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Evidence for an association between hookworm infection and cognitive function in Indonesian school children

Hastaning Sakti[1], Catherine Nokes[2], W. Subagio Hertanto[1], Sri Hendratno[1], Andrew Hall[2], Donald A. P. Bundy[2] and Satoto[1]

1 *Indonesian Partnership for Child Development, Kemitraan Indonesia Perkembangan Anak, Nutrition Department, Medical Faculty, University of Diponegoro, Jl Dr Sutomo, Central Java, Indonesia.*

2 *Partnership for Child Development, The Wellcome Trust Centre for the Epidemiology of Infectious Disease, Oxford University Oxford, UK*

Summary The association between helminth infection and cognitive and motor function was investigated in school-age children in Java, Indonesia. 432 children from 42 primary schools participated in the study. Children were stratified by age and sex into two age groups, 8–9 years and 11–13 years. Children infected with hookworm performed significantly worse than children without hookworm infection in 6 of the 14 cognitive or motor tests. After controlling for school (as a random effect) plus age, socio-economic status and parental education, sex, stunting (height-for-age < -2sd), body mass index, haemoglobin concentration and the presence of *A. lumbricoides* and *T. trichiura* infections, infection with hookworm explained significantly lower scores on tests of Fluency ($P < 0.01$), Digit-Span Forwards ($P < 0.01$), Number Choice ($P < 0.01$), Picture Search ($P < 0.03$), Stroop Colour Word ($P < 0.02$) and Mazes ($P < 0.001$). In 4 of the 6-tests (Fluency, Number Choice, Picture Search and Mazes), there was a significant interaction between hookworm infection and age ($P < 0.03$), indicating that the association between hookworm and lower test scores increased with age. No associations were observed between hookworm infection and scores in tests of Digit-Span Backwards, Corsi-Block, Stroop Colour, Stroop Interference, Free Recall, Verbal Analogies, Bead Threading or the Pegboard ($P > 0.05$). Tests associated with helminths represented various functions of working memory. No significant associations between helminth infection and motor function were observed that could not be explained by chance. The results suggest that hookworm infection can have a significant adverse effect on children's working memory which may have consequences for a child's reasoning ability and reading comprehension. Although the results are only associational, the fact that differences in cognition were observed at baseline imply that preventing infection with helminths in school-age children could be of benefit.

keywords hookworm, cognition, working memory, school children, Indonesia

correspondence: Dr Catherine Nokes, Partnership for Child Development, The Wellcome Trust Centre for the Epidemiology of Infectious Disease, Oxford University, South Parks Road, Oxford, OX1 3PS, UK. E-mail: kate.nokes@zoology.oxford.ac.uk

Introduction

School children are particularly at risk from infection with parasitic worms. Epidemiological evidence worldwide shows that school-age children are not only more likely to be infected, they are also more likely to be more heavily infected than other age groups (Anderson 1980; Bundy 1988). Since the worm burden is associated with the severity of morbidity, these children are likely to suffer most at an age when they are both growing and learning (Pollitt 1990; Nokes & Bundy 1994). While the biomedical effects of parasitic helminth infections are well described, the effects of infection on mental functioning and educational achievement are less clear, yet may be at least of equivalent importance for the individual as well as for national economic and social development (The Partnership for Child Development 1997).

Since the turn of the century geohelminth infection has been repeatedly shown to be associated with lower scores on tests of mental performance and educational achievement in school-age children (Stiles 1915; Waite & Neilson 1919; de Carneri *et al*. 1967; Kvalsvig *et al*. 1991; Nokes *et al*. 1991, 1992; Boivin *et al*. 1993; Callender *et al*. 1994; Nokes & Bundy 1994; Simeon *et al*. 1994; Levav *et al*. 1995; Sternberg *et al*. 1997b). On the other hand, cognitive or educational

benefits resulting from treatment of helminth infection are not consistent. For example, while treatment of whipworm infection led to improved scores on tests of working memory (Nokes *et al*. 1992; Simeon *et al*. 1995b) and school achievement (Simeon *et al*. 1995a), and treatment of hookworm led to improved scores on tests of children's spatial memory (Boivin *et al*. 1993), these findings were not replicated in broadly similar studies by Watkins *et al*. (1996) or Sternberg *et al*. (1997b).

Chronic undernutrition and iron deficiency anaemia are considered to mediate the effects of helminth infections on cognition, and in particular hookworm and *T. trichiura*, because both are associated with large worm burdens in school-age children (Stephenson 1987; Cooper *et al*. 1990; Lwambo *et al*. 1992) and with lower scores in tests of cognitive function (Pollitt 1990; Lozoff 1990; Simeon & Grantham-McGregor 1990; Grantham-McGregor *et al*. 1991; Nokes *et al*. 1998). However, effects on educational achievement or cognition are usually only observed where children experience chronic undernutrition (stunting in particular) or when there is anaemia and the haemoglobin concentration is less than 10 g/dl or 11 g/dl (Lozoff *et al*. 1987; Walter *et al*. 1989; Lozoff 1990; Nokes *et al*. 1998).

In Indonesia, the benefits of school health programmes are being investigated by *Kemitraan Indonesia Perkembangan Anak* (known as MITRA), the Indonesian organization which contributes to the international school-health activities of the Partnership for Child Development[1]. MITRA is providing school based interventions, including deworming, to some 150 000 children in Central Java, and offers an opportunity to evaluate the benefits of health improvements in terms of cognitive outcomes. The opportunity is particularly important because Asian children have been almost entirely neglected by previous studies of the relationship between health and cognitive function and development.

