Polyparasitism with *Schistosoma haematobium* and soil-transmitted helminth infections among school children in Loum, Cameroon

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Summary   Prevalence and abundance of *Schistosoma haematobium* and soil-transmitted helminths (STH) were assessed among a total of 1600 pupils (urine, $n = 1190$; faecal samples, $n = 1454$) attending five schools in Loum, Littoral Province, Cameroon, with the specific aim of assessing the extent of polyparasitism and the extent to which infections were focused in particular subsets of the study group. Prevalence of *S. haematobium* was 62.8% with an abundance (arithmetic mean of egg counts) of 54 eggs/10 ml urine. For the STH these were 47.7% and 619 eggs per gram of faeces (EPG) for *Trichuris trichiura*, 65.5% and 3636 EPG for *Ascaris lumbricoides*, and 1.4% and <0.1 EPG for hookworms. Most children (90.3%) were infected with at least one of these four species, the largest proportion (34.3%) carrying two species; 27.4% carried three and 1.1% carried concurrently all four species of parasites. The average number of species harboured increased with age, as did the prevalences of *S. haematobium* and *T. trichiura* but not that of *A. lumbricoides*. All STH showed marked differences in prevalence between the five schools but only *T. trichiura* varied significantly between sexes. Mean abundance of infection varied significantly between age classes, among schools and between the sexes, with females showing heavier mean EPGs for *A. lumbricoides* and *T. trichiura* and males higher mean eggs/10 ml urine for *S. haematobium* infections. A highly significant association was detected between *A. lumbricoides* and *T. trichiura*, that was not context-dependent. This was confirmed in quantitative analyses after controlling for differences in abundance between schools, sexes and age classes. A weaker context-dependent association (prevalence data) was detected between *S. haematobium* and *A. lumbricoides* (sex- and age-dependent) but quantitative associations between these two species, as well as between *S. haematobium* and *T. trichiura*, were not convincing.

keywords schistosomiasis, *Schistosoma haematobium*, soil-transmitted helminthiasis, epidemiology, Cameroon

Introduction

Human communities in the tropical regions of the World, where soil-transmitted helminth (STH) infections are endemic, are seldom afflicted by just one species. It is more usual for several STH to be present in the community concurrently and for the greatest number of subjects to carry simultaneously at least two species (Buck *et al.* 1978; Keusch & Migasena 1982; Kvalsvig 1988; Ashford *et al.* 1992; Chunge *et al.* 1995; Booth *et al.* 1998a,b), especially in developing countries where overall rates of parasitism are high. More disturbingly, recent studies have shown that for some parasites, egg counts are likely to be higher among subjects carrying mixed infections than in subjects

carrying single species infections (Booth *et al.* 1998a; Needham *et al.* 1998). This subset of the community is therefore at high risk of morbidity and, in extreme circumstances, even mortality. Moreover, Howard *et al.* (2001) reviewing the literature on parasitic associations, observed a significant geographical heterogeneity between associations although associations within smaller units such as provinces, districts and ecological zones were largely homogenous (Howard *et al.* 2002). They also recorded increasing strength of associations with increasing intensity of infection.

In Cameroon, schistosomiasis and STH infections are important parasitic diseases. Recent estimates indicate that more than 1.7 million people (of a total of 14 million

inhabitants) in the country are infected with either *Schistosoma haematobium* or *S. mansoni*, 5.6 million with *Ascaris lumbricoides*, 6.5 million with *Trichuris trichiura* and 2.6 million with hookworms (Brooker *et al.* 2000a). However, infections are unevenly distributed within the country. The highest transmission levels of schistosomiasis occur in the northern region, whereas STH infection are more prevalent in the southern part of the country (Ratard *et al.* 1990, 1991; Brooker *et al.* 2000a; Howard *et al.* 2002). Although schistosomiasis is primarily predominant in the north of Cameroon, isolated foci of high prevalence exist in the south equatorial zone of the country, with villages endemic for *S. haematobium* and *S. intercalatum* located in both Littoral and Southwest Provinces. One of the most intense transmission foci is in the town of Loum, which also represents an interesting and unique example reflecting the dynamic epidemiological evolution that may occur in schistosomiasis transmission foci over time. Indeed, before 1968, only *S. intercalatum* occurred in Loum, and subsequently this species was completely replaced by *S. haematobium* through introgressive hybridization (Tchuem Tchuenté *et al.* 1997). However, STH infections were not assessed in Loum in earlier surveys.

As part of our study programme on the epidemiology of schistosomiasis in different ecological zones in Cameroon, we conducted a detailed survey of infection with *S. haematobium* in school children, the subset of the population at highest risk of infection, attending five schools in Loum. We also quantified STH infections, as polyparasitism is known to exist in the region (Howard *et al.* 2002). A key objective of this study was to evaluate the extent to which different helminths co-occur in the community and particularly in certain subsets of the study group. These data will provide an essential background for focussed treatment and control of the parasites among children in Loum and, in the broad context, a useful resource for meta-analyses of geographical variation in associations between helminths in humans.

Materials and methods

Study site

The town of Loum lies at 4 °43′N, 9 °44′E, 100 km inland from Douala, at an altitude of about 180 m. To the north of the town Mount Koupé rises to a height of 2070 m and the source of the Mbette, a small river which passes through Loum, is located on the slopes of this mountain but is reinforced by tributaries from several springs in the town itself. Loum is situated in the equatorial zone, Cameroon-type climate, one of the wettest in the world,

with a rainy season, often heavy, of about 9 months (June–September) followed by a brief dry period.

