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Hookworm, *Ascaris lumbricoides* infection and polyparasitism associated with poor cognitive performance in Brazilian schoolchildren

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Summary

OBJECTIVE To investigate the relationship between hookworm and *Ascaris lumbricoides* infection and performance on three subsets of the Wechsler Intelligence Scale for Children – third edition (WISC-III) (Digit Span, Arithmetic and Coding) and Raven Colored Progressive Matrices.
METHODS Cross-sectional study of 210 children between the ages of 6 and 11 years in Americaninhas, Minas Gerais, Brazil. Separate proportional odds models were used to measure the association between the intensity of helminth infections and poor performance on each of the four cognitive tests.
RESULTS After adjusting for sex, age, socioeconomic status and other helminth infections, moderate-to-high-intensity hookworm infection was associated with poor performance on the WISC-III Coding subtest [OR = 3.20; 95% confidence interval (CI) = 1.43–7.17], low intensity of hookworm infection was associated with poor performance on the WISC-III Coding subtest [odds ratio (OR) = 3.71; 95% CI = 1.80–7.66] and moderate-to-high-intensity *A. lumbricoides* infection was associated with poor performance on the Raven test (OR = 2.03; 95% CI = 1.04–3.99), all in comparison with uninfected children. Children co-infected with *A. lumbricoides* infection and hookworm infection had greater odds of poor performance on some WISC-III subtests than children with only *A. lumbricoides* infection.
CONCLUSIONS These findings suggest that hookworm infection may be associated with poorer concentration and information processing skills, as measured on the WISC-III Coding subtest, and that *A. lumbricoides* infection may be associated with poorer general intelligence, as measured through the Raven Colored Progressive Matrices. This study also presents evidence that polyparasitized children experience worse cognitive outcomes than children with only one helminth infection.

keywords hookworm, *Ascaris lumbricoides,* cognitive function, polyparasitism, school children, Brazil

Introduction

Intestinal helminth infections are a major public health problem in the developing world, especially among children. The most prevalent intestinal helminths are the soil-transmitted nematodes *Ascaris lumbricoides* and the hookworms (*Necator americanus* and *Ancylostoma duodenale*) (Bethony *et al.* 2006). Worldwide, 320 million school-age children are infected with *A. lumbricoides* and 239 million with hookworm (WHO 2007). Although helminth infections rarely result in death, the morbidity they cause is significant. The annual disability-adjusted life years (DALY) lost worldwide owing to these diseases are 22.1 million for hookworm infection and 10.5 million for ascariasis (Chan 1997).

Previous observational and randomized-control studies have examined the relationship between soil-transmitted infections and cognitive function among school-aged children (Nokes *et al.* 1992; Sternberg *et al.* 1997; Hadidjaja *et al.* 1998; Oberhelman *et al.* 1998; Sakti *et al.* 1999; Ezeamama *et al.* 2005). The results from these studies suggest that helminth infections in children impair their cognitive development; however, these studies vary by geographic region examined, by helminth infections in the population, by cognitive assessments used and by research design. Moreover, in a systematic review of the effects of anthelmintic drug treatment on growth and cognitive performance in children, Dickson *et al.* (2000) found insufficient evidence that routine anthelmintic treatment improves cognitive performance. Hence, we need additional epidemiological studies on the association between helminth infection and cognitive performance, especially in under-represented geographic areas (e.g. South America). Although previous studies analysed the effect of a single helminth infection on cognitive performance (Watkins & Pollitt 1997), none analysed the impact of polyparasitism (i.e. infection with more than one helminth) on cognition in children. Our study examined whether the association between soil-transmitted helminth infections and cognitive function differs between polyparasitized children and children with only one helminth infection (mono-infection).

Methods

The data analysed in this study were collected as part of a longitudinal study of the epidemiological and immunological aspects of helminth infections in high transmission areas of Minas Gerais state, Brazil. The study was approved by the ethical review committees of the Universidade Federal de Minas Gerais (Belo Horizonte, Minas Gerais) and George Washington University Medical Center (Washington, DC). Written informed consent was obtained from all parents or guardians of participating children.

Study population

The study was conducted in the rural town of Americaninhas, located ca. 700 km from the state capital of Belo Horizonte in Minas Gerais, Brazil. A detailed description of the geographic location of the study site is available in Fleming *et al.* (2006). To be eligible for the study, children had to be between the ages of 6 and 11 years and had to attend one of seven public elementary schools located within 20 km of the commercial centre of the town. All children within the target age range at the seven schools were invited to participate. Researchers met with parents to explain the study and its risks and benefits. After parental consent, all 210 eligible children were enrolled in the study, although faecal samples for parasitological assessment were obtained from only 196. There were no statistically significant differences in the age (t-value = −1.38; $P = 0.17$) or sex ($\chi^2 = 0.11$; $P = 0.74$) of the 14 children who did not provide faecal samples and those who did. For 24 children, socioeconomic data were not collected because no one was available in the child's home to participate in a pre-scheduled, home-based socioeconomic questionnaire. There were no statistically significant differences in the age (t-value = 1.81; $P = 0.07$) or sex ($\chi^2 = 0.24$; $P = 0.62$) of the children whose socioeconomic data were collected and those whose data were not collected.

Cognitive functioning

Researchers administered the Raven Colored Progressive Matrices (Raven test) and three subtests of the Wechsler Intelligence Scale for Children – third edition (WISC-III) (Table 1) that had been previously validated for Brazilian children (Angeleni *et al.* 1999; Figueiredo 2002). The Raven test measures a person's ability to form perceptual relations and to reason by analogy independent of language and formal schooling (Raven & Raven 2003). Raw Raven test scores were converted into percentiles based on the child's age. For this study, the Digit, Arithmetic and Coding WISC-III subtests were selected for use owing to the ease of their applicability in the study population, the possibility of retesting and the facility of rating results (Cunha 2000). The WISC-III Digit Span subtest measures short-term auditory attention and memory. The WISC-III Arithmetic subtest is used to determine numerical reasoning skills of school children and their ability to concentrate and integrate spoken instructions with arithmetic knowledge. Children were tested on counting, addition, subtraction and multiplication. The WISC-III Coding subtest assesses the ability to learn unfamiliar tasks in the form of matching two sets of symbols or numbers and symbols in a given period of time (Priftera *et al.* 1998). Raw scores for each of the WISC-III subtests were converted into final scores based on age-specific norms.