The results presented here explore the associations between helminth infection and a battery of cognitive tests at baseline, before intervention. The aim of this analysis is to determine whether these associations are independent of potentially important confounders, such as socio-economic status, and whether the test results provide any indication of the specific cognitive domains which may be influenced by infection.

[1] The Partnership for Child Development – PCD – is a consortium of government agencies, institutions and donors which seeks to assess the cost and effectiveness of school based services, including deworming, as means to improve the health and education of school-age children. The work of PCD involves governments and research institutions in more than 14 countries. The Scientific Coordinating Centre is located at Oxford University. Donors to the PCD include Rockefeller Foundation, James S. McDonnell Foundation, UNDP, World Bank and the Edna McConnell Clark Foundation.

Materials and methods

Study site – selection of schools and children

The MITRA school health programme took place in Karanganyar District, Central Java, Indonesia. Of the 2262 children from 51 schools included in the overall MITRA baseline evaluation, a subsample (approximately 25% of the original sample) of 432 children from 42 primary schools were randomly selected, after stratifying by age and by sex, for more detailed biochemical examination and for an investigation of their cognitive and motor function. The children recruited for study were from two age groups: 8 and 9 years or 11, 12 and 13 years. The dates of birth of children were obtained from school registers.

Experimental design

A cross-sectional investigation was conducted from October to December 1995 of the relationship between helminth infection and children's performance on tests of cognitive and motor function. Several confounding variables were also assessed, including school, class, age, sex, educational opportunity and socio-economic status, haemoglobin and anthropometric status. The study was undertaken as part of an Indonesian Government School Health Programme, co-ordinated by the Ministry of Health. Informed consent to participate in the study was received from teachers and parents. After the study all children received treatment with a single 400 mg dose of albendazole (SmithKline Beecham, Brentford, UK).

Cognitive function and motor function tests

The tests of cognition and motor function chosen for the study met the following criteria: First, they covered a wide range of cognitive and motor functions. This was considered important since the specific cognitive domains which are adversely affected by helminths have not been defined. Second, they had been shown in previous studies to be sensitive to the effects of helminth infections or to related conditions such as hunger, undernutrition, or iron deficiency anaemia (Pollitt 1990). Third, they could be adapted to make them suitable for use with school-age children in the Javanese context. Finally, they were demonstrated to have a high test-retest reliability of > 0.70. Table 1 lists the tests included in the study, the functions they are intended to measure and the test-retest reliabilities obtained. A brief description of each test is given in the appendix.

The battery of cognitive tests was given by two testers, the motor tests by one. The batteries test took approximately 45 and 10 min per child, respectively. Both test batteries were

Table 1 Tests of cognitive and motor function used in the study and the type of function measured. *r* is the test-retest reliability (Pearsons correlation coeffecient) for the cognitive tests; where there were two testers administering the tests, the lower reliability of the two is reported

Test	*r*	Types of function measured (Working Memory Functions)
Cognitive Function		
Picture search	0.96	Visual information processing and selective attention (Central Executive)
Number choice	0.99	Speed of visual information processing, decision making (Central Executive)
Stroop	0.90	Working Memory (Central Executive)
Categorical fluency	0.71	Scanning and retrieval of memory or speed of access of knowledge (Working memory: Supervisory Attentional System, within Central Executive)
Digit-span forwards	0.74	Working memory (Phonological Loop)
Digit-span backwards	0.72	Working memory (Phonological Loop)
Corsi block	0.70	Working memory (Visual-Spatial Sketchpad)
Free recall	0.96	Long-term memory (Central Executive)
Verbal analogies	0.87	Reasoning ability
Motor function		
Pegboard	0.74	Dexterity of hands and fingers
Bead threading	0.83	Dexterity of fingers and fingers
Mazes	0.98	Visual-Motor co-ordination, memory and planning ability (Working memory: Central Executive and visual/spatial ability)

given to children individually and on the same day, in a quiet area of each school and in separate rooms where significant visual distractions had been removed. All children received a snack and a drink before testing to ensure they were not hungry during the test since hunger has been shown to affect cognitive function (Simeon & Grantham-McGregor 1989).

Biomedical measurements

All children provided a stool sample. Approximately 25 mg of stool was examined for helminth eggs under the microscope using the Kato-Katz technique. The presence and number of eggs of each helminth species was recorded. Prepared slides were read within 30 min in order to detect hookworm eggs, if present. 10% of samples were read by a second microscopist to determine the reliability of egg counts.

The egg counts usually used to define moderate to heavy infections with *T. trichiura* and hookworm are around 2000 eggs/g. However, since only 11 of 432 and 6 of 432 children were found with $>$ 2000 eggs/g of hookworm or *T. trichiura*, respectively, a lower cut-off of 500 eggs/g for both parasites was applied for defining moderate infections within this population.

Height was measured using a portable stadiometer (CMS Weighing Equipment, London, UK) to a precision of 1 mm. Weight was measured with a digital weighing scale (Soehnle, Germany) to a precision of 100 g. Children were weighed barefoot, wearing the school uniform shirt or blouse. Mid-upper arm circumference (MUAC) was measured to a precision of 2 mm with a waxed paper insertion tape (TALC, St Albans, UK) and triceps skinfold thickness was measured to a precision of 0.2 mm with Holtain calipers (CMS Weighing Equipment, London, UK).

Z-scores of height-for-age (HAZ), weight-for-age (WAZ) and weight-for-height (WHZ) were calculated using Anthro Software (Sullivan & Gorstein 1990) which uses the National Center for Health Statistics (NCHS) reference values. Weight-for-height is only calculated for girls aged up to 10 years and for boys aged under 11.5 years because of the adolescent growth spurt. Body Mass Index (BMI) which is the weight in kg divided by the height in m^2 was also calculated. For the descriptive analyses, children were defined as stunted or underweight if their respective HAZ or WAZ was < -2sd below the NCHS median.

