

Synergistic associations between hookworm and other helminth species in a rural community in Brazil

Fiona M. Fleming^{1,2}, Simon Brooker¹, Stefan M. Geiger^{2,3}, Iramaya R. Caldas², Rodrigo Correa-Oliveira², Peter J Hotez³ and Jeffrey M. Bethony^{2,3}

1 Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, UK

2 Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), Belo Horizonte, Minas Gerais, Brazil

3 Department of Microbiology and Tropical Medicine, The George Washington University, Washington DC, USA

Summary

OBJECTIVE To identify possible synergistic associations of hookworm and other helminths.

METHOD Cross-sectional survey of all households within 10 km² of Americaninhas, a rural community in Minas Gerais, Brazil. We determined the prevalence and intensity of single and multiple helminth species infection in an age-stratified sample of 1332 individuals from 335 households.

RESULTS Hookworm was the most prevalent helminth infection (68.2%), followed by *Ascaris lumbricoides* (48.8%) and *Schistosoma mansoni* (45.3%). Overall, 60.6% of individuals harboured mixed helminth infections. Multivariate analysis indicated significant positive associations for co-infection with hookworm and *S. mansoni* and for co-infection with hookworm and *A. lumbricoides*. Co-infections with hookworm and *A. lumbricoides* resulted in higher egg counts for both, suggesting a synergistic relationship between these species, although, we found important age differences in this relationship. However, the intensity of *S. mansoni* or *A. lumbricoides* co-infection did not differ from that of mono-infection.

CONCLUSION These results have implications for the epidemiology, immunology and control of multiple helminth infections. More research is needed to examine the rates of re-infection and immune responses after chemotherapy, and to what extent the effects of polyparasitism are altered by chemotherapy.

keywords hookworm, *Ascaris lumbricoides*, *Schistosoma mansoni*, epidemiology, polyparasitism, Brazil

Introduction

In 1978, Buck and colleagues published a landmark study of polyparasitism in rural communities in Chad, Peru, Afghanistan and Zaire, which indicated the frequent occurrence of concomitant parasitic infections (Buck *et al.* 1978). An increasing number of studies of helminth epidemiology have since shown that multiple helminth infections are extremely widespread (Booth *et al.* 1998; Needham *et al.* 1998; Petney & Andrews 1998; Brooker *et al.* 2000; Howard *et al.* 2002; Tchuem Tchuente *et al.* 2003; Raso *et al.* 2004). Evidence from laboratory model systems suggests that infection with one helminth may influence the outcome of infection with others (Cox 2001), with evidence suggestive of both synergism and antagonism in concurrent intestinal nematode and schistosome infections (Pritchard *et al.* 1991; Webster *et al.* 1997; Corrêa-Oliveira *et al.* 1988,2002; Cox 2001). A number of epidemiological studies have indicated that individuals with multiple helminth infections often harbour heavier

infections than individuals with single species infections (Booth *et al.* 1998; Needham *et al.* 1998; Brooker *et al.* 2000; Tchuem Tchuente *et al.* 2003). Other studies have reported that hookworm infection is positively associated with *Schistosoma mansoni* infection (Chamone *et al.* 1990; Keiser *et al.* 2002a,b; Raso *et al.* 2004) and with filarial nematode infection (Faulkner *et al.* 2005). It has also been speculated that helminth infections may adversely influence host immune responses to other parasites, especially to intracellular pathogens such as malaria (Nacher 2004). To date, most studies of polyparasitism have been undertaken among populations of school-aged children. To our knowledge, there have been only three epidemiological studies, which have investigated multiple helminth infections, both in terms of prevalence and intensity, within an entire community (Buck *et al.* 1978; Keiser *et al.* 2002b; Raso *et al.* 2004). Here, we present age-stratified data from an ongoing longitudinal study of the epidemiology and immunology of intestinal nematode and schistosome infections in Brazil. The present analysis describes the

F. M. Fleming *et al.* **Multiple helminth infections in rural Brazil**

epidemiology of single and multiple helminth infection. Emphasis is placed on age-related changes in polyparasitism and with associations that occur between the species.

Materials and methods

The study was reviewed and approved by the ethical committee of the Centro de Pesquisas René Rachou-FIOCRUZ and the National Committee for Ethics in Research (Brazil), and the ethical review boards of George Washington University (USA) and London School of Hygiene and Tropical Medicine (UK).

Study area and population

The study was conducted in Americaninhas in Minas Gerais State of southeastern Brazil. It is located in the northeast of the state, lying between 17°02'12.310" – 17°13'13.857" S and 41°20'18.334" – 41°07'39.737" W and is divided into five rural sectors and a central municipality. The Fundação Nacional de Saude (the National Health Foundation) estimates 1000 people living in the urban municipal centre and another 1000 in the surrounding rural areas. The area is hilly and characterized by a tropical altitude climate, with an average temperature of 24 °C, and experiences a long rainy season between November and March; annual rainfall is 1300–2000 mm. The majority of inhabitants are involved in rural subsistence farming, growing mainly coffee, manioc and beans. Cattle ranching is another important source of income. Houses are predominantly made from concrete or from a combination of wood and mud and have either tiles or iron sheets for roofing. Only approximately 50% of these homes have a latrine and people commonly collect their water from local springs. There is only one health post in the area with two auxiliary health workers who are paid by the municipality.