In terms of cognitive development, the Raven test measures fluid intelligence, which represents reasoning abilities, whereas the WISC-III test measures crystallized intelligence, which represents general knowledge (Flores-Mendonça & Nascimento 2007). Fluid intelligence signifies cognitive processing capability, that is, the ability to connect complex ideas, build abstract concepts and apply logic inference from general rules. Furthermore, fluid intelligence involves applying mental operations to relatively new problems without using knowledge that has been previously learned. Crystallized intelligence, on the other hand, demonstrates the extension and depth of information acquired normally through school or school-like settings. In general, crystallized intelligence is used to solve problems similar to those experienced in the past and it signifies organized schemes of information related to specific knowledge areas (Primi *et al.* 2001).

A team of psychology graduate students who were trained and monitored by a leading psychologist administered the cognitive tests. Tests were administered to each child individually in a classroom in their school. The classrooms had appropriate lighting and temperature and

Table 1 Characteristics and applications of the cognitive tests

Test	Method	Type	Skills
WISC-III Digit Span	Proctor verbally states numbers; child repeats them back in same order and in inverse order	Verbal test	Measures auditory working memory
WISC-III Coding	Child is shown a legend where numbers or signs are associated with shapes; child is presented with scenarios involving matching according to the legend; each tests gets harder/quicker	Non-verbal test Performance test	Measures short-term memory, perceptual abilities, motor coordination, speed
WISC-III Arithmetic	The proctor provides verbal narratives involving arithmetic skills; the child must perform the math problem in his/her head and then state the answer	Verbal test	Measures mathematical knowledge, mental computations, and concentration
Raven's Colored Progressive Matrices	Child is shown an incomplete geometric diagram; child has shapes that he/she decides how to complete the design	Non-verbal test	Measures the ability to learn a concept, attention, memory and provides an estimate of general intelligence and learning potential

WISC-III, Wechsler Intelligence Scale for Children – third edition.

minimal external noise. Each child was given a snack prior to test administration to minimize the effects of short-term hunger (Pollitt & Mathews 1998; Gibson & Green 2002). The Raven test was administered first and took approximately 10 min to complete, followed by the three WISC-III subtests which in total took approximately another 20 min. Children were permitted to take a break if they wished and were also informed at the beginning of the test that they could discontinue at any time.

Parasitological assessments

The presence of hookworm and *A. lumbricoides* infection was determined by the formalin–ether sedimentation technique (Neves 2003). Children positive for any helminth infection in the formalin–ether sedimentation technique were asked to provide two additional faecal samples from different days for quantification of infection by the Kato–Katz method (Katz *et al.* 1972). The intensity of infection was estimated indirectly by counting the number of eggs per gram of faeces (epg) and categorized into infection intensity thresholds. For *A. lumbricoides* infection, moderate to high intensity was defined as greater than or equal to 5000 epg and low intensity was defined as 1–4999 epg (WHO 1987). Moderate-to-high hookworm infection was defined as at least 1000 epg, and light infection was defined as fewer than 1000 epg (WHO 1991).

Infection with *Schistosoma mansoni* is not considered a primary independent variable in this study because the prevalence was relatively low (37%) compared with *A. lumbricoides* and hookworm (>71%). Schistosomiasis infection is considered as a covariate, and moderate-to-high intensity of *S. mansoni* infection was defined as 100 epg or more and low intensity infection as 1–99 epg (WHO 1993).

Socioeconomic status

Parents were asked to meet a trained researcher in the child's home for an assessment of the family's socioeconomic status (SES). A family's SES index was determined using basic questions about possessions and housing conditions according to the method of Filmer and Pritchett (2001). The index allows for the interpretation of an individual's SES status in comparison with his/her community, which is especially useful in resource-poor settings (Brooker *et al.* 2007). The lowest SES index indicates poorest socioeconomic status.

Haemoglobin assessment

Of the 196 children who submitted faecal samples, 150 also agreed to have venous blood collected by a trained phlebotomist. Haemoglobin (Hb) concentration was measured using a Coulter Model S Counter (Coulter MAXM; Beckman Coulter Inc., Fullerton, CA, USA).

Statistical methods

All statistical analyses were conducted using SAS® Version 8.2 software (SAS Institute Inc., Cary, NC, USA).

Univariable analyses were conducted to obtain descriptive information on all variables of interest in the study. Chi-square tests were used to assess whether sex was associated with hookworm or *A. lumbricoides* infection. Student's *t*-tests were used to compare mean cognitive tests scores, age, SES and Hb between children infected and not infected with hookworm and between children infected and not infected with *A. lumbricoides*.

Proportional odds models were used to quantify the association between the intensity of helminth infection and poor performance on each cognitive test. The distribution of scores on each cognitive test in the study population was divided into four ordinal groups by quartiles. The first quartile represented the poorest performance on the cognitive test and the fourth quartile represented the highest performance for each test. Each analysis modelled the odds of poor performance on a cognitive test. As opposed to using internationally established performance cut-offs, the quartile categories allowed for the comparison of children in the study population relative to their peers. The proportional odds models satisfied the proportional odds assumption (Scott *et al.* 1997; Gameoff 2007).