The town of Loum is subdivided into a number of quarters (Figure 1) and the main transmission sites of schistosomiasis have been identified in those quarters adjacent to the river Mbette and its tributaries.

Study design

In May 2000, schoolchildren from five schools in Loum were invited to participate in the study, and were registered only after the objectives of the study had been explained to them and to their parents or guardian, and full informed consent obtained. To avoid any bias, all the available children attending the schools were examined, almost all were permanent residents and no mass anthelmintic treatment had been conducted in the town before this study. Samples were obtained from 1600 pupils of whom 1454 provided a faecal sample, 1190 a urine sample and 1044 both faecal and urine samples. The composition of the study population is summarized in Table 1 by school and sex. There were significant differences in pupil numbers between the schools as well as among age groups within schools (the school × sex × age interaction in legend to Figures 3 and 4), but the sexes were well balanced within schools.

Collection of stool samples and parasitological examination

Stool and urine samples were collected from each participant and transported to a local field laboratory where all urine and stool samples were examined within 48 h. No preservatives were used. Urine samples were collected in 50 ml plastic screw-cap vials, between 10.00 and 14.00 hours. Each urine sample was agitated to ensure adequate dispersal of eggs, 10 ml of urine were filtered through a Nuclepore® filter, and the filters were examined by microscopy for the presence of eggs. Stool samples were collected in similar screw-cap vials, and examined by a single thick smear technique using a 41.7 mg Kato-Katz template. However, it should be noted that because of logistic constraints it was not always possible to examine these within 30 min and therefore hookworm prevalence and abundance is likely to be underestimated.

Statistical analysis

The frequency distribution of species richness was tested for goodness-of-fit to the null model of Janovy *et al.* (1995) by chi-square method.
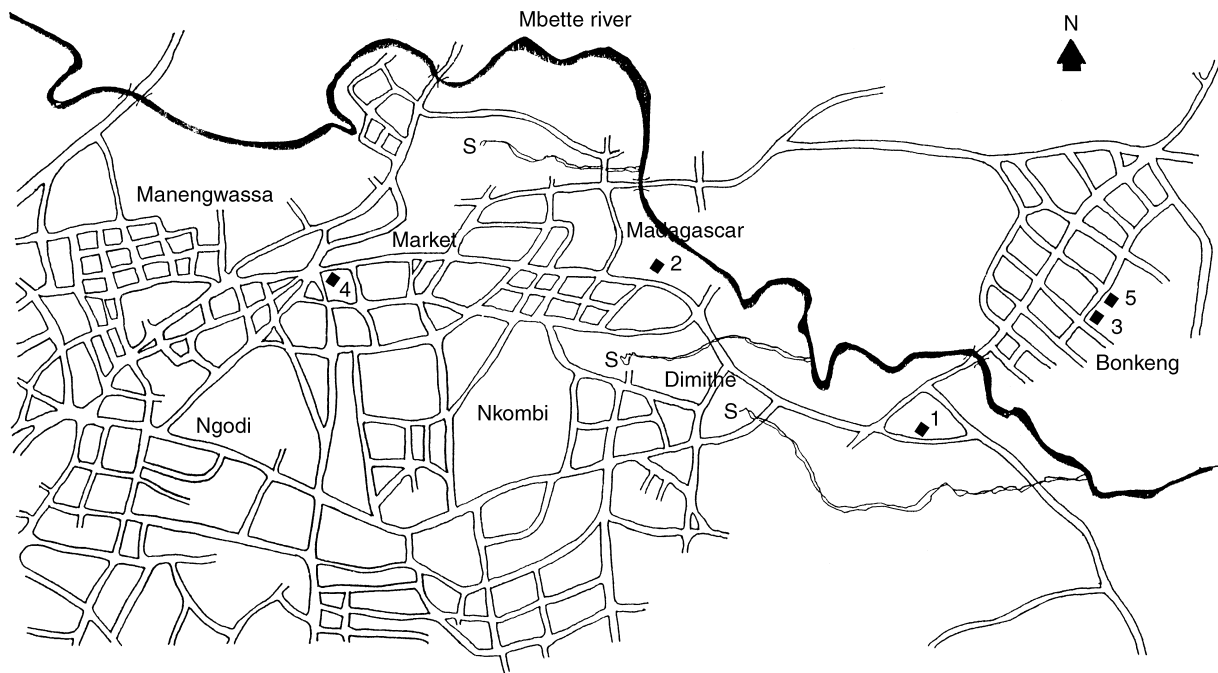
Figure 1 Map of the town of Loum in the Cameroon, showing the location of the five schools that participated in the study. 1, CBC Gare; 2, CEBCE Loum; 3, Ec. Pub. Bonkeng; 4, St J.M. Vianney; 5, St Thomas Bonkeng (♦ school; S, spring; the river is marked in bold).

Table 1 Composition of the study population by school and sex of children in Loum, Cameroon

| School number | School name | Stool examination | | | Urine examination | | |
		Males	Females	Total by school	Males	Females	Total by school
1	CBC Gare	30	26	56	28	22	50
2	CEBEC Loum B	71	71	142	73	78	151
3	Ec. Pub. Bonkeng	172	170	342	159	162	321
4	St JM Vianney	444	420	864	313	297	610
5	St Thomas Bonkeng	21	29	50	26	32	58
Total		738	716	1454	599	591	1190

Confidence limits for prevalence (percentage of subjects infected) were calculated as described by Rohlf and Sokal (1995) and prevalence was analysed by maximum likelihood techniques based on log-linear analysis of contingency tables, implemented by the software package Statgraphics Version 7.0. For each species prevalence of infection (binary factor, infected = 1, not infected = 0), school (five levels, each corresponding to one school), host age (12 levels, for analysis subjects were arranged into 12 classes corresponding to age class 1 = 5 years and less, age class 2 = 6 years etc. until age class 12 = 16 and older) and sex (two levels) were entered as factors. Beginning with the most complex model, involving all possible main effects and interactions, those combinations not contributing significantly to explaining variation in the data were eliminated stepwise, beginning with the highest-level interaction. A minimum sufficient model was then obtained, for which the likelihood ratio of chi-square was not significant, indicating that the model was sufficient in explaining the data.

