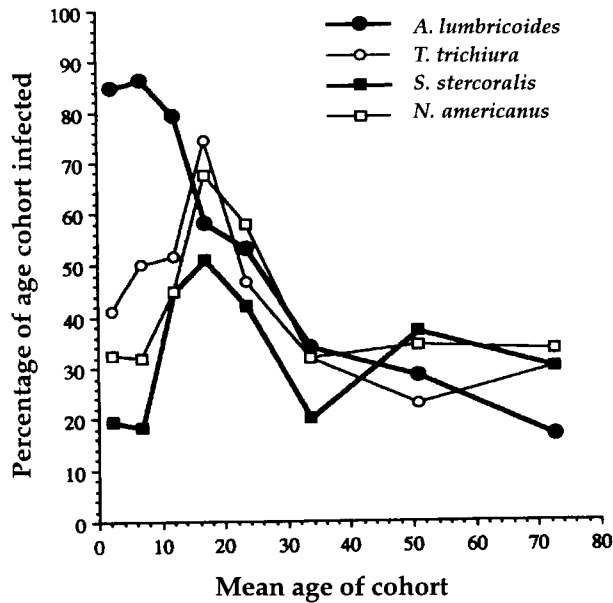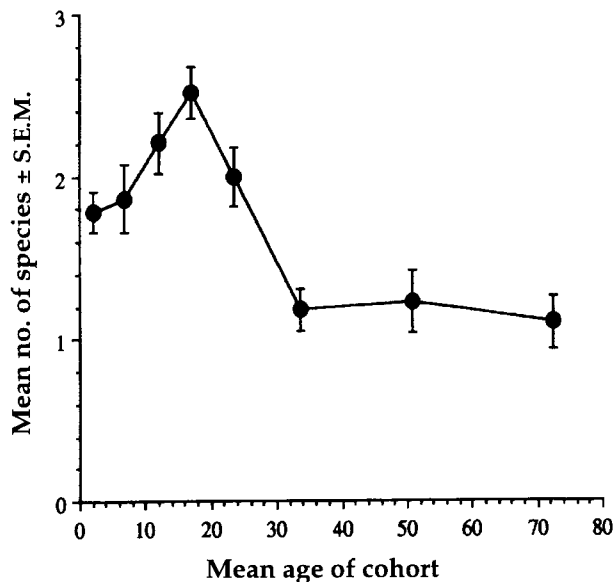
Fig. 1. Age-stratified prevalence of the four GI nematodes in the study.

Fig. 2. Age-dependent changes in the number of GI nematode species harboured by the subjects in the study.
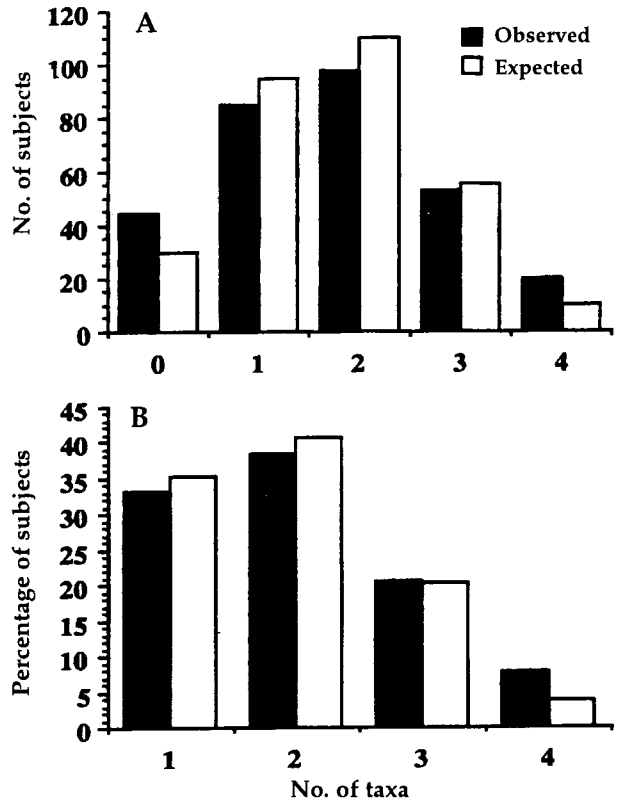