The principal analyses were conducted in three steps: first, unadjusted odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated to express the crude association between the intensity of each individual helminth infection (as defined by WHO guidelines) and performance on each cognitive test. Second, multivariable logistic regression was conducted to assess the association between the intensity of infection and performance on each cognitive test after adjustment for age, sex and SES, as these three variables have been cited as confounders in previous studies of helminth infection and cognition (Sternberg *et al.* 1997; Oberhelman *et al.* 1998; Sakti *et al.* 1999; Ezeamama *et al.* 2005). If statistically significant associations were found, the role of each of these potential confounders, along with co-existing helminth infections, in that association was explored using backwards model selection. In this step, age, sex, SES and the three helminth infections (hookworm, *A. lumbricoides* and *S. mansoni*) were included in the proportional odds model. Co-existing helminth infections were included in the model as intensity groupings (no infection, low intensity of infection, moderate-to-high intensity of infection). Three two-way interaction variables were also included to assess interaction between helminth infections. Covariates were removed from the model if the variable's Wald *P*-value in the model exceeded 0.05. The model was run again and covariates were removed until all covariates in the model had a significance level of $P = 0.05$ or less. If significant associations were found between either *A. lumbricoides* infection or hookworm infection and poor performance on any of the cognitive tests, a sub-analysis was conducted, whereby Hb was added as a covariate in the fitted model and its significance in the model was reported.

The potential role of the intensity of helminth infection among polyparasitized children was examined using proportional odds analyses. Hookworm, *A. lumbricoides* and *S. mansoni* were prevalent in the study population, and these three helminth species are considered according to three categories of intensity (none, light and moderate-to-high). Therefore, when considering overlapping burdens of the three helminth infections and their respective infection intensities, there are 27 combinations of infection and intensity to be considered. To explore the effect of polyparasitism *vs.* individual helminth infection on cognitive test performance, the distribution of the study population within these 27 polyparasitism groups was determined. To maximize the acceptability of the results given a limited sample size, the two polyparasitism groups with the largest number of children were chosen for the polyparasitism sub-analyses. Using logistic regression, the OR and 95% CI for the likelihood of poor performance on each cognitive test was estimated for a polyparasitized group versus a group with only one type of helminth infection.

Results

Table 2 shows the prevalence of hookworm, *A. lumbricoides* and *S. mansoni* infection by helminth intensity groups (no infection, low infection, moderate-to-high infection) and the mean epg of faeces for each infection in the study population. Of the 196 children who participated in the cognitive testing and provided faecal samples, 140, 142 and 72 were infected with *A. lumbricoides,* hookworm and *S. mansoni*, respectively. Only 14 children had no intestinal helminth infections, whereas 48% were co-infected with two helminth infections and 19% were co-infected with *A. lumbricoides,* hookworm and *S. mansoni.*

In the study population, the calculated SES index ranged from −2.23 to 3.77, and the mean SES index value was −0.11, with a standard deviation (SD) of 1.55 (Table 3). 107/196 study participants (54.59%) were male. The prevalence of hookworm and *A. lumbricoides* infection in males was 70.1% and 66.4%, respectively, and the prevalence of hookworm and *A. lumbricoides* infection in females was 75.3% and 77.5%, respectively. Chi-square tests showed that sex was not significantly associated with hookworm infection ($P = 0.42$) or *A. lumbricoides* infection ($P = 0.08$). Children with hookworm infection were significantly older than children without: the mean age of children with hookworm infection was 8.21 years and that of uninfected children was 7.72 years ($P = 0.04$). Children

Table 2 Prevalence and mean eggs per gram (epg) of faeces of hookworm. *Ascaris lumbricoides* and *Schistosoma mansoni* infection in the study population (n = 196)

Hookworm Mean epg = 1569			*A. lumbricoides* Mean epg = 5809			*S. mansoni* Mean epg = 164		
	#	%		#	%		#	%
Medium to high intensity (≥1000 epg)	53	27.04	Medium to high intensity (≥5000 epg)	96	48.98	Med to high intensity (≥100 epg)	36	18.37
Low intensity (1–999 epg)	89	45.41	Low intensity (1–4999 epg)	44	22.45	Low intensity (1–99 epg)	36	18.37
No infection	54	27.55	No infection	56	28.57	No infection	124	63.27

Table 3 Description of sex, age, socioeconomic status, haemoglobin and anaemia prevalence by hookworm infection status (yes/no) and by *Ascaris lumbricoides* infection status (yes/no)

		Hookworm infection			*A. lumbricoides* infection		
	Total	No	Yes		No	Yes	
Sex							
n	196	54	142		56	140	
Female # (%)	89 (45.41%)	22 (40.74%)	67 (47.18%)	*P* value	20 (35.71%)	69 (49.29%)	*P* value
Male # (%)	107 (54.59%)	32 (59.26%)	75 (52.82%)	0.42	36 (64.29%)	71 (50.71%)	0.08
			Female *vs.* Male POR (95% CI) = 0.77 (0.41–1.45)			Female *vs.* Male POR (95% CI) = 0.57 (0.30–1.08)	
Age (years)							
n	196	54	142	*P* value	56	140	*P* value
Mean ± sd	8.08 ± 1.52	7.72 ± 1.41	8.21 ± 1.54	0.04	8.04 ± 1.68	8.10 ± 1.46	0.79
SES index							
Range: −2.23 to 3.77							
n	172	50	122	*P* value	44	128	*P* value
Mean ± SD	−0.11 ± 1.55	0.55 ± 1.71	0.38 ± 1.40	<0.01	0.23 ± 1.77	−0.22 ± 1.46	0.10
Haemoglobin (mg/dl)							
n	150	49	101	*P*-value	39	111	*P*-value
Mean ± sd	12.69 ± 1.25	12.75 ± 0.91	12.66 ± 1.39	0.63	12.52 ± 1.75	12.75 ± 1.02	0.45
Anaemia							
n	150						
Anaemic # (%)	15 (10.00%)	1 (2.04%)	14 (13.86%)	*P*-value	3 (7.69%)	12 (10.81%)	*P*-value
Not anaemic # (%)	135 (90.00%)	48 (97.96%)	87 (86.14%)	0.02	36 (92.31%)	99 (89.19%)	0.58

POR, prevalence odds ratio; CI, confidence interval.

with hookworm infection also had significantly lower SES than uninfected children (P < 0.01).