Recruitment

The study area of Americaninhas is located in the municipality of Nova Oriente, NE, Minas Gerais State, Brazil. The household was the main sampling unit, with the research team visiting all houses within 10 km² of the study site to obtain informed consent using a written and verbal consent form approved by the National Ethics Committee of Brazil, the Internal Review Board, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil, and the Internal Review Board, George Washington University Medical Center, Washington DC, USA. A series of meetings were held with community members to explain the purpose and method of

the study, that participation was voluntary, and that they were able to withdraw from the study at any time. Written consent was obtained from all adult subjects and from parents or guardians of minors. Each house was assigned a unique household identification number (HHID), and each resident a unique personal identity number (PID). Only individuals meeting the following inclusion criteria were included into the study: (1) resident in the study area over the last 24 months; (2) reporting not to have received anthelmintic treatment within the last 24 months; and (3) willing and able to give informed consent to study protocol. Individuals were not included if they: (1) attended school outside the study area; (2) worked full-time outside the study area; or (3) tested positive on a pregnancy test. Females found to be pregnant during the test were excluded from treatment during their pregnancy and received treatment for all helminth infections later. The results of these tests were made available to the individual only upon request and not to family members.

Parasitology

Participating household members were informed 24 h in advance about the start of the stool sample collection and plastic containers were provided. Containers were labeled with the participant's full name, age, PID, HHID, and day of distribution. Participants were instructed to deposit one faecal sample per day into each container and return the container immediately to one of several collection points, where the sample was stored at 4 °C. Faecal samples returned later than 48 h after date of distribution were not accepted, and new containers were issued. Presence of infection was determined by using the formalin-ether sedimentation technique. Individuals positive for any helminth in the formalin-ether sedimentation technique were asked to contribute two more samples over the course of two more days to be analyzed by Kato-Katz technique for assessment of eggs per gram of faeces (infection intensity). Two slides were taken from each day's faecal sample for a total of four slides from each individual. Slides were examined within 45 min of slide preparation to avoid clearing of hookworm eggs. The arithmetic means of the four slides was calculated and then transformed according to the Kato-Katz method (Katz *et al.* 1972). Three methods were used to ensure standardization in the preparation and reading of slides: (i) the same technicians prepared and read all slides; (ii) both technicians read every tenth set of slides, with the results compared ($r = 0.89$; $P < 0.001$); (iii) the coefficient of variation for egg counts was assessed over the 2 days. A coefficient of variation above 20% was considered unacceptable ($n = 14$ individuals), and the

F. M. Fleming *et al.* Multiple helminth infections in rural Brazil

participants asked to volunteer two more faecal samples until the coefficient of variation over 2 days was below 20%. While evidence indicated the predominance of *Necator americanus* over *Ancylostoma duodenale* in Brazil (Goncalves *et al.* 1990; Kobayashi *et al.* 1995; Geiger *et al.* 2004), we examined faeces for expelled worms on three consecutive days from patients who had been treated with albendazole. The worms were washed in phosphate-buffered saline (PBS) and stored in 70% ethanol. For determination of the species, the worms were rinsed in a phenol solution (70%) and the mouthparts were examined under the microscope (400× magnification). A total number of 120 male and female worms were determined to be exclusively of the species *N. americanus*.

Data analysis

Intensity of helminth infection was expressed as arithmetic means. This is justified biologically since intensity of infection is assumed to be proportional to clinical outcomes and no saturation occurs at high intensities. For example, intensity of hookworm infection is proportional to faecal blood loss (Stoltzfus *et al.* 1996) and intensity of *S. mansoni* is proportional to periportal fibrosis. This is also justified statistically since geometric means, despite reducing the skewness of a distribution, can be biased estimators of the true population mean and give misleading indicators of the differences between groups (Fulford 1994). Definitions of heavy infection were based on thresholds of egg counts proposed by WHO: hookworm 4000 eggs/gram faeces; *A. lumbricoides* 50,000 eggs/gram faeces; and *S. mansoni* 400 eggs/gram faeces (WHO 2002). The prevalence of multiple species infection was stratified by four age classes: 0–9, 10–24, 25–39 and 40+ years. These categories were chosen to reflect the age intensity profiles (Figure 1). Expected prevalences of multiple species infections were estimated based on a simple probabilistic model, which uses overall prevalence data (Booth & Bundy 1995), and differences tested using a Chi-square test. Associations between different helminth species were investigated using a random effects logistic regression modelling. A full model was developed, forcing age group, sex and sector, as well as presence of other infections, into the model. Separate models were developed for any infection and for heavy infections. Interactions between individual species and sex and age group were tested for first and removed if non-significant ($P > 0.05$). Models were adjusted to account for non-independence of observations within households. Due to the marked skewness of egg counts, Kruskal–Wallis tests were used to assess the variation of egg counts by