Fig. 3. The distribution of subjects among uninfected, single and multiple GI nematode infection categories. A. Observed and expected numbers of uninfected subjects and subjects infected with 1–4 species in the entire study group (n = 300). B. The percentage distribution of observed and expected numbers of subjects in relation to observed (n = 256) and expected (n = 270) total sample sizes for infected individuals. Expected numbers were calculated as described in the text.

The frequency of occurrence of cases of single and multiple infections

Table 2 also presents data on the occurrence of single species infections. Eighty five individuals carried only one species of parasite, compared to the expected number of 95, the former representing 28.3% (expected = 31.6%) of the study group. The most frequent was *A. lumbricoides* accounting for 41.2% of all cases of single infections. There was little to distinguish between the other three species in this context. The observed numbers in each species category corresponded well with expected values calculated from overall prevalence rates. Despite the relatively large number of individuals in the single species infection category carrying *A. lumbricoides* (35 subjects), this exactly matched the predicted number. In contrast *N. americanus* and *T. trichiura* were both under-represented (observed vs expected, 15 vs 22 and 18 vs 23.1, respectively). Overall single-species infections showed a similar prevalence across all the age cohorts (fig. 4a), with a noticeable dip among the two cohorts corresponding to 15–29 age range, when multiple infections were more common.
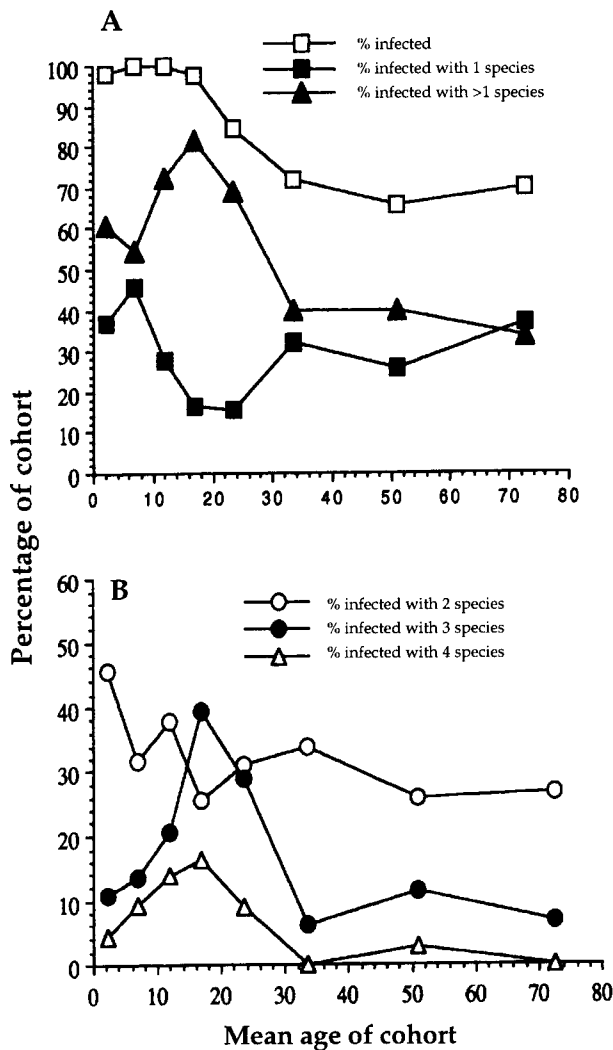
frequencies of each of the four species (χ^2=7.26, df=1, P<0.01). Fig. 4 shows that the highest prevalence of infection was encountered among the four age cohorts ≤ 19 years, among which only 2 subjects of 140 examined (combined prevalence=98.6%) were without evident infections. Nevertheless, although uninfected individuals were mostly encountered in the >30 years age cohorts, the prevalence of GI nematode infections did not decline below 65% even among the older subjects.