When comparing the mean Hb concentration between children infected with hookworm and those not infected with hookworm, there was no statistically significant difference (P = 0.63). Ten per cent of the study population (15 children) was anaemic, which is defined as having a Hb level less than 11.5 mg/dl (WHO 2001). 13.86% of children infected with hookworm were anaemic, as were 2.04% of children not infected; this difference was statistically significant (P = 0.02). 10.81% of children infected with *A. lumbricoides* were anaemic, as were 7.69% of children not infected; this difference was not statistically significant (P = 0.58).

As shown in Table 4, children with hookworm infection achieved significantly lower mean scores in all four cognitive tests than uninfected children (Digit P = 0.01, Coding P < 0.0001, Arithmetic P < 0.01, Raven P < 0.01). For *A. lumbricoides*, only the mean Raven test score in uninfected children was significantly higher than the mean test score in infected children (P = 0.02).

The highest score possible was 100 for the Raven and 19 for the Coding, Arithmetic and Digit subtests. In the current study population, the mean ± SD was 19.36 ± 17.40 for Raven, 7.02 ± 3.96 for Arithmetic, 7.93 ± 3.27 for Digit Span and 6.56 ± 3.15 for Coding (Table 4). The average range of Raven test scores for children with typical cognitive development is between 25

Table 4 Description of WISC-III Digit, Coding and Arithmetic and Raven Colored test cognitive test scores by hookworm infection status (yes/no) and *Ascaris lumbricoides* infection status (yes/no)

	Total	Hookworm infection			*A. lumbricoides* infection		
	n = 196	No *n* = 54	Yes *n* = 142		No *n* = 56	Yes *n* = 140	
	Mean ± SD	Mean ± SD	Mean ± SD	*P*	Mean ± SD	Mean ± SD	*P*
WISC Digit Span score	7.93 ± 3.27	8.85 ± 3.18	7.58 ± 3.25	0.01	8.52 ± 3.35	7.69 ± 3.22	0.11
WISC Coding score	6.6 ± 3.15	8.11 ± 3.37	5.96 ± 2.86	<0.0001	6.86 ± 3.01	6.44 ± 3.21	0.40
WISC Arithmetic score	7.02 ± 3.96	8.41 ± 4.03	6.49 ± 3.82	<0.01	7.14 ± 4.09	6.96 ± 3.92	0.78
Raven score	19.36 ± 17.40	27.52 ± 22.27	16.26 ± 14.04	<0.01	24.59 ± 20.79	17.27 ± 15.44	0.02

WISC-III, Wechsler Intelligence Scale for Children – third edition.

and 74 and the average range of WISC-III subtest scores for children with typical cognitive development is 8 to 12 (Angelini 1999; Sattler 1992). Therefore, our study population is below the expected range for cognitive development.

In unadjusted logistic regression analysis, hookworm infection was significantly associated with poor performance on every cognitive test across all infection intensity groups (moderate-to-high intensity and low intensity), with the exception of the Digit subtest, where only low intensity of hookworm infection was associated with significantly greater odds of poor test performance (Table 5). The relationship between hookworm infection and poor performance on the WISC-III Coding subtest was especially strong: children with moderate-to-high intensity hookworm infection demonstrated 3.62 greater unadjusted odds of poor performance on the Coding subtest (95% CI = 1.75–7.48) and children with low intensity of infection demonstrated a 3.81 greater odds of poor performance on the test (95% CI = 1.97–7.35) than uninfected children. In unadjusted analysis, moderate-to-high *A. lumbricoides* infection was significantly associated with poor performance on the Raven test (OR: 1.99; 95% CI = 1.09–3.61).

After adjusting for sex, age and socioeconomic status, hookworm infection remained a significant predictor of poor performance on the Coding subtest for both groups of infection intensity (Table 5). Even after controlling for these three variables, the odds of performing poorly on the Coding subtests were 3.37 (95% CI = 1.64–6.93) and 3.20 (95% CI = 1.44–7.09) times higher for low and moderate-to-high intensity of infection, respectively. Statistically significant associations for the other three cognitive tests however were no longer evident after controlling for sex, age and socioeconomic status. The association between moderate-to-high *A. lumbricoides* infection and poor performance on the Raven test remained even after controlling for sex, age and socioeconomic status: the adjusted OR for poor performance on the Raven test was 2.04 (95% CI = 1.03–4.01) for children with moderate-to-high intensity *A. lumbricoides* infection compared with uninfected children.

Using backwards model selection, a proportional odds model, including age, sex, SES and presence and intensity of co-existing helminth infections (no infection, low intensity or moderate-to-high intensity) was developed to identify the significant covariates involved in the association between hookworm infection and poor performance on the Coding test and the association between *A. lumbricoides* infection and poor performance on the Raven test (data not shown). For the association between hookworm infection and Coding subtest score, the final fitted model included SES ($P < 0.01$), sex ($P < 0.01$), and *S. mansoni* co-infection ($P = 0.01$) as significant covariates. After adjusting for these variables, the OR for both moderate-to-high hookworm intensity and low hookworm intensity and poor performance on the Coding test remained significantly higher than in uninfected children [moderate-to-high intensity: OR = 3.20 (95% CI = 1.43–7.17); low intensity: OR = 3.71 (95% CI = 1.80–7.66)]. For *A. lumbricoides*, SES remained the only statistically significant covariate ($P < 0.01$) in the final fitted model assessing the relationship between moderate-to-high infection and low Raven test scores. After controlling for SES, the OR was 2.03 (95% CI = 1.04–3.99). We found no statistically significant interactions between helminth infections in this model fitting exercise.

