Advance Access publication 13 July 2004 doi:10.1093/ije/dyh241

Multiple parasite infections and their relationship to self-reported morbidity in a community of rural Côte d'Ivoire

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Accepted 5 May 2004

Background Concomitant parasitic infections are common in the developing world, yet most studies focus on a single parasite in a narrow age group. We investigated the extent of polyparasitism and parasite associations, and related these findings to self-reported morbidity.

Methods Inhabitants of 75 randomly selected households from a single village in western Côte d'Ivoire provided multiple faecal specimens and a single finger prick blood sample. The Kato-Katz technique and a formol-ether concentration method were employed to screen faecal samples for *Schistosoma mansoni*, soil-transmitted helminths and intestinal protozoa. Giemsa-stained blood smears were analysed for malaria parasites. A questionnaire was administered for collection of demographic information and self-reported morbidity indicators.

Results Complete parasitological data were obtained for 500/561 (89.1%) participants, similarly distributed among sex, with an age range from 5 days to 91 years. The prevalences of *Plasmodium falciparum*, hookworms, *Entamoeba histolytica/E. dispar*, and *S. mansoni* were 76.4%, 45.0%, 42.2%, and 39.8%, respectively. Three-quarters of the population harboured three or more parasites concurrently. Multivariate analysis revealed significant associations between several pairs of parasites. Some parasitic infections and the total number of parasites were significantly associated with self-reported morbidity indicators.

Conclusions Our data confirm that polyparasitism is very common in rural Côte d'Ivoire and that people have clear perceptions about the morbidity caused by some of these parasitic infections. Our findings can be used for the design and implementation of sound intervention strategies to mitigate morbidity and co-morbidity.

Keywords Malaria, *Schistosoma mansoni*, soil-transmitted helminths, intestinal protozoa, polyparasitism, self-reported morbidity indicators, infection intensity, Côte d'Ivoire

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Malaria accounts for about half a billion clinical attacks each year, and kills >1 million people, mainly children under the age of 5 years living in sub-Saharan Africa.[1] In view of the rapid spread of antimalarial drug resistance, this situation is likely to worsen.[2] An estimated 2 billion people are affected by schistosomiasis and soil-transmitted helminthiasis, about 300 million of whom are concerned with associated morbidity.[3,4] In children, these parasitic infections can have adverse effects on physical growth and cognitive development.[5,6] Recent analyses suggest that >200 000 people living in sub-Saharan Africa die each year due to kidney dysfunction and haematemesis, which are consequential to sustained schistosome infections.[7] Amoebiasis is caused by the protozoan *Entamoeba histolytica*. The clinical manifestations include diarrhoea, dysentery, amoebid colitis, and liver abscess. It is estimated that the disease kills 40 000–100 000 each year.[8] Giardiasis is a disease that is caused by the protozoan parasite *Giardia duodenalis*, with ~200 million people currently infected. The severe cases manifest acute and persistent diarrhoea, malabsorption of nutrients, and impairment of children's growth and development.[9]

A common feature of the above-mentioned parasitic infections is that they are most prevalent in the developing world, particularly among the poorest segments of rural communities.[10–15] It follows that multiple parasite infections are widespread across diverse ecosystems in the tropics and subtropics. Recent cross-sectional surveys carried out in sub-Saharan Africa consistently confirmed these observations.[16–19] Lack of access to clean water and improved sanitation facilities, and inadequate personal hygiene are important underlying risk factors.[20,21]

Since individuals with multiple parasite infections are often at an elevated risk of morbidity,[22] appraisal of the extent of polyparasitism is a key measure of disease burden, and an important guide for sound control strategies. It should be noted, however, that the number of good quality studies pertaining to parasite communities in entire populations is small compared with the magnitude of this phenomenon and its public health significance. This is explained on several grounds. First, most field workers and research groups have focused on single parasite–single host interactions.[23] Second, interactions between different parasite species are complex, hence challenging to elucidate.[23] Third, there is no readily available diagnostic tool that can be applied on a single bio-fluid or tissue, thereby facilitating simultaneous accurate identification of multiple species parasitic infections. Finally, most previous studies have focused on a narrow age range (e.g. school-age children) rather than on entire populations.[15,24]

Our own cross-sectional surveys carried out in western Côte d'Ivoire confirmed that polyparasitism is very common in this part of the tropics.[18,24,25] For example, in a population sample of 260 individuals screened for *Schistosoma mansoni*, soil-transmitted helminths, and intestinal protozoa, two-thirds were found to harbour at least three parasites concurrently. Here we extend our previous work and provide an in-depth analysis of polyparasitism in a single village, with special reference to interactions between parasites and the relationship to self-reported morbidity. Study participants were screened over several days for the identification of *S. mansoni*, soil-transmitted helminths, intestinal protozoa, and *Plasmodia* infections, and individually interviewed with a questionnaire.

Materials and Methods

Study area and population

Details of the study area and the population surveyed have been described elsewhere.[26] In brief, the study was carried out in May–July 2002 in the village of Zouatta II, located 25 km east of the town of Man in western Côte d'Ivoire. Here, we focus on the cross-sectional baseline survey for assessment of the parasite community, and self-reported morbidity indicators derived from a questionnaire survey carried out simultaneously.

Cross-sectional survey and laboratory procedures

The field procedures have been described previously.[26] In summary, following the village authorities' consent to the study, a demographic survey was carried out in 75 randomly selected households, employing the Expanded Programme on Immunization (EPI) survey approach.[27] Small plastic containers were distributed in the evening, and participants were invited to collect small portions of their faeces the next morning. Faecal collection was repeated over three consecutive days. On the last day, finger prick blood samples were also obtained and thick and thin blood films were prepared on microscope slides.