Fig. 4. Age-stratified prevalence of various combinations of 0–4 species of GI nematodes.

Table 3. Relationship between intensity of infection and number of concurrently infecting species.

	Kruskal Wallis ANOVA		Spearman rank-order correlation	
	H	P	r_s	P^1
A. lumbricoides	1.197	0.75	–	–
T. trichiura	2.30	0.51	–	–
S. stercoralis	7.58	0.056	+0.236	0.019
N. americanus	7.58	0.056	–0.210	0.017

^{1}Probabilities are two-tailed since no *a priori* assumptions could be made about the relationships.

infections did not differ significantly from expected values calculated from overall prevalence data. Triple-species infections were predominantly encountered among the younger sectors of the community, peak prevalence being among the 15–19 years age cohort, 39.5% of which harboured at least three species of GI nematodes (fig. 4b). Of the 53 triple-species infections in the study, 83% were encountered among subjects ≤29 years.

Comparison of observed and expected numbers of subjects in each infection category by a 1 × 5 contingency table indicated significant departure of observed from expected figures (χ^2 = 18.968, df = 4, 0.01 > P > 0.001). Observed categories for one to three helminths were all lower than expected partially because the total of uninfected subjects was greater than expected and because there were twice as many subjects as expected carrying all four GI nematodes. Thus subjects carrying all four species of GI nematodes comprised the rarest group with only 20 (6.7% of the study group) such individuals recorded. However, from the overall frequencies of individual species in the study group we would have expected only ten persons to comprise this subset (fig. 3) and therefore quadruple-species combinations occurred twice as frequently as expected. The majority of these were encountered among the younger sectors of the community, 95% being associated with subjects ≤29 years, and a peak in the 10–19 years age cohorts.

Distribution of the four GI species across infection categories

The observed and expected distribution of subjects infected with each of the four species across the infection categories was also considered (data not shown). With the exception of *S. stercoralis* (single species category) all other species either matched exactly the predicted numbers in single and double species infections or, more often, were under-represented. Observed values for *A. lumbricoides*, *T. trichiura* and *S. stercoralis* in the triple species combination category were also lower than expected. All four species, however, were over-represented in the quadruple species combination.

Fifty seven percent of subjects had more than just one species of parasite (expected 58.4%) and there was a clear peak for the prevalence of multiple infections among the three age cohorts corresponding to the 10–29 years age range (fig. 4a). The largest group (98 subjects) comprised those carrying two species (32.7%, compared with expected 36.7%; fig. 3), but the frequencies of double-species infections did not differ significantly from expected values calculated from overall prevalence data. Double-species infections were most common among the youngest age cohort (45.7%) but then showed only a relatively slow decline to 25.7% among the 40–59 years age cohort (fig. 4b).

Fifty three subjects, representing 17.7% of the study group (fig. 3), were infected with three species of GI nematodes, matching closely the expected 55. The most frequent combination was *A. lumbricoides*, *T. trichiura* and *N. americanus* which represented 41.5% of all triple species combinations (table 4). The frequencies of triple-species

Age associated changes in intensity of infection of the 4 GI species

The intensities of infection with *A. lumbricoides* and *T. trichiura* were both affected significantly by age-cohort (K-W ANOVA, *H*=62.7, *P*<0.00001 and *H*=29.9, *P*<0.0001,

Table 4. Frequency of occurrence of double and triple species infections in the total study group and among specific infection subsets.