The concentration of haemoglobin was measured from venous blood using a Hemocue B-Haemoglobin Photometer (Hemocue Ltd, Sheffield, UK). Children were defined as anaemic if their haemoglobin concentration was $<$ 12.0 g/dl.

Children were given a structured interview individually to find out about their home environment, educational opportunities and the occupation and educational level of their parents. A socio-economic status score was calculated by adding together the scores attributed to household possessions, household utilities and the occupation of the children's parents. Household possessions (car, motorbike, TV, cow/goat, bicycle, radio, none of these) were ranked in order of cost and assigned a score of 0 (no possessions) to 3 (owned a car), giving a possible range of scores for household possessions of 0–10.5. Similarly, to calculate the household utilities score, each utility was ranked in order of cost and assigned scores as follows: water for washing, water for drinking, type of toilet, type of light and the materials used

for roof, floor and wall of house were each assigned a score between 0 and 1. Ownership of house was assigned a score between 0 and 3. The range of possible scores for household utilities was 0–10. The occupation of the mother and of the father were ranked according to income or salary and each assigned scores of between 0 and 4. The score of the mother's and father's occupation was added and divided by two to obtain the mean parental occupation score. The possible SES range of scores was therefore 0–24.5.

Educational opportunity was calculated by adding the following scores assigned to the number of school uniforms (0–2), the number of pairs of shoes (0–2), the number of books (0–3), a place for study at home (0–2) and the number of days spent studying at home per week (0–2). In addition, the mean number of years of education of the mother and the father was calculated as follows and added to the overall educational opportunity score: Not enrolled = 0; did not complete primary school education = 1; completed primary education = 2; entered and/or completed secondary education = 3; entered and/or completed higher education = 4. The range of possible educational opportunity scores was 0–15.

Data analysis

Number Choice and Picture Search tasks were log transformed forall statistical analyses to normalize the distribution of scores. For the descriptive analyses of cognitive and motor function test scores, parametric tests were used for all tests. For the descriptive analyses of the background variables such as infection status, socio-economic status and nutritional status, nonparametric tests were used because many of the background variables were not normally distributed.

For the analysis of the baseline data, Student's *t*-tests were used to compare the cognitive and motor function (a) between the two age groups and (b) between children who were infected or uninfected with hookworm. The Mann–Whitney *U*-test was used to compare the same groups in terms of the background characteristics. A one-way analysis of variance (ANOVA) was used for the comparison of test scores among children uninfected or with light (1–500 eggs/g), moderate or heavy (> 500eggs/g) infections of hookworm. Chi-square was used to compare group differences in sex, school and presence of *Ascaris lumbricoides, Trichuris trichiura*, or hookworm infections,% stunted (HAZ < − 2 sd),% undernourished (WAZ < − 2 sd) and percentage anaemic (< 12 g/dl).

Multi-variate analyses (multiple linear regressions) were conducted to control for possible confounding variables and to permit investigation of the unique effects of helminth infection. For these analyses, socio-economic status and educational opportunity were very highly correlated ($r = 0.48$) so these variables were added to give an overall score called socio-economic and education status (SEES) with a possible range of values from 0 to 39.5.

The regression model was designed to investigate the effects of hookworm and *T. trichiura* infection on cognitive and motor function after controlling for age (centred on 11.0 years), sex, SEES, stunting (HAZ < or > − 2sd), body-mass index (BMI), haemoglobin concentration, and *A. lumbricoides* infection. To account for the potential lack of independence of individuals sampled within the same school, school was treated as a cluster/primary sampling unit (PSU) using the survey routines within STATA (svy family, STATA Version 5, Texas, USA).

To investigate the possible differential effect on cognitive and motor function of hookworm infection with age, an age by hookworm infection interaction term was also offered to each model. Where the interaction between age and infection was significant, a three way interaction of stunting by age-group by hookworm infection was added to the model to investigate whether the differences in test scores with age could be explained by the differences in the proportion of stunting with age. Where the interactions were not significant, results are reported from re-running the model without the interaction terms included.

Each model was checked to ensure there were no violations of assumptions. The distribution of the standardized residuals was checked to ensure they were approximately normal. The associations between each independent variable and the outcome test scores were also checked to make sure they were adequately described by a linear term alone.

In all studies of this kind, there is the possibility that chance may be responsible for a particular significant difference which is true of all statistical analyses. There are many statistical methods available to correct the *P*-values for the number of tests performed (Rom & Connell 1994). However, we have reported unadjusted *P*-values to allow the reader access to the raw test results independently of a specific correction method. Agreement with previous findings of a significant difference increases confidence in a conclusion but an unexpected finding at the borderline of significance may well be attributed to chance.

Results

A total of 432 children participated in the study of whom 219 (51%) were female. There were 216 children in each age group (8–9 years and 11–13 years). The mean age of children was 8.9 years (sd 0.56) and 12.0 years (sd 0.67) in each age group, respectively. Helminth infection was common (43% prevalence overall). Hookworm and *T. trichiura* were the most prevalent (28% and 24%, respectively) whereas only 8% were infected with *A. lumbricoides* (Table 2).

The intensity of infection with *A. lumbricoides, T.*

Table 2 Background characteristics of children recruited to the study.

Significance values refer to nonparametric statistical comparisons between hookworm infected and uninfected children. Interviews to assess Socio-economic status and educational oppportunity were conducted with 410 children.