Faecal and blood specimens were transferred to the laboratory in the town of Man. From each faecal specimen a small amount, weighing 1–2 g, was placed in a plastic tube containing 10 ml of sodium acetate-acetic acid-formalin (SAF).[28] In addition, a single 42 mg Kato-Katz thick smear was prepared according to a standard method (Photo 1).[29] After clearing the slides for 30–45 minutes, they were examined using light microscopy by one of four experienced laboratory technicians for the presence of ova of *S. mansoni* and soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*). Thick and thin blood smears were stained with Giemsa. They were transferred to a reference laboratory in Abidjan, Côte d'Ivoire, and analysed within 4 weeks. Species-specific densities of *Plasmodia* were estimated under a light microscope at high magnification by counting the number of parasites per 200 white blood cells (WBC). If <10 parasites were found the reading was continued up to 500 WBC. These counts were converted to the number of parasites per µl of blood, assuming as standard a WBC count of 8000/µl.

The SAF-conserved faecal samples were transferred to the Swiss Tropical Institute (Basel, Switzerland). They were processed using a formol-ether concentration method,[30] and examined by experienced technicians under a light microscope at high magnification. The presence of helminth ova and intestinal protozoa were recorded, including *Blastocystis hominis, Chilomastix mesnili, Entamoeba coli, Entamoeba hartmanni, Entamoeba histolytica/E. dispar, Endolimax nana, G. duodenalis,* and *Iodamoeba bütschlii*.

Questionnaire survey

A questionnaire was developed and adapted to the current epidemiological setting after discussions with local field assistants who were designated by the village chief. The assistants were trained on how to interview household members and to fill in the questionnaire (Photo 2). It included items on the interviewee's identity and characteristics (i.e. name, age, and sex), common preventive measures against endemic diseases (e.g. sleeping under an insecticide-treated net (ITN) for malaria

prevention), self-reported water contact patterns (e.g. swimming in the rivers in the vicinity of the village) and self-reported morbidity indicators. The latter included eight diseases (chickenpox, diarrhoea, dysentery, malaria, respiratory infections, schistosomiasis, skin disease, and worm infections) and eight symptoms (abdominal pain, blood in stool, convulsions, headache, hot body, lethargy, muscle aches, and vomiting). Participants were asked whether they encountered any of these diseases or symptoms over the past 2–4 weeks.

After pre-testing, the questionnaire was administered and participants were interviewed individually. For children of age ≤5 years, their mothers or legal guardians were interviewed. The present article focuses on the self-reported morbidity indicators, excluding children aged ≤5 years.

Treatment

At the end of the epidemiological and questionnaire surveys, participants infected with *S. mansoni* were treated with praziquantel at a single oral dose of 40 mg/kg.[6] Among the remaining individuals, those who had an infection with soil-transmitted helminths were treated with a single dose of albendazole (400 mg), while the others were given poly-vitamins. Participants who complained of malaria-related symptoms and had an axillary temperature above 37.5°C were administered Nivaquine® and paracetamol, according to current national public health guidelines of Côte d'Ivoire. Our treatment protocols were approved by the internal review boards of the Swiss Tropical Institute (Basel, Switzerland) and the Centre Suisse de Recherches Scientifiques (Abidjan, Côte d'Ivoire), and received ethical clearance from the Ministry of Public Health in Côte d'Ivoire.

Analysis

Double data entry and validation was performed in EpiInfo version 6.04 (Centers for Disease Control and Prevention, Atlanta, USA), and statistical analyses were done with STATA version 7.0 (Stata Corporation, College Station, USA). Only those participants who had complete parasitological data records, namely results derived from at least two Kato-Katz thick smears, one SAF-conserved faecal sample, and one blood smear, were retained for the final analyses.

For each individual, the arithmetic mean egg count of *S. mansoni* was calculated from the Kato-Katz thick smear readings. According to WHO,[6] *S. mansoni*-positive individuals were stratified into three categories: light infections (1–100 eggs/g of faeces[epg]), moderate infections (101–400 epg), and heavy infections (>400 epg). An infection with *A. lumbricoides*, hookworm, or *T. trichiura* was defined as the presence of one or more eggs detected in the Kato-Katz thick smears and/or the formol-ether processed faecal sample. Infections with intestinal protozoa were defined by their presence in the formol-ether processed faecal sample. *Plasmodium falciparum, P. malariae*, and *P. ovale* infections were specified on the basis of blood smear examinations. Infection intensities of *P. falciparum* were stratified into four categories: 1–50, 51–500, 501–5000, and >5000 parasites/μl of blood.

Six age groups were considered: <5, 5–9, 10–14, 15–24, 25–39, and ≥40 years. To compare single parasite infections by sex and age groups, χ^2-test or Fisher's exact test were used as appropriate. Infection intensity categories of *P. falciparum* were compared by sex and age, using a χ^2-test. The frequency of polyparasitism was assessed and stratified by sex and age groups. The relationships between different infection intensity categories of *S. mansoni* and an infection with hookworm or an infection with *E. histolytica/E. dispar* were examined using a χ^2-test. Parasite associations were investigated by fitting logistic regression models for each parasite investigated with all remaining parasites employed as covariates. These models were adjusted for age and sex. A stepwise approach with backward elimination of non-significant covariates was adopted to identify the parasites significantly related to the outcome (parasite under investigation). Covariates were included at a significance level of 0.2. Adjusted odds ratios (OR), including 95% CI, were computed for those associations that resulted in *P*-values < 0.05. Finally, the same logistic regression modelling approach was used to investigate associations between a particular parasite and self-reported morbidity indicators.