Species 1	Species 2	Species 3	Expected[1] no.of subjects	Observed no.of subjects	% of all infected subjects (n=256)	% of total double/triple species infections (n=98, 53 resp.)
A. lumbricoides	T. trichiura	–	27.2	31	12.1	31.6
A. lumbricoides	S. stercoralis	–	17.2	12	4.7	12.2
A. lumbricoides	N. americanus	–	26.1	19	7.4	19.4
T. trichiura	S. stercoralis	–	11.4	8	3.1	8.2
T. trichiura	N. americanus	–	17.3	17	6.6	17.3
S. stercoralis	N. americanus	–	10.9	11	4.3	11.2
A. lumbricoides	T. trichiura	S. stercoralis	13.4	7	2.7	13.2
A. lumbricoides	T. trichiura	N. americanus	20.3	22	8.6	41.5
A. lumbricoides	S. stercoralis	N. americanus	12.9	16	6.3	30.2
T. trichuira	S. stercoralis	N. americanus	8.5	8	3.1	15.1

Expected values were calculated from observed prevalence data and were then compared with observed values for occurrence of double and triple combinations of species by 1 × 6 and 1 × 4 contingency tables respectively. For double species combinations χ^2=5.05, df=5, P=NS. For triple species combinations χ^2=3.97, df=3, P=NS.

respectively) and both declined significantly with increasing age (r_s=-0.44, n=300, P<0.00001 and r_s=-0.185, P<0.001, respectively). Peak intensity in both species was encountered in the 5–9 age cohort. The intensity of infection with S. stercoralis was also affected by age cohort (K-W ANOVA, H=17.9, P= 0.01) but the effect was weaker than in the preceding species and there was no significant correlation with age. This arose because the infections peaked among the 10–29 year age cohorts. In contrast N. americanus infections were not affected by age cohort, intensity being similar among all the age cohorts examined.

Relationship between the intensity of infection with specified parasites and number of other concurrently infecting species

We examined the hypothesis that the intensity of infection with a specific parasite is likely to be affected by the number of other taxa harboured concurrently. For each species only subjects carrying that particular species were selected and the K-W ANOVA was applied to determine whether the intensity of infection varied between subjects infected only by the species in question (one taxon), in the presence of one other species (two taxa), two other species (three taxa) or when all four were present (four taxa). The results are summarized in table 3. A significant effect of number of concurrently infecting species was detected only for S. stercoralis and N. americanus. In order to identify the direction of the relationship, the data were also subjected to analysis by the Spearman rank-order correlation test and for both of these species a significant effect was detected, but in opposite directions. Thus the intensity of infection with N. americanus was highest in subjects harbouring no other species and then declined with subjects carrying additional species. On the other hand the lightest infections with S. stercoralis were observed in subjects carrying S. stercoralis alone and the heaviest in those subjects which had all four species present.

Associations between pairs of parasites as reflected in prevalence

With four species of parasites affecting the subjects in the study, six combinations of pairs of species were possible. We made two a priori predictions, namely that A. lumbricoides should be positively associated with T. trichiura because of the mode of transmission via resistant eggs (but see also Booth & Bundy, 1992) and similarly that N. americanus should be associated positively with S. stercoralis because of the similarity in their mode of transmission via short-lived host-seeking larvae. All other combinations were examined a posteriori and significance assessed by the two-tailed test. The combinations of double infections, uncomplicated by the presence of other species, and their frequencies of occurrence are shown in table 4. The most frequent combination was A. lumbricoides and T. trichiura which represented 31.6% of all double species combinations. This combination was particularly prevalent among the younger sectors of the population, 77.4% of all cases being identified in subjects ≤29 years old, and 41.9% in the ≤9 years age cohorts. The largest discrepancy was in the combination A. lumbricoides and N. americanus which fell seven subjects below the expected number.

Associations between pairs of species were also examined for the entire data set, for each comparison ignoring the presence of other species. These data are summarized in table 5. There was a significant positive association between A. lumbricoides and T. trichiura, and between N. americanus and S. stercoralis as expected. 67.3% of all cases of the latter combination were among the 10–29 years age cohorts, but cases of this combination were otherwise encountered across the whole age spectrum. Surprisingly N. americanus also showed a significant positive association with T. trichiura, and this combination was also frequent among the 10–29 years age cohorts (70.1% of cases) but especially among the 15–19 years age cohort which accounted for 35.8% of all

Table 5. Pairwise comparisons of observed and expected frequencies for all possible double combinations of the four GI species.