Quantitative data reflecting parasite abundance within hosts are shown as arithmetic mean values, although geometric mean (GM) values are also provided for comparison because the data were highly overdispersed

(Elliott 1977; Dash *et al.* 1988) and 95% confidence limits (95% CL) are supplied. However, it should be noted that while GM provide a better estimate of the worm burdens of 'average' children, the statistical analysis fits arithmetic mean values and in some cases GM can be misleading indicators of the direction of differences between groups (Fulford 1994). Both mean values reflect the abundance of infection as defined by Margolis *et al.* (1982) and include all subjects within the specified group, infected and not infected, for which relevant data were available. The degree of aggregation in quantitative data was calculated by the index of dispersion (I = the variance to mean ratio) and the index of discrepancy (D) as described by Poulin (1993) (a value of 0 indicates an even distribution of counts across all hosts and a value of 1 indicates all parasites aggregated in a single host). Frequency distributions of individual species were also tested for goodness-of-fit to negative binomial, positive binomial and Poisson models by chi-square as described by Elliott (1977) and the negative binomial exponent k is given as appropriate.

Parasite abundance was analysed by GLIM (a statistical system for generalized linear interactive modelling; GLIM 4, PC version, Royal Statistical Society 1993) using models with negative binomial errors (Crawley 1993; Wilson & Grenfell 1997). School, and host sex (see above for levels) were entered as factors. Age was mostly entered as a factor, although models with age as a covariate were also explored and those employed were school × sex + age class. In some cases, where the iterations in GLIM would not converge satisfactorily, the models were simplified by pooling combinations of age classes as described later. The residuals from all models were checked for negative binomial distribution and were saved for analysis of interactions between species.

Quantitative associations between parasites were examined by correlation analysis (Spearman rank-order correlation test) of raw parasite data from subjects carrying both species and residuals from minimum sufficient ANOVAs in order to control for established differences between the schools, age classes and sex.

Results

Helminth species richness

Four species of helminths were recorded in the survey. The frequency distribution of species richness across this subsample of the population was compared with the distribution predicted by the null model for interactions of parasite species in an assemblage (Figure 2a; Janovy *et al.*
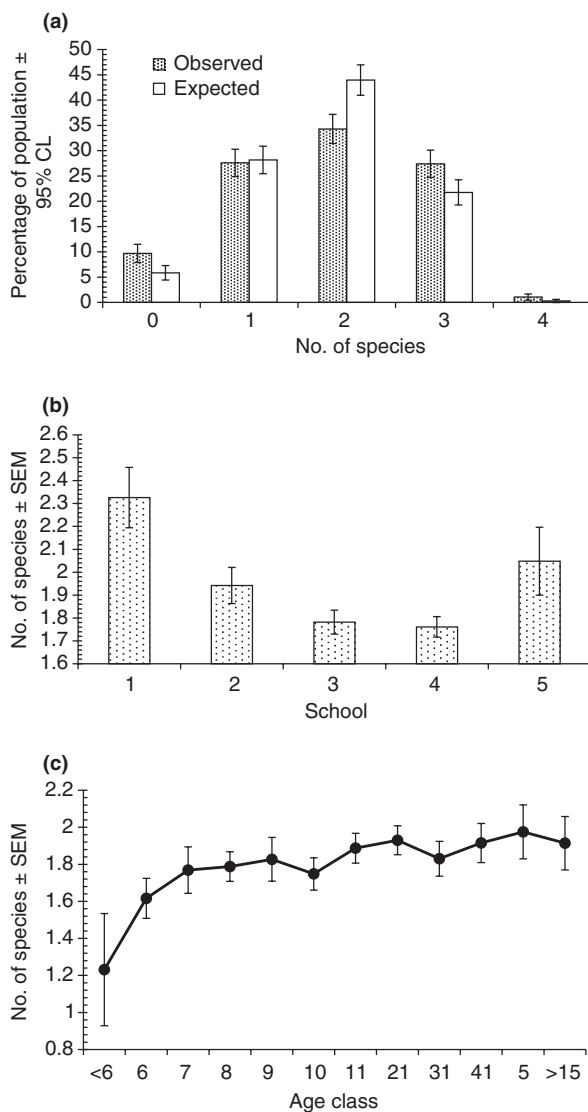
Figure 2 Analysis of species richness. (a) Frequency distribution of observed and expected (null model of Janovy *et al.* 1995) species richness ($\chi_4^2 = 82.7$, $P < 0.001$) based on 1044 subjects that provided both urine and stools. (b) Variation in species richness between schools (GLIM model = school × sex + age with Poisson errors, main effect of school, $\chi_4^2 = 12.3$, $0.025 > P > 0.01$). Schools 1–5 comprised 43, 137, 316, 506 and 42 individuals, respectively. (c) age-dependent variation in species richness (GLIM model = school × sex + age with Poisson errors, main effect of age $\chi_1^2 = 5.8$ ($0.025 > P > 0.01$). The age classes ranged from subjects ≤5 to ≥16 ages as shown and comprised 13, 73, 65, 113, 98, 131, 168, 143, 94, 71, 40 and 35 individuals, respectively.