Results

Compliance and operational results

Complete parasitological data were obtained from 500 of the 561 individuals, owing to a compliance of 89.1%. Figure 1 shows that those 61 individuals who were excluded for further analyses had only one Kato-Katz thick smear reading (n = 16), lacked a SAF-conserved faecal sample for microscopic examination of soil-transmitted helminths and intestinal protozoa (n = 16), or gave no finger prick blood sample for investigation of malaria parasites (n = 29).

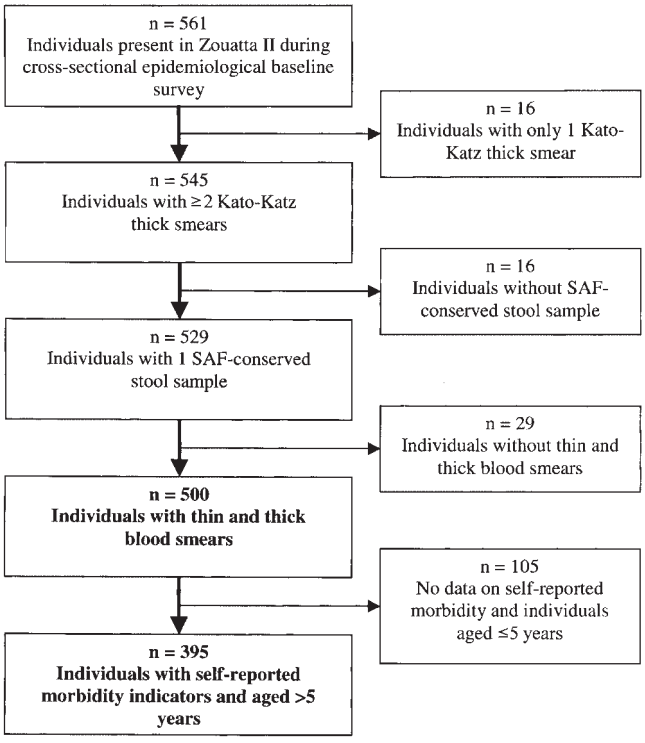

Figure 1 Compliance and study cohort for assessment of polyparasitism, parasite interactions, and associations between infection and self-reported morbidity indicators in a rural community of western Côte d'Ivoire

The final study cohort had 249 males and 251 females. The youngest participant was a newborn of 5 days and the oldest individual was 91 years. Age distribution was as follows: <5 years, n = 88 (17.6%); 5–9 years, n = 88 (17.6%); 10–14 years, n = 67 (13.4%); 15–24 years, n = 58 (11.6%); 25–39 years, n = 95 (19.0%); and ⩾40 years, n = 104 (20.8%). The number of males and females in these age groups showed no statistical significant difference ($\chi^2 = 9.82$, d.f. = 5, $P = 0.080$).

Frequencies of parasites investigated

Table 1 displays the overall prevalences and sex-related differences of each of the parasites investigated. Examination of 2–3 Kato-Katz thick smears per individual revealed eggs of *S. mansoni* in 39.8% of the participants with no significant difference among males and females. The pooled data from the Kato-Katz thick smears and the SAF-conserved faecal sample revealed an overall hookworm prevalence of 45.0%. Males were significantly more often infected with this parasite than females (53.8% versus 36.3%, $P < 0.001$). Infection prevalences of *T. trichiura* and *A. lumbricoides* were low, 6.0% and 2.0%, respectively, with no sex differences. With regard to intestinal protozoa, the highest prevalence was found for *E. coli* (64.4%). This parasite showed a borderline significant sex difference (females: 68.5%, males: 60.2%, $P = 0.053$). High infection prevalences were also found for *E. histolytica/E. dispar* and *B. hominis* with 42.2% and 41.2%, respectively. The overall prevalence of *G. duodenalis* was 10.8% with no difference among sex. A prevalence of 12.6% was found for *E. nana*. Female participants were significantly less often infected with this parasite than their male counterparts (9.6% versus 15.7%, $P = 0.040$).

More than three-quarters of the participants had an infection with *P. falciparum*, similarly distributed by sex. In addition, 11 (2.2%) individuals were found to harbour *P. malariae*, one of whom had a mixed infection with *P. falciparum*, and one individual had an infection with *P. ovale* singly.

Many of the parasites investigated showed significant associations with age categories, namely *S. mansoni* ($\chi^2 = 94.52$, d.f. = 5, $P < 0.001$), hookworm ($\chi^2 = 56.97$, d.f. = 5, $P < 0.001$), and five of the eight intestinal protozoa (*B. hominis, E. coli, E. histolytica/E. dispar, E. nana,* and *G. duodenalis*). Figure 2 depicts the age prevalence curves for each of these seven parasites.

Significant associations between infection prevalence and age groups were observed for *P. falciparum* ($\chi^2 = 16.77$, d.f. = 5, $P = 0.005$), and *P. malariae* ($\chi^2 = 32.86$, d.f. = 5, $P < 0.001$). Furthermore, both *P. falciparum* and *P. malariae* infection intensities were significantly associated with age, i.e. younger age groups showed consistently higher prevalences and infection intensities than their older counterparts. On the other hand, no significant associations were observed with sex (Table 2).

