Do Parasitic Worm Infections Impair Cognitive Development?

Matthew Jukes

Introduction

Over the course of the last century around fifty studies were published addressing the question of whether parasitic worm infections affect cognitive performance (Watkins & Pollitt 1997). Despite this sustained interest in the topic, it is still difficult to draw unequivocal conclusions. A recent review of treatment trials argued that 'there is insufficient evidence as to whether [deworming treatment] improves cognitive performance' (Dickson, Awasthi, Williamson, Demellweek & Garner 2000). The ambiguity of the evidence does not imply, of course, that parasitic worm infections do not affect cognitive function, or that treatment of children with infections cannot improve cognitive function. The ambiguity is more likely to result from the difficulty in producing clear results in a field where conducting well designed studies is expensive, time consuming and often unethical. This paper considers the key difficulties in interpreting results in this field and describes a recent study that attempted to avoid such problems. Finally, a selection of well designed studies are reviewed and conclusions are drawn as to the likely effect of parasitic worm infection on cognitive function.

Biology of Parasitic Worms

There are two broad classes of parasitic worms that have been studied in relation to their effect on cognitive development. The first consists of *geohelminths*—worms (helminths) whose eggs develop outside the human host, typically in moist soil. Three common species of geohelmiths are considered here: roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichuria*) and hookworm (of which there are two species—*Ancylostoma* *duodenale* and *Necator americanus*). The life cycle of all three types of worm all involve eggs which develop for a day to a number of weeks in moist soil. Roundworm and whipworm eggs are ingested as contaminants by human hosts and hatch in the small intestine. Whipworm then develop into larvae and migrate to the colon where they develop into adults of 3-5cm in length. Roundworm larvae burrow through the wall of the small intestine and migrate via the circulatory system to the lungs and then through the trachea and pharynx from where they are swallowed again and pass down to the small intestine where they develop into adults of around 30cm in length. Hookworm take a slightly different route to roughly the same end point. Hookworm eggs hatch in the soil into larvae which burrow through the skin around the feet of human hosts. Once inside the body hookworm migrate through the circulatory system to the lower intestine in the same manner as roundworm. Adults are 5-18mm in length. In the final phase of the life cycle of all three geohelminths, adults mate in the gut and the females produce from thousands to hundreds of thousands of eggs per day which are passed through faeces back into the soil. Geohelminths cause tissue damage and blood loss and can lead to protein energy malnutrition (PEM) with heavy loads. Iron deficiency is another common consequence of infection with geohelminths, particularly hookworm.

The second class of parasitic worms considered are the schistosomes (more commonly know as bilharzias or blood flukes). One species of shistosome (*Schistosoma haematobium*) resides in the blood vessels around the bladder and produces eggs which are passed through urine. Other species (*Schistosoma mansoni* and *Schistosoma japonicum*) reside in blood vessels around the intestines and produce eggs which passed through faeces. For all species, the eggs develop into larvae on contact with fresh water. The larva infects a species of snail in which it multiples before being released back into the water. It then burrows through the skin of a human who is in contact with the water and migrates through the blood stream to the vessels serving the bladder or intestines. Disease resulting from infection is caused primarily by the eggs that are released by the adult into tiny blood vessels. The blood vessels are damaged as eggs escape. The pathological response can lead to liver fibrosis or cirrhosis and anaemia.

Effect of Parasitic Worms on Cognitive Function

In addition to physical effects, severe and chronic infection with parasitic helminths during children's development could also have some consequences for their cognitive performance and ultimately their educational achievement. These effects on cognitive function may occur as a result of one or a combination of symptoms associated with infection. For example, one of the main consequences of infection with many species of helminth is iron deficiency anaemia[1] (IDA; Beasley *et al.* 1999; Farid 1993; Olsen Magnussen, Ouma, Andreassen & Friis 1998; Stoltzfus, Chwaya *et al.* 1997) and there is much evidence that IDA is associated with impaired cognitive performance and development (Grantham-McGregor & Ani 2000; Lozoff 1990). Furthermore, infection with hookworm and *S. haematobium* can result in poorer growth rates (Stoltzfus, Albonico, Tielsch, Chwaya & Savioli 1997; Warren *et al.* 1993) and this may also be a route by which infection leads to impaired performance because undernutrition affects cognitive development and educational achievement (Mendez & Adair 1999; Simeon & Grantham-McGregor 1990).

Whilst these and other pathways provide the potential for helminth infection to impair cognitive function, evidence remains equivocal as to whether this impairment actually takes place. The need for the research reported here arises from the inconsistent results found so far in this field. Broadly, there have been two different methodological approaches to address the issue of whether worm infections affect cognitive function: observational studies involving the analysis of correlations between worm infections and cognitive function; and intervention studies where the cognitive performance in infected children and a control group is observed for a period after deworming treatment is given. The latter methodology of treatment trials has generally been favoured because it has the potential to demonstrate cause and effect in the relationship between helminth infection and cognitive function. However, in the treatment trials conducted so far

[1] Abbreviations used in this paper are: BMI – body mass index, epg – eggs per gram; IDA –iron deficiency anaemia; HAZ – height-for-age z-score; Hb –haemoglobin; NCHS – National Center for Health Statistics; MUAC – mid-upper arm circumference; SES – socioeconomic status; SEES – socioeconomic and educational status.

only one study *(Nokes, Grantham McGregor, Sawyer, Cooper, Robinson et al.* 1992) has produced a clear main effect of anthelmintic treatment on cognitive function. Other studies have found improvements after anthelmintic treatment only for subgroups of the study population (Nokes *et al.* 1999; Simeon, Grantham McGregor, Callender, & Wong 1995; Simeon, Grantham McGregor & Wong 1995) or no improvement at all (Gardner, Grantham McGregor & Baddeley 1996; Sternberg, Powell, McGrane & Grantham McGregor 1997). These mixed results have led to some scepticism as to whether helminth infection affects cognitive function (Dickson *et al.* 2000). However, there are other explanations for the failure to find effects in the majority of treatment studies. First, it is quite possible that helminth infection impairs cognitive function in a way that cannot be remedied simply by removing the infection or that a different subset of cognitive functions may recover after treatment from those that are impaired by the initial infection. This may happen in a number of ways. For example, treatment may improve a child's attentiveness in the short term, which in turn allows the child to benefit more from teaching in the long term. Thus, we may be able to measure changes in attention shortly after treatment but not be able to detect improvements in other cognitive abilities or educational achievement for months or years after treatment. Alternatively, helminth infection may inflict long-term damage to cognitive functions in addition to affecting performance in cognitive tests concurrently, as a result of general lassitude and morbidity in the child. Treatment may remove the morbidity and improve performance but not remove the long-term impairment to cognitive function.