Haemoglobin

Haemoglobin was added as a continuous variable to the fitted logistic regression models that were obtained using backwards model selection. Of the 150 children for whom there were Hb data, two lacked SES data; therefore, the analyses with Hb included only 148 children. When Hb was added to the hookworm-Coding test regression model, the *P* value associated with Hb in the model was not statistically

Table 5 Poor performance in cognitive tests among children in rural Brazil; unadjusted and adjusted associations

		Unadjusted OR (95% CI) n = 196	Adjusted for age, sex and socioeconomic status OR (95% CI) n = 172
Cognitive test	**Hookworm infection**		
WISC – Digit Span	None	1.00	1.00
	Low intensity	**2.40 (1.28, 4.47)**	1.77 (0.89, 3.51)
	≥ Moderate intensity	1.84 (0.92, 3.68)	1.23 (0.58, 2.65)
	Any intensities	**2.17 (1.22, 3.88)**	1.55 (0.82, 2.92)
WISC – Coding	None	1.00	1.00
	Low intensity	**3.81 (1.97, 7.35)**	**3.37 (1.64, 6.93)**
	≥ Moderate intensity	**3.62 (1.75, 7.48)**	**3.20 (1.44, 7.09)**
	Any intensities	**3.73 (2.02, 6.91)**	**3.30 (1.69, 6.47)**
WISC – Arithmetic	None	1.00	1.00
	Low intensity	**2.30 (1.24, 4.28)**	1.48 (0.75, 2.93)
	≥ Moderate intensity	**2.28 (1.14, 4.54)**	1.32 (0.62, 2.83)
	Any intensities	**2.30 (1.29, 4.08)**	1.42 (0.75, 2.66)
Raven	None	1.00	1.00
	Low intensity	**2.02 (1.09, 3.74)**	1.54 (0.78, 3.05)
	≥ Moderate intensity	**2.78 (1.39, 5.58)**	2.14 (1.00, 4.60)
	Any intensities	**2.28 (1.28, 4.05)**	1.76 (0.94, 3.33)
Cognitive test	***A. lumbricoides* infection**		
WISC – Digit Span	None	1.00	1.00
	Low intensity	1.50 (0.73, 3.07)	1.45 (0.66, 3.16)
	≥ Moderate intensity	1.64 (0.90, 3.00)	1.46 (0.74, 2.87)
	Any intensities	1.60 (0.91, 2.81)	1.45 (0.77, 2.76)
WISC – Coding	None	1.00	1.00
	Low intensity	0.82 (0.40, 1.71)	1.05 (0.47, 2.33)
	≥ Moderate intensity	1.69 (0.92, 3.10)	1.85 (0.93, 3.69)
	Any intensities	1.34 (0.76, 2.36)	1.52 (0.80, 2.91)
WISC – Arithmetic	None	1.00	1.00
	Low intensity	0.65 (0.32, 1.33)	0.56 (0.25, 1.24)
	≥ Moderate intensity	1.64 (0.91, 2.98)	1.54 (0.76, 3.03)
	Any intensities	1.22 (0.70, 2.13)	1.12 (0.59, 2.11)
Raven	None	1.00	1.00
	Low intensity	1.14 (0.56, 2.32)	1.10 (0.51, 2.41)
	≥ Moderate intensity	**1.99 (1.09, 3.61)**	**2.04 (1.03, 4.01)**
	Any intensities	1.65 (0.94, 2.90)	1.66 (0.87, 3.13)

Bold numbers indicate statistical significance ($P < 0.05$).
OR, odds ratio; CI, confidence interval.

significant ($P = 0.74$) and when Hb was added to the *A. lumbricoides*-Raven model, the P value associated with Hb in the model was not statistically significant ($P = 0.70$).

Polyparasitism

Children with moderate-to-high *A. lumbricoides* burden and low hookworm burden formed the largest polyparasitized group ($n = 31$) (Table 6), whereas children with moderate-to-high intensity *A. lumbricoides* infection and moderate-to-high intensity hookworm infection represented the second largest polyparasitized group ($n = 23$). Separate logistic regression analyses were conducted to estimate poor performance on each cognitive test as compared with two reference groups: (i) children with only *A. lumbricoides* infection, of any intensity ($n = 20$); and (ii) children with only hookworm infection, of any intensity ($n = 23$). Children with a moderate-to-high *A. lumbricoides* burden and a light hookworm burden had 4.63 greater odds of poor performance on the WISC-III Arithmetic subtest than children with only *A. lumbricoides* infection (95% CI = 1.56–13.79). This association remained statistically significant after controlling for age, sex and SES (OR = 6.67; 95% CI = 1.73–25.74). The odds of poor performance on the WISC-III Arithmetic subtest were also higher among children with moderate-to-high intensity

Table 6 Unadjusted odds for poor performance on cognitive tests in polyparasitized children compared with children with only one helminth infection

	Polyparasitism group 1 ≥ Moderate intensity of *Ascaris lumbricoides* Low intensity of hookworm No *Schistosoma mansoni* infection *n* = 31		Polyparasitism group 2 ≥ Moderate intensity of *A. lumbricoides* ≥ Moderate intensity of hookworm No *S. mansoni* infection *n* = 23	
Reference groups	Only *Ascaris* Infection *n* = 20 Unadjusted OR (95% CI) *P* value	Only hookworm infection *n* = 23 Unadjusted OR (95% CI) *P* value	Only *Ascaris* infection *n* = 20 Unadjusted OR (95% CI) *P* value	Only hookworm infection *n* = 23 Unadjusted OR (95% CI) *P* value
WISC – Digit	1.51 (0.54, 4.22) *P* = 0.43	1.04 (0.39, 2.78) *P* = 0.94	0.93 (0.32, 2.72) *P* = 0.89	0.71 (0.25, 2.04) *P* = 0.53
WISC – Coding	2.31 (0.81, 6.62) *P* = 0.12	1.23 (0.46, 3.31) *P* = 0.68	2.58 (0.81, 8.19) *P* = 0.11	1.28 (0.44, 3.74) *P* = 0.66
WISC – Arithmetic	4.63 (1.56, 13.79)* *P* = 0.01	1.73 (0.64, 4.66) *P* = 0.28	2.35 (0.78, 7.06) *P* = 0.13	1.03 (0.36, 2.93) *P* = 0.95
Raven	1.20 (0.44, 3.30) *P* = 0.73	1.10 (0.42, 2.91) *P* = 0.85	1.25 (0.43, 3.67) *P* = 0.68	1.23 (0.43, 3.50) *P* = 0.70

*After controlling for age, sex and socioeconomic status, the odds ratio and corresponding 95% CI are 6.67 and (1.73, 25.74).

