Imperial College London

Biostatistical Analysis of the Schistosomiasis Control Initiative Programme on *Schistosoma* Prevalence, Intensity and Associated Morbidity

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Dedicated to my parents

John and Angeliki Koukounari

Abstract

Background*:** Schistosomiasis remains one of the most prevalent parasitic diseases in developing countries. ***Goal and objectives*:** The potential relationship between *Schistosoma mansoni* and anaemia, was examined using Schistosomiasis Control Initiative (SCI) data on Ugandan children and a randomized clinical trial implemented by colleagues from the London School of Hygiene and Tropical Medicine (LSHTM) in Western Kenya. The impact of large-scale administration of chemotherapy on *Schistosoma haematobium* infection and associated morbidity was also evaluated using SCI data on Burkinabe children. Evaluation and validation of field applicable tools, such as ultrasound, for the cost-effective diagnosis of schistosomiasis morbidity using SCI baseline data from Malian children was another aim of this thesis. Furthermore, in combination with the ultrasound scans, microscopic examination of urine for detection of *S. haematobium* eggs, dipsticks for detection of haematuria, tests for circulating antigens and serology tests were examined for the cost-effective assessment of *S. haematobium* prevalence in an adult's dataset from Ghana. ***Methods: Biostatistical analysis of afore mentioned data was applied. ***Principal findings*:** Results from SCI and LSHTM studies suggest that anaemia is associated with schistosomiasis in African children, and that such anaemia shows a significant improvement following chemotherapy. Results from the SCI study in Burkina Faso suggested that even a single round of mass chemotherapy can have a substantial impact on *S. haematobium* infection and its associated morbidity in children. Microscopy and haematuria dipsticks were suggested as sensitive and specific indicators of prevalence of *S. haematobium* infection in Ghanaian adults. Furthermore, ultrasound global scores were demonstrated to be valuable markers in children for morbidity caused by *S. haematobium* infection, but ultrasonographic examination is not a reasonable substitute for microscopy or dipsticks for determining the prevalence of this infection. ***Significance:*** Finally, the key findings from all the studies presented, emphasizing how these relate to one another, are discussed.

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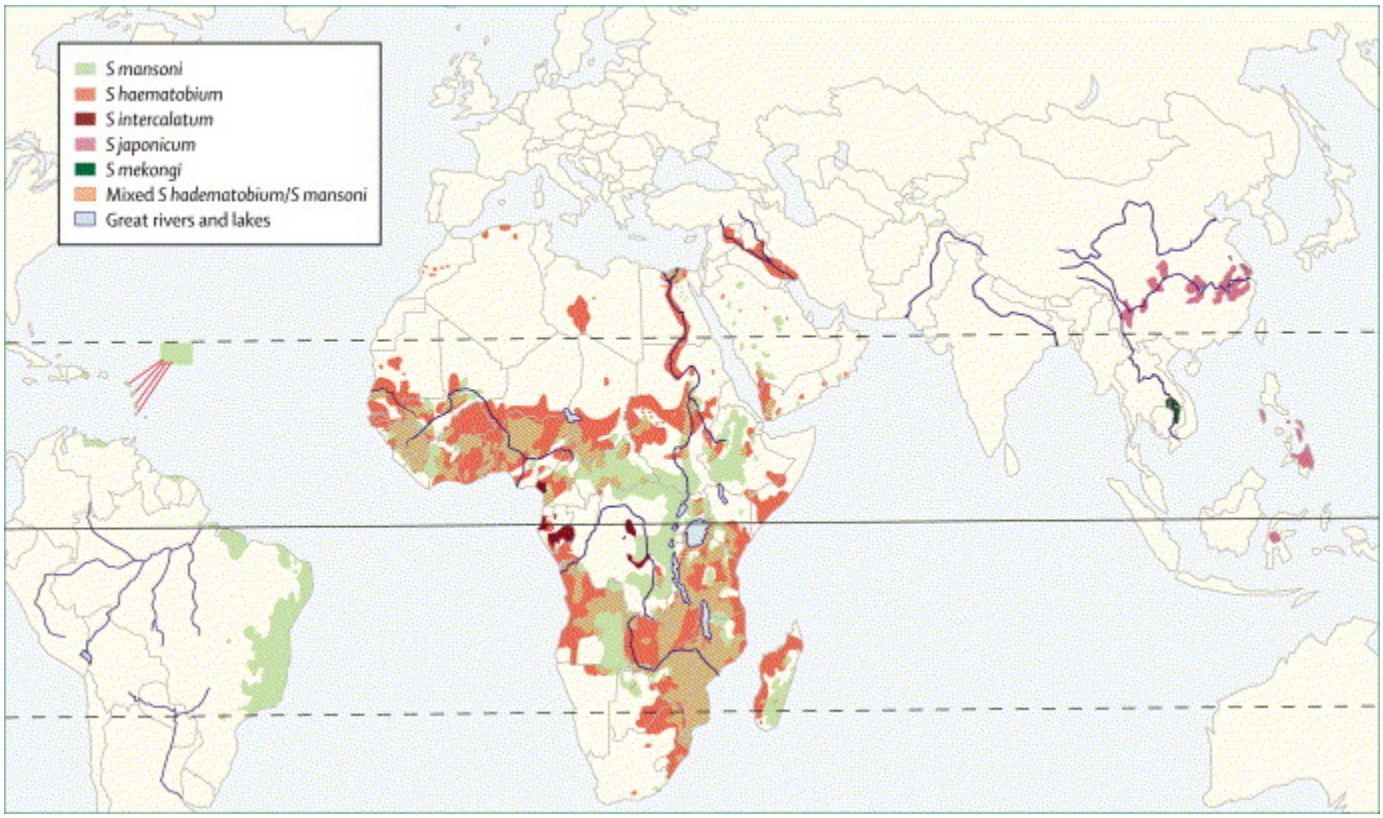
Chapter 1: General introduction

Schistosomiasis or Bilharzia as it is sometimes referred to, remains one of the most prevalent parasitic diseases in developing countries (Engels et al., 2002). After malaria, schistosomiasis is the most important tropical disease in terms of human morbidity with significant economic and public health consequences (WHO, 1993). Recently, at least three studies have also indicated that schistosomiasis is a much more serious public health problem than currently perceived. A meta-analysis of performance-related symptoms and disability-linked morbidities in schistosomiasis patients (King et al., 2005) suggest a 4 to 30 times greater chronic disability than originally estimated using disability-adjusted life years (DALYs). Another study made similar observations about the frequency of clinical morbidity and also indicated an annual mortality of 280,000 cases directly attributable to schistosomiasis in sub-Saharan Africa (van der Werf et al., 2003), far greater than the 15,000 p.a. worldwide estimated by the World Health Organization (WHO, 2004). Furthermore, the most recent estimate in mid-2003 of 779 million people at risk and 207 million people infected with schistosomiasis translates to increases of 12.6 % and 7.2 % respectively (Steinmann et al., 2006) when compared with estimates from the mid-1990's (Chitsulo et al., 2000).

The main forms of human schistosomiasis are caused by five species of flatworm in the genus *Schistosoma*, within the class trematodes which are: *Schistosoma haematobium*, which infects the urinary tract, *Schistosoma mansoni*, *Schistosoma japonicum, Schistosoma intercalatum and Schistosoma mekongi* which all infect the intestinal tract. The studies presented in this thesis are confined to the first two species of schistosomes that are prevalent in humans in sub-Saharan Africa (*S. haematobium* and *S. mansoni*) conferring the greatest burden on human health (see Figure 1.1 for global distribution of schistosomiasis).

Figure 1.1 Global distribution of schistosomiasis



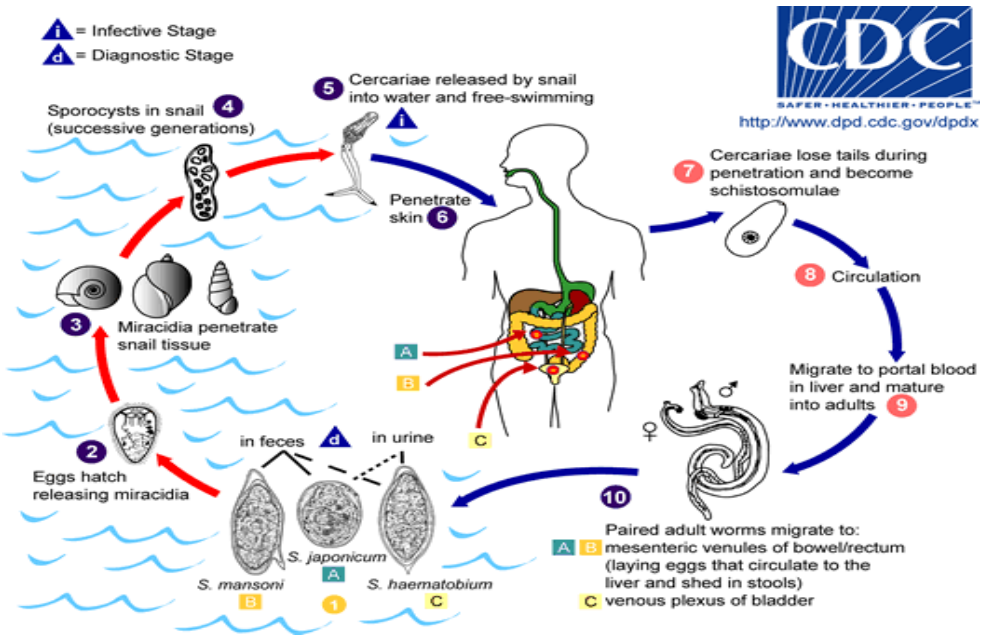
Main foci: *S mansoni*—much of sub-Saharan Africa, northeast Brazil, Surinam, Venezuela, the Caribbean, lower and middle Egypt, the Arabic peninsula; *S haematobium*—much of sub-Saharan Africa, Nile valley in Egypt and Sudan, the Maghreb, the Arabian peninsula; *S japonicum*—along the central lakes and River Yangtze in China; Mindanao, Leyte, and some other islands in the Philippines; and small pockets in Indonesia; *S mekongi*—central Mekong Basin in Laos and Cambodia; *S intercalatum*—pockets in west and central Africa. (From Gryseels et al., 2006).

1.1 Life cycle

Schistosomes have indirectly transmitted life-cycles involving obligatory alternation of generations between sexual reproduction in a mammalian host, such as a human, and asexual reproduction within a molluscan (freshwater snail) host. In fresh water, free-living schistosome larvae (miracidia) hatch from eggs passed out in the urine or stool, depending on the species, which then invade the intermediate snail hosts. There are different genera of snails according to the different schistosome species presented in the studies of this thesis. More precisely, intermediate host of *S. haematobium* are snails of the genus *Bulinus* while intermediate host of *S. mansoni* are snails of the genus *Biomphalaria.* Following the penetration in the snail, the

miracidium develops into sporocysts which in over about four to six weeks develop into stages infective to the mammalian definitive host through asexual replication where thousand of cercariae are produced. These cercariae are then released into the water, where they can infect by penetration of the skin, for example, children playing in the water; women collecting water or engaging in domestic chores; and men swimming, fishing, and irrigating crops. During the penetration process the cercariae lose their tale and transform into the larval stage: the schistosomulum which migrate to the lungs in three to four days. After penetration in the pulmonary capillaries schistosomula are carried to the systemic circulation and to the portal system. In the hepatic circulation schistosomes mature to adult, and in pairs they migrate to the vesical plexus (*S. haematobium*) and to the mesenteric veins (*S. mansoni*). Embryonated eggs are excreted in urine and/or faeces after approximately seventy days (*S. haematobium*) and thirty five days (*S. mansoni*) (Molyneux, 2006;Webster et al., 2008) (see Figure 1.2 for an illustrated summary of the human schistosomiasis life cycle). It should be noted that the duration of the life cycle of human schistosomiasis depends on the schistosome species examined, on environmental factors such as temperature and water quality as well as on the number of miracidia that enter and survive in a snail.

Figure 1.2 The Life Cycle of human schistosomiasis



Eggs are eliminated with feces or urine (1). Under optimal conditions the eggs hatch and release miracidia (2), which swim and penetrate specific snail intermediate hosts (3). The stages in the snail include 2 generations of sporocysts (4) and the production of cercariae (5). Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host (6), and shed their forked tail, becoming schistosomulae (7). The schistosomulae migrate through several tissues and stages to their residence in the veins ((8), (9)). Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species (10). Lifecycle and annotations are adapted from the Center for Disease Control and Prevention (CDC) website http://www.dpd.cdc.gov/dpdx/HTML/Schistosomiasis.htm (accessed August 2008).