Second, cognitive impairments probably result from many years of living with a disease and so we would be surprised if this cumulative effect could be reversed with a few months of administering deworming tablets. No study has followed the recovery of cognitive function for more than six months after treatment. Perhaps a longer-term course of treatment is required to find improvements in cognitive function (Bundy & Peto 2000), or perhaps a psychological intervention is required, in addition to deworming, to remediate cognitive impairment (Drake, Jukes, Sternberg & Bundy 2000; Sternberg *et al.* 1997).

Third, the negative results of many studies may be due to the quality of instruments used to detect change in cognitive function. Studies have been conducted so far in Africa, Asia, Central America and the Caribbean but have exclusively used tests developed in North America or Europe. Some or all of these tests may have been inappropriate and insensitive measures of cognitive function in local populations. Alternatively, cognitive tests may not have captured performance on the key cognitive domains that are affected by parasitic infection.

In order to overcome the first of these three problems (the possibility that different cognitive functions recover after treatment from those that were infected in the first place) treatment studies need to be complemented by cross-sectional observational studies. This methodology provides the only way to examine the relationship between cognitive function and parasitic worm infection without simultaneously altering that relationship. Thus, these studies can tell us which cognitive functions are affected by parasitic infection, not just those that recover after treatment. Such studies cannot establish a causal relationship between infection and cognitive ability, but if sufficient care is taken to control for a wide range of possible confounders they may allow us to infer the likely profile of cognitive abilities that are affected by helminth infection in a way that treatment trials cannot.

The most likely confounding factor to affect studies of helminth infection and cognition is poverty (Seifer 2001) and yet the majority of cross-sectional studies have failed to control for socioeconomic status (SES) (Watkins & Pollitt 1997). Only six studies have controlled for SES (Gardner *et al.* 1996; Levav, Mirsky, Schantz, Castro & Cruz 1995; Sakti *et al.* 1999; Simeon, Callender, Wong, Grantham McGregor & Ramdath 1994; Simeon, Grantham McGregor & Wong 1995; Sternberg *et al.* 1997) which may affect cognitive function either independently or as a further index of poverty. There is clearly a need for cross-sectional studies that make a comprehensive attempt to control for all candidate confounders in order to provide evidence to complement that from treatment trials. The first aim of our research efforts was to conduct such a well-controlled associational study to meet this need. This study is reported elsewhere (Partnership for Child Development 2002) and summarised here. Other shortcomings with previous studies, discussed above (the short term nature of previous treatment trials and the inappropriateness of tests), were also addressed in our research project and our findings will be discussed in forthcoming publications (Grigorenko, Sternberg, Ngorosho, Jukes & Bundy submitted; Sternberg *et al.* in press).

Methods and Subjects

Study Population

The study took place in 10 schools in the coastal area of Bagamoyo District and Kibaha District, Tanzania, about 70 km north of Dar es Salaam. Children were aged between 9 and 15 years and spoke Swahili at home and at school. Of 1476 children who were eligible for the study and who returned consent forms. 906 of these were randomly selected for parasitological screening.

After completing the larger study of which the current investigation was a part, all children received anthelmintic treatment according to their infection status. Children with haemoglobin levels (Hb) of <80 g/L were given iron treatment. A local doctor was attached to project throughout to monitor the health of children in participating schools. Any illnesses identified by the project nurse were treated or referred to the project doctor and district hospital.

Experimental Design

Between May and August 1997, a comparison was made between children with moderate-heavy hookworm ($\geq$400epg) and/or S. haematobium ($\geq$50eggs/10ml) infections and those without significant helminth infection, on their performance on tests of cognitive and motor function and educational achievement.

As part of a larger study, half of the eligible children were randomly selected for parasitological screening after stratifying within school and grade. Of those who were screened, all children with moderate-heavy infection were recruited. For every second moderately-heavily infected child recruited in a class, an uninfected child in the same school grade and of the same sex was selected to serve as a control.

Approval for this study was obtained from the Tanzania Ministry of Health and Ministry of Education and Culture at national, regional, district and ward level and also by the schools and teachers participating in the study. Ethical clearance was obtained from the Institute of Child Health, London, UK and the Tanzania Food and Nutrition Centre, Dar es Salaam, Tanzania. Children and parents had the study explained to them in Swahili and signed informed consent was obtained from all children and their parents before measurements began.

Psychometric Tests

A battery of educational tests measuring reading, spelling and arithmetic skills (detailed in the Appendix) was given to all children by two testers. With the exception of oral arithmetic, which was administered individually, tests were given to groups of about 15 children in their classroom. The educational tests were completed before other psychometric tests were given. Half the children were also given tests measuring a range of cognitive and motor functions (see Appendix) by four testers. The test battery took 30 to 45 minutes per child to complete. Tests were presented in a fixed order to children and were organised so that tests placing the highest demands on children were administered towards the end of the battery when children were familiar with the testing situation.

Both educational achievement tests and tests of cognitive function were developed over a period of one year prior to the start of the study to ensure face validity and content validity of the tests (correlations amongst tests and between school achievement and tests follow the predicted pattern), to ensure that children were familiar and comfortable with all testing materials and to ensure a test-retest reliability of at least .7 for each test. A novel test of reading ability was developed as part of this study (Alcock *et al.* 2000). All other tests had been adapted and chosen because they measured a range of abilities, had been shown in previous studies to be sensitive to the effects of hunger, undernutrition, IDA, other chronic illnesses or helminth infection. Children were tested in Kiswahili by testers who were fluent in this language. All children received a snack and a drink before testing to ensure they were not hungry during the test since short-term hunger has been shown to affect cognitive function (Pollitt & Mathews 1998).

Explanatory Variables

Children's date of birth was recorded from the school register. Children's sex, class and school attended were also recorded. Children were also given a structured interview individually to find out about their home environment and their educational opportunities—characteristics that might affect their cognitive function and educational achievement.

Biomedical Variables

The number of *S. haematobium* eggs found in 10ml of urine, and the number of hookworm eggs per gram of stool were used as proxy measures of the intensity of parasitic worm infections. Measures were also taken of nutritional indices (height, weight, mid upper arm circumference and skinfold thickness), haemoglobin levels, ferritin concentration (an indicator of iron depletion), C-reactive protein (an indicator of an acute infection which may also lead to elevated ferritin levels independent of any depletion of iron), and also malaria parasite density (see Partnership for Child Development 2002 for methods)