Parasite community

Among the 500 study participants, only 51 (10.2%) were not infected with any of the intestinal parasites (*S. mansoni*, soil-transmitted helminths, and intestinal protozoa). After inclusion of *P. falciparum*, only nine individuals had no infection. There

Table 1 Overall infection prevalence of each parasite investigated and sex-related differences among 500 study participants in the village of Zouatta II, western Côte d'Ivoire

Parasite	Prevalence of infection			
	Overall (95% CI)	Females ($n = 251$)	Males ($n = 249$)	P-value[a]
Schistosoma mansoni	39.8 (35.5, 44.1)	38.3	41.4	0.507
Soil-transmitted helminths				
Hookworm	45.0 (40.6, 49.4)	36.3	53.8	<0.001
Trichuris trichiura	6.0 (3.9, 8.1)	5.6	6.4	0.690
Ascaris lumbricoides	2.0 (0.8, 3.2)	1.6	2.4	0.515
Intestinal protozoa				
Entamoeba coli	64.4 (60.2, 68.6)	68.5	60.2	0.053
Entamoeba histolytica/E. dispar	42.2 (37.9, 46.5)	40.6	43.8	0.478
Blastocystis hominis	41.2 (36.9, 45.5)	41.4	41.0	0.915
Entamoeba hartmanni	23.2 (19.5, 26.9)	25.5	20.9	0.222
Iodamoeba buetschlii	21.2 (17.6, 24.8)	22.3	20.1	0.542
Chilomastix mesnili	14.2 (11.1, 17.3)	14.7	13.7	0.728
Endolimax nana	12.6 (9.7, 15.5)	9.6	15.7	0.040
Giardia duodenalis	10.8 (8.1, 13.5)	10.0	11.7	0.544
Plasmodia				
Plasmodium falciparum	76.4 (72.7, 80.1)	75.3	77.5	0.560
Plasmodium malariae	2.2 (0.9, 3.5)	2.0	2.4	0.750
Plasmodium ovale	0.2 (-0.2, 0.5)	0.4	0.0	1.000[b]

[a] P-value based on χ^2-test.

[b] P-value based on Fisher's exact test.

were 49 individuals (9.8%) with a mono-infection, mainly infants and young children. Three-quarters of the participants harboured three or more parasite species concurrently. There were 11 individuals with a parasite community of 8 species, 4 individuals with 9, and one individual with 10 different species. Figure 3 shows a frequency of species of parasites from male and female participants, which was similar ($\chi^2 = 10.84$, d.f. = 10, $P = 0.370$).

Figure 4 shows that parasite communities among different age groups varied considerably. In general, younger age groups harboured fewer parasite species when compared with older age groups ($\chi^2 = 150.22$, d.f. = 50, $P < 0.001$).

Parasite associations

Table 3 summarizes all significant associations between a particular parasite and any other parasite, sex, and age group. Infections with *S. mansoni* showed significant positive associations with hookworm ($P = 0.003$) and *C. mesnili* ($P = 0.047$). Concurrently, hookworm infections were positively associated with *S. mansoni* ($P = 0.006$). In addition, this parasite showed a significant positive association with *E. coli* ($P = 0.001$). A highly significant positive association was found between *A. lumbricoides* and *T. trichiura* ($P < 0.001$). The latter further showed a significant association with *G. duodenalis* ($P = 0.005$). Significant associations were also observed between *E. histolytica/E. dispar* and *E. coli* ($P < 0.001$), between *G. duodenalis* and *E. hartmanni* ($P = 0.027$), and between *P. falciparum* and *P. malariae* ($P < 0.001$).

Table 4 shows that there was a highly significant association between hookworm infections and the intensity of *S. mansoni* infections ($\chi^2 = 34.59$, d.f. = 3, $P < 0.001$). Thus, high OR were observed when the number of hookworm infections was compared between *S. mansoni*-negative individuals and those with either a moderate (OR = 5.30) or a heavy *S. mansoni* infection (OR = 3.32). In addition, there was a highly significant association between *E. histolytica/E. dispar* infections and

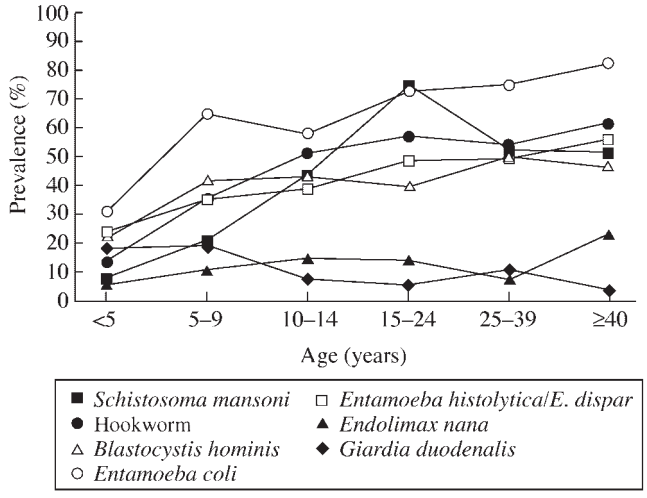

Figure 2 Age prevalence curves

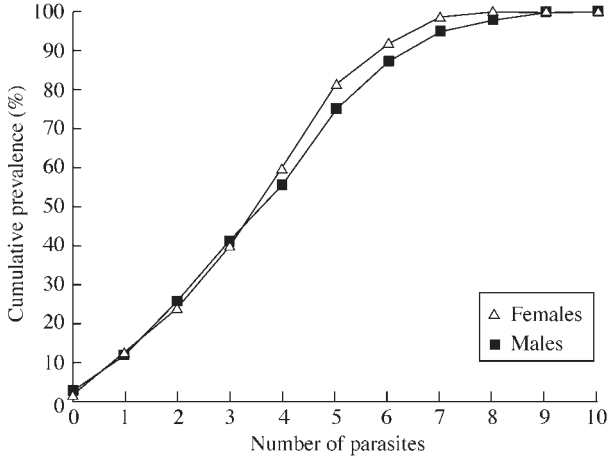

Figure 3 Cumulative frequency (%) of polyparasitism among 500 individuals from the village of Zouatta II, western Côte d'Ivoire, stratified by sex. *Plasmodium malariae* and *P. ovale* were excluded from the analysis