1.2 Schistosomiasis morbidity

The schistosome life cycle has three phases in the human host, and all have their clinical implications. The first phase is host penetration by cercaria, which is usually manifested as a transient (hours) itchy eruption that occurs soon after exposure and is known as swimmer's itch or cercarial dermatitis.

The second phase is schistosomulae tissue migration and maturation and is associated with transient (a few weeks to months) hypersensitivity, which gives rise to the syndrome of acute schistosomiasis, known as 'Katayama' fever. The disease starts suddenly with fever, fatigue, myalgia, malaise, non productive cough, eosinophilia, and patchy infiltrates on chest radiography. Abdominal symptoms can develop later, caused by the migration and positioning of the mature worms. Most patients recover spontaneously after two to ten weeks, but some develop persistent and more serious disease with weight loss, dyspnoea, diarrhoea, diffuse abdominal pain, toxaemia, hepatosplenomegaly and widespread rash.

The third phase is the chronic one where main lesions are due not to the adult worms but to eggs that are trapped in the tissues during the peri-vesical or peri-intestinal migration or after embolisation in the liver, spleen, lungs, or cerebrospinal system (Gryseels et al., 2006). Human infection with *S. mansoni* is associated with chronic hepatic and intestinal fibrosis, whilst *S. haematobium* infections can lead to ureteric and bladder fibrosis, calcification of the urinary tract and bladder cancer due to the retention of schistosome eggs within tissues and inflammatory response to them (Vennervald et al., 2000).

1.3 Schistosomiasis control

There is currently no effective vaccine against human schistosomiasis.

The tendency to urinate and defecate in and around water is an important behavioural characteristic of humans on which relies the schistosome parasite. The most striking fact relevant to control is that if human behaviour could be changed to stop contamination of water bodies with urine and faeces containing schistosome eggs, then transmission of the parasite would cease. This fact is central to many longer-term control efforts, including the education of children, to reduce water contamination (1st stage of life cycle in Figure 1.2).

Environmental sanitation and safety of supply water constitute essential forms of schistosomiasis control. Water resource development and projects, as well as the consequent concentrations of human settlements and increased water contact, lead to heavy worm infections in people, more eggs reaching the water, and greater human pathology. The provision of safe water supplies and latrines is obviously useful, but for the prevention of schistosomiasis, safe contact sites are also needed (Fenwick, 2006a).

Furthermore, within the snail, the parasite develops and multiplies asexually (3rd, 4th and 5th stages of life cycle in Figure 1.2). Particularly snails of the *Bulinus* and *Biomphalaria* molluscan genera thrive in areas frequented by humans. Indeed these species seem to favour habitats polluted with human excreta and the detritus of everyday living. The intricate relationship between snail and schistosome make this part of the life cycle vulnerable to control activities, and there is a long history of attempted snail control using chemicals (molluscicides) to kill snails as well as biological control of snails. However, despite the clear evidence that the population of snails can be much reduced through molluscicides, the latter are not specific to killing just molluscs. For example, fish are also killed and therefore this measure of control can have deleterious impact on the environment and biodiversity.

In areas where schistosomiasis is highly endemic therefore, the present goal is to mitigate the burden of the disease by controlling morbidity (Fenwick & Webster 2006). Chemotherapy with

praziquantel (PZQ) is the mainstay for schistosomiasis control, and will remain the drug of choice for several years.

For instance, Egypt's schistosomiasis control programme has achieved great success and a major impact on schistosomiasis prevalence and morbidity by using mass drug administration with praziquantel since 1996. In May 2001, the World Health Assembly adopted resolution 54.19 affirming schistosomiasis and soil transmitted helminthiasis as public health priority and setting the target of providing treatment to at least 75 % of school-age children at risk of morbidity by 2010 (Colley et al., 2001). Since 2003 further, major efforts have being launched by the Schistosomiasis Control Initiative (SCI), the initial aim of which was to assist six sub-Saharan African countries to develop sustainable national schistosomiasis morbidity control programmes, through the provision and monitoring of praziquantel treatment, to 15 million children and other high risk groups over a period of five years. A primary objective of these SCI-supported control programmes was to achieve, and hence also demonstrate, a quantifiable reduction in schistosome-associated morbidity as a consequence of such chemotherapeutic intervention (Fenwick 2006b).

1.4 Monitoring and evaluation and further research

Since control operations require monitoring and evaluation strategies, the focus on morbidity control calls for careful validation of the direct and indirect indicators of schistosome-related morbidity (Hatz et al., 1990). As there is no agreement regarding the list of sequelae that should be included in morbidity assessments (Michaud et al. 2004), further research into reliable markers for this purpose can contribute to better targeting of individuals at higher risk. Furthermore, good estimates of these specific epidemiological parameters, and knowledge of their associated uncertainty, would help towards implementing the optimal design of future control programmes for schistosomiasis and other parasitic diseases in general, as well as the proper evaluation of the current ones. Kabatereine and others (Kabatereine et al., 2005) have also stressed the necessity to provide epidemiological descriptions of schistosomiasis on a countrywide basis in order to move towards control efforts while Wiest, 1996 emphasized the necessity of accurate assessment of morbidity in endemic communities and the determination of the impact of different interventions on schistosomiasis infection in order that governments and

authorities make the right decisions given the often limited resources for the control of this parasitic disease.

1.5 Diagnosis

Diagnosis is central to all aspects of schistosomiasis. Decisions on individual and community treatment, estimations on prognosis and assessment of morbidity, evaluation of chemotherapy and control measures all build on the results from diagnostic tests.

Schematically, three different categories of approaches for the diagnosis of schistosomiasis are known. Direct parasitological methods detect schistosome eggs in urine, stool or the rectal mucosa, and histological methods disclose schistosomula, adults worms or eggs in tissue biopses. Indirect methods, relying on clinical, biochemical or immunological disease markers, detect pathology typically or frequently associated with schistosome infections. Finally, immunological methods measure the immune response to certain schistosome antigens or the concentration of parasite-derived antigens in blood or urine (Jordan et al., 1993). Some of these available direct parasitological, indirect and finally immunological diagnostic methods are evaluated throughout this thesis.

1.6 Thesis aims and structure

The majority of the studies presented in this thesis (Chapters 2, 4 and 5) are targeted towards evaluation of recent mass human chemotherapy programmes through biostatistical analysis of surveillance and monitoring data of children provided from the country teams on *Schistosoma* intensity and associated morbidity. Positive relationships between the intensity of infection and clinical manifestations as well as organ pathology are more pronounced in children in the early stages of the disease, whereas in adults, severe pathology may occur during a low intensity of infection (Richter, 2003). Thus using mainly data from school-aged children (6 to 14 and 10-21 years old) here, pre-treatment baseline morbidity levels within these risk populations are defined and characterized (Chapters 2, 3, 4 and 5) so that any subsequent changes in morbidity caused by the intervention can be accurately determined (Chapters 2 and 4). Data used throughout all this PhD thesis are uniquely detailed and together with appropriate statistical analysis should add

significantly to the gap which exists because of the limited large scale community screening of morbidity and rare broad geographic comparisons (Balen et al., 2006). Indeed, further studies of geographical variations in morbidity have been proposed as one of the priorities for future research in schistosomiasis (WHO, 2000; Boisier et al., 2001).

Data used in Chapters 2, 3, 4 and 5 are organized in hierarchical structure, with clustering within units. More precisely, children cluster in the same school and schools cluster within districts. In general, individuals who belong to the same 'unit' may share common genetic, behavioural, or social risk factors of disease. They may also have similar exposures to environmental factors. The health outcomes of two individuals within the same unit will therefore correlate more highly than those of two individuals from different units (Carabin et al., 2003). In addition, there are longitudinal cohorts while relations between individual and 'supra-individual' determinants are of particular interest, especially for investigating the reasons of variation between areas (Mauny et al., 2004). In statistical terms, data used in all the afore mentioned Chapters of this PhD thesis raise the issue of correlated data analysis which can be tackled with hierarchical modeling. Initially popular in the social sciences, many hierarchical modeling studies are now published in health sciences (Shouls et al., 1996; Verheij, 1996; Bosma et al., 2001; Pickett & Pearl, 2001; Chaffin et al., 2007; Heijink et al., 2008; Ohman-Strickland et al., 2008; Szyszkowicz, 2008; Yu et al., 2008), as well as in infectious human diseases (Myers et al., 1996; Broome et al., 1999; Tinsman et al., 2001; da Silveira et al., 2005; Bortz & Nelson, 2006; Huang et al., 2006; Labbe & Verotta, 2006; Voss et al., 2007; Halkitis et al., 2008) although it is noteworthy that few as yet deal with parasitic diseases infecting humans (Mauny et al., 2004; Pion et al., 2006; Wang et al., 2006; Levy et al., 2007; Werneck et al., 2007).

Therefore, in this thesis hierarchical models are used extensively both in a frequentist and Bayesian framework in order to identify and characterise potential "markers" for predicting short or long-term morbidity from *S. haematobium* and *S. mansoni* infections (Chapters 2, 3, 4 and 5). Such models also aim to identify potential causal relationships and associations between pathologic changes such as macrohematuria and ultrasound scans and clinical manifestations such as intensity of infection induced by schistosomiasis egg excretion in stool or urine samples, (this approach would thereby provide 'direct morbidity' markers: Chapters 4 and 5) because

within defined age groups, intensity of infection is expected to be associated with morbidity (Richter, 2003). Another aim of these models is to investigate nutritional consequences of schistosome infection and potential manifestations of schistosomiasis such as anaemia (this approach would thereby provide 'indirect morbidity' markers: Chapters 2, 3 and 4).

Evaluation and validation of field applicable tools, such as ultrasound, for the cost-effective diagnosis of schistosomiasis morbidity in the context of large-scale schistosomiasis control programmes is another aim of this thesis (Chapters 5 and 6). Furthermore, in combination with the ultrasound scans, microscopic examination of urine for detection of *S. haematobium* eggs, dipsticks for detection of haematuria, tests for circulating antigens and serology tests are examined for the rapid and cost-effective assessment of *S. haematobium* prevalence in an adult's dataset. The matter of effective diagnosis of schistosomiasis is of increasing importance, particularly with respect to the recent paper by King and others (2006) and the current progress of the treatment programmes. One solution may therefore relate to the need for more sophisticated statistical methods to be developed and utilized in order to obtain more reliable empirical estimates of sensitivities and specificities of various diagnostic tests. In this thesis, latent class analysis models are explored and applied to achieve such aims (Chapter 6).

Chapters 2-6 have appeared or will shortly appear as papers in scientific journals and are included in this PhD thesis with slight modifications. More precisely, Chapters 2 and 5 have been published in the *American Journal of Tropical Medicine and Hygiene*, Chapter 3 has been published in the *International Journal for Parasitology*, Chapter 4 has been published in the *Journal of Infectious Diseases* and Chapter 6 will shortly be published in the *American Journal of Tropical Medicine*; the original papers can be found in the appendix of this PhD thesis. Because it was intended that each chapter could be read by itself, some overlap between chapters is unavoidable.

The thesis concludes with a general discussion of the novel results highlighted by the statistical analysis applied in all previous chapters (Chapter 7).

Chapter 2: Morbidity indicators of *Schistosoma mansoni*: relationship between infection and anaemia in Ugandan schoolchildren before and after praziquantel and albendazole chemotherapy

Summary

This study examines the potential relationship between *Schistosoma mansoni* and anaemia, using data obtained by the Schistosomiasis Control Initiative (SCI) before (baseline) and one year after (follow-up of) a chemotherapeutic treatment programme in Uganda. Changes in haemoglobin (Hb) levels in 2,788 children in relation to their *S. mansoni* and/or hookworm infection intensity category and baseline anaemia status were analyzed. At baseline, significant predictors of childhood anaemia were intensities of *S. mansoni* and hookworm infection. At follow-up, moderate or heavy hookworm as well as heavy *S. mansoni* infections were important. Children heavily infected with *S. mansoni* or hookworm had significantly lower Hb levels at baseline compared to those not infected. Among anaemic children at the baseline survey, a significant increase in Hb counts of 0.83 g/dl after treatment was found. These results suggest that anaemia is associated with schistosomiasis and hookworm in Ugandan children, and that such anaemia shows a significant improvement after chemotherapy.

Key words: hierarchical models, anaemia, Uganda, *Schistosoma mansoni*, hookworm

Note: A modified version of this chapter has been published as: Koukounari A, Fenwick A, Whawell S, Kabatereine NB, Kazibwe F, Tukahebwa EM, Stothard JR, Donnelly CA, Webster JP (2006) *Am J Trop Med Hyg* 75, 278-86. See publications by the candidate (Appendix).