1995) and differed significantly. Figure 2a shows that more than expected subjects carried three and four worm infections or were not infected.

Mean species richness

The overall mean number of species of helminths harboured per host was 1.8 ± 0.03, with a variance to mean ratio of 0.52. There was a significant difference between the schools (Figure 2b) with children attending schools 1 and 5 (CBC Gare and St Thomas Bonkeng) carrying significantly more species of helminths than those attending the other schools. There were no other significant effects when age was entered as a factor. However, models with age as a covariate (Figure 2c) indicated a significant positive increase in the number of species with increasing age (gradient 0.021 ± 0.009).

Prevalence of infection

The overall prevalence of *S. haematobium* was 62.9% ± 2.7 (*n* = 1190). As Figure 3a shows there was a highly significant effect of age (the age × infection interaction), prevalence increasing from 42.9% among the youngest children (<5) and rising to peak (81.3 ± 11.04%) among the 15-year-old class. There was also a significant difference between the schools. The lowest prevalence was at St JM Vianney G1, the school with the most subjects (51.3% of study group) and the highest at CBC Gare, the school with the fewest subjects (4.2% of study group). Overall there was a significant effect of sample size on prevalence ($r_s = -0.9$, $n = 5$, $P = 0.037$). There was no difference between the sexes (prevalence in males = 62.1% ± 3.9; females = 63.6% ± 3.9).

Only 21 subjects carried the hookworm *Necator americanus* (1.4%) and so no statistical analysis was possible. In both *A. lumbricoides* and *T. trichiura* there were significant differences between the schools (Figures 4 and 5), but only *T. trichiura* showed varying prevalence between the sexes (males = 68.6% ± 3.4, females = 62.4% ± 3.5) and age classes (Figure 5a).

Abundance of infection

As with prevalence there were too few cases of *N. americanus* infection to analyse statistically. All three remaining helminths showed overdispersed distributions that conformed to the negative binomial model (Table 2).

The overall mean for *S. haematobium* egg counts was 54.3 ± 4.14 (GM = 7.1, CL 6.23–8.11) with a range from 0 to 1628 eggs/10 ml. Abundance of infection was highly dependent on host age (Figure 3a), rising irregularly from the youngest age classes and peaking among the 15-year age class. Abundance also differed between the sexes (males = 63.8 ± 7.0, females = 44.6 ± 4.3) and schools but interpretation was confounded by a highly significant

(a)

(b)
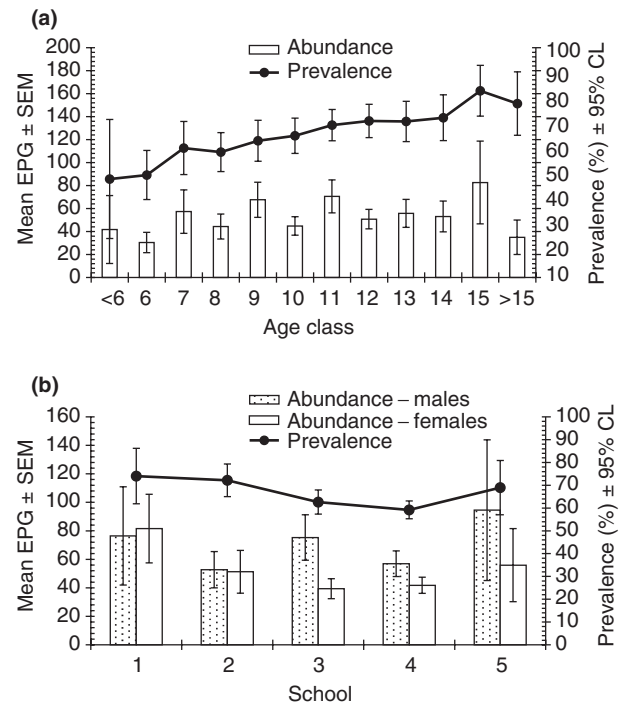
Figure 3 Prevalence and abundance of *Schistosoma haematobium*. (a) Variation in relation to age. For prevalence, age × infection, $\chi^2_{11} = 44.2$, $P < 0.0001$; For abundance, main effect of age, $\chi^2_{11} = 71.07$, $P < 0.0005$. The age classes ranged from subjects ≤5 to ≥16 ages as shown and comprised 14, 83, 71, 132, 116, 154, 184, 160, 109, 82, 48 and 37 individuals, respectively. (b) Variation between schools and sex. For prevalence, school × infection, $\chi^2_4 = 21.74$, $P = 0.0002$; for abundance, main effect of school, $\chi^2_4 = 27.43$, $P < 0.0005$; main effect of sex, $\chi^2_1 = 22.16$, $P < 0.0005$; interaction between school and sex, $\chi^2_4 = 34.69$, $P < 0.0005$. Additional significant terms in the minimum sufficient model for prevalence included a three-way interaction, school × sex × age, $\chi^2_{44} = 67.5$, $P = 0.013$. Test of the goodness-of-fit of the minimum sufficient model gave $\chi^2_{104} = 115.3$, $P = 0.2$. See text for full explanation and Table 1 for *n*.

interaction between school × sex (Figure 3b). The latter arose because the mean egg counts were higher among boys than girls in schools 3, 4 and 5 (accounting for 83.1% of the study group), similar in school 2 and marginally lower in school 1.