A. lumbricoides and moderate-to-high intensity hookworm infection (*n* = 23) than among children with only *A. lumbricoides* infection (*n* = 20), although this was not statistically significant (OR = 2.35, 95% CI = 0.78–7.06).

For further examination, the odds of performing poorly on each cognitive test among all children co-infected with *A. lumbricoides* and hookworm, regardless of intensity of infection, as compared with children with only one infection, was calculated. Children co-infected with any intensity of both *A. lumbricoides* and hookworm (*n* = 68) had 2.62 greater odds of poor performance on the WISC-Coding subtest than children with only *A. lumbricoides* infection (95% CI = 1.03–6.70). However, the odds of poor performance on the WISC-Coding test among children co-infected with any intensity of *A. lumbricoides* and hookworm compared with children with only hookworm infection (*n* = 23) were not statistically significant (OR = 1.16; 95% CI = 0.49–2.80). Logistic regression analyses with the same groups and the other three cognitive tests did not yield any statistically significant results. Children co-infected with any intensity of *A. lumbricoides* and hookworm did have a greater odds of poor performance on the WISC-Arithmetic subtest as compared with children with only *A. lumbricoides* infection, although this result was not statistically significant (OR = 2.33; 95% CI = 0.95–5.75).

Discussion

This study provides evidence that infection with hookworm or *A. lumbricoides* is associated with poor performance on tests of cognitive functioning among school children in rural Brazil. Even after controlling for sex, age, socioeconomic status and other helminth infections, hookworm infection was associated with poor performance on the WISC-III Coding test, and moderate-to-high intensity of *A. lumbricoides* infection was associated with poor performance on the Raven test. This is also the first study to present evidence suggesting that polyparasitized children experience worse cognitive outcomes than children with only one helminth infection.

These results are consistent with findings from other epidemiological studies and randomized controlled trials examining the relationship between helminth infections and cognitive function among children (Nokes *et al.* 1992; Hadidjaja *et al.* 1998; Oberhelman *et al.* 1998; Sakti *et al.* 1999; Ezeamama *et al.* 2005). In a study of children between the ages of 8 and 13 years in Indonesia, Sakti *et al.* (1999) reported that hookworm infection was independently associated with lower scores on six WISC subtests. A randomized controlled trial of *A. lumbricoides*-infected children who were treated with mebendazole demonstrated greater improvements in their Raven test scores than in children who received placebo (Hadidjaja *et al.* 1998). These observations support our findings that hookworm infection is associated with lower WISC scores and that moderate-to-high *A. lumbricoides* infection is associated with lower Raven test scores.

Ascaris lumbricoides infection is associated with reduced food consumption, malabsorption of carbohydrates, protein and fat, and intestinal tract damage (Crompton & Nesheim

2002). In an analysis of anthropometric measures in the same study population in Americaninhas, Brazil, *A. lumbricoides* infection was associated with growth stunting in children (Jardim-Botelho *et al.* 2008). Stunted physical growth among children is linked with chronic malnutrition (WHO 1995). According to Raven (2000), intelligence is comprised of educative ability, which refers to the ability to analyse complex situations, and reproductive ability, which refers to memory and the ability to reproduce information. These abilities are especially affected by biological and neurological factors and by incidental learning (Cunha 2000). It is possible that chronic malnutrition is an environmental determinant that leads to reduced educative and reproductive abilities, and therefore, poor performance on the Raven test, among children with heavy *A. lumbricoides* infections. Another analysis has supported the notion that growth stunting and chronic malnutrition are related to poor performance on Raven tests (Tarleton *et al.* 2006).

The intestinal blood loss caused by adult hookworms is a well-known cause of iron-deficiency anaemia in endemic populations (Crompton & Nesheim 2002). The distribution of oxygen to the brain via Hb in red blood cells is an important factor in the cognitive development of children, as the brain requires adequate amounts of oxygen for its aerobic metabolism (Brody 1994; Bourre 2006). The WISC-III subtests are best interpreted through the four components of information processing: input, integration, storage and output (Kaufman 1994). The Coding and Arithmetic subtests, in particular, measure a child's ability to concentrate during each of these information processing components and also the speed with which he or she is able to completely process the information (Kaufman 1994). Lower levels of Hb have been linked with reduced ability to work and concentrate (WHO 2001). Therefore, it is likely that low Hb levels and the resulting anaemia are an important underlying mechanism that contribute to this study's finding that hookworm infection is linked with poorer performance on WISC-III subtests, especially the Coding subtest. Previous studies support the concept of low Hb as the linking biological mechanism between hookworm infection and cognitive delays (Stoltzfus *et al.* 2001; Kordas *et al.* 2004). Specific to our findings with the WISC subtests, a cross-sectional study of anaemia and cognition among Mexican school children found that children with lower Hb concentration had performed poorer on WISC subtests than children with higher Hb concentrations (Kordas *et al.* 2004). Performance on the WISC-Coding subtest in particular was associated with lower iron deficiency, which can lead to low Hb levels (Kordas *et al.* 2004).