2.1 Introduction

Anaemia is a common problem throughout the world and of enormous public health concern in developing countries, but its aetiology is very complex making the effect of any one factor difficult to assess. Malaria plays a key causative role for anaemia among young African children, although HIV infection, haemoglobinopathies, intestinal helminths, in particular that of hookworm infection, poor nutritional status and micronutrient deficiencies are also likely to make important additional contributions (Crawley, 2004). Schistosomiasis is a parasitic disease of profound medical and veterinary importance, second only to malaria in terms of parasite-induced human morbidity and mortality, with some 600 million people exposed and 200 million infected at any time throughout the tropical world (Engels et al., 2002). The relative role of schistosomiasis as a causative agent for anaemia, however, particularly as compared to that known for hookworm infection, remains controversial (Stephenson & Holland, 1987; Stephenson, 1993; Olsen et al., 1998; Nokes et al., 1999; Coutinho et al., 2005). Preston and Dargie (1975), for instance, provided convincing evidence that schistosomiasis causes anaemia in experimental animals (Preston & Dargie, 1975), although, whilst Foy and Nelson (1963), did acknowledge that anaemia was generally associated with heavy schistosome infections, they doubted that early or light infections were of importance (Foy & Nelson, 1963). There is, nevertheless, a general consensus for the need for further epidemiological research into the potential role of schistosomiasis as a causative agent for anaemia (Foy & Nelson, 1963; Latham et al., 1983).

Since 2003, the Schistosomiasis Control Initiative (SCI) has assisted six sub-Saharan African countries with the objective to develop sustainable national schistosomiasis morbidity control programmes and reach at least 75 % of school-aged children and other high-risk group, through mass deworming using praziquantel for schistosomiasis and albendazole for intestinal helminths. Such control programmes thereby provide a unique opportunity to assess the potential role of schistosomiasis and/or other intestinal helminths as causative agents of anaemia morbidity, together with the potential ameliorative impact of chemotherapy on a large scale. This may be particularly pertinent in terms of identifying and evaluating morbidity associated with *S. mansoni* (intestinal schistosomiasis), which is frequently very difficult to assess and quantify precisely

except in the most severe or late chronic cases (Lengeler et al., 1991; Utzinger et al., 1998; Brouwer et al., 2003; Vennervald & Dunne, 2004). Moreover, because demonstration of successful morbidity control is feasible only if there are reliable morbidity markers capable of showing reversion within the time-frame of disease surveillance, it is vital to identify *S. mansoni* morbidity indicators for sustainable disease control and for evaluating the success of intervention (Richter, 2003; Booth et al., 2004).

Using uniquely detailed data arising from the Ugandan National Schistosomiasis Control Programme, before and one year following praziquantel and albendazole chemotherapy, here the aim of this chapter was to evaluate the potential relationship between *S. mansoni* infection, anti-helminthic chemotherapy, and anaemia as well as to assess the extent to which schistosomiasis is a cause of anaemia.

2.2 Materials and methods

2.2.1 Study sites, sampling and cohort design. Parasitological and morbidity data were collected on a cohort of 4351 Ugandan children, aged 6 to 14 years old, randomly-sampled from 37 schools situated in eight districts just before and one year following implementation (2003 and 2004 respectively), as part of ongoing monitoring and evaluations of the SCI programme in Uganda. The eight districts were selected to represent the Albert Nile (Nebbi, Arua, and Moyo), Lake Victoria (Bugiri, Busia and Mayuge) and Lake Albert basins (Masindi and Hoima). Further details on these districts and their inhabitants are provided elsewhere (Kabatereine et al., 1999; Kabatereine et al., 2003).

Schools were chosen on the basis of existing *S. mansoni* data for schoolchildren from Uganda (SCI-National Survey of Bilharziasis) and parasitological stratification with different categories of infection prevalence, classified as low (< 10 %), medium (11-50 %) and high prevalence (> 50 %) within each district, which also allowed pooling in order to reach sample sizes technically detailed elsewhere (Brooker et al., 2004). Fixed cohort structure was recruited at each school to allow comparisons to be made across schools and districts. Required sample sizes were calculated using the EpiSchisto software tool (http://www.schoolsandhealth.org/epidynamics.htm) using an expected reduction in mean intensity of 60 % (*S. mansoni*) following chemotherapy to achieve 80% statistical power and a significance level of 5 %. The value of 60 % was chosen as a conservative estimate of the expected reduction over a two-year period (two annual treatments). An overall drop-out rate of 40 % over the course of the monitoring period was also allowed.

As it was not logistically possible to survey all schools within the same month, surveys were staggered. Children were identified at follow-up using a named roll call as well as retrieval of SCI individual treatment cards with unique code identifier given to the children the previous year as well as hardcopy of a group cohort photograph to ensure that children remember to which group they were reassigned. Before the one year follow-up visit a pre-survey team re-registered the children and also sensitized the children to ensure that as many children as possible in the cohorts were at school the day of survey. All children enrolled into this study were interviewed

and examined by appropriately trained Ministry of Health field workers. Administration of praziquantel and albendazole was according to WHO guidelines (praziquantel 40mg/Kg and single 400 mg albendazole tablet). Ethical clearance was obtained from the Ugandan National Council of Science and Technology and Imperial College London. For ethical reasons, it was not appropriate to include any untreated control groups in the study design.

2.2.2 Parasitological and anaemia morbidity data. A single stool sample was collected from each individual and 41.7 mg processed to make duplicate Kato-Katz slides for microscopic determination of schistosome and/or hookworm infection and, where applicable, egg per gram counts. For comparative reasons between the two successive years of study, where the second measurement of the Kato-Katz smear was missing, *S mansoni* prevalence and individual intensities were calculated using a single thick Kato-Katz smear although we are aware that we miss a certain proportion of infections for assessment of individuals' infection status, by this way. Anaemia was defined (according to WHO guidelines), as a dichotomous variable taking the value 1 for children from 5 to 11 years old with Hb less than 11.5 g/dl and for children between 12 and 14 years old with Hb less than 12.0 g/dl. Further details for other indicators included and methodology followed in the SCI questionnaires are given elsewhere (Brooker et al., 2004). All Kato-Katz smears were read within one hour of preparation so that hookworm eggs could be easily seen.

S. haematobium is known not to be endemic in these areas of Uganda (Stothard et al., 2006). However, for absolute confirmation, urine from all children sampled was also tested for micro-haematuria using haemastix dipsticks and all urine sampled from children from the 11-year-old cohort was pooled for concentration of schistosome eggs using a Pitchford & Visser funnel and no *S. haematobium* egg was observed. Therefore it is almost certain that *S. haematobium* was absent within this cohort. The prevalence of *Trichuris trichura* and *Ascaris lumbricoides* is unevenly distributed in the country with prevalence greatest in south-western Uganda and such infections were also examined from the faeces of all children sampled (Kabatereine et al., 2005).

Nutritional assessment data (i.e. weight and height) and blood samples for haemoglobin levels (Hb) were obtained from each individual by the finger-prick method, as this provides a sufficient sample for accurate Hb measurement using a Hemocue photometer (Parker et al., 1997).

2.2.3 Statistical analyses. Because of clustered data with a natural hierarchical structure, linear hierarchical models were chosen in order to model changes in Hb levels from baseline to follow-up and hierarchical logistic regression models in order to model the risk of being anaemic. Additionally, because schistosomiasis occurs focally, heterogeneity in the degree of endemicity among schools has to be accounted for (Van der Werf et al., 2003). In such circumstances, ordinary least squares regression can overestimate the precision associated with an analysis yielding spuriously statistically significant results (i.e. a type 1 error). Hierarchical models permit combination of exposures to group and individual factors which is important in epidemiological analyses of infectious disease data (Verbeke & Molenberghs, 2000; Mauny et al., 2004). The hierarchical logistic regression models were fitted to data coming from children with complete records at each time point of interest. Due to variable cohort recovery success, attributable to frequent population movements within Uganda, the remainder of the statistical analyses were restricted to children with complete records from both time-points of interest, and no replacements were used for missing subjects. Summary reports, figures and analyses are based on data from all available subjects. A comparison between the baseline characteristics of children successfully followed-up and those that dropped out in the second year of the study was performed through chi-square tests with regard to all the parameters examined in this study. Frequency tables were obtained using SAS V8 (SAS Institute Inc., Cary, NC, USA). A forward selection procedure was used for the evaluation of the remaining variables in the final models.

An association of anaemia with parasitic infections (schistosome and hookworm) was studied after simultaneously adjusting for potential confounders in the statistical models used. Indices of the anthropometric status of the studied children based on the 1978 CDC/WHO growth reference curves were computed using the Nutstat program within Epi Info V 3.3. Body Mass Index is considered an indicator of acute under-nutrition (thinness or wasting) and is generally associated with failure to gain weight or a loss of weight (Gorstein et al., 1994). The Z-score cut-off point recommended by WHO, CDC, and others to classify low anthropometric levels is 2 SD units

below the reference median for this specific index. Cut-off of -2 Body Mass Index Z-scores (BMIZ) were calculated to classify underweight children and finally this categorical variable was incorporated in all models as a potential predictor. Initially, the impact of parasitic infections, sex, and nutritional status as defined from Z-scores at baseline and follow-up was examined using two-level multivariate logistic regression models with level-one: the children and level-two: the schools. Three-level multivariate logistic regression models were also tested with level-three: the districts but as it was proved that the districts were rather homogeneous in the risk of being anaemic, this random effect was not finally included in the models. Nutritional status was included in the baseline hierarchical logistic regression model as generally malnutrition in children is the consequence of a range of factors that are often related to poor food quality, insufficient food intake and severe and repeated infectious diseases, or frequently some combinations of the three. The same variable (BMI Z-score at baseline) was included in the hierarchical logistic regression model referring to 1-year post-praziquantel and albendazole treatment, as in this study the duration of this period did not produce a nutritional impact. Finally, in both hierarchical logistic regression models the BMI Z-score at baseline was not significant, and therefore it was omitted from these models. Age was included in both hierarchical logistic regression models (baseline and follow-up) as a categorical variable.

Two separate hierarchical linear modeling analyses were carried out to determine any change in the children's Hb levels in relation to their schistosomiasis and/or hookworm infection intensity category, controlling at the same time for age and sex and anaemia status (as defined above). Children's Hb levels at baseline and 1-year post-treatment were modeled through three-level hierarchical models where level-one represented the two periods of interest; level-two: the children and level-three: the schools. The validity of the distributional assumptions of this model was examined using plots of level one, level two and level three residuals against their normal scores (see Figures 2.1, 2.2 and 2.3). To take into account the paired data structure, a dummy variable corresponding to the second year of study was included as a covariate in the model. The aim of this model was to quantify the adjusted overall change of Hb from baseline to follow-up and to quantify average Hb counts of different groups of children at baseline.

Changes in Hb levels in relation to their baseline schistosomiasis and/or hookworm infection intensity category and anaemia status from baseline to 1-year follow-up were modeled through two-level hierarchical models with level-one: the children and level-two: the schools. The aim of this approach was to compare the average change in Hb counts over the two examined periods between different groups of children. Baseline anaemia status was also included in the explanatory part of the model to be able to examine rises in Hb levels in anaemic and not anaemic subjects as earlier recommended (Latham et al., 1983). It was attempted to control for the fact that by adjusting for baseline anemia the effect of moderate or heavy infection for *S.mansoni* or hookworm may be underestimated if the type of anemia in the uninfected group is predominantly mild and self-limiting. Therefore, the two-way interaction terms of intensity of *S. mansoni* and of hookworm infections with anaemia status were also tested, and, as none of these were significant, they were then omitted from the model. To test the statistical significance of the fixed effects, Wald tests were used, whilst likelihood ratio tests were performed for the random effects. All of the hierarchical models presented in this study are random intercepts models with multiple independent variables and were obtained using Mlwin (Multilevel Models Project, Institute of Education, London, UK).

2.3 Results

Because of drop-outs during the second year of the study, baseline and follow-up data concerning the Hb counts were available from 2,788 children, aged 6 to 14 years old, from 36 schools out of the 37 schools initially visited at baseline, over the period 2003 and 2004 inclusive. In school Tonya in Hoima district, Hb counts were measured neither at baseline nor at follow-up so this specific school was not included in this analysis. Table 2.1 presents the health indicators of children surveyed during this period. Over the 12 months between examinations, overall prevalence significantly decreased for single *S. mansoni*, hookworm and *A. lumbricoides* infections as well as for co-infections of *S. mansoni* and hookworm and finally for anaemia. For both years of the study the prevalences of severe anaemia were negligible and therefore these cases were not examined further here. Similarly, overall point prevalences, at both time points, for *T. trichura* and *A. lumbricoides* were very low. The arithmetic mean intensities for all subjects (negatives and positives) for *S. mansoni* and hookworm infections decreased significantly 1-year post-praziquantel and albendazole treatment. A significant increase in haemoglobin concentration was also observed during the study period.