The egg counts for *A. lumbricoides* varied from 0 to 101 376 EPG, with a mean of 3636.2 ± 253.5 EPG (GM = 37.2, CL = 30.06–46.06). Attempts to analyse the abundance of *A. lumbricoides* were complicated because GLIM would not converge in models based on 12 age classes. However, a model with four age classes (original 12 classes were merged into 3-year ranges) resolved satisfactorily, and indicated that only the main effects were
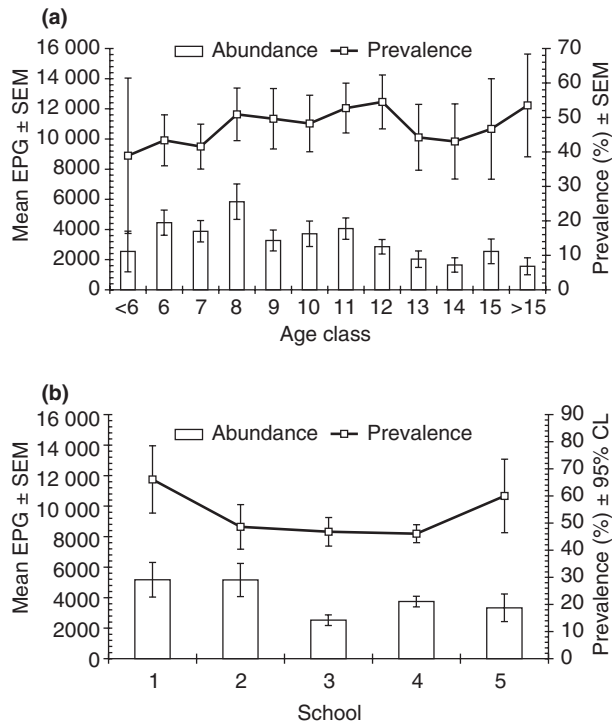
Figure 4 Prevalence and abundance of *Ascaris lumbricoides*.
(a) Variation in relation to age. Prevalence did not vary significantly with age; for abundance, main effect of age, $\chi^2_3 = 59.53$, $P < 0.0005$. The age classes ranged from subjects ≤5 to ≥16 ages as shown and comprised 18, 173, 219, 165, 125, 143, 184, 156, 104, 79, 45 and 43 individuals, respectively. (b) Variation between schools. For prevalence, school × infection, $\chi^2_4 = 11.8$, $P = 0.019$; for abundance, main effect of school, $\chi^2_4 = 23.48$, $P < 0.0005$; main effect of sex, $\chi^2_1 = 8.75$, $0.005 > P > 0.001$. Additional significant terms in the minimum sufficient model for prevalence included a three-way interaction; school × sex × age, $\chi^2_{44} = 76.99$, $P = 0.0015$. Test of the goodness-of-fit of the minimum sufficient model gave $\chi^2_{115} = 108.9$, $P = 0.6$. See text for full explanation and Table 1 for *n*.

significant. Hence, the abundance of *A. lumbricoides* differed significantly between the age classes (Figure 4a) with a clear peak of abundance among the 8-year-old pupils, followed by a reduction to the oldest age class. With both age and school taken into account, females had higher average EPGs than males (mean EPG for males = 3333.3 ± 316.0 and for females 3948.3 ± 298.6), although the GM values of the raw data were very similar (males = 38.5, females = 36.0). As Figure 4b shows, children in schools 1 and 2 had the highest mean egg counts and those in school 3 the lowest (GM egg counts for schools 1–5 were 192.4, 42.4, 32.0, 32.8 and 97.7, respectively).
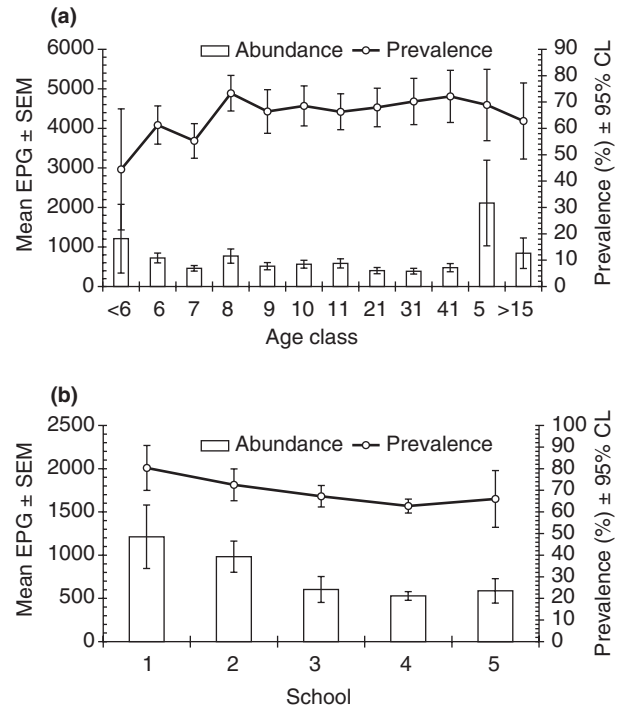
Figure 5 Prevalence and abundance of *Trichuris trichiura*.
(a) Variation in relation to age. For prevalence, age × infection, $\chi^2_{11} = 22.9$, $P = 0.018$; for abundance, main effect of age, $\chi^2_{11} = 479.01$, $P < 0.0005$. The number of subjects within age classes as in Figure 4. (b) Variation between schools. For prevalence, school × infection, $\chi^2_4 = 12.53$, $P = 0.014$; for abundance, main effect of school, $\chi^2_4 = 17.22$, $0.005 > P > 0.001$; main effect of sex, $\chi^2_1 = 4.88$, $0.05 > P > 0.025$; for *n* see Table 1. Additional significant terms in the minimum sufficient model for prevalence included a three-way interaction, school × sex × age $\chi^2_{44} = 76.99$, $P = 0.0015$ and a two-way interaction, sex × infection, $\chi^2_1 = 6.17$, $P = 0.013$. Test of the goodness-of-fit of the minimum sufficient model gave $\chi^2_{103} = 105.95$, $P = 0.4$. See text for full explanation and Table 1 for *n*.