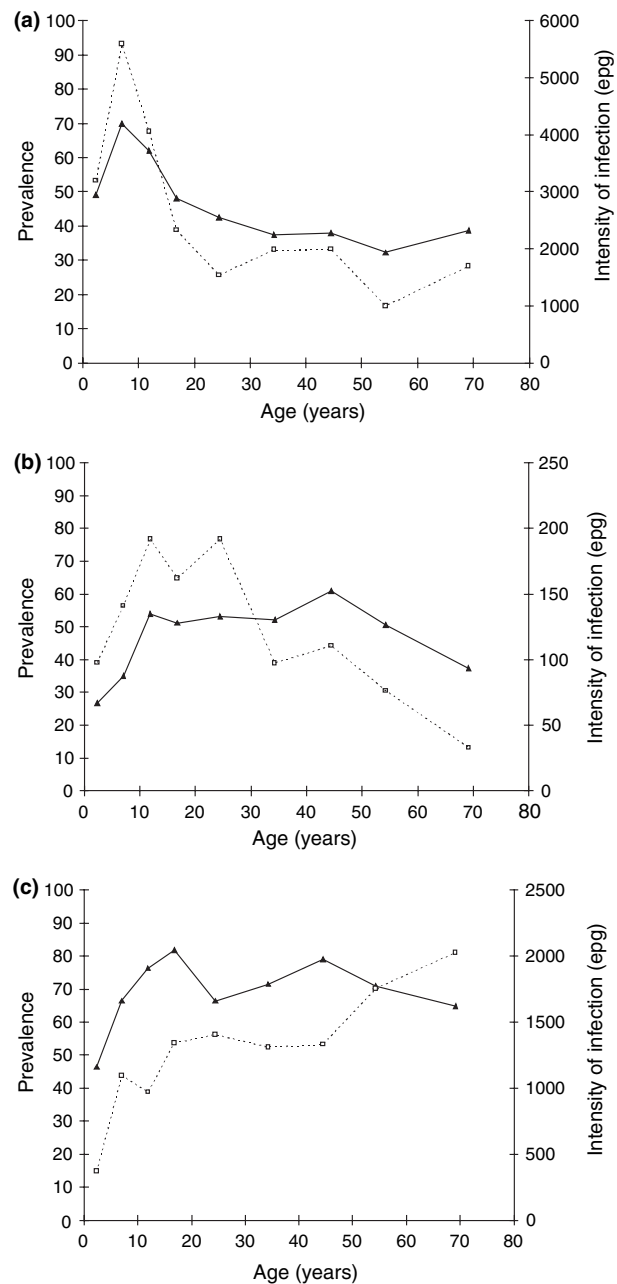


Figure 1 Age-prevalence and intensity curves for helminth infection among 1332 individuals in Americaninhas, Minas Gerais, Brazil 2004. (a) *A. lumbricoides*, (b) *S. mansoni* and (c) hookworm. Solid line indicates prevalence and dashed line indicates intensity of infection.

multiple species infection, stratified by age group. Analysis was undertaken using Stata 8.2 (College Station, TX, USA).

Results

A total of 1332 individuals from 335 households were included in the study, of these individuals 82.7% were infected with at least one species of helminth. The overall prevalence of hookworm infection was 68.2%, the prevalence of *S. mansoni* was 45.3%, the prevalence of *A. lumbricoides* was 48.8%, the prevalence of *Trichuris trichiura* was 1.1% and the prevalence of infection of *Enterobius vermicularis*, *Hymenolepis nana* and *Taenia* spp. were respectively, 0.8%, 0.2% and 0.2%. The prevalence of heavy hookworm infection was 8.3%, the prevalence of heavy *S. mansoni* infection was 8.1%, and the prevalence of heavy *A. lumbricoides* infection was 18.2%.

Prevalence and intensity of *A. lumbricoides* rose to a peak in the 5–10 years age group and declined thereafter (Figure 1a). Prevalence of hookworm infection rose with age until the 15–20 years age group and stayed constant thereafter. Prevalence of *S. mansoni* infection also rose with age until the 10–15 years age group where it remained constant until peaking in the 40–45 years age group. Intensity of *S. mansoni* infection peaked in the 10–15 years age group and again in the 20–25 years age group and declined thereafter, whereas intensity of hookworm infection rose with increasing age (Figure 1b, c).

The prevalence of single and multiple species infection overall and by age group is shown in Table 1. Overall, of those infected with a helminth, 73.3% of individuals harboured mixed infections. The most common combinations were infection with hookworm and *A. lumbricoides* and with hookworm and *S. mansoni*. Only 4.6% of infected individuals were concurrently infected with *A. lumbricoides* and *S. mansoni*, which was significantly

lower than would be expected by chance alone (6.5%) ($\chi^2 = 9.9$, $P = 0.001$). The observed percentage of individuals with all three species of infection was 19%, significantly higher than the expected value of 13.8% ($\chi^2 = 13.0$, $P = 0.0003$), and the prevalence of dual infection with hookworm and *A. lumbricoides* was also significantly higher than expected by chance (19.9% vs. 16.7%, $\chi^2 = 4.6$, $P = 0.03$). The prevalences of different species combinations are shown to vary by age. Table 2 presents the results of the logistic regression analysis and shows that, while controlling for age-group, sex and sector, hookworm was significantly positively associated with *A. lumbricoides* and with *S. mansoni*. This was true both for infections *per se* and for heavy infections. In contrast, no significant associations were observed between co-infections *A. lumbricoides* and *S. mansoni*.

Overall, the mean intensity of hookworm infection significantly increased according to the multiplicity of species infection: hookworm only = 1220 epg; hookworm and *A. lumbricoides* = 1827 epg; hookworm and *S. mansoni* = 1888 epg; all three species = 2023 epg ($P = 0.0002$). Intensity of *A. lumbricoides* also significantly differed by multiple species infections, with individuals infected with *A. lumbricoides* and *S. mansoni* harbouring the lightest infections: *A. lumbricoides* only = 6323 epg; *A. lumbricoides* and hookworm and = 6551 epg; *A. lumbricoides* and *S. mansoni* = 4304 epg; all three species = 5330 epg ($P = 0.003$). Individuals infected with *A. lumbricoides* and *S. mansoni* also had the lowest intensities of *S. mansoni*, although group differences had only borderline significance: *S. mansoni* only = 208 epg; *S. mansoni* and hookworm = 338 epg; *S. mansoni* and *A. lumbricoides* = 163 epg; all three species = 266 epg ($P = 0.07$). When investigating these patterns by age

Table 1 Observed and expected prevalence of single and multiple helminth infection among 1332 individuals in Americaninhas, Minas Gerais, Brazil in 2004.