	Uninfected (n = 311)		Infected (n = 121)		
	Mean or %	sd	Mean or %	sd	$P<$
Sex (% girls)	55.6		38.0		**0.001**
T. trichiura (% infected)	18.6		38.0		**0.001**
A. lumbricoides (% infected)	5.1		14.9		**0.001**
>1 helminth spp (% infected)	4.5		49.6		**0.001**
HAZ (% <-2sd)	44.1		54.5		**0.05**
WAZ (% <-2sd)	30.9		47.1		**0.002**
Haemoglobin (% < 12g/dl)	17.7		19.8		0.6
Hookworm intensity (eggs/g)	0	0	994	3054	–
T. trichiura intensity (eggs/g)	115	668	162	634	**0.002**
A. lumbricoides intensity (eggs/g)	1351	13727	654	2816	0.1
Socio-economic status	12.9	2.7	11.8	2.7	**0.001**
Educational opportunity	6.5	1.5	5.7	1.2	**0.001**
Height-for-age Z-score (HAZ)	−1.86	0.98	–2.15	0.87	**0.007**
Weight-for-age Z-score (WAZ)	−1.63	0.72	−1.83	0.69	**0.004**
Body mass index (kg/m^2)	15.2	1.4	15.3	1.7	0.5
Mid-upper arm circumference (mm)	180	17	179	21	0.1
Triceps skinfold thickness (mm)	7.5	2.2	6.9	2.4	**0.001**
Haemoglobin (g/dl)	12.8	1.1	12.8	1.0	0.6

trichiura and to a lesser extent, hookworm, was generally low in both age groups according to WHO (1981). The maximum egg count for *T. trichiura* was 8740 (7000–10 000 are commonly used cut-offs for heavy *T. trichiura* infections). There was no significant difference between age groups in the prevalence or intensity of hookworm, *T. trichiura* or *A. lumbricoides*.

Older children had a significantly lower height-for-age (mean (sd) – 2.1 (0.95)) than younger children (– 1.8 (0.95) $P < 0.001$) and the proportion of older children classified as stunted (HAZ < − 2sd of NCHS standard) was also significantly higher than in younger children (54% and 39%, respectively, $P < 0.001$). However, weight-for-age was similar in both groups (averaging 35%), and while older children had significantly higher body mass indices, MUAC and triceps skinfold thickness (TSKF), this is expected because these measures of anthropometric status and body fat are not standardized for age.

Younger children had a significantly lower haemoglobin (Hb) concentration than older children (mean (sd) 12.4 (1.0) and 13.1 (1.1), respectively, $P < 0.0001$). However, the mean haemoglobin of both age groups was quite high and only 7.4% of children in the youngest age group and 1.4% of children in the oldest age group were iron deficient anaemic (Hb < 11 g/dl). There was no significant difference between older and younger children in the percentage of girls, the socio-economic status or educational opportunity.

On the whole, children infected with hookworm were significantly more likely to also be infected with *A. lumbricoides* and *T. trichiura*. Children infected with hookworm also had a significantly lower socio-economic status and educational opportunity than uninfected children. There were also significant differences in anthropometric status between uninfected children and those infected with hookworm. Infected children had a significantly lower height-for-age ($P < 0.01$), weight-for-age ($P < 0.01$) and were thinner (TSKF $P < 0.001$) than uninfected children (Table 2).

These differences in anthropometric status between infected and uninfected children were usually significant in the younger age group only – infected younger children compared to uninfected children had significantly lower height-for-age (− 2.1 (0.9) and − 1.7 (0.9), respectively, $P < 0.05$), weight-for-age (− 1.9 (0.6) and − 1.6 (0.7), respectively, $P < 0.001$) and were thinner (BMI $P < 0.02$; MUAC $P < 0.001$; TSKF $P < 0.01$) whereas in the older age group, differences in anthropometric status between infected and uninfected children were only observed in TSKF ($P < 0.02$). Children infected or uninfected with hookworm had a similar intensity of infection with *A. lumbricoides*, and similar BMI, MUAC and iron status ($P < 0.05$) (Table 2).

The association between hookworm infection and cognitive and motor function scores was examined for the younger and the older children separately and together (Table 3). Older children scored significantly higher than younger children in all tests ($P < 0.05$). Results from the bivariate analyses indicated that the association between hookworm infection and lower scores on tests of cognition and motor function were mostly observed in the older children only. Older

Table 3 Cognitive and motor function test scores between children infected and uninfected with hookworm. Significance values refer to *t*-tests for comparing test scores between infected and uninfected children within each age group. No. children completing Stroop was 317 owing to some children being either unable to read or colour blind. Number Choiceand Picture Search *t*-test comparisons conducted on log transformed scores

	Children aged 8 and 9 years					Children aged 11, 12 and 13 years				
	Uninfected ($n = 164$)		Infected ($n = 52$)			Uninfected ($n = 147$)		Infected ($n = 69$)		
Variable	Mean	sd	Mean	sd	$P <$	Mean	sd	Mean	sd	$P <$
Cognitive function										
Fluency	16.9	5.2	15.7	4.5	0.1	22.1	5.8	18.9	4.6	**0.001**
Digit-span forwards	4.1	1.4	3.9	1.1	0.4	4.9	1.5	4.1	1.4	**0.001**
Digit-span backwards	3.0	1.4	2.9	1.4	0.5	3.7	1.3	3.3	1.3	**0.03**
Corsi block score	9.8	2.5	9.8	2.6	1	11.3	2.5	11.1	2.7	**0.8**
Number choice	362.0	72.1	372.7	83.6	0.4	266.5	47.9	290.8	52.0	**0.001**
Picture search	135.4	37.6	140.9	31.6	0.2	100.1	18.4	107.1	16.0	**0.002**
Stroop colour	144.6	40.4	137.1	42.6	0.4	180.2	46.1	155.3	39.4	**0.001**
Stroop colour-word	52.7	17.8	43.8	17.8	**0.014**	62.9	18.7	54.7	19.1	**0.01**
Stroop interference	91.9	35.8	93.3	36.7	0.8	117.3	39.5	100.6	40.3	**0.012**
Free recall total	25.8	6.9	25.5	6.5	0.8	29.0	6.9	27.2	6.1	0.07
Verbal analogies	4.7	1.9	4.1	1.8	0.06	6.1	2.1	5.7	1.7	0.2
Motor function										
Mazes score	10.6	3.4	9.0	3.8	**0.005**	12.4	3.2	10.8	3.9	**0.002**
Bead threading	15.6	2.5	15.4	2.0	0.7	17.9	2.3	17.5	2.4	0.3
Pegboard total	59.2	7.7	58.8	9.7	0.7	70.1	7.7	68.1	6.5	0.07