Table 2.1 Health characteristics of children in 2003 to 2004 successfully followed up for one year.

	2003	2004	p-values
Parasitology[*]			
% infected with *S. mansoni* (n = 2,619)	43.9 (42.0 - 45.8)[¶]	28.8 (27.1 - 30.5)	<0.001
% infected with hookworm (n = 2,617)	52.1 (50.2 - 54.0)	24.0 (22.4 - 25.7)	<0.001
% infected with *T. trichura* (n = 2,617)	2.3 (1.8 - 2.9)	2.3 (1.7 - 2.9)	0.917
% infected with *A. lumbricoides* (n =2,613)	2.4 (1.8 - 3.0)	1.5 (1.0 - 1.9)	0.006
% co - infected with *S. mansoni* and hookworm	23.9 (22.2 - 25.5)	8.3 (7.2 - 9.4)	<0.001
Mean *S. mansoni* intensity (epg)	239.3 (215.8 - 262.7)	75.7 (64.6 - 86.7)	<0.001
Mean hookworm intensity (epg)	307.2 (253.8 - 360.6)	79.4 (66.5 - 92.3)	<0.001
Haematology (n = 2788)			
Mean haemoglobin (g/dl)	11.4 (11.4 - 11.5)	11.7 (11.6 - 11.7)	<0.001
% anaemic	50.0 (48.1 - 51.8)	45.8 (44.0 - 47.7)	<0.001
% severely anaemic[§]	0.3 (0.1 - 0.5)	0.2 (0.0 - 0.3)	0.405

[*] Sample sizes are provided into parentheses for each examined outcome

[§] Severe anaemia: Hb<7 g/dl

[¶] (…-…) are 95% CI's

Table 2.2 shows that there were significant differences between the children that were successfully followed up and the drop-outs with reference to their age, sex, home district and schistosomiasis infection. Children of age 6 and 11 years old or above as well as children living in Arua and Mayuge proved most difficult to recruit into the cohort in the second year of the study. The largest recovery cohort failure was observed in children heavily infected with *S. mansoni* at baseline.

The results of the hierarchical multivariate logistic regression model for the probability of a child being anaemic at baseline are presented in Table 2.3. This model included intensities of *S. mansoni* and hookworm infections and the analysis controlled for age, and sex, allowing also for assessment of the extent of between-school variation in anaemia prevalence. At follow-up, there were very few children who were heavily infected with hookworm and therefore the convergence of the fitting algorithm in some of the models led to merge the heavy and moderate hookworm intensities into one category. Moreover, there were very few children that were 9 and 10 years old (only 2 children were 9 and none of these was 10 years old) within the cohort with these ages who caused problems to some of the models fits and again we decided to exclude them from all analyses. This was performed in all models presented in this study so that they can be comparable. Also for a similar reason, different intensities of co-infections of schistosomiasis and hookworm are not specifically examined here as overall there were too few co-infected children falling in different categories of intensities of the two infections in this cohort to support model fitting.

Table 2.3 shows that children moderately or heavily infected with *S. mansoni* were marginally significantly more likely to be anaemic than those uninfected with *S. mansoni*. Children moderately or heavily infected with hookworm were significantly more likely to be anaemic than those uninfected with hookworm. The school-level variance at the bottom of the same table shows that schools differed significantly in the baseline prevalence of anaemia observed. Whether the effects of the intensities of schistosomiasis and hookworm infections varied by age, sex or state of weight were also tested. For this reason the relevant two-way interaction terms

were incorporated in the model and as none of these were significant, these estimates are not presented here.

Table 2.2 Comparison of children successfully followed up and drop outs

Variable	Categories	Drop outs	Followed up	p-values[f]
Intensity of S. mansoni infection	Uninfected	786 (50.3%)	1512 (54.2%)[*]	0.034
	Lightly Infected[a]	245 (15.7%)	426 (15.3%)	
	Moderately Infected[b]	171 (10.9%)	314 (11.3%)	
	Heavily Infected[c]	295 (18.9%)	440 (15.8%)	
	Missing	66 (4.2%)	96 (3.4%)	
Intensity of hookworm infection	Uninfected	736 (47.1%)	1289 (46.2%)	0.527
	Lightly Infected[d]	724 (46.3%)	1330 (47.7%)	
	Moderately/Heavily Infected[e]	37 (2.4%)	72 (2.6%)	
	Missing	66 (4.2%)	97 (3.5%)	
Age	>=11 years old	422 (27.0%)	707 (25.4%)	<0.001
	8 years old	360 (23.0%)	739 (26.5%)	
	7 years old	352 (22.5%)	683 (24.5%)	
	6 years old	401 (25.7%)	654 (23.5%)	
	Missing	28 (1.8%)	5 (0.2%)	
Sex	Male	723 (46.3%)	1406 (50.4%)	<0.001
	Female	702 (44.9%)	1380 (49.5%)	
	Missing	138 (8.8%)	2 (0.1%)	
Anaemia	Not Anaemic	754 (48.2%)	1395 (50.0%)	0.260
	Anaemic	809 (51.8%)	1393 (50.0%)	
	Missing	-	-	
District	Nebbi	148 (9.5%)	414 (14.9%)	<0.001
	Arua	74 (4.7%)	44 (1.6%)	
	Bugiri	218 (14.0%)	380 (13.6%)	
	Busia	188 (12.0%)	440 (15.8%)	
	Hoima	233 (14.9%)	322 (11.6%)	
	Masindi	212 (13.6%)	366 (13.1%)	
	Mayuge	261 (16.7%)	332 (11.9%)	
	Moyo	229 (14.7%)	490 (17.6%)	
	Missing	-	-	

[a]lightly infected with *S. mansoni:*1--99 epg, [b]moderately infected with *S. mansoni*: 100--399 epg, [c] heavily infected with *S. mansoni*: >=400 epg,
[d]lightly infected with hookworm: 1--1999 epg, [e]moderately/heavily infected with hookworm: >=2000 epg,
[f]p-value for chi-square test, [*]column percentages are given in the parentheses.

Table 2.3 Adjusted odds ratios from hierarchical multivariate logistic regression model for baseline prevalence of anaemia (n = 2,682)

Fixed effects			
Variable	**Categories**	**Adjusted odds ratio (95% CI)**	**p-values**
Intensity of S. mansoni infection at baseline	Uninfected	1	
	Lightly Infected[a]	0.958 (0.753 - 1.219)	0.727
	Moderately Infected[b]	1.274 (0.962 - 1.686)	0.091
	Heavily Infected[c]	1.309 (0.993 - 1.725)	0.056
Intensity of hookworm infection at baseline	Uninfected	1	
	Lightly Infected[d]	1.142 (0.965 - 1.352)	0.123
	Moderately/Heavily Infected[e]	2.219 (1.312 - 3.752)	0.003
Age at baseline	>=11 years old	1	
	8 years old	1.464 (1.175 - 1.823)	<0.001
	7 years old	1.824 (1.453 - 2.290)	<0.001
	6 years old	2.212 (1.755 - 2.788)	<0.001
Sex	Male	1	
	Female	0.908 (0.774 - 1.064)	0.230
Random effects	σ^2	S.E.	
School-level variance	0.291	0.083	

[a]lightly infected with *S. mansoni* at baseline:1--99 epg, [b]moderately infected with *S. mansoni* at baseline: 100--399 epg,
[c] heavily infected with *S. mansoni* at baseline: >=400 epg,
[d]lightly infected with hookworm at baseline: 1--1999 epg, [e]moderately/heavily infected with hookworm: at baseline >=2000 epg

Results from the hierarchical logistic regression model for the probability of a child being anaemic at 1-year follow-up are presented in Table 2.4. Children heavily infected with *S. mansoni* at follow-up were significantly more likely than uninfected children to be anaemic. Age, gender and moderate or heavy hookworm infections were also significant predictors. Finally, schools varied in the prevalence of anaemia observed at follow-up.

Table 2.4 Adjusted odds ratios from hierarchical multivariate logistic regression model for follow-up prevalence of anaemia (n = 2,696)

Fixed effects			
Variable	**Categories**	**Adjusted odds ratio (95% CI)**	**p-values**
Intensity of schistosomiasis infection at follow-up	Uninfected	1	
	Lightly Infected[a]	1.158 (0.905 - 1.483)	0.244
	Moderately Infected[b]	1.203 (0.866 - 1.672)	0.272
	Heavily Infected[c]	1.567 (1.038 - 2.365)	0.032
Intensity of hookworm infection at follow-up	Uninfected	1	
	Lightly Infected[d]	1.037 (0.842 - 1.276)	0.732
	Moderately/Heavily Infected[e]	4.289 (1.468 - 12.530)	0.008
Age at follow-up	>=12 years old	1	
	9 years old	0.697 (0.560 - 0.868)	0.001
	8 years old	0.760 (0.607 - 0.950)	0.015
	7 years old	1.184 (0.941 - 1.489)	0.147
Sex	Male	1	
	Female	0.837 (0.713 - 0.983)	0.029
Random effects	σ^2	S.E.	
School-level variance	0.367	0.100	

[a]lightly infected with *S. mansoni* at follow-up*:*1--99 epg, [b]moderately infected with *S. mansoni* at follow- up: 100--399 epg, [c] heavily infected with *S. mansoni* at follow-up: >=400 epg,
[d]lightly infected with hookworm at follow-up: 1--1999 epg, [e]moderately/heavily infected with hookworm at follow-up: >=2000 epg

Table 2.5 contains the estimates of the three-level hierarchical model for Hb counts before and after praziquantel and albendazole treatment. Normal plots of the different levels-residuals appeared fairly linear (see Figures 2.1, 2.2 and 2.3), which suggested that the assumption of normality and the selection of a linear hierarchical model were reasonable. An overall increase of 0.061 g/dl in the Hb level after chemotherapeutic treatment was not significant (p=0.115). Children heavily infected with *S. mansoni* had significantly lower Hb counts (0.322 g/dl), (p<0.001) compared to uninfected children at baseline. Children with moderate or heavy hookworm infection at baseline had significantly lower Hb counts (0.595 g/dl), (p<0.001) than those not infected after controlling for *S. mansoni* intensity, age, sex and anaemia status. The random effects variance components indicate that much of the variation is between children within a school, but there was also statistically significant variation between schools. The total variance is 1.758, the sum of the three variance components [$Var(v_{0k})$, $Var(u_{0jk})$ and $Var(\varepsilon_{0ijk})$]. Of the total variability in Hb counts, 0.122/1.758=6.94% was situated at the school level while 0.551/1.758=31.34% arose between children within a school.

Table 2.5 Estimates from three-level hierarchical model for haemoglobin counts before and after chemotherapeutic treatment (n = 2,682)

Fixed effects	**Parameter**	**Coefficient (S.E.)**	**p-values**
Intercept	γ_{00}	11.854 (0.083)	<0.001
Baseline Intensity of schistosomiasis infection (Reference category' Uninfected')			
Lightly Infected*	γ_{01}	-0.073 (0.055)	0.183
Moderately Infected	γ_{02}	-0.153 (0.068)	0.024
Heavily Infected	γ_{03}	-0.322 (0.072)	<0.001
Baseline Intensity of hookworm infection (Reference category' Uninfected')			
Lightly Infected	γ_{04}	-0.046 (0.041)	0.262
Moderately/Heavily	γ_{05}	-0.595 (0.139)	<0.001
Gender (Reference category Male)			
Female	γ_{06}	0.097 (0.042)	0.115
Baseline Age (Reference category >=11 years old)			
8 years old	γ_{07}	-0.319 (0.052)	<0.001
7 years old	γ_{08}	-0.509 (0.053)	<0.001
6 years old	γ_{09}	-0.615 (0.069)	<0.001
Effect of follow-up (relative to baseline) -Linear Coefficient of time	γ_{10}	0.061 (0.039)	0.115
Random effects			
Level-3 (i.e. between schools) variance			
$\sigma_v^2 = Var(v_{0k})$		0.122 (0.032)	
Level-2 (i.e. between children within a school) variance			
$\sigma_u^2 = Var(u_{0jk})$		0.551 (0.038)	
Level-1 (i.e. measurement occasions within a child) variance			
$\sigma_\varepsilon^2 = Var(\varepsilon_{0ijk})$		1.085 (0.034)	
Deviance		**15398.82**	

*Categories of intensities of infections are identical as for those in previous tables