Species 1[1]		A. lumbricoides		T. trichiura		S. stercoralis		N. americanus	
		Observed[2]	Expected	Observed	Expected	Observed	Expected	Observed	Expected
A. lumbricoides	+			49.4	43.7[3]	34	33	47.5	42.7
A. lumbricoides	–			37.0	43.7	31.9	33	37.0	42.7
T. trichiura	+	61.1	54[3]			32.8	33	51.2	42.7[4]
T. trichiura	–	48.5	54			33.1	33	36.1	42.7
S. stercoralis	+	55.6	54	43.4	43.7			55.6	42.7[5]
S. stercoralis	–	53.2	54	43.8	43.7			36.3	42.7
N. americanus	+	60.2	54	52.3	43.7[4]	43.0	33[5]		
N. americanus	–	49.4	54	37.2	43.7	25.6	33		

1. For species 1 + = present, – = absent.
2. Data are presented as percentages for ease of comparison. Statistical tests, χ^2 2 × 2 contingency table, were carried out on the raw data.
3. $\chi^2=4.68$, $P <0.05$
4. $\chi^2=6.83$, $P <0.01$
5. $\chi^2=10.03$, $P <0.01$

Table 6. Analaysis of correlation between the intensity of infection in all possible double combinations of the four GI species.

Species 1	Species 2	All subjects carrying combination[1]			Subjects carrying only specified combination[2]		
		r_s	n	P	r_s	n	P
A. lumbricoides	T. trichiura	0.264	80	0.009[3]	0.400	31	0.013[3]
A. lumbricoides	S. stercoralis	0.278	55	0.04	0.493	12	0.104
A. lumbricoides	N. americanus	0.219	77	0.055	0.156	19	0.523
T. trichiura	S. stercoralis	0.147	43	0.35	–0.278	8	0.505
T. trichiura	N. americanus	0.319	67	0.008	0.582	17	0.014
N. americanus	S. stercoralis	0.203	55	0.069[3]	0.598	11	0.026[3]

[1]Data for all subjects carrying the combination specified, including subjects infected >2 species.
[2]Data for subjects carrying only the combination specified and no other species.
[3]One-tailed tests for these comparisons. Two-tailed for all others.

cases. In each combination the associations were positive in so far as a greater number of subjects than expected harboured both species concurrently.

Associations between pairs of parasites as reflected in intensity

In order to determine whether the associations between species were also reflected in terms of intensity of infection, data from subjects carrying specified pairs of parasites were examined in two separate analyses (table 6). The first included all subjects with relevant combinations of just two species, including subjects carrying other species (i.e. those infected with three and four species) and the second considered only subjects with specified combinations and no other species. Each was analysed by the Spearman rank-order test. The results in table 6 show that our two predictions were borne out, but unexpectedly, the strongest association was found between T. trichiura and N. americanus.

Discussion

The study reported in this paper was based on individuals who presented at the two major hospitals in Port Harcourt for a variety of reasons, mostly connected with emergency illnesses and accidents, and who therefore comprised a selected subset of the population as a whole, only on the basis that they required treatment during the period of the study. This approach of using hospital patients to obtain data on the prevalence of parasitic diseases has been employed previously (Cowper & Woodward, 1961; Obiamiwe, 1977). It is important to note that none of our patients came to hospital for the specific treatment of GI nematode infections nor symptoms related to the diseases associated with these nematodes. The subjects were equally balanced as far as sexes were concerned and came from all age groups in the range <1 through to >60 years. They were chosen randomly, although an attempt was made throughout to balance

groups for age and gender. Because the subjects were registered in hospitals by trained staff, we are totally confident that their individual records were reliably registered. We are also confident that three separate examinations of each stool using two different methods gave accurate results for prevalence and that our semi-quantitative measurements of parasite eggs/larvae reflected relative differences in worm burden. Overall, we feel that the individuals who comprised the study population represented a cross-section of the communities living in the metropolis of Port Harcourt and its suburbs.