Table 2 Measures of aggregation for individual species of helminths

Species	k ± SEM*	I†	D‡
Schistosoma haematobium	0.22 ± <0.01	376.0	0.808
Ascaris lumbricoides	0.13 ± <0.01	25700.4	0.642
Trichuris trichiura	0.30 ± <0.01	6252.6	0.789

* Negative binomial exponent.
† Index of dispersion = variance to mean ratio.
‡ Index of discrepancy (Poulin 1993).

For *T. trichiura* egg counts ranged from 0 to 42 000 with a mean of 618.9 ± 51.6 (GM EPG = 42.4, CL = 36.21–49.57). There was a significant age effect, mostly because

of the peak egg count among the 15 year age class, but with very little variation in the mean values among the other age classes (Figure 5a). The peak among the 5-year-old children arose because of a relatively small sample ($n = 18$) with two children who had relatively heavy infections (15 120 and 5280 EPGs) that skewed the data disproportionately upwards. There was also a significant difference in abundance between the schools (Figure 5b), with pupils attending schools 1 and 2 showing the highest abundance of *T. trichiura* (GM for schools 1–5 were 147.4, 84.3, 41.5, 34.8 and 50.8, respectively), and a weaker but significant effect of host sex (mean for males = 603.2 ± 59.2 and for females = 635.1 ± 85.2; GM for males = 49.7, females = 36.0).

Interaction between species based on prevalence data

Two log-linear models for co-occurrence were examined: the first included *S. haematobium*, *A. lumbricoides* and *T. trichiura* in a subset of the population that provided both stools and urine ($n = 1044$) and the second was based on a larger subset that provided stools and hence was restricted to *A. lumbricoides* and *T. trichiura*

($n = 1454$). The results (Table 3) indicate only one highly significant interaction between helminths that was not confounded by context, i.e. between *A. lumbricoides* and *T. trichiura*. The prevalence of *T. trichiura* among subjects with *A. lumbricoides* was 80.7% ± 3.4 and 80.3% ± 3.0 for the smaller and larger data sets respectively, compared with 55.6% ± 4.3 and 52.1% ± 3.6 among subjects without *A. lumbricoides*. Model 1 also incorporated a significant interaction between *S. haematobium* and *A. lumbricoides*, but this was dependent on sex and age of the host and significance was considerably lower than the more convincing co-occurrence of *T. trichiura* with *A. lumbricoides*.

Interaction between species based on abundance data

Table 4 summarizes multiple correlation analysis on abundance data for the three species of helminths, employing data from subjects carrying both species in each pair-wise comparison. A highly significant correlation was found between the abundance of infection with *A. lumbricoides* and *T. trichiura* and a weaker correlation

Table 3 Minimum sufficient maximum likelihood statistical model incorporating school, sex and age and either two or three species of helminths. Each line in the upper compartment represents a significant interaction that therefore forms part of the final model

Principal interactions in explaining variation in data*			Test of individual effects		
Extrinsic factors	Intrinsic factors	Parasites	χ^2	d.f.	P†
Model 1					
	Sex, age	*Schistosoma haematobium, Ascaris lumbricoides*	20.52	11	0.0387
School	Sex, age		72.48	44	0.0044
		Ascaris lumbricoides, Trichuris trichiura	77.40	1	<0.0001
School		*Ascaris lumbricoides*	13.44	4	0.0093
School		*Schistosoma haematobium*	20.17	4	0.0005
Model 2					
School	Sex, age		76.97	44	0.0015
		Ascaris lumbricoides, Trichuris trichiura	131.03	1	<0.0001
	Age	*Trichuris trichiura*	22.93	11	0.0181
	Sex	*Trichuris trichiura*	6.17	1	0.013
School		*Trichuris trichiura*	12.53	4	0.0138
Goodness-of-fit of the minimum sufficient model specified by the interactions listed above					
Model 1			583.701	758	1.0‡
Model 2			311.553	341	0.872‡

Model 1 is based on 1044 subjects who provided both urine and faecal samples for analysis. Model 2 is based on 1454 subjects that provided faecal samples.
* School, five levels (five schools); age, 12 levels (12 age classes); sex, two levels (male and female); infection, two levels (infected or uninfected).
† Probability that excluding the effect will make a significant change to the model.
‡ Probability that the data do not differ significantly from the minimum sufficient model described by the interactions listed.

Table 4 Analysis of quantitative associations between species. Each test was carried out on subjects carrying both species in the pair specified

Combination	n*	Raw data		Residuals¶	
		r_s†	P‡	r_s	P
Schistosoma haematobium + *Ascaris lumbricoides*	359	0.145	0.006	0.117	0.027
Schistosoma haematobium + *Trichuris trichiura*	464	0.064	NS§	0.114	0.015
Ascaris lumbricoides + *Trichuris trichiura*	557	0.451	<0.0001	0.389	<0.0001

* Number of subjects carrying both species.
† Spearman's correlation coefficient.
‡ Probability (two-tailed). Applying the Dunn–Sidak correction for multiple comparisons, we accept $P = 0.0169$ as the cut-off for rejecting the null hypothesis (Sokal & Rohlf 1981).
§ Not significant.
¶ Residuals from minimum sufficient ANOVAS in GLIM as explained in the text.