Figure 2.1 Normal plot of level-one residuals of hierarchical model in Table 2.5

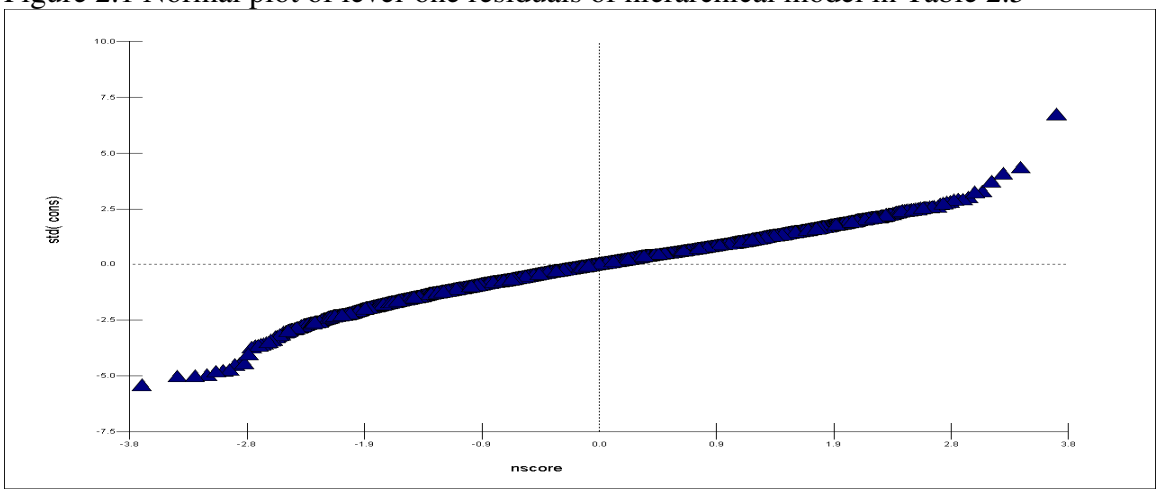


Figure 2.2 Normal plot of level-two residuals of hierarchical model in Table 2.5

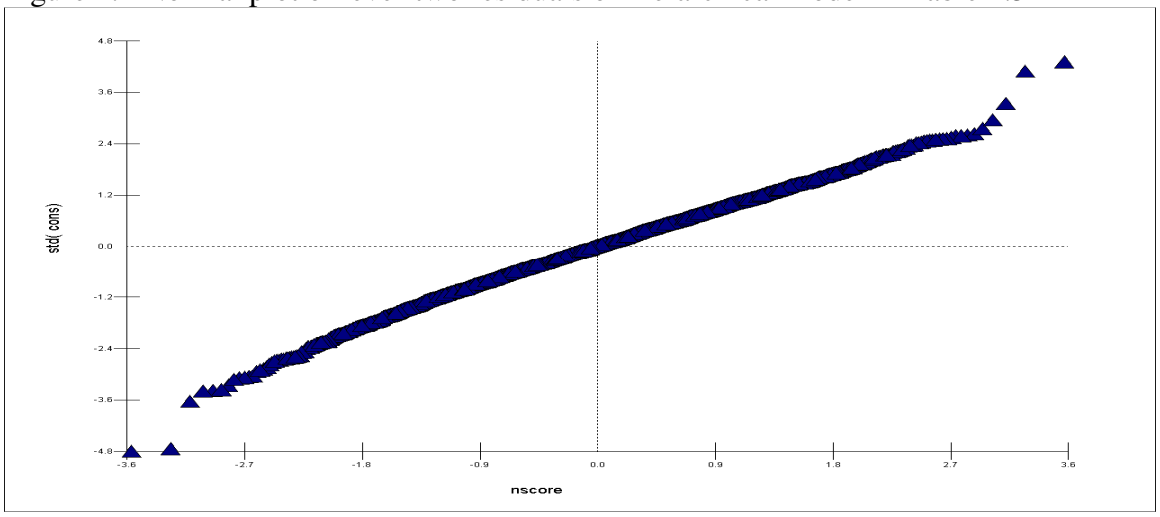


Figure 2.3 Normal plot of level-three residuals of hierarchical model in Table 2.5

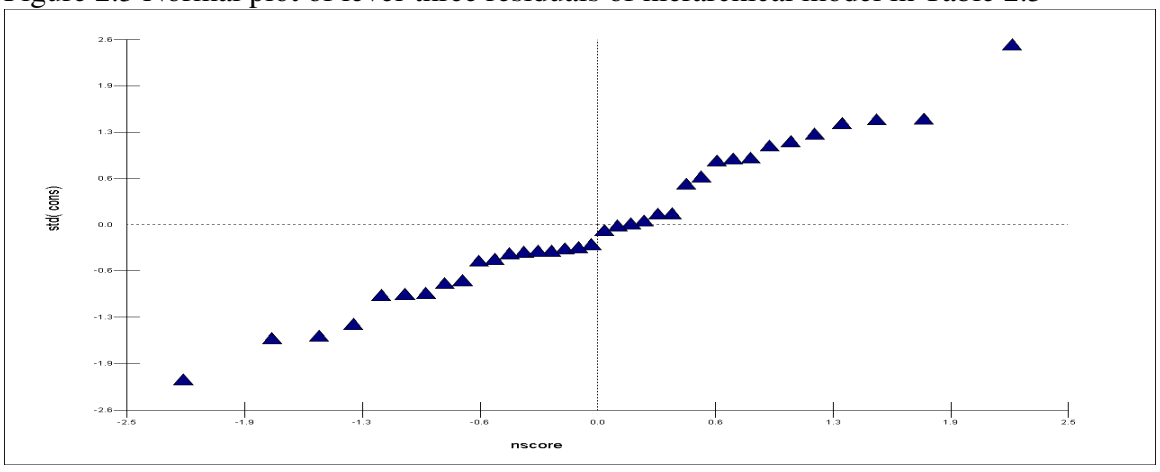
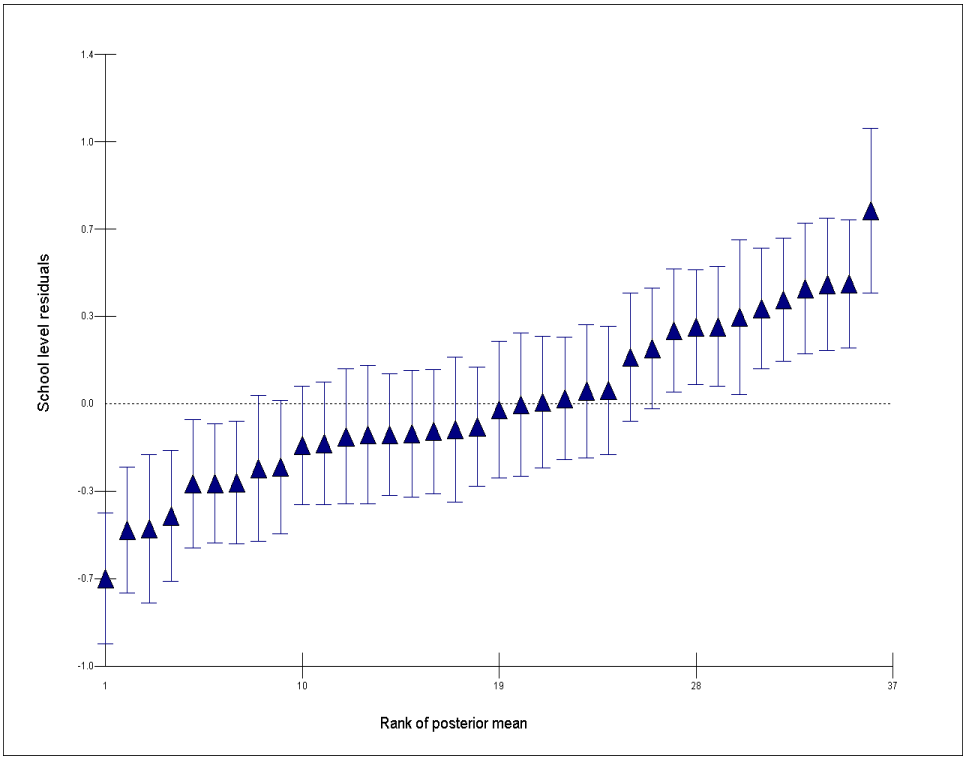


Figure 2.4 shows 36 level-three residuals plotted one for each school with the schools ordered according to the rank of their posterior mean. It indicates that most of them have approximately average Hb counts that cannot be distinguished statistically. The school with the lowest intercept residual (at the bottom left) and mean Hb count was Kibiro school in Hoima district. At baseline the *S. mansoni* and hookworm point prevalences in this school were 94.87% and 10.26%, while at follow-up these decreased to 87.18% and 2.56%, respectively. The school with the highest intercept and mean Hb count was Arua Public in the Arua district. The raw data for this school showed that *S. mansoni* and hookworm prevalence at baseline were 22.86% and 25.71% and decreased to 14.29% and 2.86% respectively at follow-up.

Figure 2.4 Caterpillar plot: 36 level-three residuals from model presented in Table 2.5
(Bars represent 95% CIs and the triangles level-three residuals)



Finally, Table 2.6 contains the estimates of the two-level hierarchical model for the change of Hb counts between the two years of the study. It indicates that most risk groups had a relatively

slowed reduction or actual increase in Hb counts after praziquantel and albendazole chemotherapy compared to the reference group (baseline uninfected, non-anaemic, >=11-year-old). More precisely, children heavily infected with *S. mansoni* at baseline had a smaller decrease by 0.229 g/dl in their Hb counts in comparison with the uninfected with *S. mansoni*. Among children found to be anaemic at the baseline survey, Hb counts increased significantly by 0.834 (i.e. 1.240 – 0.406) g/dl after treatment.

Table 2.6 Estimates from two-level hierarchical model for the change of haemoglobin counts (n = 2,682)

Fixed effects	**Parameter**	**Coefficient (S.E.)**	**p-values**
Intercept	γ_{00}	-0.406 (0.103)	<0.001
Baseline intensity of schistosomiasis infection (Reference category' Uninfected')			
Lightly Infected*	γ_{01}	-0.061 (0.076)	0.428
Moderately Infected	γ_{02}	-0.004 (0.090)	0.963
Heavily Infected	γ_{03}	0.229 (0.089)	0.010
Baseline intensity of hookworm infection (Reference category' Uninfected')			
Lightly Infected	γ_{04}	0.021 (0.054)	0.694
Moderately/Heavily Infected	γ_{05}	0.274 (0.160)	0.087
Gender (Reference category Male)			
Female	γ_{06}	0.030 (0.050)	0.552
Baseline Age (Reference category >=11 years old)			
8 years old	γ_{07}	0.015 (0.070)	0.824
7 years old	γ_{08}	0.028 (0.071)	0.694
6 years old	γ_{09}	-0.161 (0.073)	0.028
Baseline anaemia(Reference category non anaemic children)			
Anaemic	γ_{10}	1.240 (0.052)	<0.001
Random effects		***Variance components***	
Level-2 (i.e. between schools) variance			
$\sigma_u^2 = Var(u_{0jk})$		0.180 (0.048)	
Level-1 (i.e. between children within a school) variance			
$\sigma_\varepsilon^2 = Var(\varepsilon_{0ijk})$		1.660 (0.046)	
Deviance		**9076.5**	

*Categories of intensities of infections are identical as for those in previous tables

2.4 Discussion

Friedman and others recommended that future research aimed to quantify a relationship between schistosomiasis and anaemia should include an adequate follow-up period and include individuals with a range of infection intensities (Friedman et al., 2005). To my knowledge, this study represents the first longitudinal examination into the occurrence of anaemia in a cohort of children infected with *S. mansoni* and hookworm in Uganda using a uniquely detailed large dataset arising from the National Control Schistosomiasis Programme within the country. The aim of this chapter was to examine the relationship of *S. mansoni* and hookworm infection to anaemia and Hb levels while the effectiveness of practical interventions to control or reduce the severity of such anaemia was also evaluated.

Helminth infections at a young age may induce, amongst other factors, pro-inflammatory mediators that are detrimental to protein metabolism, appetite and erythropoiesis and the WHO therefore recommends the use of anti-schistosome treatment (Crawley, 2004). In Uganda, it has been proven that drug distribution in schools is excellent and community-directed treatment is a feasible health approach for mass drug distribution in poor remote communities (Kabatereine et al., 2006). This study provides further convincing evidence to show that young children may benefit from deworming in terms of increased Hb and consequently reduced anaemia levels. In particular, whilst these data clearly demonstrate that, while there was association between anaemia and both schistosomiasis and hookworm infection at baseline, by the follow-up data, the probability of anaemia was only associated with heavy *S. mansoni* and moderate or heavy hookworm infections (Tables 2.3 & 2.4).