Species combination	Number positive	Percentage of infections	Percentage of population		χ^2	P value	Observed by age groups (years)			
			Observed	Expected			0–9	10–24	25–39	40+
Any single infection	294	26.7	22.1				24.8	19.8	18.7	24.2
Hookworm only	152	13.8	11.4	20.6	41.6	<0.0001	8.8	10.7	11.6	15.2
<i>A. lumbricoides</i> only	81	7.4	6.1	7.8	3.1	0.080	12.4	4.3	1.5	3.9
<i>S. mansoni</i> only	61	5.5	4.6	8.0	13.4	<0.0001	3.6	4.8	5.6	5.1
Any double infection	554	50.3	41.6				37.6	48.9	39.3	38.9
Hookworm and <i>A. lumbricoides</i>	265	24.1	19.9	16.7	4.6	0.03	26.2	22.7	12.1	13.8
Hookworm and <i>S. mansoni</i>	238	21.6	17.9	17.1	0.3	0.610	6.0	21.4	24.2	23.9
<i>A. lumbricoides</i> and <i>S. mansoni</i>	51	4.6	3.8	6.5	9.9	0.001	5.4	4.8	3.0	1.2
Triple infection	253	23.0	19.0	13.8	13.0	0.0003	16.1	22.1	19.7	17.9
Any mixed infection	807	73.3	60.6				53.7	71.0	59.0	56.8

Parasite	Association	Adjusted OR (95% CI)*	P-value
Hookworm	<i>A. lumbricoides</i>	3.59 (2.67, 4.82)	<0.001
	<i>S. mansoni</i>	2.95 (2.19, 3.98)	<0.001
Heavy hookworm	Heavy <i>A. lumbricoides</i>	2.29 (1.35, 3.90)	0.002
	Heavy <i>S. mansoni</i>	4.13 (2.43, 6.99)	<0.001
<i>A. lumbricoides</i>	Hookworm	3.65 (2.71, 4.91)	<0.001
	<i>S. mansoni</i>	0.99 (0.75, 1.29)	0.917
Heavy <i>A. lumbricoides</i>	Heavy hookworm	2.29 (1.34, 3.92)	0.002
	Heavy <i>S. mansoni</i>	0.59 (0.32, 1.11)	0.105
<i>S. mansoni</i>	Hookworm	3.01 (2.22, 4.06)	<0.001
	<i>A. lumbricoides</i>	0.98 (0.75, 1.28)	0.881
Heavy <i>S. mansoni</i>	Heavy hookworm	4.19 (2.45, 7.14)	<0.001
	Heavy <i>A. lumbricoides</i>	0.59 (0.32, 1.09)	0.091

* OR = Odds ratio for infection adjusted by age group, sex and presence of other infections, and clustering by household; CI = Confidence Interval.

group important differences were revealed. Although, mono-infected individuals had lower hookworm intensities in each age group, hookworm intensity differed significantly by species combinations only in the age categories 10–24 and 25–39 years (Kruskal–Wallis test of equality of populations: $P = 0.027$ and $P = 0.015$) (Figure 2c). Similarly, although individuals infected with *S. mansoni* and *A. lumbricoides* had the lowest intensity of *A. lumbricoides* infection, intensity only differed by species combinations among 25–39 years old ($P = 0.009$) (Figure 2b). For *A. lumbricoides* there were no significant differences in intensity by species combinations between the age groups, although individuals infected with *S. mansoni* and *A. lumbricoides* consistently had the lowest intensities (Figure 2a).

Discussion

In the tropics it is common for a single individual to be infected with several parasite species at the same time (Brooker *et al.* 2000; Tchuem Tchuenté *et al.* 2003; Raso *et al.* 2004; Faulkner *et al.* 2005). Here, we show that, among all age groups, co-infection with schistosomes and intestinal nematodes is extremely common in rural Brazil. This finding confirms those studies conducted among school-aged children (Booth *et al.* 1998; Needham *et al.* 1998; Brooker *et al.* 2000; Tchuem Tchuenté *et al.* 2003; Faulkner *et al.* 2005).

Our analysis showed that there was a significant positive association between co-infection with hookworm and *S. mansoni* and with hookworm and *A. lumbricoides*. The positive association for co-infection with hookworm and *S. mansoni* is in keeping with previous studies in Brazil (Chamone *et al.* 1990) and Côte d'Ivoire (Keiser

Table 2 Associations between helminth species among 1332 individuals in Americaninhas, southeastern Brazil in 2004.

et al. 2002a,b; Raso *et al.* 2004). We further show that intensity of hookworm infection typically increased with the multiplicity of infection. Taken together, these results suggest a synergistic relationship between hookworm and other helminths, as has been observed elsewhere (Chamone *et al.* 1990). This observation is also compatible with immunological data from hookworm patients showing that antibodies reacting with crude antigen extracts from hookworms cross-react with egg and adult worm antigen extracts from *S. mansoni* (Pritchard *et al.* 1991; Corrêa-Oliveira *et al.* 1988, 2002). Similar antibody cross-reactivity between *N. americanus* and *S. mansoni* has been observed in murine models (Timothy *et al.* 1992). However, even though antigenic cross-reactivity was reported in this experimental model, no protective immunity was achieved by heterologous challenge infection (Timothy *et al.* 1992). Corrêa-Oliveira *et al.* (1988,2002) found that individuals from areas co-endemic for *S. mansoni* and hookworm had significantly lower proliferative responses to crude hookworm antigen extracts than individuals from hookworm mono-endemic areas.