Table 2 Number of individuals (%) with different infection intensities of *Plasmodium falciparum*, stratified by sex and age (n = 489; individuals infected with *P. malariae* only [$n = 10$] or *P. ovale* only [$n = 1$] were excluded)

Variable	Infection intensity of *P. falciparum* (parasites/μl blood)					χ^2	*P*-value
	0	1–50	51–500	501–5000	>5000		
Sex							
Male	51 (10.4)	23 (4.7)	111 (22.7)	50 (10.2)	9 (1.8)		
Female	56 (11.5)	18 (3.7)	106 (21.7)	54 (11.0)	11 (2.2)	1.31	0.860
Age (years)							
<5	7 (1.4)	5 (1.0)	26 (5.3)	25 (5.1)	15 (3.1)		
5–9	10 (2.0)	6 (1.2)	33 (6.7)	35 (7.2)	4 (0.8)		
10–14	12 (2.5)	4 (0.8)	29 (5.9)	21 (4.3)	1 (0.2)		
15–24	16 (3.3)	10 (2.0)	26 (5.3)	5 (1.0)	0		
25–39	30 (6.1)	9 (1.8)	45 (9.2)	11 (2.2)	0		
≥40	32 (6.5)	7 (1.4)	58 (11.9)	7 (1.4)	0	129.00	<0.001
Total	107 (21.9)	41 (8.4)	217 (44.4)	104 (21.3)	20 (4.1)		

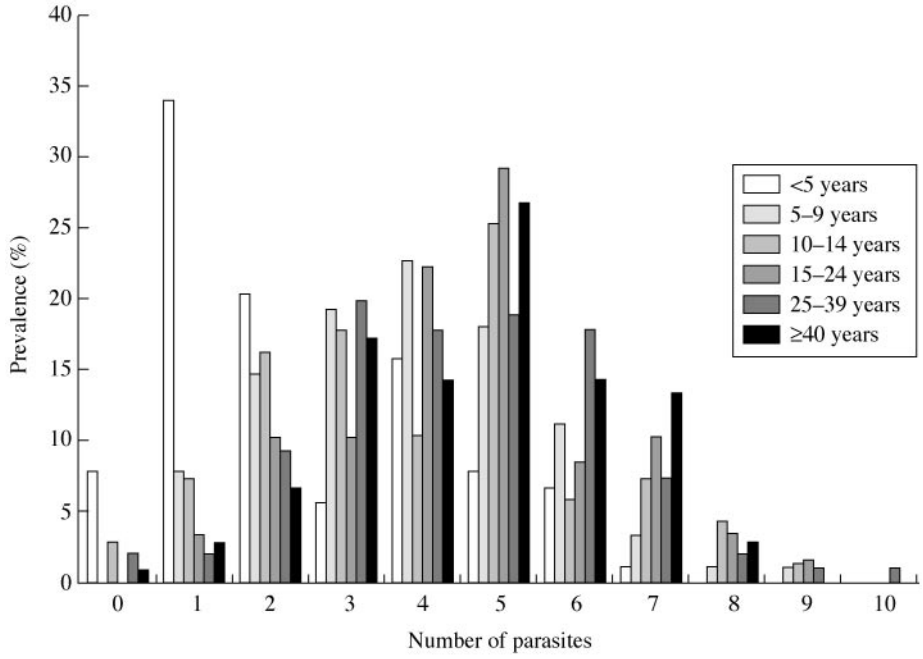

Figure 4 Frequency distribution of parasitic infections among 500 individuals from the village of Zouatta II, western Côte d'Ivoire, stratified by age. *Plasmodium malariae* and *P. ovale* were excluded from the analysis

S. mansoni infection intensities ($\chi^2 = 21.53$, d.f. = 3, $P < 0.001$). While 36.2% of the *S. mansoni*-negative participants were infected with *E. histolytica/E. dispar*, 76.5% of those individuals with heavy *S. mansoni* infections concurrently harboured *E. histolytica/E. dispar*.

Parasite community and self-reported morbidity indicators

Self-reported morbidity indicators were available from 73 of the 75 households. Since children aged ⩽5 years did not respond to the questions themselves, they were excluded for these analyses. On the other hand, those 17 children aged >5 years who had questions responded to by their mothers or guardians were retained for these analyses. The final study cohort consisted of 395 individuals (Figure 1). Table 5 summarizes the results from the multivariate analyses, with an emphasis on significant associations between an infection with a particular parasite and self-reported morbidity indicators after adjusting for sex and age group. Infection with *S. mansoni* was strongly associated with abdominal pain (OR = 2.51, 95% CI: 1.47, 4.29) and dysentery (OR = 2.12, 95% CI: 1.28, 3.51). On the other hand, *S. mansoni*-infected individuals were significantly less likely to report diarrhoea (OR = 0.55, 95% CI: 0.34, 0.89), which is partially explained by other covariates. There was evidence of a positive association between *T. trichiura* infections and blood in stool (OR = 3.46, 95% CI: 1.20, 10.02). Individuals infected with *P. falciparum* were more likely to report convulsions than their non-infected counterparts (OR = 4.84, 95% CI: 1.10, 21.29). In contrast, *P. falciparum*-infected individuals were less likely to report lethargy (OR = 0.43, 95% CI: 0.21, 0.92), but age played a very important role in this association.