Table 5 Analysis of quantitative associations between species. Mean egg counts in subjects in relation to presence or absence of each of *Schistosoma haematobium*, *Ascaris lumbricoides* and *Trichuris trichiura*

	n	*Schistosoma haematobium*		*Ascaris lumbricoides*		*Trichuris trichiura*	
		AM ± SEM*	GM† (95% CL)	AM ± SEM*	GM† (95% CL)	AM ± SEM*	GM† (95% CL)
Schistosoma haematobium+	661			3746 ± 366	60.6 (44.3–82.8)	725 ± 92.7	57.7 (45.9–72.4)
Schistosoma haematobium−	383			3027 ± 501	21.4 (14.2–32.1)	529 ± 90	35.9 (26.5–48.5)
Ascaris lumbricoides+	519	64 ± 7	9.6 (7.9–11.6)			996 ± 129	126.7 (100.2–160.3)
Ascaris lumbricoides−	525	41 ± 5	5.2 (4.3–6.4)			314 ± 37	18.4 (14.2–23.7)
Trichuris trichiura+	711	56 ± 6	8.0 (6.8–9.5)	4810 ± 420	99.5 (73.4–134.8)		
Trichuris trichiura−	33	45 ± 7	5.4 (4.2–7.0)	647 ± 138	5.8 (3.9–8.4)		

* Arithmetic mean ± standard error of the mean (SEM).
† Geometric mean with lower and upper 95% confidence limits (CL).

between *A. lumbricoides* and *S. haematobium*. These relationships may have arisen because of concentration of particular species in some of the schools in the study or in particular age classes or among one of the sexes. Therefore, to control for school, host age and sex we also correlated the residuals from minimum sufficient ANOVAS described in the earlier sections. The correlation between *A. lumbricoides* and *T. trichiura* remained highly significant, although the correlation coefficient was now lower. That between *A. lumbricoides* and *S. haematobium* it was no longer significant. However, the correlation between *T. trichiura* and *S. haematobium* now showed marginal significance.

Finally, the mean EPGs for each of the species in subjects infected or not infected with *S. haematobium*, *A. lumbricoides* and *T. trichiura* were calculated (Table 5). For this, children that had provided both urine and faecal samples were used ($n = 1044$). It can be seen clearly from Table 5 that in each case, whether arithmetic mean or GMs are considered, subjects infected with the named species (left column) have a higher mean EPG for both of the other species in the study compared with subjects not carrying the named species. In some cases these differences are marked, notably in *T. trichiura* × *A. lumbricoides*. However, as relationships could have been exaggerated or arose through infections being focussed in particular schools, age groups or sexes, the confounding effects were controlled for by examining the residuals from minimum sufficient ANOVAS (Figure 6). Although in each case infections with heterologous species were higher when *S. haematobium*, *A. lumbricoides* or *T. trichiura* were present, the effect was strongest in the relationship between *A. lumbricoides* and *T. trichiura*. Relationships involving *S. haematobium* and STH, were less marked.

Discussion

This study demonstrates that mean species richness increases with age, across the age classes and differs among

L.-A. Tchuem Tchuenté *et al.*   **Polyparasitism in Cameroon**

(a)

(b)

(c)
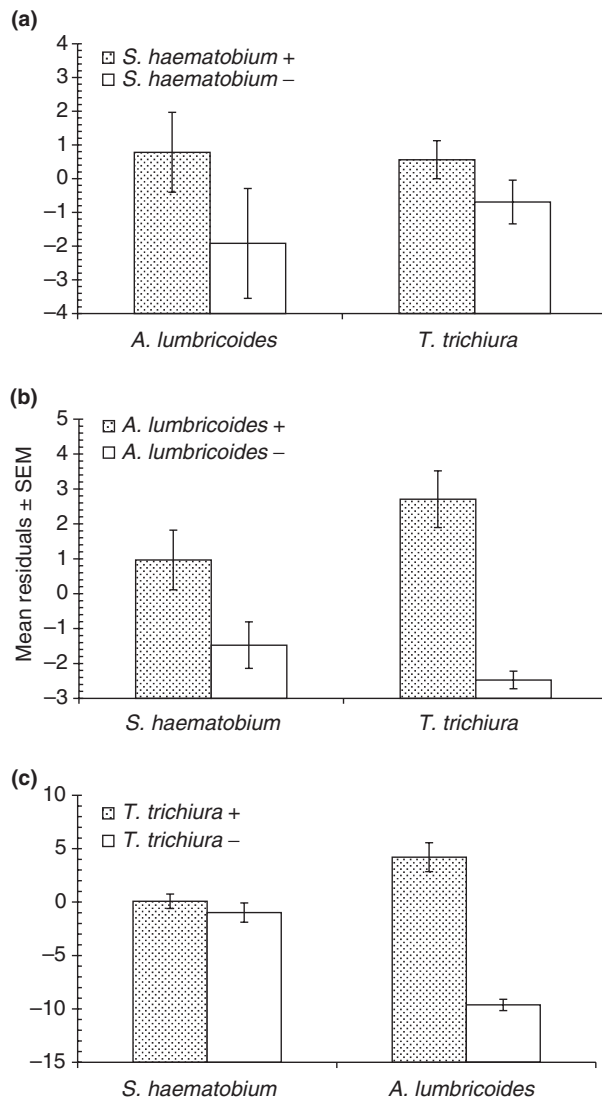
Figure 6 Subjects infected with one helminth species carry heavier infections with other species compared to those without the first species. (a) Subjects with and without *Schistosoma haematobium*; (b) subjects with or without *Ascaris lumbricoides*; (c) subjects with and without *Trichuris trichiura*.