Immunological mechanisms, such as the differential activation of T helper cell subsets by parasitic stages, may explain the synergisms and antagonisms observed during helminth co-infections in this study. Mice with an underlying *S. mansoni* infection were found to have a T helper 2-dominant immune response that could alter the disease outcome of different intestinal nematode species, e.g., *Strongyloides venezuelensis* (Yoshida *et al.* 1999) and *T. muris* (Curry *et al.* 1995). Protective effects might vary between different parasitic species, acting at different sites (skin, lung and gut) and different developmental stages such as third stage infective larvae (L3) (Yoshida *et al.*

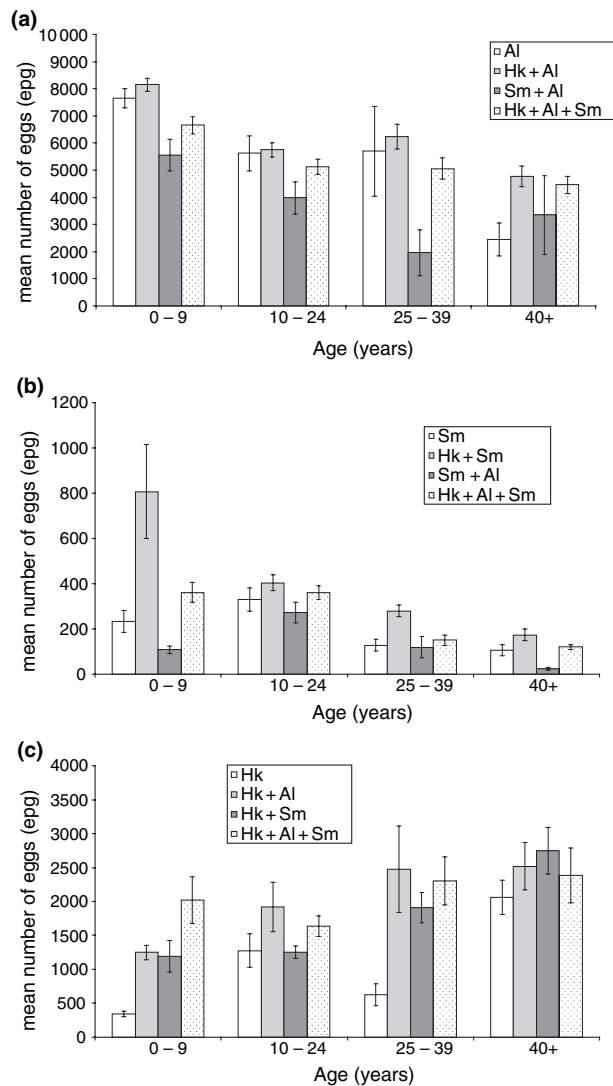
F. M. Fleming *et al.* Multiple helminth infections in rural Brazil

Figure 2 Mean intensity of infection among 1332 individuals in Americaninhas, Minas Gerais, Brazil, stratified by multiplicity of infection and age group. (a) *A. lumbricoides*, (b) *S. mansoni* and (c) hookworm. Columns indicate arithmetic mean intensity of infection and vertical bars indicate standard error of the mean.

1999) or adult worms (Curry *et al.* 1995). In *N. americanus* infection, increased levels of interleukin (IL)-5 secreted by peripheral blood mononuclear cells upon stimulation with crude adult hookworm antigen extract correlated with resistance to reinfection, indicating a role for Th2 cytokine responses in protection against hookworm reinfection (Quinnell *et al.* 2004a).

Interestingly, individuals in our study co-infected with hookworm and other strong Th2 cytokine (including IL-

5) inducing helminth such as *A. lumbricoides* or *S. mansoni* had higher intensities of infection than individuals mono-infected with hookworm, pointing to a possible synergistic effect on the immune response to these helminths during hookworm co-infection. As such, it is possible that co-infection with hookworm, with a reduced cellular reactivity (Corrêa-Oliveira *et al.* 2002) and the secretion of immunomodulatory molecules (Chow *et al.* 2000; Loukas & Procvic 2001; Hsieh *et al.* 2004), subverts or overrides the mechanisms which would enable an age-acquired decline in infection intensity seen in other helminth infections. In this context, it is important to mention the possible role of recently described T-regulatory cells (Tr1), and their secretion of downmodulatory cytokines, e.g. IL-10 or TGF- β , which seem to participate in the regulation of the host's immune response (Maizels *et al.* 2004). For experimental schistosomiasis, these T-regulatory cells were found to be the major source of IL-10 (Hesse *et al.* 2004). Whether the observed production of IL-10 is stimulated directly by the presence of the parasites and/or their products, or represents a natural cross-regulatory mechanism initiated by the host remains to be established.