Data Analysis

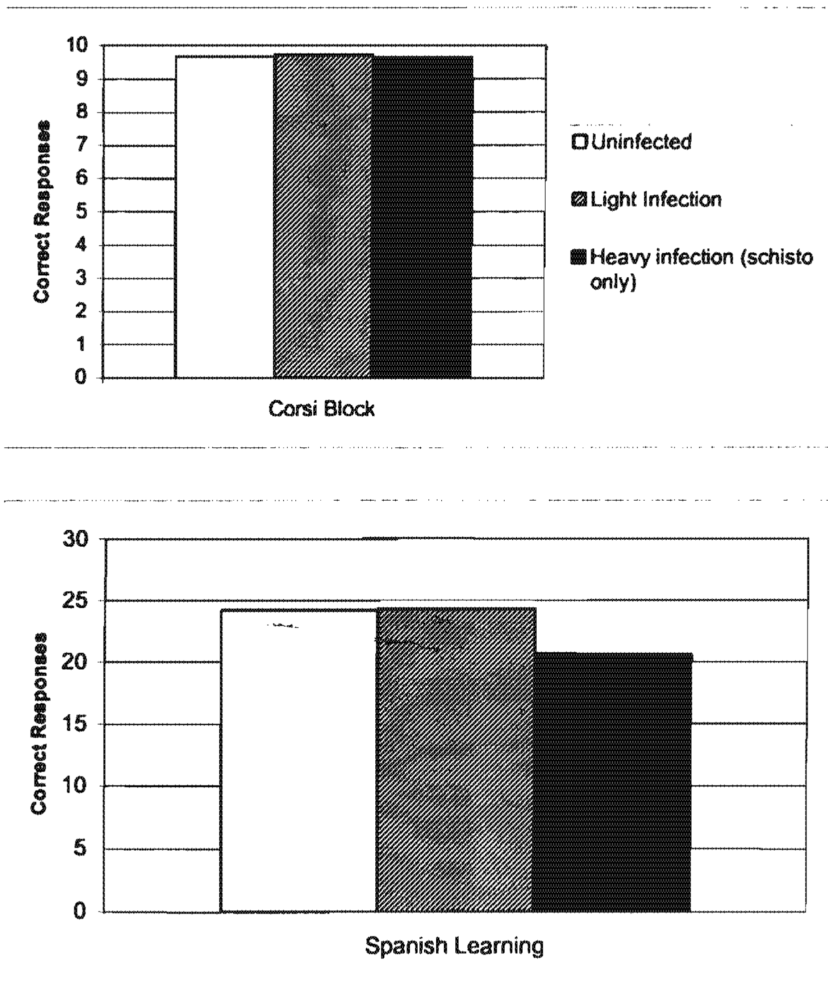
The distributions of all cognitive and education test scores were examined for normality and transformed where necessary. Socioeconomic and education status (SEES) data was reduced by factor analysis with varimax rotation to five factors. There were two factors related to economic wealth -: one related to quality of *house* and larger possessions and another related to smaller *possessions*—and also three school-related factors including possession *books*, state of school *uniform* possession of other school *equipment*. Intensity of helminth infection was coded into categorical variables representing intensity above and below certain thresholds. *S. haematobium* infection was coded into three categories: uninfected or lightly infected, moderately infected and heavily infected, with thresholds at 50 eggs/10ml and 500 eggs/10ml. Hookworm infection was coded into a binary variable with infection above or below the threshold of 400 eggs per gram. The category of heavy infection with hookworm (> 4000 eggs / gram; Montresor, Crompton, Bundy, Hall & Savioli 1998) included only 9 children (only 5 of whom did cognitive tests), which was considered insufficient to include in analyses as a separate group.

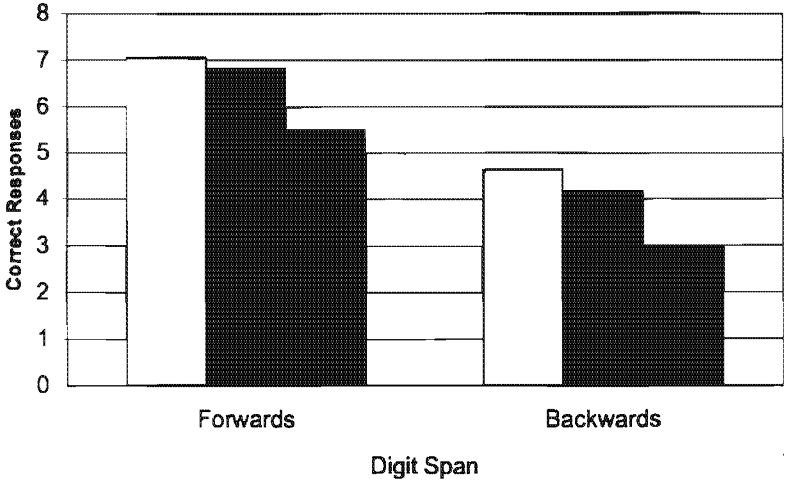
Multiple linear regressions were performed to analyse the effect of helminth infection on cognitive function and educational achievement controlling for possible confounding variables. Multilevel modelling using the Mlwin program (Rasbash *et al.* 2000) was used to control for random effects at both child and school levels. Separate analyses were conducted for each cognitive and educational test score. Regression equations were constructed as follows. First, age and sex were entered into the regression equation. Second, three dummy variables were offered to control for the variation between the four testers who conducted the cognitive tests. The third step involved controlling for potentially confounding factors. Infection with Plasmodium spp and all five SEES variables were offered. In the fourth step all potentially mediating variables (the nutrition and other health variables HAZ, BMI, Hb, MUAC, C-reactive protein concentration, ferritin concentration) were offered. In this and all stepwise regression procedures used in the analysis, variables were admitted into the equation at the $p = .05$ level. After all potentially confounding and mediating variables were offered in the regression equation, the categorical helminth infection variables were entered. Finally, interaction terms between all helminth infection variables and each of age, body mass index, and height-for-age were offered to the regression. [Previous studies have shown that both age (Sakti *et al.* 1999)—and nutritional status (Simeon, Grantham McGregor, Callender *et al.* 1995; Simeon, Grantham McGregor & Wong 1995)—can moderate the effect of parasitic infection on cognitive function].

Results

904 children were screened for their parasitological status. 272 children qualified for the moderately-heavily infected group and 117 for the uninfected control group. All of these 389 children were scheduled to take education achievement tests and 223 were scheduled to take tests of cognitive function. Complete records were available for 338 and 203 of children who did the educational and cognitive test batteries respectively, representing an average loss of 13% resulting mostly from absenteeism on one or more of the days of testing.