children infected with hookworm scored significantly lower than uninfected children in Fluency ($P < 0.001$), Digit-Span Forwards ($P < 0.001$) and Backwards ($P < 0.03$), all three scores on the Stroop ($P < 0.02$), Number Choice ($P < 0.001$), Picture Search ($P < 0.002$) and Mazes ($P < 0.002$). Younger children infected with hookworm scored significantly lower than uninfected children only in tests of Stroop Colour Word ($P < 0.02$) and Mazes ($P < 0.005$).

The association between the intensity of hookworm infection or of *T. trichiura* infection on cognition and motor function was investigated using analysis of variance, but use of Scheffe's post hoc comparisons indicated that all the differences in test scores between groups could be explained by comparing infected and uninfected children alone. Thus, all subsequent analyses simply compared children with or without helminth infection.

The results from the regression analyses (Table 4) supported the results from the bivariate analyses reported above. After accounting for possible clustering within school and controlling for age, SEES, sex, stunting, BMI, haemoglobin concentration and the presence of *A. lumbricoides*, the presence of hookworm infection significantly explained the lower scores in six of the 12 tests given to children: Fluency ($P < 0.01$), Digit-Span Forwards ($P < 0.01$), Number Choice ($P < 0.01$), Picture Search ($P < 0.03$), Stroop Colour Word ($P < 0.02$) and Mazes ($P < 0.001$). In two of the tests – Stroop Colour Word and Mazes (Figure 1b) – hookworm infection significantly predicted lower test scores in children of all ages ($P < 0.02$) (Table 4). However, in the remaining four tests, the effect of hookworm on test scores increased with age; the age × hookworm interaction was significant for the tests of Fluency ($P < 0.002$ – Figure 1a), Digit-Span Forwards ($P < 0.01$), Number Choice ($P < 0.01$) and Picture Search ($P < 0.03$) (Table 4). Figures 1a and b provide examples of the two types of relationship with age of hookworm infection on lower test scores. To investigate whether the significantly higher prevalence of stunting in the older children could explain why the effect of hookworm infection on test scores increased with age, a three-way interaction of stunting × age × hookworm infection was included in the regression analyses. The interaction was not significant in all four cases.

Other variables which significantly predicted lower scores on several of the cognitive and motor tests were low SEES ratings, stunting and age (Table 4). The presence of *T. trichiura* significantly explained the lower scores on the test of Fluency ($P < 0.01$) and the presence of *T. trichiura* and *A. lumbricoides* significantly explained the lower scores on the test of Bead Threading ($P < 0.02$) (Table 4). No significant association of helminth infection was observed for the tests of Digit-Span Backwards, Corsi Block, Stroop Colour and Interference, Free Recall, Verbal Analogies and Pegboard.

Table 4 Results of regression analysis, to investigate whether hookworm infection significantly predicted cognitive and motor function scores after accounting for clustering within school and after controlling for age, sex, SEES, stunting (HAZ (2sd)), BMI, haemoglobin concentration and infection with *A. lumbricoides* and *T. trichiura*. Results only shown for those tests where a significant effect of helminth infection on scores was observed. 95% Confidence Intervals are given only for the main variables of interest – hookworm and the age × hookworm interaction. n/o: means the interaction term had been shown to be not significantly related to test score and was therefore not offered in the model presented.

	Fluency		Digit-span forwards		Number Choice		Picture search		Stroop colour word		Mazes		Bead threading	
	b	*P* <	*b*	*P* <	*b*	*P* <	*b*	*P* <	*b*	*P* <	*b*	*P* <	*b*	*P* <
Hookworm infectiong (0/1)	−1.8	0.008	−0.43	0.009	0.03	0.007	0.02	0.03	−6.1	0.018	−1.5	0.001	−0.22	0.4
Lower 95% CI	−3.2		−0.74		0.007		0.002		−11.2		−2.3			
Upper 95% CI	−0.5		−0.12		0.04		0.04		−1.1		−0.7			
Age × hookworm interaction	−0.88	0.002	−0.22	0.009	0.01	0.006	0.01	0.03	n/o	n/o	n/o	n/o	n /o	n/o
Lower 95% CI	−1.41		−0.38		0.004		0.001							
Upper 95% CI	−0.34		−0.06		0.02		0.016							
Age	1.69	0.001	0.27	0.001	−0.04	0.001	−0.04	0.001	3.4	0.001	0.6	0.001	0.77	0.001
Stunting HAZ (0 = < −2sd 1 = > −2)	0.82	0.2	0.32	0.03	−0.02	0.03	−0.02	0.04	0.49	0.1	0.7	0.06	−0.21	0.3
Sex	−0.42	0.6	−0.04	0.8	0.01	0.2	0.01	0.2	−6.9	0.005	1.7	0.001	−0.14	0.5
SEES	0.22	0.002	0.06	0.001	0	0.001	0	0.003	0.86	0.001	0.11	0.01	0.05	0.1
BMI	0.16	0.4	−0.03	0.6	0	0.5	−0.01	0.06	1.12	0.1	−0.08	0.6	−0.19	0.02
Haemoglobin (g/dl)	−0.31	0.2	0.06	0.4	0	0.8	0	0.4	−1.29	0.2	0.01	1	0.07	0.6
T. trichiura (g/dl)	−0.67	0.01	0.01	1	0.01	0.4	0.02	0.2	0.62	0.8	−0.27	0.5	−0.58	0.02
A. lumbriocides infection (0/1)	1.3	0.2	−0.1	0.8	−0.02	0.2	−0.01	0.3	0.27	1	0.48	0.4	0.6	0.02
Constant	16.9	0.00	2.8	0.03	0.001	2.26	0.001	40.7	0.008	9	0.01	18.4	0.001	
Model adj *r*-squared	0.23	0.001	0.14	0.001	0.45	0.001	0.41	0.001	0.18	0.001	0.16	0.001	0.23	0.001