Here we also show that that individuals co-infected with *A. lumbricoides* and *S. mansoni* have lower infection intensities for each species than individuals mono-infected with each species, suggestive of antagonism between *A. lumbricoides* and *S. mansoni* infection. Previous studies of *A. lumbricoides* infections in humans have shown that Th-2-type immune responses were associated with reduced worm burden and age-related protective immune responses (Turner *et al.* 2003). A similarly strong induction of Th-2 responses has been observed with the start of *S. mansoni* egg deposition in intestinal tissues in experimental animal models (Grzych *et al.* 1991; Pearce *et al.* 1992). As such, this markedly similar immune modulation may account for the decrease in intensity of infection observed in individuals co-infected with *S. mansoni* and *A. lumbricoides* compared to individuals mono-infected with each helminth. This has also been proposed for experimental infections with *A. suum* (Frontera *et al.* 2003). However, many other factors may also contribute to these parasite interactions as well, and we plan to further investigate the immune responses of individuals who are mono- and co-infected for hookworm, *A. lumbricoides*, and *S. mansoni* in future studies.

An alternative explanation for the observed associations is the similar and different transmission routes of the different helminth species. Transmission of hookworm and *A. lumbricoides* occurs through exposure to soil contaminated with free-living infective stages and is influenced by several factors, including micro-climatic suitability,

F. M. Fleming *et al.* Multiple helminth infections in rural Brazil

sanitation and hygiene, and environmental contamination with human excreta (Olsen *et al.* 2001; Keiser *et al.* 2002a; Traub *et al.* 2004). Schistosomiasis, in contrast, is a water-borne infection and transmission is based on infective water bodies and human water contact. Information on individual and household specific risk factors was not collected and this hypothesis remains to be further investigated.

In the tropics polyparasitism is extremely common. Our results show that infection with one helminth can profoundly affect the intensity of infection with another helminth, e.g., heavy hookworm infection results in heavy infection with *S. mansoni* and vice versa. It may be that the powerful immunomodulatory mechanisms of helminths have important effects on susceptibility not only to other helminth infections but to other infectious diseases in general, including implication for the response to vaccination: e.g., the response to Bacillus Calmette-Guerin (BCG) vaccination in the tropics (Elias *et al.* 2001; Brooker *et al.* 2004; Quinnell *et al.* 2004b) and the live oral cholera vaccine CVD 103-HgR (Cooper *et al.* 2001). Our cross-sectional study provides an indication of the presumably cumulative effect of single and multiple species infection. Longitudinal studies are now underway in the study area to examine the rates of re-infection and immune responses following chemotherapy, and thus will provide information on how much of the effects of polyparasitism is altered by chemotherapy.

Acknowledgement

We are very grateful to inhabitants of Americaninhas who kindly participated in the study. We are also most appreciative of the hard work of all of the field staff, which made this analysis possible, and deserve many thanks. Fieldwork was financially supported by the Human Hookworm Vaccine Initiative (HHVI) of the Sabin Vaccine Institute, and the Bill and Melinda Gates Foundation. SB is supported by a Wellcome Trust Advanced Training Fellowship (073656), JB is supported by an International Research Scientist Development Award (IRSDA) (K01 TW00009) from the John E. Fogarty International Center, NIH, and FF was supported by a Chadwick Trust travel award.

References

- Booth M & Bundy DAP (1995) Estimating the number of multiple-species geohelminth infections in human communities. *Parasitology* **111**, 645–653.
- Booth M, Bundy DAP, Albonico M, Chwaya H & Alawi K (1998) Associations among multiple geohelminth infections in school-children from Pemba Island. *Parasitology* **116**, 85–93.
- Brooker S, Miguel E, Moulin S, Luoba A, Bundy D & Kremer M (2000) Epidemiology of single and multiple species of helminth infections among school children in Busia District, Kenya. *East African Medical Journal* **77**, 157–161.
- Brooker S, Bethony J & Hotez PJ (2004) Human hookworm infection in the 21st century. *Advances in Parasitology* **58**, 197–288.
- Buck AA, Anderson RI & MacRae AA (1978) Epidemiology of poly-parasitism: I. Occurrence, frequency, and distribution of multiple infections in rural communities in Chad, Peru, Afghanistan, and Zaire. *Annals of Tropical Medicine and Parasitology* **29**, 61–70.
- Chamone M, Marques CA, Atuncar GS, Pereira AL & Pereira LH (1990) Are there interactions between schistosomes and intestinal nematodes? *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 557–558.
- Chow SC, Brown A & Pritchard D (2000) The human hookworm pathogen *N. americanus* induces apoptosis in T lymphocytes. *Parasite Immunology* **22**, 21–29.
- Cooper PJ, Chico M, Sandoval C *et al.* (2001) Human infection with *Ascaris lumbricoides* is associated with suppression of the interleukin-2 response to recombinant cholera toxin B subunit following vaccination with the live oral cholera vaccine CVD 103-HgR. *Infection and Immunity* **69**, 1574–1580.
- Corrêa-Oliveira R, Dusse LM, Viana IR, Colley DG, Santos Carvalho O & Gazzinelli G (1988) Human antibody responses against schistosomal antigens. I. Antibodies from patients with *Ancylostoma*, *Ascaris lumbricoides* or *Schistosoma mansoni* infections react with schistosome antigens. *American Journal of Tropical Medicine and Hygiene* **38**, 348–355.
- Corrêa-Oliveira R, Golgher DB, Oliveira GC *et al.* (2002) Infection with *Schistosoma mansoni* correlates with altered immune responses to *Ascaris lumbricoides* and hookworm. *Acta Tropica* **83**, 123–132.
- Cox FEG (2001) Concomitant infections, parasites and immune responses. *Parasitology* **122**, S23–S38.
- Curry AJ, Else KJ, Jones F, Bancroft A, Grenis RK & Dunne DW (1995) Evidence that cytokine-mediated immune interactions induced by *Schistosoma mansoni* alter disease outcome in mice concurrently infected with *Trichuris muris*. *The Journal of Experimental Medicine* **181**, 769–774.
- Elias D, Wolday D, Akuffo H, Petros B, Bronner U & Britton S (2001) Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guerin (BCG) vaccination. *Clinical & Experimental Immunology* **123**, 219–225.
- Faulkner H, Turner J, Behnke J *et al.* (2005) Associations between filarial and gastrointestinal nematodes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99**, 301–312.
- Frontera A, Carron A, Serrano FJ, Roepstorff A, Reina D & Navarrete I (2003) Specific systemic IgG1, IgG2 and IgM responses in pigs immunized with infective eggs or selected antigens of *Ascaris suum*. *Parasitology* **127**, 291–298.
- Fulford AJ (1994) Dispersion and bias: can we trust geometric means? *Parasitology Today* **10**, 446–448.
- Geiger SM, Massara CL, Bethony J, Soboslay PT & Correa-Oliveira R (2004) Cellular responses and cytokine production in