However, whereas the first hierarchical model of Hb counts (Table 2.5) showed that treatment arrests the drop in Hb, it suggests that without iron replacement, Hb levels probably will not rise. Additionally, the second hierarchical model (Table 2.6) revealed a drop in Hb counts in the comparison group of baseline uninfected, non-anaemic, >=11-year-old children. One could speculate that this could reflect, for example an increased malaria infection rates at follow-up and/or a bad harvest during the second year of this study perhaps explained from the fact that there were more than 1.6 million internally displaced persons in Uganda's north and east where malnutrition is one of the most pressing health concerns as a result of fighting between

government forces and rebels from the Lord's Resistance Army (Nullis-Kapp, 2004). It is likely that inadequate dietary iron was another factor contributing to this observed decline. These results confirm the findings of Taylor et al (2001) in South Africa where through a randomized controlled trial provided two different anthelminthic treatment regimens twice at 6-monthly intervals combined with iron supplementation for one year in groups of 428 primary-school pupils (Taylor et al., 2001). These authors observed a significant decrease in Hb levels of pupils receiving triple dose of albendazole and praziquantel only and a significant increase in Hb levels of pupils receiving three doses of these drugs and iron supplementation. Such combined results thereby strengthen the argument of Stoltzfus et al (1998) that deworming programs should be combined with increased iron intake through supplementation, fortification or improved diet in order to reduce the incidence of anaemia substantially (Stoltzfus et al., 1998).

Moreover, data from Beasley et al (1999) in rural Tanga, Tanzania and Friis et al., 2003 in western Kenya suggest that children most benefited from anthelminthic treatment in terms of increased Hb levels were those who were anaemic at baseline. From the second hierarchical model of Hb counts presented in the study of this chapter (Table 2.6) a significant increase in the change of Hb counts and a stronger effect for anaemic children compared to non-anaemic was also found. This same model also showed a slower reduction in Hb counts for heavily infected children with *S. mansoni* and moderately or heavily infected children with hookworm compared to uninfected after treatment, which suggests that the effect of anthelminthic treatment on Hb was mediated by reductions in intensities of *S. mansoni* and hookworm infections.

Ethical reasons as well as the operational reality of the national control programme did not permit the inclusion of a control (i.e. untreated) group. Consequently, the study design did not allow estimation of the absolute impact of treatment only the relative impact in different groups. Therefore, in order to provide substantial support for the plausibility of the impact of the intervention, the epidemiological findings from the models presented in this study have now been further validated with quantitative predictions arising from mathematical models with very promising results (Kabatereine et al., 2007). The high percentage of drop outs in the group of heavily infected children with *S. mansoni* (Table 2.1) due to frequent population movements within Uganda, might also add some bias to the epidemiological findings of this study.

Moreover, although malaria is a well known cause of anaemia and *Plasmodium falciparum* is almost holoendemic in the examined population here, this factor as well as dietary iron intake were not examined, but it is highly recommended that this information to be included in future evaluation follow-ups (Friedman et al., 2005). Indeed the variance components of the linear hierarchical models (Tables 2.5 & 2.6) have shown that there remains substantial variability which is not explained by the models, and this might well be due to malaria and iron deficiency not being taken into account here. In addition to these, variables referring to mosquito control at the school/village level were not available, and future studies may benefit from their incorporation. The hierarchical model did, however, show that Kibiro school in Hoima district, with significantly lower mean Hb count compared to the overall mean Hb count, had also very high *S. mansoni* prevalence, possibly due to its proximity to Lake Albert, the major source of transmission. On the other hand, the school with significantly higher mean Hb count compared to the overall mean Hb count was situated in the northern part of the country, where *S. mansoni* and hookworm prevalences were moderately low. These results agree with previous work on the distribution of *S. mansoni* in Uganda, which indicates that highest schistosomiasis prevalences are found close to the eastern shores of Lakes Albert and Victoria while areas of low or zero prevalence are found in the northeast of the country (Kabatereine et al., 2004).

To conclude, the results of the study presented in this chapter, which derive from a large-scale national control programme, suggest that *S. mansoni* and hookworm infections may be related to anaemia in specific districts of Uganda and chemotherapy with praziquantel and albendazole may reduce anaemia. Anaemia is therefore likely to represent a valuable marker for morbidity caused by heavy infection with *S. mansoni*, provided that other likely causes, hookworm, dietary iron intake and malaria are taken into consideration.

Chapter 3: Relationships between *Schistosoma mansoni, Plasmodium falciparum* infections and anaemia in Kenyan schoolchildren: a Bayesian hierarchical modeling approach

Summary

Anaemia is multi-factorial in origin and disentangling its aetiology remains problematic, as has been demonstrated in Chapter 2 of this PhD thesis. In the current Chapter, I report cross-sectional data on *Schistosoma mansoni* infection, haemoglobin (Hb), malaria parasitaemia, hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura* infections, as well as undernutrition, among 1,523 schoolchildren enrolled in classes 5 and 6 (aged 10 to 21 years) in 30 primary schools in western Kenya. Bayesian hierarchical modelling was used to investigate putative relationships. Children with a heavy *S. mansoni* or with *Plasmodium falciparum* infection, stunted children and girls were found to have lower haemoglobin concentrations. Children heavily infected with *S. mansoni* were also more likely to be anaemic compared with uninfected children. The findings of this study in combination with the findings of Chapter 2, further highlight the importance of intestinal schistosomiasis and/or malaria as contributors to reduced Hb levels among schoolchildren and help guide the implementation of integrated school health programmes in areas of differing parasite transmission.

Keywords: Bayesian hierarchical models, anaemia, Kenya, *Schistosoma mansoni*, hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, malaria

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3.1 Introduction

As discussed in Chapter 2, anaemia remains one of the most intractable public health problems in Africa, contributing to a quarter of Africa's nutrition-related Disability Adjusted Life Years (DALYs) lost (World Health Organization, 2002). Several studies have highlighted the contribution of parasitic diseases to childhood anaemia. Recent meta-analyses of malaria intervention trials among African children, for example, provide compelling evidence that both symptomatic and asymptomatic malaria contributes to anaemia (Geerligs et al., 2003; Korenromp et al., 2004). The effect of hookworm infection is also well documented, with risk of anaemia correlated with intensity of infection (Stephenson, 1993; Olsen et al., 1998; Brooker et al., 1999); in contrast, the contributory role of schistosomiasis remains unclear (Desai et al., 2005b; Friedman et al., 2005a) as also discussed in Chapter 2. However, there are surprisingly few published studies describing the relative contribution of all the different parasitic infections mentioned above in populations of school-aged children (Olsen et al., 1998; Tatala et al., 1998; Friis et al., 2003; Leenstra et al., 2003; Leenstra et al., 2004; Desai et al., 2005a; Friedman et al., 2005b). Investigation of this issue is particularly relevant for anthelminthic treatment programmes through school health programmes. In particular, the same suite of school-based interventions will not be relevant everywhere, and the selection of intervention options will need to be guided by an informed understanding of the epidemiology of parasite-related anaemia (Crawley, 2004), as well as of the geography of infection (Brooker et al., 2007).

The aim of the present study was to examine the relationships of haemoglobin (Hb) concentration and anaemia with *S. mansoni* infection using a similar statistical methodological approach as the one employed in Chapter 2 (i.e. hierarchical modeling) controlling at the same time for hookworm, *Ascaris lumbricoides*, *Trichuris trichiura* and malaria infections in school children in western Kenya but within a Bayesian framework. Adjustment for nutritional and socioeconomic status (SES) has also been taken into account here as they might influence anaemia risk (Ong'echa et al., 2006). By employing a Bayesian approach for the statistical modeling of the Hb counts and of anaemia prevalence, the model specification via Markov chain Monte Carlo (MCMC) algorithms offers flexibility in fitting complex models and enables

estimates for the whole distribution of the unknown parameters, including point and interval estimates, to be derived.

3.2 Materials and methods

3.2.1 Study sites, sampling and design. Data were collected between February and March 2005 in 30 primary schools in Bondo district in western Kenya. Previous studies in western Kenya have reported a medium prevalence of *S. mansoni* and *A. lumbricoides* infections (Brooker et al., 2000; Thiong'o et al., 2001) and a high prevalence of hookworm and *T. trichiura* infections. Malaria transmission is intense and perennial (Beier et al., 1994), with two seasonal peaks, March-May and November-December, following the long and short rainy seasons respectively.

This study used cross-sectional, baseline data from a stratified, cluster-randomised placebo-controlled trial of the impact of antimalarial intermittent preventive treatment (IPT) among schoolchildren in order to compare and validate results reported in Chapter 2. The trial design and protocol of the current study are described elsewhere (Clarke et al., 2008). Briefly, sample size was estimated on the basis of the expected impact of IPT on anaemia, using the methods for cluster-randomised trial design proposed by Hayes and Bennett (1999). The 30 study schools were randomly selected from primary schools in Usigu and Maranda Divisions with ≥ 150 pupils in total and > 15 pupils per class and located more than 5 km from the shores of Lake Victoria, so as to minimise the effect of *S. mansoni* which is generally only prevalent along the shoreline (Brooker et al., 2001; Handzel et al., 2003). No stratification by intestinal nematode was undertaken because of their relatively homogeneous distribution (Handzel et al., 2003) but schools were stratified according to past school examination performance. This study presents data from the baseline survey on a sub-sample of children enrolled in classes 5 and 6 (age range 10 to 21 years) for whom complete data on anaemia, helminth infection, malaria parasitaemia, nutritional status and SES were available.

Ethical clearance for the current study was obtained from the ethics committee of the Kenyatta National Hospital, Kenya and from the London School of Hygiene and Tropical Medicine, UK. Permissions were obtained from the Ministry of Education, and the district education and health authorities, and headteachers. Prior to the start of the study, a series of meetings were held in participating schools to explain the nature and purpose of the trial and to obtain individual informed parental consent from the parents or legal guardians of children enrolled in study schools.

3.2.2 Parasitological and anaemia morbidity data.

A slide was declared negative after examination of 100 high-powered fields. Stool samples provided by each child were examined microscopically using the semi-quantitative Kato-Katz technique and intensity of infection was expressed as eggs/gram of faeces. Malaria parasite prevalence and parasite densities were estimated in Giemsa-stained thick blood films, assuming an average white blood cell count of 8,000 per µl, with species identification carried out on Giemsa-stained thin films. Finger-prick blood samples were obtained from all children to assess Hb levels and malaria parasitaemia. Haemoglobin was measured in the field using a portable photometer (Haemocue, Angelholm, Sweden).

Height was measured to the nearest 0.1 cm using a Leicester portable fixed base stadiometer (Chasmors, UK) and weight was measured to the nearest 0.1 kg using an electronic balance. A simple questionnaire was administered to pupils to obtain data on key socio-economic variables including: structure of the house, type of overall light, ownership of bicycle, use of bednet as well as education of the child's guardian.

3.2.3 Statistical analyses. An index of SES was constructed from asset and education variables using principal component analysis (Filmer & Pritchett, 2001). Data were available for 1453 (92%) of the 1577 children in 30 schools. Analysis was done using the PROC PRINCOMP command in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). For the index of SES, the first principal component explained 28% of the variance in the asset and education variables with the greatest weight given to the presence of a permanent house structure (0.43), and the lowest weight to the presence of a traditional house structure (-0.39), respectively. Weights for each

variable were derived from the first principal component and applied to each child to derive a SES index (Table 3.1). Children were then assigned to a group on the basis of their value on the index. Following the approach of Filmer and Pritchett (2001), children were classified into a 0-39 percentile, 40-79 percentile and upper 20 percentile, which are referred to as 'most poor', 'poor' and 'least poor', respectively.

Table 3.1 Scoring Factors and summary statistics for variables entering the computation of the first principal component

Asset & education variables	Scoring Factors	Mean	SD
Own metal roof	0.386	0.666	0.472
Own cement floor	0.423	0.283	0.451
Traditional house structure	-0.389	0.328	0.470
Semi-permanent house structure	-0.015	0.396	0.489
Permanent house structure	0.425	0.277	0.448
Original light of house/Electric- Generator	0.133	0.021	0.142
Original light of house/Pressure	0.045	0.019	0.138
Original light of house/Hurricane	0.297	0.385	0.487
Original light of house/Tin	-0.343	0.575	0.495
Highest education of who you live with/Primary	-0.200	0.418	0.493
Highest education of who you live with/Secondary	0.135	0.302	0.459
Highest education of who you live with/Post-secondary	0.123	0.038	0.191
Highest education of who you live with/Schooled	0.046	0.170	0.376
Own bicycle	0.157	0.788	0.409
Own bednet	0.120	0.095	0.293

Each variable takes the value 1 if true, 0 otherwise. Scoring factor is the 'weight' assigned to each variable in the linear combination of the variables that constitute the first principal component. The first eigenvalue is 4.76; the second eigenvalue is 2.72; the proportion of variance explained is each eigenvalue divided by the sum of the eigenvalues.; based on the obtained results and a screeplot, it was decided to retain one principal component. SD stands for standard deviation.