The total number of parasites harboured in an individual was strongly associated with self-reported itching illnesses ($\chi^2 = 19.15$, d.f. = 10, $P = 0.038$), and malaria ($\chi^2 = 18.54$, d.f. = 10, $P = 0.046$).

Discussion

The town of Man and surrounding villages and settlements in western Côte d'Ivoire are endemic for *S. mansoni* and soil-transmitted helminth infections, particularly hookworms.[31,32] Consequently, since 2000, district health authorities have implemented a programme to improve access to treatment with praziquantel and albendazole, primarily targeted to the school-age population, with the aim of controlling morbidity among this high-risk group. Unfortunately, due to socio-political unrest commencing in September 2002, treatment campaigns had to be interrupted for more than a year. Previous surveys among schoolchildren and entire communities in several villages in the region of Man also showed that polyparasitism is a common phenomenon.[18,24,25,33] Here, we confirm these observations for the village of Zouatta II; high frequencies of *S. mansoni*, hookworms, *P. falciparum*, and several intestinal protozoa were found, and polyparasitism was very common. In fact, <2% of the 500 study participants had no infection, whereas three-quarters of them were infected with three or more species. The extent of polyparasitism was similar by sex, but was strongly associated with age. Infants and young children were less likely to harbour multiple parasite species when compared with older age groups, probably explained by lower levels and shorter durations of exposure.

Our results were obtained from a cross-sectional survey, employing standardized field procedures, and quality-controlled laboratory procedures that are widely used in population-based epidemiological surveys in the tropics.[6,34] For example, infections with *S. mansoni* were diagnosed with the Kato-Katz

Table 3 Association between a particular parasite investigated and sex, age group, and any of the remaining parasites among 500 study participants from the village of Zouatta II, western Côte d'Ivoire

Parasite	Association	Adjusted odds ratio (95% CI)	*P*-value
Schistosoma mansoni	Hookworm	1.83 (1.22, 1.75)	0.003
	Chilomastix mesnili	1.75 (1.01, 3.04)	0.047
	Age group	1.46 (1.29, 1.64)	<0.001
Soil-transmitted helminths			
Hookworm	*Entamoeba coli*	2.07 (1.33, 3.23)	0.001
	Schistosoma mansoni	1.79 (1.18, 2.68)	0.006
	Age group	1.32 (1.17, 1.48)	<0.001
	Sex	0.40 (0.27, 0.60)	<0.001
Ascaris lumbricoides	*Trichuris trichiura*	15.86 (3.93, 63.99)	<0.001
Trichuris trichiura	*Ascaris lumbricoides*	14.60 (3.74, 57.09)	<0.001
	Giardia duodenalis	3.88 (1.52, 9.90)	0.005
Intestinal protozoa			
Entamoeba histolytica/E. dispar	*Entamoeba coli*	4.56 (2.86, 7.27)	<0.001
	Age group	1.17 (1.04, 1.31)	0.008
Entamoeba hartmanni	*Iodamoeba bütschlii*	2.56 (1.57, 4.15)	<0.001
	Entamoeba coli	2.24 (1.29, 3.90)	0.004
	Giardia duodenalis	2.06 (1.07, 3.97)	0.030
Entamoeba coli	*Entamoeba histolytica/E. dispar*	4.36 (2.68, 7.08)	<0.001
	Chilomastix mesnili	3.58 (1.60, 7.99)	0.002
	Endolimax nana	2.55 (1.17, 5.55)	0.018
	Sex	1.82 (1.17, 2.86)	0.009
	Hookworm	1.79 (1.12, 2.88)	0.018
	Age group	1.32 (1.15, 1.50)	<0.001
Endolimax nana	*Entamoeba coli*	3.14 (1.49, 6.60)	0.003
	Sex	0.48 (0.27, 0.85)	0.012
	Chilomastix mesnili	0.31 (0.12, 0.84)	0.021
Iodamoeba bütschlii	*Entamoeba hartmanni*	2.46 (1.52, 3.99)	<0.001
	Entamoeba coli	1.79 (1.02, 3.14)	0.043
Giardia duodenalis	*Trichuris trichiura*	3.40 (1.28, 9.04)	0.014
	Entamoeba hartmanni	2.15 (1.09, 4.23)	0.027
	Age group	0.67 (0.55, 0.81)	<0.001
Chilomastix mesnili	*Entamoeba coli*	4.18 (1.99, 8.76)	<0.001
	Schistosoma mansoni	1.84 (1.09, 3.11)	0.023
	Endolimax nana	0.36 (0.14, 0.96)	0.041
Blastocystis hominis	*Iodamoeba bütschlii*	1.73 (1.12, 2.69)	0.014
	Age group	1.16 (1.05, 1.29)	0.004
Plasmodia			
Plasmodium falciparum	Age group	0.74 (0.65, 0.85)	<0.001
	Plasmodium malariae	0.01 (0.002, 0.11)	<0.001
Plasmodium malariae	Age group	0.25 (0.10, 0.64)	0.004
	Plasmodium falciparum	0.01 (0.001, 0.13)	<0.001

Table 4 Relationship between the intensity of *Schistosoma mansoni* infections and the presence/absence of hookworm or the presence/absence of *Entamoeba histolytica/E. dispar* infections among 500 study participants from Zouatta II, western Côte d'Ivoire

	***Schistosoma mansoni* infection intensity**[a]				χ^2	*P*-value
	Negative	Light	Moderate	Heavy		
Hookworm positive	107	69	38	11		
Hookworm negative	194	62	13	6		
Odds ratio	1.00	2.02	5.30	3.32	34.59	<0.001
E. histolytica/E. dispar positive	109	57	32	13		
E. histolytica/E. dispar negative	192	74	19	4		
Odds ratio	1.00	1.36	2.97	5.72	21.53	<0.001

[a] Negative: 0 epg, light: 1–100 epg, moderate: 101–400 epg, heavy: >400 epg.