Figure 1. Relationship between Memory-based Tasks and Helminth Infection




Correct Responses
0
1
2
3
4
5
6
7
8
Forwards
Backwards
Digit Span

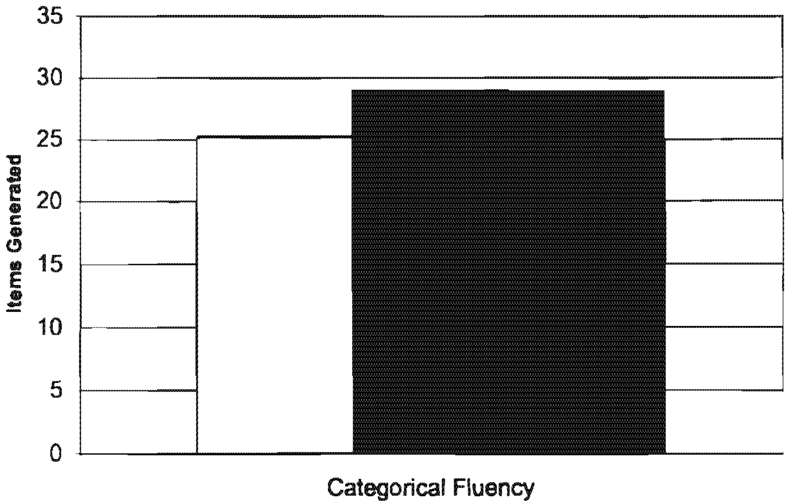
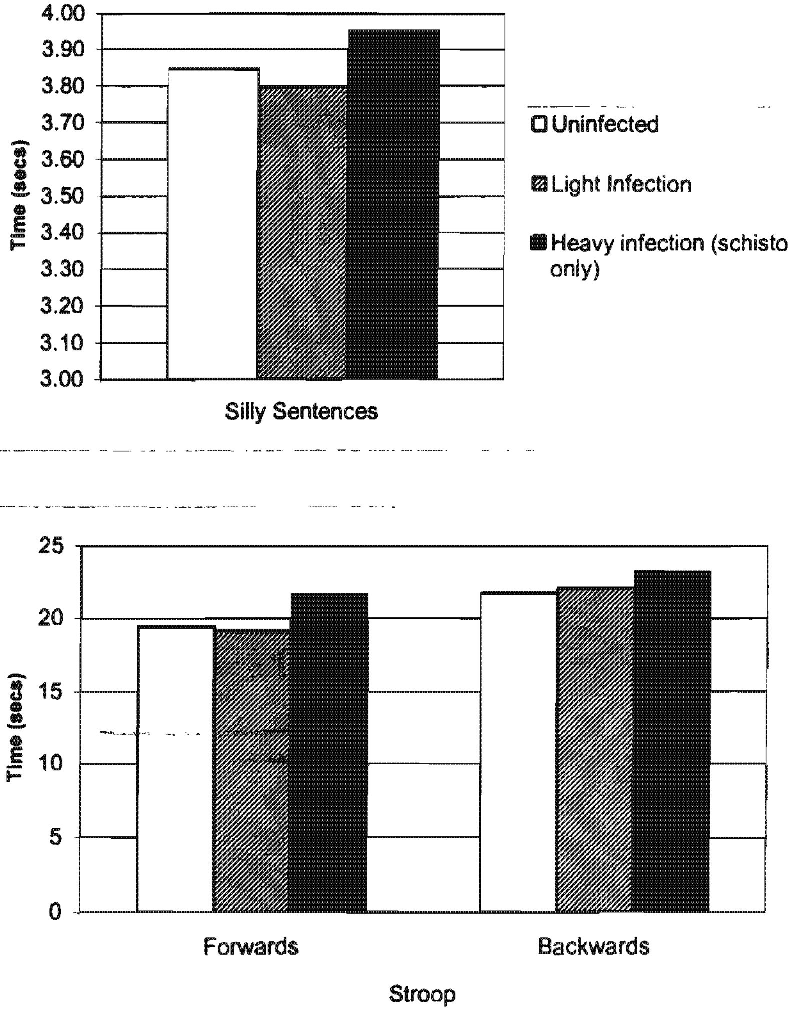
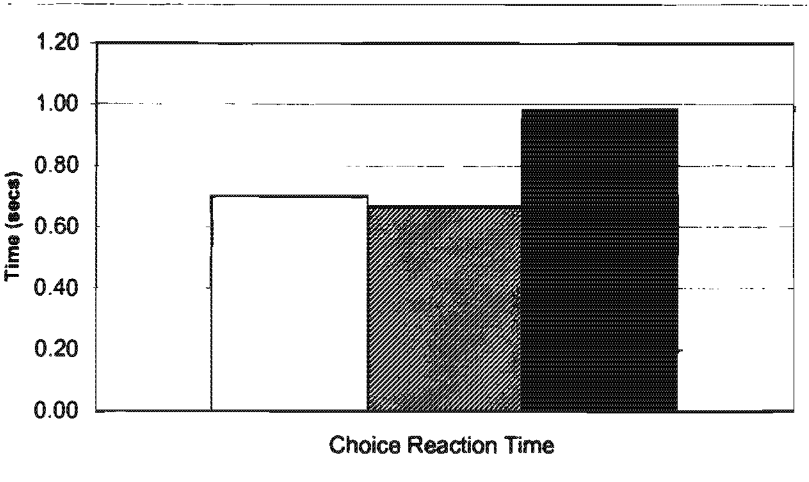
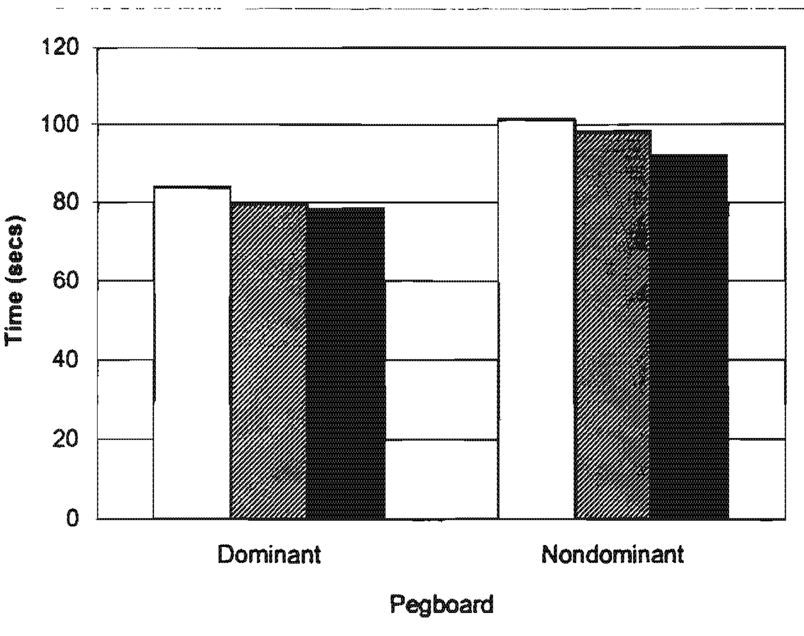

Items Generated
0
5
10
15
20
25
30
35
Categorical Fluency

Figure 2. Relationship Between Timed Tasks and Helminth Infection




1.20
1.00
0.80
0.60
0.40
0.20
0.00
Time (secs)
Choice Reaction Time


120
100
80
60
40
20
0
Time (secs)
Dominant
Nondominant
Pegboard

Associations with Parasitological Status

Figures 1 and 2 show the relationship between cognitive function and parasitic infection[2]. The most consistent pattern of results from regression analyses was observed for heavy infection with *S. haematobium*. This was associated with lower scores in four of the eleven tests of cognitive function[3]: Digit Span Forwards (p = .005, difference between heavily-infected and uninfected groups = 1.34 or 0.70 standard deviations[4]), Digit Span Backwards (p = .005, diff. = 2.16 or 1.16 SDs), Silly Sentences (p = .001, diff. = 0.298 secs or 1.04 SDs) and Choice Reaction Time (p = .001, diff. = 0.775 secs or 2.92 SDs). For Silly Sentences, the relationship resulted from interactions between heavy schistosome infection and height-for age (p = .001). This was because children who were both heavily infected with *S. haematobium* and stunted (HAZ < -2) had longer mean reaction times on the Silly Sentences test than other children (see Table 1).