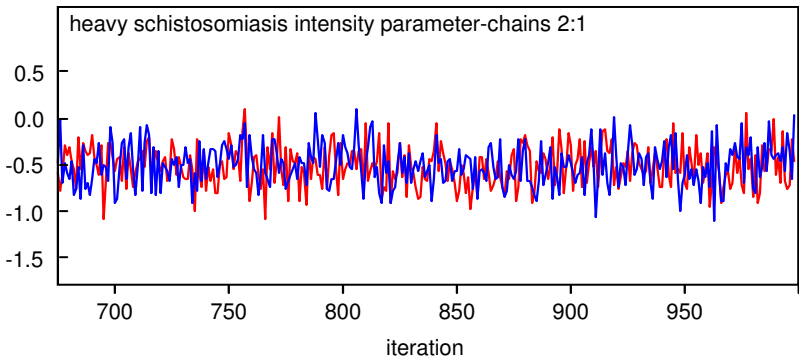
Anthropometric indices were calculated on the basis of the 2000 Centres for Disease Control and Prevention (CDC) Growth Charts, and analysed as binary variables. Children were classified as stunted if z-scores of height-for-age were less than 2 SD below the CDC median. Body mass index, which is weight (kg)/height $(cm)^2$, was also calculated and a cut-off of -2 Body Mass Index Z-scores (BMIZ) were calculated to classify underweight children. Data management and bivariate relationships between mean Hb concentration and key predictors examined were obtained using SAS V 9.1 (SAS Institute Inc., Cary, NC, USA).

Bayesian hierarchical modelling was used to assess associations between anaemia, Hb and parasitic infection, including children and schools as random effects drawn from some common

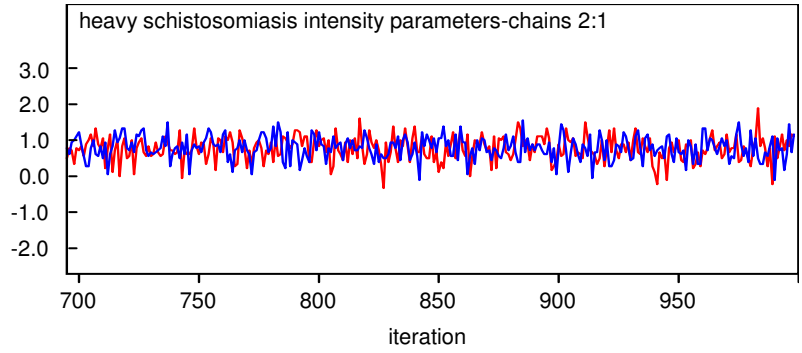
prior distribution with unknown parameters. Children within each school as well as the results from different schools were treated as 'exchangeable' in the sense that their joint probability densities should be invariant to permutations of the indexes (Gelman et al., 2004).

In Bayesian analysis, the proposed model of the observed data is combined with the prior distribution of all the unknown model parameters to give the posterior distributions for all unknowns. MCMC methods (Gilks et al., 1996) are used to sample from the posterior distributions of the unknown parameters. Analysis was conducted using WinBUGS which employs the Gibbs sampler to form the posterior distribution for each unknown parameter by drawing samples from their full conditional distributions (Spiegelhalter et al., 2003) to fit models. An advantage of the Bayesian hierarchical approach is that prior information can be incorporated in the model in probabilistic form. However, in the absence of any prior knowledge about the model parameters, the choice of non-informative improper priors is dictated by pragmatic conditions (Diggle et al., 2002). Model convergence was evaluated on the basis of inspection of sample traces, which all showed a reasonable degree of convergence to a stationary distribution. Figure 3.1 illustrates two examples of such trace plots for the parameters of heavy intensity of *S. mansoni* infection: a) in the linear hierarchical Bayesian model for the Hb counts (see Figure 3.1a) and b) in the logistic hierarchical Bayesian model for the anaemia risk (see Figure 3.1b). Trace plots for the rest of the estimated parameters in both Bayesian hierarchical models also, eventually reached stationary distributions.

Figure 3.1 Trace plots for the parameters of heavy intensity of *S.mansoni* infection in final Bayesian hierarchical models



a. Trace plot (or history plot) of the parameter for heavy intensity of *S.mansoni* infection from linear hierarchical Bayesian model presented in Table 3.2. It is apparent from this plot that the processes from both chains (blue and red lines) look stationary which strengthens our belief that the MCMC simulations of the assumed model have converged.



b. Trace plot (or history plot) of the parameter for heavy intensity of *S.mansoni* infection from logistic hierarchical Bayesian model presented in Table 3.3. It is apparent from this plot that the processes from both chains (blue and red lines) look stationary which strengthens our belief that the MCMC simulations of the assumed model have converged.

Bayesian normal hierarchical models were fitted on Hb because there is a two-level data structure applied to cross-sectional data. Individual subjects were classified at the lower level for older children with data on helminth infection and age range 10-21 years old was classified by cluster at the higher level. It was assumed that individual i (=1,…,n) - where n = 1,523 can belong in any of j (=1,…,$n2$) - where $n2$ = 30 schools. Hb at a child level may be affected by those childrens' characteristics (age, sex, intensities of helminth infections, nutritional and socio-economic status) but may also vary according to which school these children are enrolled in. Specifically for Hb, a random intercepts normal model was used which can be written as:

$$Hb_{ij} \sim normal(\mu_{ij}, \tau),$$

$$\text{with } \mu_{ij} = X_{ij}\beta + u_j \text{ and } \tau \sim gamma(0.001, 0.001),$$

$$u_j \sim normal(0, \tau_{u_j}) \text{ and } \tau_{u_j} \sim gamma(0.001, 0.001).$$

where μ_{ij} is given by the sum of the product of X_{ij} with β - this constitutes the fixed part of the model, and u_{j-} constitutes the random part of the model. More precisely, *Xij* is a vector of individual-level characteristics, β is a vector of k estimated parameter coefficients and u_j is the error term at the school-level which represents each school's difference from the overall population mean as its mean is set to 0. τ represents the *precision* (1/variance) of the normal distribution of the response Hb whereas τ_{uj} represents the *precision* (1/variance) of the normal distribution of the u_j's. For both variance components, the usual practice of specifying a gamma prior distribution to the corresponding precision parameters which is proper and close to being uniform on log (τ), has been followed.

For the vector of the k estimated parameter coefficients it was assumed:

$$p(\beta_k) \propto 1$$

Where $p(\beta_k)$ symbolizes the prior distribution of the k estimated parameters and is proportional to 1.

In order to examine the prevalence of anaemia with associated covariate vectors, the single outcome of the probability that a child is anaemic was chosen, as estimated by a hierarchical Bayesian logistic regression model. The survey responses $anaemia_{ij}$ were labeled as 1 for children i being anaemic (if Hb < 11.0 g/dL) in school j and 0 otherwise; they were modeled independently with $\Pr(\text{anaemia}_{ij}=1)=\text{logit}^{-1}((X_{ij})\beta)$. The analysis presented is based on a prior distribution for β that is independent and locally uniform in the k parameters; that is $p(\beta_1, \ldots, \beta_k) \propto 1$. Specifically for the risk of being anaemic, a random intercepts logit link model was used which can be written as:

$$anaemia_{ij} \sim Bernoulli(p_{ij}),$$

$$\text{with } logit(p_{ij}) = X_{ij}\beta + u_j \text{ and } \tau \sim gamma(0.001,0.001),$$

$$u_j \sim normal(0, \tau_{u_j}) \text{ and } \tau_{u_j} \sim gamma(0.001,0.001)$$

where p_{ij} is the probability of being anaemic. The rest of the notation remains identical to the normal hierarchical Bayesian models which were employed for the analysis of the Hb levels. In these aforementioned regression models, the same predictors of anaemia prevalence as those in the models of Hb counts were used.

To compare model complexities and goodness of fit, the deviance information criterion (DIC) of (Spiegelhalter et al., 2002) was also monitored.

3.3 Results

Data on Hb, helminth infection and malaria parasitaemia were available for 1,523 children, aged 10 to 21 years old in the 30 schools (Table 3.2). Overall 13.5%, (95% confidence interval (CI): 11.8-15.2) of these children were anaemic at the time of survey and the mean Hb concentration was estimated to be 12.43 g/dL (95% CI: 12.35-12.50).

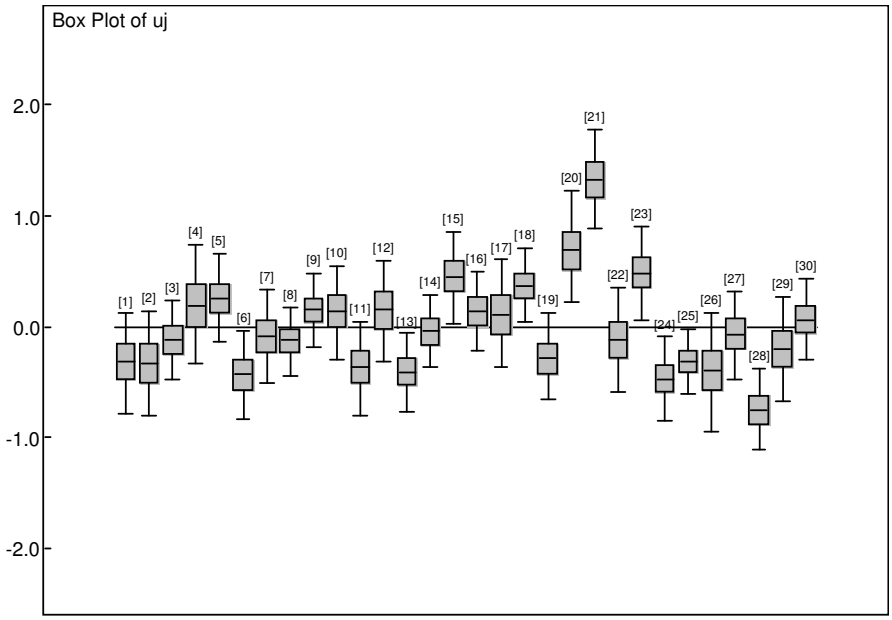
76.9% of children were infected with at least one parasitic helminth infection. Hookworm was the most prevalent helminth infection (47.3%); 14.1% were infected with *S. mansoni,* 23.7% with *A. lumbricoides* and 12.9% with *T. trichiura.* A total of 34.7% of children were infected with *Plasmodium falciparum* with a further 0.3% having mixed infections with *P. falciparum* and *Plasmodium malariae*. The prevalences of *P. falciparum,* hookworm and *S. mansoni* were, respectively, 35.8%, 41.2% and 12.4% in the 10-12 years old age group, 34.4%, 51.2% and 14.7% in the 13-15 years old age group and 25.9%, 46.3% and 20.4% in the older age group (i.e. >= 16 years old).

3.3.1. Bayesian hierarchical normal model of haemoglobin. Table 3.3 contains the posterior means and 95 % credible intervals of the final two-level Bayesian normal

hierarchical model for the Hb of children with complete data on all covariates. According to this model, the posterior mean for the overall mean Hb was estimated to be 12.52 g/dL (95% Bayesian credible interval (BCI): 12.28 to 12.76). On average, girls had lower mean Hb compared with boys by 0.18 g/dL. Older children tended to have higher mean Hb than children aged 10-12 years. Stunted children compared with non-stunted children had lower mean Hb by 0.34 g/dL. From the 95 % BCIs of all the parasitic infections, only children heavily infected with *S. mansoni* had significantly lower mean Hb by an average of 0.51 g/dL, (95% BCI: -0.94 to -0.10) and children infected with malaria had significantly lower mean Hb by an average of 0.16 g/dL, (95% BCI: -0.32 to -0.01) compared with uninfected children, respectively. Although there was no evidence of a significant effect of the intensities of single hookworm, *T. trichiura* or *A. lumbricoides* infection, adjustment for these was still allowed and thus they were finally included in the model. The two-way interaction terms of intensities of helminth infections mentioned before, were also included and tested in the models in order to check for the effect of helminth co-infections on the mean Hb. As none of these terms, as well as the SES, were found to be significantly associated with Hb levels and/or anaemia, they were omitted from the final model. The random effects variance components indicate that most of the variance is between children within a school: of the total variability in Hb, 9.9 % (0.225/2.256) occurred at the school level while 90.0 % (2.031/2.256) occurred between children within a school.