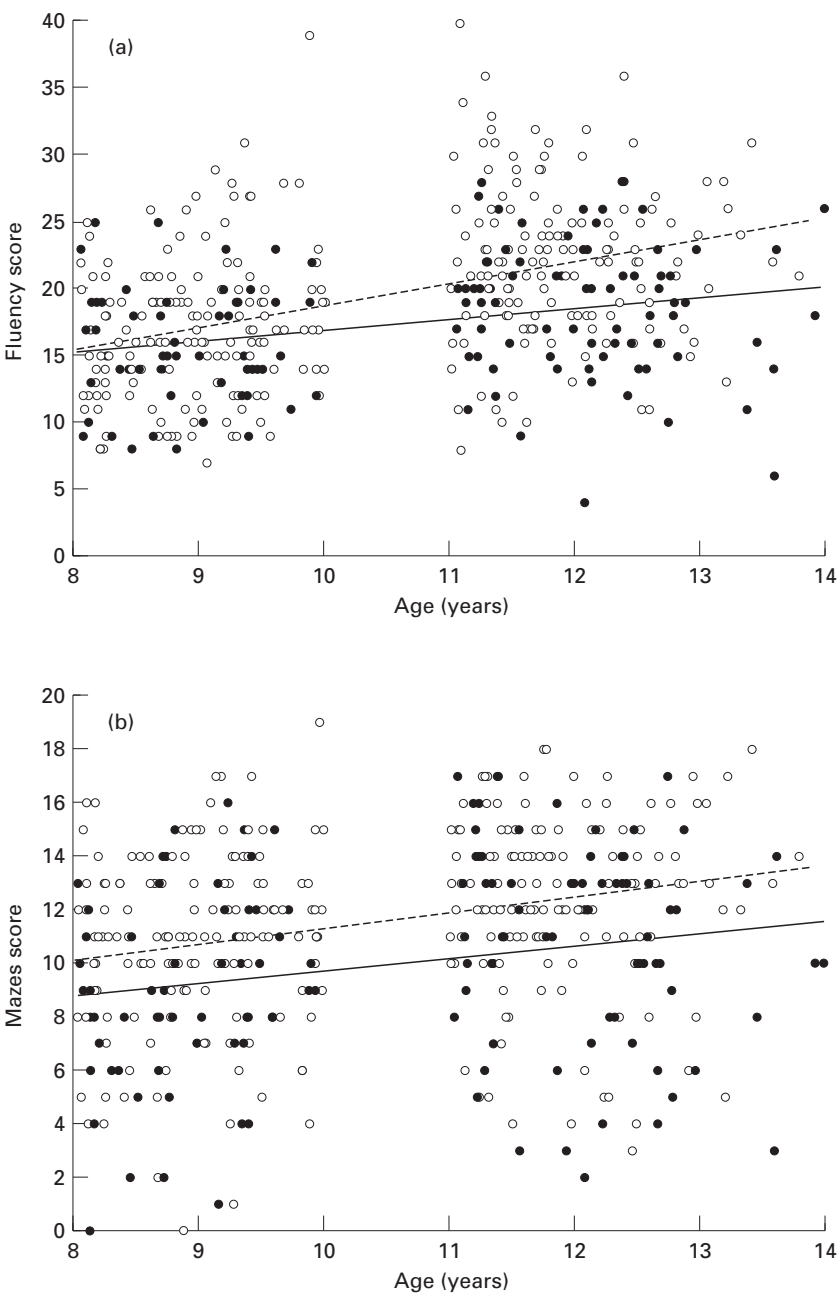

Figure 1 Two examples of tests showing the two types of relationship observed between infection status, age and score. (a) Fluency: where the association between hookworm infection and lower test scores increased significantly with age. (b) Mazes: where the association between hookworm infection and lower test scores was independent of age. ○ Uninfected; ● Infected; ·····Uninfected predicted; ——— Infected predicted.

Discussion

Lower cognitive test scores were most apparent amongst children with hookworm rather than other helminth infections probably because the prevalence and/or intensity of infection with *A. lumbricoides* and *T. trichiura* were too low to cause significant morbidity or be of public health importance. The occasional significant effect on test scores of *T. trichiura* and *A. lumbricoides* may be attributable to chance.

Children infected with hookworm had significantly lower scores than uninfected children on tests of Fluency, Digit-Span Forwards, Number Choice and Picture Search, Stroop Colour Word and Mazes. Apart from performance on the Stroop and Mazes tests, the association between hookworm infection and lower scores increased with age. These effects were significant after accounting for possible clustering

within schools and controlling for several confounding factors including sex, socio-economic status, educational opportunity, anthropometric status, haemoglobin concentration and the presence of other parasitic infections.