Table 1. Breakdown of the interaction between height-for-age and heavy *S. haematobium* infection for the Silly Sentences test. Mean reaction time is shown in sec.

		Height-for-age z score	
		Below -2	Above -2
S. haematobium	<500 eggs/10ml	3.82	3.80
infection	>500 eggs/10ml	4.11	3.80

For two further cognitive tests—Stroop Compatible (p = .038) and Incompatible (p = .025)—the association between heavy schistosomiasis and poorer performance approached significance.

[2] Results are presented more fully in Partnership for Child Development (2002)

[3] Throughout cognitive test analyses a revised significance level (α = .010) was used following Bonferroni corrections for multiple tests with correlated outcome measures (Sankoh, Huque & Dubey 1997).

[4] Calculated from the estimated coefficient (b) in the final regression equation.

There were fewer and less consistent relationships between cognitive outcomes and moderate levels of infection. For *S. haematobium*, moderate infection was associated with higher cognitive test scores for the Verbal Fluency test ($p < .01$, diff. = 3.52 items or 0.46 SDs). Moderate hookworm infection was not significantly related to any of the cognitive tests. In addition, there were no significant associations with either worm infection and any of the three education tests.

Discussion

This study provides strong evidence that infection with *Schistosoma haematobium* can impair cognitive function. Associations were found between heavy schistosomiasis and impaired performance in 4 of the 11 cognitive tests independent of confounding and mediating factors.

Cross sectional studies such as this one are problematic because we cannot be certain that the relationship between schistome infection and cognitive function is a causal one, rather than an association mediated by a third (unmeasured) factor. However, as argued in the introduction, cross sectional studies, when designed and interpreted with care, address questions that treatment studies cannot and thus are worth pursuing. A number of aspects of the current study support the argument that there is a genuine causal relationship between schistosomiasis and cognitive impairment.

First, care was taken to measure and control for all variables that may be correlated with both cognitive performance and level of schistosome infection. Thus, the associations found were independent of confounding factors such as socioeconomic status, age, sex, malaria infection and other chronic infections, and variables potentially responsible for mediating the effect of parasitic infection on cognitive function such as nutritional indices and iron status.

Perhaps a more persuasive argument that there is a genuine causal relationship between cognitive function and helminth infection comes from the profile of cognitive abilities impaired by helminth infection. We found that having a heavy infection of schistosomiasis was associated with a drop in performance in the Digit Span Forwards and Backwards tests of 0.7 SDs and 1.16 SDs respectively and with increased reaction time in the Silly Sentences and Choice Reaction Time tasks of 1.04 and 2.92 SDs respectively. No other tests were negatively affected by heavy schistosomiasis. Particularly striking in this pattern of results is that there are large differences from one test to another in the relationship with helminth infection. In particular, the effect on the Choice Reaction Time task approaches 3 SDs—an extraordinarily large effect for a psychometric test—whilst other tests show more moderate or nonsignificant effects. This is not the pattern we would expect if the effect were based simply on the fact that poor performance in cognitive tests and high parasitic loads tend to co-occur in the poorest children. On the contrary, socioeconomic status—the environmental factor having by far the largest influence on performance in psychometric tests—tends to have an equal effect on all domains of cognitive function (Seifer 2001).

This argument highlights an important, and often overlooked, consideration when interpreting studies of parasitic infection and cognitive function. When assessing the impact of parasitic infection on a battery of cognitive tests in a particular study, the nature of the cognitive tests affected—and not merely the number of cognitive tests affected—is the most useful guide as to whether there is a genuine causal effect. Consistency across studies in the type of cognitive tests affected, particularly when supported by theoretical considerations, is a powerful indicator that parasitic worms have an effect on cognitive function.

Table 2 summarises results from all studies whose design allows us to make strong conclusions about the effect of parasitic worm infection on cognitive function. For intervention studies, this includes those with an experimental design involving a placebo group. Cross sectional studies are included if associations between worm infections and cognitive function cannot be easily dismissed as resulting from confounding factors. In most cases this is because confounding factors have been measured and controlled for statistically. For each study, results are broken down by cognitive test. Across different studies cognitive tests attempting to measure the same function in similar but slightly different formats have been included in the same column (e.g. 'number search' and 'picture search' have been group together as 'visual search'). Cognitive tests have also been grouped according to the cognitive domain they are aiming to assess. However, this should be seen merely as a rough guide; cognitive domains are difficult to define and most tests assess performance in one or more domains. In each column, a √ indicates that a significant effect was found for a particular test, and a X indicates that no significant effect was found. Blank cells indicate that the test was not included in the study.

Let us look first at the cognitive tests that were found to be sensitive to parasitic infection in the current study: Digit Span Forwards, Digit Span Backwards, Choice Reaction Time and Silly Sentences. In all the studies reported in Table 2, 4 out of 16 found an impact of worm infection on the Digit Span Forwards tests, 2 out of 8 found an effect for the Digit Span Backwards, 3 out of 3 for the Choice Reaction Time task and 2 out of 3 for Silly Sentences. This presents a mixed pattern of results. However, the current study found an effect only for children with the heaviest parasitic loads. What if we restrict our analysis of other studies to those in which the treatment group (for intervention studies) or the infected group (for cross sectional studies) consisted of children with heavy worm loads (Nokes, Grantham McGregor, Sawyer, Cooper, Robinson *et al.* 1992; Partnership for Child Development 2002) and to those studies where the relationship between infection intensity and cognitive performance was analysed (Kvalsvig, Cooppan & Connolly 1991; Levav *et al.* 1995; Nokes *et al.* 1999; Sakti *et al.* 1999)? (That is, excluding those studies where infected and uninfected children were compared irrespective of infection intensity). Here we find that 3 out of 5 studies found an effect for Digit Span Forwards, 2 out of 3 for Digit Span Backwards, 2 out of 2 for Choice Reaction Time and 1 out of 1 for Silly Sentences. This analysis indicates that the domains of verbal short term memory and speed of processing are two of the key domains affected by parasitic worm infection. It also suggests that effects on cognitive function are apparent only for children with the heaviest worm loads.