The combined prevalence of the four species of GI nematodes (85.3% of subjects carried at least one species) was much as expected from previous studies, considering that a proportion of the individuals came from affluent sectors of the community with better quality housing. In village as opposed to urban communities a higher prevalence would have been expected (Udonsi, 1983, 1984) particularly with *N. americanus*, conditions for trans-mission in the former environment being more conducive than in the urban centres. The prevalence of *S. stercoralis* was relatively high in comparison to studies in other parts of the world but consistent with previous reports for this region of Nigeria (Udonsi & Morgan, 1985). The present analysis concentrated on the proportion of the study group falling into single and multiple worm infections, on age-related distribution of the parasites individually and in combinations and on the co-occurrence of the four species of parasites. We were able to predict the number of persons who should have been infected with at least one species of parasite from the observed frequencies for each species and found that combined prevalence should have been higher than that detected (270 [90%] vs 256 [85.3%] individuals respectively). Since prediction of the number of infected individuals depended on observed prevalence for each species, we infer that combined prevalence was less than expected because more individuals than expected were less prone to infection and because of associations between combinations of parasites.

Expected and observed data both indicated that double species infections should have been and were the most commonly encountered combination as a whole (fig. 3) and for each of the individual species. Most double species combinations, uncomplicated by the presence of other species, were much as expected, even following compensation for differences between observed and expected sample sizes. This agrees with the data of Kvalsvig (1988) who found that among primary school children in South Africa, affected by 14 species of protozoan as well as helminth parasites, the most frequent combination was for two species to be present. In our study, double-species infections were most common in the <5 years age group, followed by a slowly declining frequency into the older age cohorts (fig. 4b). However, Kan (1989) reporting on prevalence of GI nematodes among plantation workers and their families in West Malaysia, found that single species infections were more common, although in his study the prevalence for each species was lower than that which we recorded (36.4% for *T. trichiura*, 33.9% for *A. lumbricoides* and 15.6% for hookworms). The numbers of triple-species combinations were similar to expected numbers but quadruple-species infections were more common than expected, both combinations being

aggregated in the ≤29 year age cohorts with a peak prevalence in 15–19 years age cohort. The shift towards four species combinations was also apparent after compensation for differences between expected and observed numbers of infected subjects, through expression of data as a percentage of the total infected group in each case (n=270 and 256 respectively). This population, therefore, harboured a greater number of individuals than expected who developed no patent infection as well as those who were predisposed to combined infection by all four parasite species: the former were mostly adults ≥30 years old, the latter teenagers and young adults ≤29 year age. The triple- and quadruple-species combinations, in particular, were aggregated in the ≤29 years age cohorts.

In an attempt to clarify these conclusions we sought to identify the species mainly responsible for the interactions between combinations of parasites. *A. lumbricoides* was the most frequently encountered of the four GI nematodes, particularly prevalent in the ≤14 year age cohorts, and not surprisingly therefore, was the most common among subjects carrying just one species (41.2%). Single species infections with *A. lumbricoides* were twice as common as those of the other parasites (table 2) and among persons infected with *Ascaris*, proportionally more were found in the single species infection category than for the other three species (21.6%) but the numbers underlying the latter were expected. However, *A. lumbricoides* was encountered more frequently than expected from overall prevalence data among subjects with *T. trichiura* (table 5) and there were marginally more individuals than expected for the combination *A. lumbricoides* and *T. trichiura* (31 vs 27.2) in the absence of other species (table 4). In fact, this combination was the most common among subjects carrying two species (31.6% of all double species infections), in agreement with, but lower than, that reported by earlier authors (Kan, 1989–60.6%; Kan 1984– 95.7–100%). The combination was also frequently encountered in the very young age cohorts (≤9 years). Whilst, perhaps surprisingly in view of the above, the intensity of *A. lumbricoides* infections did not increase with the number of concurrently residing species (table 3), there was a positive association with intensity of *T. trichiura* (table 6). Both species are transmitted by eggs which take time to embryonate to the infective stage once voided in host faeces and whose environmentally resistant trans-mission stages require to be ingested for infections to be initiated. Whilst the parasites differ in their basic reproductive rates and in their fecundities, the related transmission strategies generate similar age-intensity and age-prevalence profiles in affected communities with peaks of both parameters among children and teenagers (Bundy, 1990), as was found in the present study, and the two species are often encountered in association with each other (Booth & Bundy, 1992). It is very likely, therefore, that the transmission of *A. lumbricoides* and *T. trichiura* were linked in our communities, because of the high risk of exposure and susceptibility of the ≤29 years, but particularly the 5–14, years age cohorts to infection. This was also reflected in the high prevalence of all four species in these sectors of the community, i.e. the aggregation of the triple and quadruple-species infections among these age cohorts. The association between *A. lumbricoides* and *T. trichiura* is consistent with the data of Haswell-Elkins *et al.*