The reasons why the effects of helminth infection on cognition appeared to be stronger in the older children is difficult to pin-point. One possible explanation may be that the prevalence of infection with hookworm was greater in the older children than in the younger (32% and 24%, respectively, $P < 0.07$). Another possibility is that a significantly larger proportion of the older than the younger children were stunted (HAZ < -2 sd), perhaps suggesting that chronically undernourished children are more vulnerable to the adverse effects of helminth infection or that older children are more affected because they have been infected for longer. However, this explanation is not adequate in explaining the results because when the interaction between stunting, age and hookworm was investigated in the regression models, it was not significant.

On the other hand, the effects of helminth infection might have been predicted to be greater in younger children because (a) they are still developing physically and cognitively, and (b) they were significantly more anaemic than older children, and anaemia is associated with hookworm infection and poorer performance on tests of cognitive function. However, although the younger children were more anaemic, their haemoglobin levels were still reasonably high (mean 12.4; sd 1) and only 16 of 216 children had Hb levels of < 11 g/dl (WHO 1972). Thus only 7% of children had haemoglobin levels below the cut-off at which a significant effect of iron status on cognition is usually observed (Lozoff *et al*. 1987; Walter *et al*. 1989; Nokes *et al*. 1998).

Since the regression models only predicted between 14 and 45% of the total variance, it is possible that some other factor, perhaps correlated with height-for-age but not height-for-age itself, was responsible for giving stronger effects in the older children. A plausible explanation is that this reflects positive secular trends within Indonesia. During the past decade there is some evidence that socio-economic and nutritional status as well as school environment have been steadily improving. If this is the case, the older children in this study, compared with the younger children, have been exposed to more disadvantaged environmental and nutritional conditions, and for a longer period of time. While this may be reflected in their relatively lower height-for-age, it may be expressed in other ways not measured in this study. Experience of such disadvantaged conditions may result in a cumulative deficit in cognitive function over many years which may be greater in those children with the additional stress of infection (Sternberg *et al*. 1997a). In contrast, among the younger children the effects of hookworm on cognition may have been buffered by the relatively higher standard of living and nutritional status they have experienced during their lifetime. Thus although some effects on cognition were observed in both age groups, the effects were seen more clearly in the older children.

The functions which were most affected were memory-based and specifically represented the components of the Working Memory Model (Baddeley 1992). This adds to an emerging hypothesis that helminth infection adversely affects working memory (Nokes *et al*. 1999) and specifically tests of Fluency (Nokes *et al*. 1992; Simeon *et al*. 1995b), Digit-Span Forwards (Boivin *et al*. 1993; Sternberg *et al*. 1997b) and Digit-Span Backwards (Nokes *et al*. 1992).

Working memory refers to a brain system that provides temporary storage and manipulation of the information necessary for more complex cognitive tasks such as language comprehension, learning and reasoning (Waters & Caplan 1996). Within the system, there is an Attentional Controller/Central Executive which is supplemented by two subsidiary systems called the Phonological Loop and the Visual-Spatial Sketchpad. The Phonological Loop is assumed to be responsible for maintaining speech-based information. The Digit-Span test provides a good example of this role and was significantly adversely affected by hookworm and *T. trichiura* infections in this study even after controlling for confounding variables including nutritional and socio-economic status.

The Visual-Spatial Sketchpad is assumed to perform a similar function in setting up and manipulating the visuo-spatial imagery. While the Corsi Block test provides a good example of this function, this task was not adversely affected by hookworm infection in this study. However, since there was an effect of hookworm infection on the Mazes task which also has a visuo-spatial component, it is possible that the Visual-Spatial Sketchpad within the working memory model was also affected. If this is the case, it would support the findings of Boivin *et al*. (1993) in Zaire, where benefits of hookworm treatment were observed for children's spatial memory.

The Central Executive is thought to be a type of attentional controller (Baddeley 1996). Of the 6 tasks which could be considered measures of the Central Executive, all but one (Free Recall) were adversely affected by hookworm or *T. trichiura* infection after controlling for confounding variables. The tests adversely effected were: the Stroop test which provides a good example of the attentional controlling function; Number Choice and Picture Search where performance could be affected through reducing children's capacity to attend to information (Baddeley 1996); and Fluency and the Mazes tests which capture the Supervisory Attentional System of the Central Executive because good performance relies on a child maintaining goals and resisting distractions in order to either recall names from long-term memory, as in the case of Fluency, or to plan ahead as in the case of the Mazes.

Of the three motor tasks measured in this study, perfor-

mance on only one task, Mazes was significantly affected by hookworm infection. Since this is also a test of Central Executive function, it is a reasonable interpretation that no effect of hookworm infection on motor function was observed in this study.

Why working memory, as opposed to other cognitive domains, might be affected by helminth infection is unclear. Central Executive tasks in particular involve the frontal lobe, a part of the brain which seems to continue developing through to adolescence (Goldman-Rakic 1984), and is thus still developing during the period when children are most likely to be infected with helminths. Whatever the mechanism, if working memory is affected, it could have broader implications for the educational achievement and development of a child, not least because of the underlying association of working memory with complex real-world tasks such as reasoning ability and reading comprehension (Waters & Caplan 1996).