children from five schools in Loum. It established that *S. haematobium* and STH are very common infections among schoolchildren in the town and provides strong evidence that at least two of the species, the soil-transmitted nematodes *A. lumbricoides* and *T. trichiura* not only co-occur, but that infections with each are heavier in the presence of the other and that they are not dependent on host age, sex nor school. Furthermore, it confirms the complete replacement of *S. intercalatum* by

S. haematobium in this focus through introgressive hybridization (Tchuem Tchuenté *et al.* 1997).

Children attending school 1 (CBC Gare) were clearly the worst off, with the highest prevalence and abundance of *S. haematobium*, *A. lumbricoides* and *T. trichiura*. School 2 (CEBEC Loum B) was second in the order for the two STH, although school 5 (St Thomas Bonkeng) showed higher abundance of *S. haematobium*. This school-related distribution pattern of parasites could be explained, at least for schistosomiasis, by the location of the schools in relation to the hydrographical network. School 1 is completely surrounded by the Mbette river, running directly across the town, and its tributaries (Figure 1). School 2 is partly surrounded, whereas schools 3, 4 and 5 are located further, with school 4 being the most distant from the river and its tributaries. Assuming that most children attend the nearest school, and that the closer the school location to water, the higher the water contact, then it is logical that children attending school 1 were the most infected, and those attending school 4 the least infected. Interestingly, the malacological surveys of the Mbette river and its tributaries showed that the snails *Bulinus truncatus* (intermediate host for *S. haematobium*) and *B. forskalii*, were mainly found in the tributaries, suggesting that transmission occurs primarily in these tributaries rather than in the main river. The differences between school 3 and school 5 are difficult to explain as both are very close together. One of the possible reasons could be the difference in the relative social status of the families sending children to these schools, with pupils attending school 5 originating from relatively poorer families. Indeed, it is known that parasitic diseases are strongly associated with poverty, and infections are most common among the poorest and most disadvantaged sectors of the community (Drake *et al.* 2000).

The average value for *S. haematobium* eggs/10 ml of urine was higher among boys than girls. However, this sex effect was reversed for STH with girls showing higher mean EPGs in both cases, although the differences were not marked. At this stage, it is unclear whether this difference can be attributed to differences in host exposure or host susceptibility (Flores *et al.* 2001).

Most children were infected with at least one parasite species but the average number of species harboured (mean species richness) increased with age and consequently many children, particularly in the older age groups, harboured multiple species infections. The analyses showed a highly significant association between *A. lumbricoides* and *T. trichiura*, which corroborates previous work in other endemic regions (Booth & Bundy 1992; Brooker *et al.* 1999, 2000b; Flores *et al.* 2001) as well as in

Cameroon (Howard *et al.* 2002). The positive association between *A. lumbricoides* and *T. trichiura* is likely explained by their common transmission pattern, and could be favoured by behavioural factors. Indeed, *A. lumbricoides* and *T. trichuria* are both transmitted by the faecal–oral route, the eggs of both species are resistant to environmental hazards and can persist for some time in the environment, in dust, soil and on vegetables. Social and behavioural factors that lead to infection with one species will increase the probability of infection with the other species (Booth & Bundy 1995). Perhaps surprisingly, it appeared from our results that there was a significant interaction between *S. haematobium* and *A. lumbricoides*, but this was dependent on sex and age of the host and significance was considerably weaker than the more convincing co-occurrence of *T. trichiura* with *A. lumbricoides*. Such positive associations between *S. haematobium* (which has a different transmission pattern, i.e. infection by percutaneous route) and *A. lumbricoides* and/or *T. trichuria* are unusual. Indeed, comparisons of various published data sets indicate that schistosome and geohelminth infections are usually independently distributed among hosts when they are both present in a community (Brooker *et al.* 1999; Lwambo *et al.* 1999; Keiser *et al.* 2002).

Consistent with other studies we found that subjects carrying any one of the three species were more prone to heavy infections with other species. When bias arising from school, age and sex effects was controlled for, the strongest association was again between *T. trichiura* and *A. lumbricoides*, and very clearly individuals with high intensity of infection with one of these species were significantly more likely to experience high intensity of infection with the other. Similar findings were reported by Needham *et al.* (1998) and Brooker *et al.* (2000b) who showed that subjects with multiple species infections had higher egg counts of each species than those with single species infections. All these observations suggest the occurrence of synergistic interactions in concurrent infections and/or a predisposition to polyparasitism (multiple infections) and heavy infections in some individuals. Predisposition in both schistosomiasis (Tingley *et al.* 1988) and STH infections (Keymer & Pagal 1990) is well established and there is evidence of a genetic basis in some species (Abel & Dessein 1997; Williams-Blangero *et al.* 1999, 2002).

The very high prevalences and abundances of parasitic worm infections observed in Loum raise serious concerns about the resulting morbidity in the schoolchildren in this community. Previous studies have shown that there is an association between the degree of morbidity and the intensity of infections (Ramdath *et al.* 1995), and the degree of morbidity may be related also to the number of different species harboured (Booth *et al.* 1998a). Therefore, it is likely that school children in Loum are subject to significant nutrition, education, developmental and productivity constraints as a consequence of their heavy infections and poor health. In particular, those children with heavy multiple infections are likely to suffer exacerbated morbidity (Drake & Bundy 2001), and their identification will be important for the control of these parasites in the community. A school-based control programme has been launched already in this locality, with emphasis on a multiple species strategy by combining control of both schistosome and STH infections, using two drugs: praziquantel and mebendazole. This multispecies control approach is recognized as the most cost-effective, and future work will evaluate its impact on the transmission levels in this extremely high endemic focus.

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