Whilst these results raise some interesting psychological questions, their most important implications are in the field of public health. Parasitic worm infections have a great impact on the health and education of school-age children but can be treated simply and cheaply. To keep one child free of worms for a year costs as little as $0.23 for intestinal worms (including hookworm), and $0.79 for urinary schistosomiasis when existing education structures are used to deliver the drugs (Partnership for Child Development 1999). Such school-based health services are part of a package of measures put forward in a recent international initiative on school health: Focussing

Resources on Effective School Health (FRESH; World Bank 2000) . This framework for school health programmes includes cost-effective measures that reach the poorest children, including health-promoting policies, improved water and sanitation, school-based health services (such as the administration of deworming treatment) and skills-based health education. In an ideal world, all children should have the right to develop in the absence of parasitic disease. In a world of priorities and limited resources, data such as those presented in the paper are essential to convince government and donors of the damage being done to children's education as a result of allowing them to grow up carrying parasitic infections. Children are a nation's greatest resource, and have the potential to make great contributions economically and in other ways to a nation's future. It is only by investment in school health programmes to ensure that children develop free of disease that this potential will be realised.

Appendix

Cognitive and Motor Function Tests

A brief description of the each of the tests used in the study, the function measured and the scoring procedure is given:

Digit Span Forwards and Backwards

Children repeat increasingly longer strings of numbers immediately after the examiner has read them out. In the forwards test the numbers are repeated in the same order as they are read out, and in the backwards test, in the reverse order. For both tests there are three trials at each level, starting with three digits in the forward condition and two digits in the backwards condition. The test is discontinued if a child fails all three trials at one level. The score is the total number of correct answers. This is a test of verbal working memory. The backwards test is also thought to test executive function.

Grooved Pegboard

The time taken for children to place 25 grooved pegs into a board is recorded. The first trial is completed with the dominant hand and the second with the nondominant hand. This is a test of psychomotor function.

Corsi Block

The test is a visuo-spatial equivalent to the Digit Span Forwards test. There are nine blocks positioned on a board. The examiner points to the blocks in increasingly longer strings and the children have to replicate the pointing sequence. There are three trials at each level, starting with three blocks, and the test is discontinued if a child fails all three trials at one level. This is a test of visuo-spatial working memory.

Verbal Fluency

This has two trials of one minute in which children name as many animals and then as many foods as they can. One point is given for each answer (disregarding duplicates) and totalled. The test is designed to measure the scanning and retrieval of long-term memory. There is also a speed of processing component.

Stroop

The test was an adaptation of the Stroop test. It consists of four trials each consisting of one page with 48 boxes containing either a tick (3) or a cross (8). There are two trials with 'compatible' instructions, where children are asked to touch each box and say as quickly as possible whether it contains a tick or a cross. There are also two 'incompatible' trials where children do the reverse, saying, 'tick' if the box contains a cross and saying 'cross' if the box contains a tick. The time taken to complete each trial and the number of errors made were scored and from these the time taken to complete 48 boxes without error was calculated. (Where errors were made the time was divided by the proportion of correct answers.) The adjusted times were totalled and averaged separately for both trials on the compatible or the incompatible tests. The test is designed to measure executive function (Baddeley 1992).

Spanish Vocabulary Learning

Adapted from a French Vocabulary Learning test by Baddeley designed for Jamaican children (Baddeley, Gardner & Grantham McGregor 1995), this test consists of 16 familiar pictures whose names were to be learnt in Spanish. Spanish is not taught in school, is easy for testers to pronounce and is not spoken in East Africa so was considered equally unfamiliar to all children. Initially, children were told the names of four pictures and then asked to point to the correct pictures when the tester repeated the names. After two correct trials, four more pictures were named and they were then asked to point to the eight pictures in turn when they were named. This was repeated introducing four new pictures every time the child pointed correctly to all named pictures on two consecutive trials. A total of eight trials was given. The score was the total number of correct responses. It was designed to measure paired-associate learning.

Computerised Tests

There were two computerised tests requiring the following equipment: Apple Mac portable computer, Psyscope software, a button box and speakers. Computers were unfamiliar to all the children in the study so the equipment was hidden prior to use and care was taken to introduce it in a sensitive and appropriate manner. Children were familiar with radios and audio speakers and most were comfortable with the setup.

Auditory Choice Reaction Time

Children's reaction time was measured when choosing which of two pictures matched an auditory stimulus. Above the red button on the button box was placed a picture of a dog and above the green button, a picture of a chick. Children were asked to press the red (dog) button as quickly as possible after hearing the sound of a dog ('woof woof') and the green (chick) button after hearing the sound of a chick ('cheep cheep'). The auditory stimuli (dog or chick) were presented in random order. The practice trial contained 10 stimuli and the main test 60 stimuli. The computer recorded each reaction time and calculated the mean reaction time for all responses excluding incorrect responses and those with reaction times greater than three standard deviations above or below the mean.

Silly Sentences

The Silly Sentences test was computerised and based on the Silly Sentences task (Baddeley, Emslie & Nimmo-Smith 1992). Two lists of 40 questions requiring either a 'yes' or 'no' response, were presented to children over the speakers. Each child was randomly assigned one of the two lists. For example, children were required to answer the question as quickly as possible by pressing the appropriate button on the button box. There were two practice trials of 6 and 10 questions. In addition, to assist children, a tick symbol was placed above the green button for 'yes' and a cross symbol above the red button for 'no'. The test score was the mean reaction time for correct answers, excluding responses longer than three standard deviations above the mean response time. The test was designed to measure auditory speed of processing.

Educational Achievement Tests

1. Reading Tests

These tests were developed specifically for the study. Traditional reading tests proved unsuitable because Kiswahili is a regularly spelt language which means that children can correctly read words out loud without understanding their meaning. Three tests were developed and presented in increasing order of difficulty. The letter reading and word reading tasks were given to all children. Sentence reading was given to all children scoring ≥ 9 (hits – false alarms) or ≥ 21 (total score) on the word reading task. The scores (hits – false alarms) for the letter, word and sentence reading tests were added together to produce a composite reading score. Note that a hit is when a real letter/word/sentence is correctly identified as real. A false alarm is when a false letter/word/sentence is incorrectly identified as real.

(a) Letter reading. Children had to discriminate letters and nonletters by putting a tick or a cross next to the test item. There were 12 letters and 12 nonletters presented in random order. Letters that resembled Arabic letters were excluded during piloting since many children in the study also attended Koranic school. A standard guessing correction was applied by subtracting the number of false alarms from the number of hits. The maximum possible score was therefore 12 and a score of chance was 0.

(b) Word reading. Children had to discriminate words from nonwords in the same way as for the letter reading task. There were 12 words and 12 nonwords. Words were taken from school reading books. A guessing correction was again used, hence the maximum possible score was 12 and a score of chance was 0. There were two parallel versions of this test and children were randomly assigned to receive either Version A or Version B.