In conclusion, these results suggest that helminth infection, particularly infection with hookworm, is associated with lower scores on tests of cognitive function and perhaps with tests of working memory. Although the evidence presented is associational, the lower scores on tests of cognitive function observed in children infected with hookworm compared with uninfected children remained significant after controlling statistically for confounding variables. Thus the results imply that preventing hookworm infection in this population of Indonesian schoolchildren will be beneficial. Whether there are also benefits to children's mental or motor function resulting from treating helminth infection is an important issue and one which is currently being addressed by the MITRA programme.

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Appendix

The Cognitive and Motor Test Battery.

A brief description of the tests used in the study is given below.

Fluency

Each child is asked to name as many foods, and afterwards, as many animals as he/she can think, as quickly as possible. One minute is allowed for each category. The score is the total number of different foods and animals mentioned within the time limit. The task measures a child's long-term semantic memory and performance may depend on the Supervisory Attentional System of the Central Executive in working memory (Baddeley 1992).

Digit-Span Forwards and Backwards

Digit-Span is part of the Wechsler Intelligence Scale for Children (WISC-R. Wechsler 1974). Increasingly longer strings of numbers are read at the rate of one number per second, and immediately after each trial, the child is required to recall and repeat the string of numbers either forwards or backwards. The trials begin with 3 and 2 digits for the forwards and backwards tasks, respectively, and increase by one digit at each level. There are two trials at each level and the task is stopped when an error is made in two consecutive trials. The score is the total number of correct trials. Both tests represent auditory working memory (phonological function) but the backwards test also has an immediate processing element.

Corsi Block

The Corsi Block is essentially a visual version of the Digit-Span Forwards test. It consists of a board approximately 8×8 inches with nine 1.5 inch square blocks fixed to it in a random pattern. In each trial the tester points to the individual blocks in increasingly longer sequences and immediately afterwards the child is asked to point to the blocks in the same order as the tester. The trials begin with a sequence of 3 blocks and

increase by one block at each level. There are three trials at each level and the task is stopped when an error is made on two consecutive trials. The score is the total number of correct trials. The test represents visual working memory (Visual/Spatial Sketchpad).

Stroop

The Stroop consists of two tasks. The Colour task consists of a card with the names of four colours (tan, red, blue and green) typed repeatedly on the page. The task was adapted so the Javanese names of the colours were used. The names of the colours are typed in different colours (tan, red, blue or green) and the child is asked to read the word that is written thereby ignoring the colour in which the word is printed. Thus, if the word 'green' is printed in red ink, the correct answer is 'green'. The score was the total number of correct answers given in 2 min. In the Colour-Word task, the child is asked to read the colour that the word is printed in and not the word itself. Thus if the word 'green' is printed in red, the correct answer is 'red'. The score was the total number of correct answers given in 2 min. The Stroop Effect, termed Interference, can be calculated by subtracting the score on the Colour Word task from the score on the Colour Task. Before starting the test, each child was shown a page with four rectangles of the colours used in the Stroop task and asked to name the colours of the rectangles. Children were also shown a page with the names of the colours printed in black and asked to read the words on the page. A child who failed either task did not proceed with the test. The Stroop test measures Central Executive function of working memory (Baddeley 1992). The Interference score is a measure of inhibition control.

Free Recall

The test comprised three lists of 20 Javanese words in 4 subject categories: food, family relations, clothes and parts of the body, each of 5 items. The words were the same in each list but presented in a different, random order. The list of words was read by the tester at a rate of one word/second and immediately afterwards, the child was given one minute to recall all the words in the list s/he could remember. The task was then repeated and given a total of three times. The score used for this study was the total number of words correctly recalled over all three trials. The test measures auditory long-term memory.

Number Choice

On the page are printed, in rows, pairs of numbers ranging from 1 to 9. The child was asked to strike through the larger of the two numbers in each pair. There are three test pages which are given separately. The time taken to complete each test page is recorded. The score was adjusted to the total time taken to complete all three trials plus the average number of errors made per minute taken to do the task. Thus a high score indicates a poor performance. The test measures speed of visual processing and decision making.

Picture Search

Printed in rows on the left hand side of the page is one 'target' picture of a a face, raging bull or snake for example. On the right-hand side of the page next to each target picture are eight other pictures. The child was asked to strike through any picture on the right-hand side of the page which is the same as the target picture. There may be one, two or three pictures which match the target picture on each row. Each page is timed. The adjusted score was the total time taken to complete all three trials plus the average number of errors made per minute taken to do the task. Thus a high score indicates a poor performance. The test measures visual speed of processing and capacity for focused attention.

Verbal Analogies

Each child was read a total of 10 analogy puzzles which were presented in order of increasing difficulty and asked to complete the sentence. For example, 'Fire goes with hot, the same way as ice goes with _____? (correct answer = cold)'. The test was adapted to ensure there were no ambiguities in the items after translating to Javanese. The score was the total number of correct responses. The task measures ability to make analogies.

Mazes

The Mazes is part of the WISC-R (Wechsler 1974). Mazes of increasing complexity are drawn on a page and children are required to draw the path from the outside of the maze to the centre. The task requires some psychomotor skills and also some planning ability. It is both a motor function task and a task representing executive function and visual spatial aspects of working memory. The test was scored in accordance with the WISC-R guidelines.

Pegboard

The Pegboard consists of a board with holes into which pegs are placed. The number of pegs placed in the holes of the board in a fixed time period is recorded. For the first task the pegs are placed with the hand the child uses for writing. In the second task the child uses the other hand. In the third task both hands are used, and in the last task the child assembles several pegs and washers of different shapes and in a fixed order in the holes on the board. The test is designed to measure motor function, specifically manual dexterity.

Bead Threading

Each child was asked to thread as many coloured beads onto a piece of string as they could in one minute. The score is the total number of beads which were threaded. It is a task of hand and finger dexterity, and considered to be a culturally appropriate task of motor function.