Table 5 Relationship between parasitic infections and self-reported morbidity indicators among 395 study participants from Zouatta II, western Côte d'Ivoire (analysis adjusted for sex and age groups; *Plasmodium malariae* and *P. ovale* were excluded from the models)

Parasite	Association	Adjusted odds ratio (95% CI)	*P*-value
Schistosoma mansoni	Abdominal pain	2.51 (1.47, 4.29)	0.001
	Dysentery	2.12 (1.28, 3.51)	0.004
	Age group	1.28 (1.10, 1.49)	0.002
	Diarrhoea	0.55 (0.34, 0.89)	0.015
Soil-transmitted helminths			
Hookworm	Itching illnesses	1.85 (1.04, 3.30)	0.036
	Age group	1.20 (1.03, 1.39)	0.016
	Sex	0.36 (0.23, 0.55)	<0.001
Trichuris trichiura	Blood in stool	3.46 (1.20, 10.02)	0.022
	Schistosomiasis	0.13 (0.03, 0.50)	0.003
Intestinal protozoa			
Entamoeba histolytica/E. dispar	Age group	1.22 (1.06, 1.40)	0.006
Entamoeba hartmanni	Respiratory problems	1.88 (1.15, 3.08)	0.012
	Abdominal pain	1.80 (1.01, 3.21)	0.046
	Malaria	0.50 (0.26, 0.97)	0.039
Entamoeba coli	Age group	1.34 (1.14, 1.57)	<0.001
Endolimax nana	Blood in stool	0.48 (0.26, 0.90)	0.022
Iodamoeba bütschlii	Blood in stool	2.09 (1.28, 3.41)	0.003
	Lethargy	0.50 (0.28, 0.89)	0.019
Chilomastix mesnili	Headache	5.25 (1.08, 25.39)	0.039
	Diarrhoea	2.30 (1.18, 4.49)	0.014
	Age group	1.31 (1.05, 1.63)	0.018
	Hot body	0.37 (0.17, 0.78)	0.009
Giardia duodenale	Muscle aches	2.29 (1.03, 5.12)	0.043
	Age group	0.62 (0.47, 0.81)	0.001
Plasmodium falciparum	Convulsions	4.84 (1.10, 21.29)	0.037
	Age group	0.80 (0.67, 0.96)	0.015
	Lethargy	0.43 (0.21, 0.92)	0.028

technique, examining multiple faecal samples. Individuals with <2 Kato-Katz thick smear readings were excluded from further analyses, because of the chances of having missed some light infections.[35–37] Diagnosis of soil-transmitted helminths was based on multiple Kato-Katz thick smears plus a single formol-ether processed faecal sample. This approach is superior to the Kato-Katz technique alone, since hookworm eggs tend to dissolve promptly once the thieved faecal samples are placed on the microscope slides and are covered with glycerine-socked cellophane paper.[38] Indeed, while multiple Kato-Katz thick smears revealed an estimated hookworm prevalence of 35.4% for the present population sample, pooling these results with the formol-ether processed faecal examinations augmented the prevalence to 45.0%. The results of the intestinal protozoa are based on a single formol-ether processed faecal sample. Repeated faecal examinations increase the sensitivity of this diagnostic test,[39,40] hence we can assume that the prevalences reported here are underestimating the 'true' prevalences. Similarly, examination of a single thick and thin blood smear per individual is likely to have underestimated *Plasmodia* prevalence rates.

Taken together, the extent of polyparasitism, reported to be very high in this village of Côte d'Ivoire, could be even higher. However, it is conceivable that mainly light infections were missed; hence the impact on morbidity and co-morbidity of these undetected infections is likely to be small. No attempt was made to concurrently assess bacteria and viruses pathogenic to the intestine, which are also prevalent in populations living in the developing world and presumably contribute substantially to morbidity and mortality (for a recent review see Thapar & Sanderson[41]). It would be desirable to have a tool with a high sensitivity and a high specificity for accurate diagnosis of multiple species parasitic infections, which in turn would enhance our understanding of interactions between different parasites. For example, it was recently shown in Senegal that helminth-free individuals had the same level of protection against clinical malaria attacks as that provided by the sickle-cell trait.[42] Accurate diagnosis is thus of considerable relevance for understanding morbidity and co-morbidity patterns. Recognizing the limitations of current diagnostic tools for capturing polyparasitism, we are investigating whether proton nuclear magnetic resonance (^{1}H-NMR)-based metabonomics can be developed to fill this gap. Recent advances with this approach in the field of chronic diseases (e.g. coronary heart disease)[43] might hold promise for infectious diseases.