(c) Sentence reading. The test was based on the Silly Sentences task (Baddeley *et al.* 1992) and was intended to measure speeded comprehension for better readers. Children had to discriminate silly from true sentences e.g. ('Do goats lay eggs?' or 'Is your hand attached to your arm?') The total number of sentences presented in the task was 125 and the time allotted was 5 minutes, which was designed to ensure no child would complete the task. Children were tested in groups of up to 10. Using a guessing correction based on hits minus false alarms, gave a maximum score of 63 and a chance score of 0.

2. Spelling

To test children's spelling children were read out a total of 50 words and asked to spell the word on the sheet of paper they were given. The maximum score was 50.

3. Arithmetic

There were 2 arithmetic tests—one written and one oral. The oral arithmetic test was easier and given individually to all children in Grade 2 and those children in Grade 3 who scored ≤ 6 on the written arithmetic test. The test covered basic numerical understanding including number recognition, counting and simple addition and subtraction of numbers one or two digits long. The score was the total number correct and the maximum score was 15. The written arithmetic was given in groups and tested more advanced numerical skills of numbers two to six digits long. The score was the total number correct and the maximum score was 30. Children not qualifying to do the oral arithmetic test were awarded the maximum score on this test of 15. Scores on the two tests were added together to get an overall score on arithmetic of 45.

Partnership for Child Development
Department of Infectious Disease Epidemiology
Imperial College School of Medicine
Norfolk Place, London

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References

Alcock, KJ, C Nokes, F Ngowi, C Musabi, A Mbise, R Mandali, D Bundy & A Baddeley 2000. The Development of Reading Tests for Use in a Regularly Spelt Language. *Applied Psycholinguistics* 21,4.

Baddeley, A 1992. Is working memory working? The fifteenth Bartlett Lecture. *Quarterly Journal of Experimental Psychology: Human Experimental Psychology* 44a1:1-31.

Baddeley, A, H Emslie & I Nimmo-Smith 1992. *The Speed and Capacity of Language Processing (SCOLP) Test: Manual*. Bury St. Edmonds: Thames Valley Test Company.

Baddeley, A, JM Gardner & S Grantham-McGregor 1995. Cross-cultural Cognition: Developing Tests for Developing Countries. *Applied Cognitive Psychology*.

Beasley, NM, AM Tomkins, A Hall, CM Kihamia, W Lorri, B Nduma, W Issae, C Nokes & DA Bundy 1999. The Impact of Population Level Deworming on the Haemoglobin Levels of Schoolchildren in Tanga, Tanzania. *Trop Med Int Health* 4,11:744-750.

Bundy, D & R Peto 2000. Treatment for Intestinal Helminth Infection. *BMJ,* 321,7270:1224.

Dickson, R, S Awasthi, P Williamson, C Demellweek & P Garner 2000. Effects of Treatment for Intestinal Helminth Infection on Growth and Cognitive Performance in Children: Systematic Review of Randomised Trials. *BMJ* 320,7251:1697-1701.

Drake, LJ, MCH Jukes, RJ Sternberg & DAP Bundy 2000. Geohelminthiasis (ascariasis, trichuriasis and hookworm): Cognitive and Developmental Impact. *Seminars in Pediatric Infectious Disease* 11,4:1-9.

Farid, Z 1993. Schistosomes with Terminal-spined Eggs: Pathological and Clinical Aspects. In P Jordan, G Webb & R Sturrock (eds): *Human Schistosomiasis*. Wallingford: CAB International.

Gardner, JM, S Grantham-McGregor & A Baddeley 1996. Trichuris Trichiura Infection and Cognitive Function in Jamaican School Children. *Ann Trop Med Parasitol* 90,1:55-63.

Grantham-McGregor, S & C Ani 2000. *A Review of Studies on the Effect Iron Deficiency has on Cognitive Development in Children*. WHO.

Grigorenko, E, R Sternberg, D Ngorosho, M Jukes & D Bundy (forthcoming). Effects of Antiparasitic Treatment on Dynamically-Assessed Cognitive Skills. *Child Development*.

Hadidjaja, P, E Bonang, MA Suyardi, SA Abidin, IS Ismid & SS Margono, 1998. The Effect of Intervention Methods on Nutritional Status and Cognitive Function of Primary School Children Infected with Ascaris Lumbricoides. *Am J Trop Med Hyg* 59,5:791-795.

Kvalsvig, JD, RM Cooppan & KJ Connolly 1991. The Effects of Parasite Infections on Cognitive Processes in Children. *Ann Trop Med Parasitol*, 85,5:551-568.

Levav, M, AF Mirsky, PM Schantz, S Castro & ME Cruz 1995. Parasitic Infection in Malnourished School Children: Effects on Behaviour and EEG. *Parasitology* 110,Pt 1:103-111.

Lozoff, B 1990. Has Iron Deficiency been Shown to Cause Altered Behaviour in Infants? In Dobbing, J (ed): *Brain, Behaviour and Iron in the Infant*. London: Springer-Verlag.

Mendez, MA & LS Adair 1999. Severity and Timing of Stunting in the First Two Years of Life Affect Performance on Cognitive Tests in Late Childhood. *J Nutr* 129,8:1555-1562.

Montresor, A, DWT Crompton, DAP Bundy, A Hall & L Savioli 1998. *Guidelines for the Evaluation of Soil-transmitted Helminthiasis and Schistosomiasis at Community Level. A guide for Managers of Control Programmes*. Geneva. WHO/CTD/SIP/98.1: World Health Organisation.

Nokes, C, S Grantham-McGregor, AW Sawyer, ES Cooper & DA Bundy 1992. Parasitic Helminth Infection and Cognitive Function in School Children. *Proc R Soc Lond B Biol Sci* 247,1319:77-81.

Nokes, C, S Grantham-McGregor, AW Sawyer, ES Cooper, BA Robinson & DA Bundy 1992. Moderate to Heavy Infections of Trichuris Trichiura

Affect Cognitive Function in Jamaican School Children. *Parasitology* 104,Pt 3:539-547.

Nokes, C, ST McGarvey, L Shiue, G Wu, H Wu, DA Bundy & GR Olds 1999. Evidence for an Improvement in Cognitive Function Following Treatment of Schistosoma Japonicum Infection in Chinese Primary Schoolchildren. *Am J Trop Med Hyg,* 60,4:556-565.

Olsen, A, P Magnussen, JH Ouma, J Andreassen & H Friis 1998. The Contribution of Hookworm and other Parasitic Infections to Haemoglobin and Iron Status among Children and Adults in Western Kenya. *Trans R Soc Trop Med Hyg* 92,6:643-649.

Partnership for Child Development 1999. The Cost of Large-scale School Health Programmes which Deliver Anthelminthics to Children in Ghana and Tanzania. *Acta Tropica* 73:183-204.

Partnership for Child Development 2002. Heavy Schistosomiasis Associated with Poor Short-term Memory and Slower Reaction Times in Tanzanian School Children. *Tropical Medicine and International Health* 7,2.