(1987), Robertson *et al.* (1989) and Booth & Bundy (1992) but contrasts with that of Croll & Ghadirian (1981) and Barnish & Ashford (1990), neither study finding evidence of an association between these species.

N. americanus showed the greatest departure from expected numbers among single species infections (table 2) indicating that proportionally more cases were located among multiple species combinations (88.3% of *N. americanus* cases were either two-, three- or four- species infections cf expected 82.7%). However, the intensity of *N. americanus* infections declined significantly as the number of concurrently infecting species increased (table 3) and thus on average the most intense hookworm infections were encountered among subjects carrying no other species. This might suggest a negative interaction with one or a combination of the other species, but no such relationship was identified when each of the other species was considered in pairwise comparisons in patients concurrently infected with hookworms, either in the presence (table 5) or absence (table 4) of other concurrently infecting species. In fact, as predicted, *N. americanus* was found more frequently than expected in association with *S. stercoralis* (table 5). Although only 11 subjects carried the combination and no other species, a significant positive association between the intensity of these two species was identified (table 6). The association between the skin penetrating *N. americanus* and *S. stercoralis* was expected because in communities where both parasites are available, individuals who do not wear shoes are likely to become infected with both following frequent exposures on peridomestic farmlands (Killewo *et al.*, 1991). Nevertheless, our data contrast with those reported by Akogun (1989) who found that in an area of Nigeria where overall prevalence of GI nematodes was lower than in our study (39%), *S. stercoralis* was never encountered in multiple worm combinations.

Unexpectedly, an association of hookworms with *T. trichiura* was evident in both the frequency of occurrence of *N. americanus* among subjects with *T. trichiura* (table 5), although this was not apparent among subjects with just these two and no other parasites (table 4), and in the strong positive relationship when the semi-quantitative egg count data were analysed (table 6). This finding contrasts with earlier reports that the distribution of hookworms is largely independent of *T. trichiura* (Haswell-Elkins *et al.*, 1987; Booth & Bundy, 1992) but is consistent with the data of Robertson *et al.* (1989) who concluded that positive associations with other GI nematodes were most evident in subjects carrying *T. trichiura*. In our study *T. trichiura* was also encountered more frequently than expected among multiple worm combinations than in single species infections (observed 86.3% vs expected 82.4%) and it is likely that the association between *N. americanus* and *T. trichiura* arose primarily through their co-occurrence in the 10–29 years old subset of the population (70.1% of the *N. americanus*–*T. trichiura* combination was detected in this age group) showing high frequency of three- and four-species combinations.

Finally, this study has drawn attention to the high frequency of multiple GI nematode infections among the individuals of communities in the metropolis of Port Harcourt, probably arising through associations between parasites, some of which can be explained through the similarities in their modes of transmission and others which cannot. We conclude that overall prevalence data for individual species predicted reasonably accurately the proportion of individuals who carried single, double and triple infections, with a tendency to over estimate the actual numbers involved in most cases. Prediction of combined prevalence and of those carrying quadruple infections was compounded by the greater than expected frequency of occurrence of uninfected subjects, particularly among adults ≥30 years, as well as those carrying all four parasites, predominantly teenagers and young adults ≤29 years.

Acknowledgements

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