The present study also confirms previous results obtained elsewhere in Côte d'Ivoire,[18,24] and in Brazil,[44,45] as a significant positive association was found between *S. mansoni* and hookworm infections. In addition, our recent observation that schoolchildren with higher infection intensities of *S. mansoni* are at higher risk of a concurrent hookworm infection is confirmed here for an entire community.[24] In this epidemiological setting, the interactions are likely to be ecological in nature, largely explained by the lack of clean water and sanitation.[24,32] In turn, improving access to safe drinking water and enhanced excreta disposal entails opportunities for sustainable control of schistosomiasis, soil-transmitted helminthiasis, and diarrhoeal diseases among other benefits.[21,41,46] In future work, emphasis should also be placed on the nutritional level of study participants living

in the developing world, as this is an important underlying risk factor by which intestinal parasites inhibit growth and development.[47,48]

Another interesting finding of the present study is the strong association between an infection with *E. histolytica/E. dispar* and the infection intensity of *S. mansoni*. This strengthens the evidence of this protozoa-helminth interaction, which had already been reported in Egypt.[49] Since light microscopy fails to separate between *E. histolytica* and *E. dispar*, it would be interesting to carry out species-specific diagnosis. Only the former parasite is pathogenic, but it is common that the latter is the predominant species.[50] In eastern Côte d'Ivoire, for example, a recent cross-sectional survey among schoolchildren revealed a ratio of *E. histolytica* to *E. dispar* of 1:46.[51] If we assume that a similar ratio occurs in western Côte d'Ivoire, hence only very few cases of *E. histolytica* were actually present, this might explain the lack of any significant associations between *E. histolytica/E. dispar* and self-reported morbidity indicators. In the population sample studied here, there was a positive association between *A. lumbricoides* and *T. trichiura* infections, which is in agreement with previous studies from different epidemiological settings.[15,52]

Our study also confirms previously reported associations between a particular parasitic infection and self-reported morbidity. For example, abdominal pain and dysentery were strongly associated with *S. mansoni*, which is of considerable relevance for rapid screening of high-risk populations as a means of cost-effective interventions, e.g. mass administration of praziquantel.[53] However, care is needed in the interpretation of the associations between a particular parasitic infection and any of the self-reported morbidity indicators, because polyparasitism was so common. In other words, the majority of the study participants harboured multiple parasite species concurrently; hence it is difficult to separate out which one is responsible for the self-reported morbidity over the past 2–4 weeks.

We conclude that multiple species parasitic infections are the norm rather than the exception in this community of rural Côte d'Ivoire, as is probably the case elsewhere in developing countries. Consequently, we speculate that capturing polyparasitism can serve as a basis for measuring the dynamics of morbidity and co-morbidity following specific and comprehensive interventions. In turn, this is of importance for informed decision-making with a view towards integrated control to reduce overall morbidity within a population. For example, chemotherapy-based morbidity control should only be viewed as the initial stage in a more comprehensive control approach, emphasizing preventive measures. In the population studied here, only a tiny proportion currently sleep under ITN and house constructions are inadequate to effectively prevent the entrance of mosquitoes. Access to clean water and improved sanitation facilities are lacking. Consequently, effective information, education, and communication campaigns readily adapted to this setting could raise awareness of sound preventive measures against some of the major health problems. For example, house screening, closing eaves, and sleeping under ITN will significantly reduce exposure to malaria vectors,[54] and provision of clean water and installation of improved sanitation facilities will address the root ecological problem of schistosomiasis, as well as other intestinal parasites.[21,41,46] Such a multi-stage approach could ultimately form the basis for transmission control of selected pathogens, which in turn will contribute to poverty alleviation.

Acknowledgements

We are grateful to the village authorities of Zouatta II, the village chief's designated field assistants—Oulaï Innocent, Séponh Bernard, Séyouo Anatole, Blé Victor, Poté Kouao Apolinaire, Mahan Mathias, Djinhin Monique, and Thes Larissa—for their dedication during the epidemiological survey and questionnaire administration, and all study participants. We thank the laboratory technicians—Alphonse Allangba, Abdoulay Fondio, Kouassi L. Lohourignon, Brou Sosthène, and Mamadou Traoré—for their commitment in the field-work and behind the bench. We acknowledge Touho Gaston, community health worker from the neighbouring village of Fagnampleu, for his extraordinary contribution to the present work. Thanks are addressed to Dr Hanspeter Marti and his team at the Swiss Tropical Institute for diagnosis of intestinal protozoa. This investigation received financial support from the Claire Sturzenegger-Jean Favre Foundation. Giovanna Raso is partially supported by a fellowship from the Roche Research Foundation. Barbara Matthys is grateful for financial support by the Integrated Project 4 (IP4) 'Health and Well-being' of the NCCR North-South: 'Research Partnerships for Mitigating Syndromes of Global Change', which is funded by the Swiss National Science Foundation (SNSF). Jürg Utzinger acknowledges financial support from SNSF (Project No. PPOOB–102883).

KEY MESSAGES

- Multiple species parasitic infections are common in the developing world, but the bulk of previous research has focused on a single parasite in a narrow age group.
- We screened 500 individuals (age range from 5 days to 91 years) from a single village in western Côte d'Ivoire over consecutive days for *Schistosoma mansoni*, soil-transmitted helminths, intestinal protozoa, and *Plasmodia* infections, employing standardized, quality-controlled methods.
- In this sample, three-quarters of the population were infected with at least three parasites concurrently.
- Significant associations were found between different pairs of parasites, and some parasitic infections, as well as the total number of parasites, were positively correlated with self-reported morbidity indicators.
- Interventions are needed that address the root behavioural and ecological causes of these parasitic infections to reduce the intolerable burden caused by these parasites.

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Photo 1 Kato-Katz thick-smear preparation for subsequent microscopic examination for *Schistosoma mansoni* and soil-transmitted helminth infections

Photo 2 Training session on how to interview household members and to complete the questionnaires