In this study, Hb was not a significant predictor of low cognitive test scores and did not statistically confound the association between the helminth infection and poor test performance in both statistical models into which Hb was added as a covariate. Low Hb concentrations among children in resource-poor settings are caused by nutritional deficiencies, helminth infections and other diseases (WHO 2001). Adjusting for Hb concentration in statistical analyses allows for the control of these multiple contributory factors. In our study population, hookworm infection and Hb were strongly correlated, likely owing to the heavy blood loss caused by adult hookworms in the intestine (Crompton & Nesheim 2002). Therefore, in this population, it may be more appropriate to consider Hb concentration as part of the causal chain between hookworm infection and poor cognitive test performance, as opposed to considering Hb concentration as a potential confounder. Additional research, including quantification of faecal Hb, which can be used to approximate the relative contribution of hookworm infection on low Hb concentration, may aid in clarifying the role of Hb as an underlying cause of the association between hookworm infection and poor cognitive test performance.

A previous study in Americaninhas, Brazil, suggested that synergism between multiple helminth species within human hosts may have important epidemiological and immunological implications for polyparasitized individuals (Fleming *et al.* 2006). We attempted to assess the effects of polyparasitism on cognitive function. To do so, a method was developed whereby poor performance on cognitive tests was compared between groups of polyparasitized children and groups of children with only one helminth infection. The finding that children with both *A. lumbricoides* and hookworm infection performed worse on WISC-III subtests, especially the Arithmetic and Coding subtests, than children with only *A. lumbricoides* infection supports our overall finding that hookworm infection is related to poor concentration, distractibility resistance and cognitive quickness. It is interesting that the only statistically significant OR of poor performance on the WISC-III Arithmetic subtest was noted in children with moderate-to-high *A. lumbricoides* and light hookworm infection, and not in the group of children with moderate-to-high *A. lumbricoides* and moderate-to-high hookworm infection. This may be attributed to the small sample size of children with moderate-to-high *A. lumbricoides* infection and moderate-to-high hookworm infection. A key limitation in the polyparasitism sub-analysis were the small sample sizes. Analyses in larger study populations would allow for a better understanding of the association between polyparasitism and cognitive functioning in children.

Another limitation is that, as this was a cross-sectional study, causal inferences cannot be made on the relationship between helminth infections and cognitive development (Ezeamama *et al.* 2005). Randomized controlled trials are needed to study such casual relationships. Another important limitation is that total intelligence, as defined by Wechsler, could not be calculated because not all the WISC-III subtests were administered (Kaufman 1994). We chose the three subtests that were most applicable to children living in rural environments with limited educational opportunities – Digit, Coding, and Arithmetic – which allowed us to focus on assessing the childrens' learning capability and school experiences (Cunha 2000). Our study incorporated the Raven Colored Test, which allowed for an overall assessment of intellectual capability using a non-verbal method appropriate for the study population's environment. Residual confounders may exist in our analysis, such as domestic factors that mediate cognitive development. The dynamics of the family, especially the mother–child relationship, are associated with children's social behaviour, emotional, status and reasoning, conditions that impact on cognitive growth (Peterson & Skevington 1988; Adrián *et al.* 2007).

In summary, this study provides evidence of statistically significant associations between hookworm and *A. lumbricoides* infection and poor performance on tests of cognitive development among children in rural Brazil. A follow-up study is currently underway to assess whether there is any change in cognitive performance after treatment with anthelminthic drugs. Our study population in Americaninhas, Brazil, had substandard mean scores for typical cognitive development as assessed by the WISC-III subtests and the Raven Colored Test. Mean scores on the Raven test however were similar to those reported in other rural areas of Brazil, indicating that the results from this study may be applicable to other *A. lumbricoides* and hookworm endemic areas in Brazil (Santos *et al.* 2002).

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References

Adrián JE, Clemente RA & Villanueva L (2007) Mothers' use of cognitive state verbs in picture-book reading and the development of children's understanding of mind: a longitudinal study. *Child Development* **78**, 1052–1067.

Angeleni AL, Alves ICB, Custódio EM, Duarte J LM & Duarte WF (1999) *Manual Matrizes Progressivas Coloridas de Raven: escala especial*. Centro Editor de Testes e Pesquisas em Psicologia, São Paulo.

Bethony J, Brooker S, Albonico M *et al.* (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* **367**, 1521–1532.

Bourre JM (2006) Effects of nutrients (in food) on the structure and function of nervous system: update on dietary requirements for brain. Part 1: micronutrients. *Journal of Nutrition, Health & Aging* **10**, 377–385.

Brody T (1994) *Nutritional Biochemistry*. Academic Press, San Diego.

Brooker S, Jardim-Botelho A, Quinnell RJ *et al.* (2007) Age-related changes in hookworm infection, anaemia and iron deficiency in an area of high *Necator americanus* hookworm transmission in south-eastern Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **101**, 146–154.

Chan MS (1997) The global burden of intestinal nematode infections – fifty years on. *Parasitology Today* **13**, 438–443.

Crompton DW & Nesheim MC (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. *Annual Review of Nutrition* **22**, 35–59.

Cunha JA (2000) *Psicodiagnóstico-V- Revisa e ampliada*. Artmed, Porto Alegre.

Dickson R, Awasthi S, Williamson P, Demellweek C & Garner P (2000) Effects of treatment for intestinal helminth infection on growth and cognitive performance in children: systematic review of randomised trials. *British Medical Journal* **320**, 1697–1701.

Ezeamama AE, Friedman JF, Acosta LP *et al.* (2005) Helminth infection and cognitive impairment among Filipino children. *American Journal of Tropical Medicine and Hygiene* **72**, 540–548.

Figueiredo VLM (2002) *WISC-III: Escala de Inteligencia Wechsler para Crianças: Manual. Adaptação e Padronização de uma amostra Brasileira*. Casa do Psicólogo, São Paulo.

Filmer D & Pritchett L (2001) Estimating wealth effects without expenditure data – or tears: an application to educational enrolment in states of India. *Demography* **38**, 115–132.