F. M. Fleming *et al.* **Multiple helminth infections in rural Brazil**

- post-treatment hookworm patients from an endemic area in Brazil. *Clinical and Experimental Immunology* **136**, 334–340.
- Goncalves JF, Tanabe M, Medeiros F de P *et al.* (1990) Parasitological and serological studies on amoebiasis and other intestinal parasitic infections in the rural sector around Recife, northeast Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo* **32**, 428–435.
- Grzych JM, Pearce EJ, Cheever A *et al.* (1991) Egg deposition is the major stimulus for the production of Th 2 cytokines in murine schistosomiasis mansoni. *Journal of Immunology* **146**, 1322–1327.
- Hesse M, Piccirillo CA, Belkaid Y *et al.* (2004) The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. *Journal of Immunology* **172**, 3157–3166.
- Howard SC, Donnelly CA, Kabatereine NB, Ratard RC & Brooker S (2002) Spatial and intensity-dependent variations in associations between multiple species helminth infections. *Acta Tropica* **83**, 141–149.
- Hsieh GC-F, Loukas A, Wahl AM *et al.* (2004) A secreted protein from the human hookworm *N. americanus* binds selectively to NK cells and induces IFN- γ production. *Journal of Immunology* **173**, 2699–2704.
- Katz N, Chaves A & Pelligrino J (1972) A simple device for quantitative stool thick-smear technique in *schistosomiasis mansoni*. *Revue Instituto Medicina Tropical* **14**, 817–820.
- Keiser J, N'Goran EK, Singer BH, Lengeler C, Tanner M & Utzinger J (2002a) Association between *Schistosoma mansoni* and hookworm infections among schoolchildren in Cote d'Ivoire. *Acta Tropica* **84**, 31–41.
- Keiser J, N'Goran EK, Traore M *et al.* (2002b) Polyparasitism with *Schistosoma mansoni*, geohelminths, and intestinal protozoa in rural Cote d'Ivoire. *Journal of Parasitology* **88**, 461–466.
- Kobayashi J, Hasegawa H, Forli AA *et al.* (1995) Prevalence of intestinal parasitic infection in five farms in Holambra, São Paulo. *Revista do Instituto de Medicina Tropical de Sao Paulo* **37**, 13–18.
- Loukas A & Prociv P (2001) Immune responses in hookworm infections. *Clinical Microbiology Review* **14**, 689–703.
- Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD & Allen JE (2004) Helminth parasites-masters of regulation. *Immunology Reviews* **201**, 89–116.
- Nacher M (2004) Interactions between worm infections and malaria. *Clinical Reviews in Allergy Immunology* **26**, 85–92.
- Needham C, Kim HT, Hoa NV *et al.* (1998) Epidemiology of soil-transmitted nematode infections in Ha Nam Province, Vietnam. *Tropical Medicine and International Health* **3**, 904–912.
- Olsen A, Samuelsen H & Onyango-Ouma W (2001) A study of risk factors for intestinal helminth infections using epidemiological and anthropological approaches. *Journal of Biosocial Science* **38**, 569–584.
- Petney TN & Andrews RH (1998) Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *International Journal of Parasitology* **28**, 377–393.
- Pearce EJ, Caspar P, Grzych JM, Lewis FA & Sher A (1992). Downregulation of Th 1 cytokine production accompanies induction of Th 2 responses by a parasitic helminth *Schistosoma mansoni*. *The Journal of Experimental Medicine* **173**, 159–162.
- Pritchard DI, Quinnell RJ, McKean PG *et al.* (1991) Antigenic cross-reactivity between *N. americanus* and *Ascaris lumbricoides* in a community in Papua New Guinea infected predominantly with hookworm. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 511–514.
- Quinnell RJ, Pritchard DI, Raiko A, Brown AP & Shaw MA (2004a) Immune responses in human necatoriasis: association between interleukin-5 responses and resistance to reinfection. *Journal of Infectious Diseases* **190**, 430–438.
- Quinnell RJ, Bethony J & Pritchard DI (2004b) The immunoepidemiology of human hookworm infection. *Parasite Immunology* **26**, 443–454.
- Raso G, Luginbuhl A, Adjoua CA *et al.* (2004) Multiple parasite infections and their relationship to self-reported morbidity in a community of rural Cote d'Ivoire. *The International Journal of Epidemiology* **33**, 1092–1102.
- Stoltzfus RJ, Albonico M, Chwaya HM *et al.* (1996) Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. *American Journal of Tropical Medicine and Hygiene* **55**, 399–404.
- Tchuem Tchuenté LA, Behnke JM, Gilbert FS, Southgate VR & Vercruyse J (2003) Polyparasitism with *Schistosoma haematobium* and soil-transmitted helminth infections among school children in Loum, Cameroon. *Tropical Medicine and International Health* **8**, 975–986.
- Timothy LM, Coulson PS, Behnke JM & Wilson RA (1992). Cross-reactivity between *N. americanus* and *Schistosoma mansoni* in mice. *International Journal of Parasitology* **22**, 1143–1149.
- Traub RJ, Robertson ID, Irwin P, Mencke N & Andrew Thompson RC (2004) The prevalence, intensity and risk factors associated with geohelminth infection in tea-growing communities of Assam, India. *Tropical Medicine and International Health* **9**, 688–701.
- Turner JD, Faulkner H, Kamgno J *et al.* (2003) Th 2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. *Journal of Infectious Diseases* **188**, 1768–1775.
- Webster M, Correa-Oliveira R, Gazzinelli G *et al.* (1997) Factors affecting high and low human IgE responses to schistosome worm antigens in an area of Brazil endemic for *Schistosoma mansoni* and hookworm. *American Journal of Tropical Medicine and Hygiene* **57**, 487–494.
- World Health Organization (2002) *Prevention and Control of Schistosomiasis and Soil-Transmitted Helminthiasis*. WHO Technical Report Series 912, Geneva.
- Yoshida A, Maruyama H, Yabu Y, Amano T, Kobayakawa T & Ohta N (1999) Immune response against protozoal and nematodal infection in mice with underlying *Schistosoma mansoni* infection. *Parasitology International* **48**, 73–79.