Pollitt, E & R Mathews 1998. Breakfast and Cognition: An Integrative Summary. *Am J Clin Nutr* 67,4:804s-813s.

Rasbash, J, W Browne, H Goldstein, M Yang, I Plewis, M Healy G Woodhouse, D Draper, I Langford & T Lewis 2000. *A User's Guide to MLwiN, V2.1.* London: Multilevel Models Project, Institute of Education, University of London.

Sakti, H, C Nokes, WS Hertanto, S Hendratno, A Hall, DA Bundy & Satoto 1999. Evidence for an Association between Hookworm Infection and Cognitive Function in Indonesian School Children. *Trop Med Int Health* 4,5:322-334.

Sankoh, A, M Huque & S Dubey 1997. Some Comments on Frequently used Multiple Endpoint Adjustment Methods in Clinical Trials. *Statistics in Medicine* 16:2529-2542.

Seifer, R 2001. Socioeconomic Status, Multiple Risks, and Development of Intelligence. In Sternberg, RJ & EL Grigorenko (eds): *Environmental Effects on Cognitive Abilities*. Mahwah, New Jersey: Lawrence Erlbaum Associates, Inc.

Simeon, D, J Callender, M Wong, S Grantham-McGregor & DD Ramdath DD 1994. School Performance, Nutritional Status and Trichuriasis in Jamaican Schoolchildren. *Acta Paediatr* 83,11:1188-1193.

Simeon, D & S Grantham-McGregor 1990. Nutritional Deficiencies and Children's Behaviour and Mental Development. *Nutrition Research Reviews* 3:1-24.

Simeon, DT, S Grantham-McGregor, JE Callender & MS Wong 1995. Treatment of Trichuris trichiura infections improves growth, spelling scores and school attendance in some children. *J Nutr, 125*(7), 1875-1883.

Simeon, DT, S Grantham-McGregor & MS Wong 1995. Trichuris Trichiura Infection and Cognition in Children: Results of a Randomized Clinical Trial. *Parasitology* 110,Pt 4:457-464.

Sternberg, R, E Grigorenko, D Ngorosho, E Tuntufye, A Mbise, C Nokes, M Jukes & D Bundy (in press). Assessing Intellectual Potential in Rural Tanzanian School Children. *Intelligence.*

Sternberg, RJ, C Powell, P McGrane & S Grantham-McGregor 1997. Effects of a Parasitic Infection on Cognitive Functioning.

Stoltzfus, RJ, M Albonico, JM Tielsch, HM Chwaya & L Savioli 1997. School-based Deworming Program Yields Small Improvement in Growth of Zanzibari School Children after one Year. *Journal of Nutrition* 127,11:2187-2193.

Stoltzfus, RJ, HM Chwaya, JM Tielsch, KJ Schulze, M Albonico & L Savioli 1997. Epidemiology of Iron Deficiency Anaemia in Zanzibari Schoolchildren: The Importance of Hookworms. *American Journal of Clinical Nutrition* 65,1:153-159.

Warren, K, D Bundy, R Anderson, A Davis, D Henderson, D Jamison, N Prescott & A Senft 1993. Helminth Infection. In Jamison, D, W Mosley, A Measham & J Bobadilla (eds): *Disease Control Priorities in Developing Countries.* New York, NY: Oxford University Press.

World Bank 2000. *Focusing Resources on Effective School Health: A FRESH Start to Enhancing the Quality and Equity of Education.* Washington, DC: World Bank.

Table 2. Summary of the effect of parasitic worm infections on specific cognitive tests in selected studies.

		1	2	3
	Author	Sakti et al. (1999)	PCD (2002)	Kval.svig et al. (1991), study 1
	Design	Cross Sectional	Cross Sectional	Cross Sectional
	Country	Indonesia	Tanzania	South Africa
	Sample size	I=311 U=121	I=272 U=117	L1=34 L2=26 L3=36
	Age	8-13	9-14	Primary School
	Treatment length (months)			
	Species	Hookworm	*S. haematobium*	Geohelminths
	Parasitic load in infected children	Intensity	Heavy	Parasitic Load Index
Short term Memory	Forwards	✓	✓	
	Digit Span Backwards	X	✓	
	Corsi Block	X	X	
Long term memory	Fluency	✓	X	
	Language learning	X		
	Free Recall			
	Trail making			
Executive	Stroop Colour-Word	✓	X	
Language	Comprehension			
	Vocabulary			
Problem Solving	Verbal Analogies	X		
	Raven CPM			
	MFFT Easy			
Speed of Processing	Silly Sentences		✓	
	Number Choice	✓		
	Visual Search	✓		X

10	9	8	7	6	5	4
Nokes et al. (1999)	Nokes et al. (1992)	Sternberg et al. (1997)	Gardner et al. (1996)	Simeon et al. (1995)	Levav et al. (1995)	Kvalsvig et al. (1991), study 2
Treatment	Treatment	Cross Sectional	Cross Sectional	Cross Sectional	Cross Sectional	Cross Sectional
China	Jamaica	Jamaica	Jamaica	Jamaica	Ecuador	South Africa
T=92 P=89	T=62 P=41C=56	T=66 P=67 C=63	I=97 U=48	I=189 U=100	I=103 U=27	N=100
5-15	9-12	9-11	10-11	7-10	9-14	10
14	9					
S. japonicum	*T. trichura*	*T. trichura*	*T. trichura*	*T. trichura*	*A. lumbricoides*	Helminths
Intensity	Moderate-heavy	Moderate	Moderate	Moderate	Intensity	Parasitic Load Index
X	✓	✓	X	X	X	
	✓	X	X			
X						
✓1	✓		X	✓		
			X	✓		
✓2		X				X
					X	
					✓	
	X					
					✓	
		✓				
	X					
			✓			
				X		
X		X		✓	X	

14	13	12	11
Hadidjaja et al. (1998)	Sternberg et al. (1997)	Gardner et al. (1996)	Simeon et al. (1995)
Treatment	Treatment	Treatment	Treatment
Indonesia	Jamaica	Jamaica	Jamaica
P=148 U=86	I=133 U=63	T=49 P=48 C=48	T=96 P=93 C=100
6-8	9-11	10-11	7-10
22	10	14	14
A. lumbricoides	*T. trichura*	*T. trichura*	*T. trichura*
Prevalence	Moderate	Moderate	Moderate
X	X	X	X
X	X	X	
		X	✓1
		X	X
	X		
	X		
✓4			
		X	
			X
	X		X

I=Infected, U=Uninfected, T=Treatment, P=Placebo, C=Uninfected control, L1-3 = measure of multiple infection and load

PCD = Partnership for Child Development

1=improvement only for children with poor nutritional status
2=improvement for children with heaviest infection
3=differences between infected and uninfected only for easy tasks
4=Mebendezole alone improved cognitive scores but mebendezole + health education did not.