Fleming FM, Brooker S, Geiger SM *et al.* (2006) Synergistic associations between hookworm and other helminth species in a rural community in Brazil. *Tropical Medicine and International Health* **11**, 56–64.

Flores-Mendonça CE & Nascimento E (2007) Condição cognitiva de crianças de zona rural. *Estudos de Psicologia* **24**, 13–22.

Gameoff M (2007) *Using the Proportional Odds Model for Health-Related Outcomes: Why, When, and How with Various SAS® Procedures*. Paper 205-30. Available: http://www2.sas.com/proceedings/sugi30/205-30.pdf>. Accessed on 8 June 2007

Gibson EL & Green MW (2002) Nutritional influences on cognitive function: mechanisms of susceptibility. *Nutrition Research Reviews* **15**, 169–206.

Hadidjaja P, Bonang E, Suyardi MA, Abidin SA, Ismid IS & Margono SS (1998) The effect of intervention methods on nutritional status and cognitive function of primary school children infected with *Ascaris lumbricoides*. *American Journal of Tropical Medicine and Hygiene* **59**, 791–795.

Jardim-Botelho A, Brooker S, Geiger SM *et al.* (2008) Age patterns in undernutrition and helminth infection in a rural area of Brazil: associations with Ascarisiasis and Hookworm. *Tropical Medicine and International Health* **13**, 458–467.

Katz N, Chaves A & Pelligrino J (1972) A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. *Revista Instituto Medicina Tropical* **14**, 397–400.

Kaufman A (1994) *Intelligence Testing with the WISC-III*. John Wiley and Sons, New York.

Kordas K, Lopez P, Rosado JL *et al.* (2004) Blood lead, anemia, and short stature are independently associated with cognitive performance in Mexican school children. *Journal of Nutrition* **134**, 363–371.

Neves DP (2003) *Parasitologia Humana*. Editora Atheneu, São Paulo.

Nokes C, Grantham-McGregor SM, Sawyer AW, Cooper ES & Bundy DA (1992) Parasitic helminth infection and cognitive function in school children. *Proceedings of The Royal Society: Biological Sciences* **22**, 77–81.

Oberhelman RA, Guerrero ES, Fernandez ML *et al.* (1998) Correlations between intestinal parasitosis, physical growth, and psychomotor development among infants and children from rural Nicaragua. *American Journal of Tropical Medicine and Hygiene* **58**, 470–475.

Peterson C & Skevington S (1988) The relation between young children's cognitive role-taking and mothers' preference for a conflict-inducing childrearing method. *Journal of Genetic Psychology* **149**, 163–174.

Pollitt E & Mathews R (1998) Breakfast and cognition: an integrative summary. *American Journal of Clinical Nutrition* **67**, 804s–813s.

Priftera A, Weiss L & Saklofske DH (1998) The WISC-III in Context. In: *WISC-III: Clinical Use and Interpretation* (eds A Priftera & D Saklofske) Academic Press, San Diego.

Primi R, Santos AAA, Vendramini CM *et al.* (2001) Competências e habilidades cognitivas: diferentes definições dos mesmos construtos. *Psicologia: Teoria e Pesquisa* **17**, 151–159.

Raven J & Raven J (2003) Raven Progressive Matrices. In: *Handbook of Nonverbal Assessment* (ed S McCallum) Kluwer Academia Plenum Publishers, Boston.

Sakti H, Nokes C, Hertanto WS *et al.* (1999) Evidence for an association between hookworm infection and cognitive function in Indonesian school children. *Tropical Medicine & International Health* **4**, 322–334.

Santos DN, Borges AP, Sanders PP & Almeida CA (2002) Epidemiology of schoolchildren's cognitive development in Jequié, Bahia States, Brazil: assessment procedures and general results. *Caderno de Saude Publica* **18**, 723–733.

Sattler JR (1992) *Assessment of Children: WISC-III and WPPSI-R Supplement*. Jerome M. Sattler, San Diego.

Scott SC, Goldberg MS & Mayo NE (1997) Statistical assessment of ordinal outcomes in comparative studies. *Journal of Clinical Epidemiology* **50**, 45–55.

Sternberg RJ, Powell C, McGrane P & Grantham-McGregor S (1997) Effects of a parasitic infection on cognitive functioning. *Journal of Applied Experimental Psychology* **3**, 67–76.

Stoltzfus RJ, Kvalswig JD, Chwaya HM *et al.* (2001) Effects of iron supplementation and antihelmintic treatment on motor and language development of preschool children in Zanzibar: double-blind, placebo controlled study. *British Medical Journal* **323**, 1–8.

Tarleton JL, Haque R, Mondal D, Shu J, Farr BM & Petri WA Jr (2006) Cognitive effects of diarrhea, malnutrition, and *Entamoeba histolytica* infection on school age children in Dhaka, Bangladesh. *American Journal of Tropical Medicine and Hygiene* **74**, 475–481.

Watkins WE & Pollitt E (1997) 'Stupidity or worms': do intestinal worms impair mental performance? *Psychological Bulletin* **121**, 171–191.

WHO (1987) Prevention and control of intestinal parasitic infections. *WHO Technical Report Series* **749**.

WHO (1991) *Hookworm Infection and Anemia: Approaches to Prevention and Control*. WHO, Geneva.

WHO (1993) The Control of Schistosomiasis. *WHO Technical Report Series* **830**.

WHO (1995) Physical status: the use and interpretation of anthropometry. *WHO Technical Report Series* **854**.

WHO (2001) *Iron Deficiency Anaemia: Assessment, prevention, and control – a guide for programme managers*. WHO, Geneva.

WHO (2007) *Partners for Parasite Control: Geographical distribution and useful facts and stats*. Available: http://www.who.int/wormcontrol/statistics/geographical/en/index.html. Accessed 15 March 2007.

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