Corresponding Authors

Simon Brooker, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. E-mail: simon.brooker@lshtm.ac.uk.

Jeffrey M. Bethony, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), Belo Horizonte, Minas Gerais, Brazil. E-mail: jeff@cpqrr.fiocruz.br.

Associations synergétiques entre l'ankylostome et d'autres espèces d'helminthes dans une communauté rurale du Brésil

OBJECTIF Identifier les associations synergétiques possibles entre l'ankylostome et les autres helminthes.

MÉTHODE Une étude transversale incluant toutes les habitations sur 100 km² autour de Americaninhas, une communauté rurale à Minas Gerais au Brésil. Nous avons déterminé la prévalence et l'intensité de l'infection à une ou plusieurs espèces d'helminthes dans un échantillon d'âge stratifié comportant 1332 individus provenant de 335 habitations.

RÉSULTATS L'ankylostome était l'helminthe le plus prévalant dans les infections (73.3%), suivi du *Schistosoma mansoni* (45.3%) et *Ascaris lumbricoides* (48.8%). Au total, 60.6% des individus étaient porteurs d'infections mixtes à helminthes. Une analyse multivariée a indiqué des associations significatives pour la coinfection ankylostome et *S. mansoni* et pour la coinfection ankylostome et *A. lumbricoides*. Les coinfections à ankylostome et *A. lumbricoides* résultaient en un nombre élevé des œufs des deux espèces, quoique nous avons trouvé une différence dans les âges pour cette relation. Cependant, l'intensité de la coinfection avec *S. mansoni* ou *A. lumbricoides* ne différait pas de celle de la monoinfection.

CONCLUSION Ces résultats ont des implications dans l'épidémiologie, l'immunologie et le contrôle des infections mixtes aux helminthes. Des recherches plus poussées sont nécessaires pour examiner le taux de réinfection et les réponses immunitaires suite au traitement, et à quel point les effets de polyparasitisme sont altérés par la chimiothérapie.

Mots clés ankylostome, *Ascaris lumbricoides*, *Schistosoma mansoni*, épidémiologie, polyparasitisme, Brésil

Asociaciones sinérgicas entre uncinarias y otras especies de helmintos en una comunidad rural en Brasil

OBJETIVO Identificar posibles asociaciones sinérgicas entre uncinarias y otros helmintos.

MÉTODO Estudio de corte transversal de todas las casas dentro de 100 km² de Americaninhas, una comunidad rural de Minas Gerais, Brasil. Determinamos la prevalencia y la intensidad de infecciones únicas o múltiples en una muestra estratificada por edad de 1332 individuos provenientes de 335 hogares.

RESULTADOS Las uncinarias fueron la infección helmíntica más prevalente (73.3%), seguidas por *Schistosoma mansoni* (45.3%) y *Ascaris lumbricoides* (48.8%). En total, 60.6% de los individuos portaban infecciones helmínticas mixtas. El análisis multivariado indica una asociación positiva para la coinfección con uncinarias y *S. mansoni* así como para la co-infección con uncinarias y *A. lumbricoides*. Co-infecciones con uncinarias y *A. lumbricoides* resultaban con conteos de huevos más altos para ambos, sugiriendo una relación sinérgica entre estas dos especies, aunque encontramos diferencias en edad importantes en esta relación. Sin embargo, la intensidad de co-infección de *S. mansoni* o *A. lumbricoides* no se diferenciaba de la de la mono-infección.

CONCLUSIÓN Estos resultados tienen implicaciones en la epidemiología, inmunología y el control de infecciones helmínticas múltiples. Es necesario realizar más estudios para determinar las tasas de re-infección y respuesta inmune después de recibir quimioterapia, así como para averiguar hasta que punto los efectos del poli-parasitismo son alterados por la quimioterapia.

Palabras clave Uncinarias, *Ascaris lumbricoides*, *Schistosoma mansoni*, epidemiología, poli-parasitismo, Brasil