This is also illustrated by Figure 3.2 which presents a box plot of the level-two residuals among older children for each school, and indicates that most schools had similar Hb levels that could not be distinguished statistically, thereby confirming the suitability of assumptions for the chosen final model. Interestingly, mean Hb was substantially higher in one school (#21) relative to other schools.

Figure 3.2 Box plot of school-level residuals from a Bayesian hierarchical model for Hb counts among older children.



Each box plot represents a school-level residual in this study. Numbers above each box plot are label identifiers for each school. This is a plot in which the posterior distributions of all u2s are summarised side by side. Boxes represent inter-quartile ranges and the solid black line at the (approximate) centre of each box is the mean of each specific u2; the arms of each box extend to cover the central 95 per cent of the distribution - their ends correspond, therefore, to the 2.5 % and 97.5 % quantiles. The horizontal straight line in the middle of the graph represents the overall mean of the u2s which is set to 0.

Table 3.2 Mean Hb concentration and prevalence of anaemia according to infection status and other characteristics in 1,523 schoolchildren aged 10-21 years (univariate analysis).

Variable	**Children No (%)**	**Mean Hb level, g/dL (95% CI)**	**Anaemia prevalence (%) (95% CI)**
Sex			
Male	786 (51.6)	12.51 (12.40 to12.61)	13.2 (10.9 to 15.6)
Female	737 (48.4)	12.34 (12.23 to12.45)	13.7 (11.2 to 16.2)
Age			
10-12 years old	573 (39.0)	12.29 (12.17 to 12.41)	13.1 (10.3 to 15.9)
13-15 years old	896 (61.099)	12.52 (12.42 to 12.62)	13.8 (11.6 to 16.1)
>=16 years old	54 (3.7)	12.44 (12.08 to 12.80)	11.1 (2.7 to 19.5)
Classification of SES (n=1404[b])			
Least poor	281 (20.01)	12.42 (12.24 to 12.61)	12.81 (8.90 to 16.72)
Poor	560 (39.89)	12.45 (12.34 to 12.58)	13.93 (11.06 to 16.79)
Most poor	563 (40.10)	12.40 (12.28 to 12.53)	13.14 (10.35 to 15.93)
Classification of BMIZ			
Not underweight	1430 (93.9)	12.44 (12.36 to 12.52)	13.2 (11.4 to 14.9)
Underweight	93 (6.1)	12.22 (11.92 to 12.52)	18.3 (10.4 to 26.1)
Classification of HAZ			
Not stunted	1323 (86.9)	12.48 (12.40 to 12.56)	12.8 (11.0 to 14.6)
Stunted	200 (13.1)	12.10 (11.88 to 12.31)	18.0 (12.7 to 23.3)
Intensity of hookworm infection[c]			
Not Infected	803 (52.7)	12.42 (12.31 to 12.53)	14.0 (11.6 to 16.3)
Lightly Infected	691 (45.4)	12.45 (12.35 to 12.56)	12.9 (10.4 to 15.4)
Moderately Infected	15 (1.0)	12.21 (11.60 to 12.83)	13.3 (0.0 to 30.5)
Heavily Infected	14 (0.9)	11.87 (11.34 to 12.40)	14.3 (0.0 to 32.6)
Intensity of *S. mansoni* infection[c]			
Not Infected	1309 (86.0)	12.44 (12.36 to 12.53)	13.2 (11.4 to 15.1)
Lightly Infected	91 (6.0)	12.41 (12.11 to 12.71)	11.0 (4.6 to 17.4)
Moderately Infected	76 (5.0)	12.54 (12.21 to 12.87)	13.2 (5.6 to 20.8)
Heavily Infected	47 (3.0)	11.79 (11.28 to 12.30)	25.5 (13.1 to 38.0)
Intensity of *T.trichiura* infection[c]			
Not Infected	1326 (87.1)	12.41 (12.33 to 12.50)	13.4 (11.6 to 15.3)
Lightly Infected	188 (12.3)	12.47 (12.26 to 12.69)	14.4 (9.4 to 19.4)
Moderately Infected	8 (0.5)	13.26 (12.30 to 14.23)	0.0 NA[a]
Heavily Infected	1 (0.1)	12.80 NA[a]	0.0 NA[a]
Intensity of *A. lumbricoides* infection[c]			
Not Infected	1162 (76.3)	12.46 (12.37 to 12.55)	13.6 (11.6 to 15.6)
Lightly Infected	236 (15.5)	12.34 (12.16 to 12.52)	13.1 (8.8 to 17.5)
Moderately Infected	125 (8.2)	12.27 (12.04 to 12.50)	12.8 (6.9 to 18.7)
Heavily Infected	0 (0.0)	0.0 NA[a]	0.0 NA[a]
Malaria spp. infection			
Not Infected	989 (69.9)	12.49 (12.40 to 12.58)	12.8 (10.8 to 14.9)
Infected	534 (35.1)	12.31 (12.18 to 12.44)	14.6 (11.6 to 17.6)

[a]NA, not available; [b] Socio-economic status or SES data were missing for 119 children; [c]Intensity of helminth infection was classified in light, moderate and heavy according to WHO recommended thresholds: *Schistosoma mansoni* infection, 1-99, 100-399 and ≥ 400 eggs per gram of faeces (epg); hookworm, 1-1,999, 2,000-3,999 and ≥ 4,000 epg; *Trichuris trichiura,* 1-999, 1,000-9,999 and ≥ 10,000 epg; and *Ascaris lumbricoides*, 1-4,999, 5,000-49,999 and ≥ 50,000 epg. BMIZ stands for Body Mass Index Z-score; HAZ stands for Height for Age Z-score and CI, confidence interval.]

Table 3.3 Estimated posterior mean differences in mean Hb concentration for the effects of selected explanatory variables from a final Bayesian hierarchical model (n = 1,523).

Variable	**Mean**	**95% BCI** [a]
Fixed part of the model		
Intercept	12.520	(12.280 to12.760)
Sex (Reference category: 'Male')		
Female	-0.183	(-0.330 to -0.036) [b]
Age (Reference category: '10-12 years old')		
13-15 years old	0.222	(0.064 to 0.377) [b]
>=16 years old	0.417	(0.012 to 0.832) [b]
Classification of BMIZ (Reference category:' Not wasted')		
Wasted	-0.244	(-0.544 to 0.062)
Classification of HAZ (Reference category:' Not stunted')		
Stunted	-0.347	(-0.564 to -0.128) [b]
Intensity of hookworm infection (Reference category: 'Not Infected')		
Lightly Infected	0.050	(-0.102 to 0.197)
Moderately Infected	-0.310	(-1.048 to 0.419)
Heavily Infected	-0.516	(-1.277 to 0.233)
Intensity of *Schistosoma mansoni* infection (Reference category: 'Not Infected')		
Lightly Infected	-0.113	(-0.417 to 0.189)
Moderately Infected	0.068	(-0.268 to 0.414)
Heavily Infected	-0.513	(-0.942 to -0.097) [b]
Intensity of *Trichuris trichiura* infection (Reference category: 'Not Infected')		
Lightly Infected	0.105	(-0.121 to 0.336)
Moderately Infected	0.834	(-0.195 to 1.857)
Heavily Infected	0.110	(-2.649 to 2.890)
Intensity of *Ascaris lumbricoides* infection (Reference category: 'Not Infected')		
Lightly Infected	-0.164	(-0.372 to 0.047)
Moderately Infected	-0.206	(-0.480 to 0.073)
Malaria spp infection (Reference category: 'Not Infected')		
Infected	-0.159	(-0.315 to -0.009) [b]
Random part of the model		
Level-2 (i.e. between schools) variance	0.225	(0.114 to 0.413)
Level-1 (i.e. between children within a school) variance	2.031	(1.889 to 2.186)

[a]95% Bayesian credible intervals (BCIs) are different from classical 95% confidence intervals (CIs) in various ways, one of which is: in their interpretation: we say there is a 95% probability that the true parameter lies in a 95% BCI where this is certainly not the interpretation of a 95% CI. In a long series of 95% CIs, 95% of those should contain the true parameter value - unlike the Bayesian interpretation we cannot give a probability for whether a particular CI contains the true value

[b]These are significant differences compared with the reference category in the sense that the probability is at least 95% that these parameters lie within the BCI, which is significant.

BMIZ stands for Body Mass Index Z-score; HAZ stands for Height for Age Z-score

3.3.2. Bayesian logistic regression model of anaemia. Table 3.4 presents the hierarchical logistic regression model of anaemia risk and shows that only children with heavy *S. mansoni* intensities were more likely to be anaemic, defined as Hb < 11.0 g/dL, compared with uninfected children (Odds ratio: OR = 2.3, 95% BCI: 1.1 to 4.3; Table 3.3). There was no evidence that other predictors were significantly associated with the risk of anaemia.

Table 3.4 Estimated posterior odds ratios for prevalence of anaemia (Hb < 110g/L) for the effects of selected explanatory variables from final Bayesian hierarchical logistic regression model (*n* = 1,523).

Variable	**Odds ratio**	**95% BCI**
Fixed part of the model		
Main effects		
Sex (Reference category: 'Male')		
Female	1.073	(0.787 to 1.405)
Age (Reference category: '>=10-12 years old')		
13-15 years old	1.095	(0.804 to 1.462)
>=16 years old	0.688	(0.218 to 1.547)
Intensity of hookworm infection (Reference category: 'Not Infected')		
Lightly Infected	0.895	(0.645 to 1.208)
Moderately Infected	1.156	(0.121 to 3.708)
Heavily Infected	1.165	(0.132 to 3.861)
Intensity of *Schistosoma mansoni* infection (Reference category: 'Not Infected')		
Lightly Infected	0.825	(0.360 to 1.442)
Moderately Infected	1.004	(0.426 to 1.969)
Heavily Infected	2.292	(1.070 to 4.258) [a]
Intensity of *Ascaris lumbricoides* infection (Reference category: 'Not Infected')		
Lightly Infected	1.060	(0.680 to1.584)
Moderately Infected	1.057	(0.582 to 1.748)
Malaria spp infection (Reference category: 'Not Infected')		
Infected	1.136	(0.821 to 1.540)
Random part of the model		
Level-2 (i.e. between schools) variance	0.288	(0.088 to 0.630)

[a] this is a significant odds ratio of heavily infected children with *S. mansoni* compared with uninfected children in the same sense as denoted in Table 3.2.

3.4 Discussion

Helminth infections, undernutrition and malaria have a large impact on the survival and quality of lives of school-aged children in Africa. Understanding the direct and indirect consequences of these factors on lower Hb levels and anaemia is important, as findings may help guide the suite of school-based interventions in endemic areas where polyparasitism is the norm (Raso et al., 2004; Pullan & Brooker, 2008). Analysis in this Chapter found evidence that heavy intensity of *S. mansoni* infection, being stunted and malaria parasitaemia were significantly associated with lower mean Hb (Table 3.3), although only heavy intensity of *S. mansoni* infection was significantly associated with the risk of anaemia among schoolchildren over 10 years of age (Table 3.4). Such results underscore current efforts to control helminths and malaria as part of integrated school health programmes (Brooker et al., 2008).

Although the cross-sectional design hampers the interpretation of the findings reported in this study, especially the direction of causality, the use of hierarchical Bayesian modelling allows the incorporation of both individual- and school-level factors, the omission of one or the other leading to biased estimates (Congdon, 2001). Furthermore, the results of this study are consistent with previous studies which report similar associations (Stoltzfus et al., 1997b; Olsen et al., 1998; Leenstra et al., 2004; Desai et al., 2005a) as well as with the findings presented in Chapter 2 where I also found statistically significant associations of heavy intensities of *S. mansoni* infection with mean lower Hb and anaemia prevalence before treatment.

Potential mechanisms through which *S. mansoni* may contribute to anaemia include: (i) blood loss caused by the rupture of blood vessels surrounding the intestine by the spined schistosome eggs; (ii) splenic sequestration; (iii) autoimmune hemolysis; and (iv) anaemia of inflammation which is typically characterized by decreased RBC production induced by pro-inflammatory cytokines (Friedman et al., 2005a; Tolentino and Friedman, 2007). In addition, it is possible that the importance of *S. mansoni* infection is likely to be greater than estimated here since only schools located more than 5 km from the lake shore were sampled to purposively minimize confounding by *S. mansoni* in the intervention trial.

By contrast, hookworm infection, a major attributable factor for anaemia in schoolchildren in other areas of East Africa (Stoltzfus et al., 1997a; Lwambo et al., 2000) including Uganda where in Chapter 2 I also found statistically significant associations of moderate and heavy intensities of hookworm infections with lower mean Hb levels and anaemia prevalence, was not associated either with lower Hb or with anaemia in the present study. The current finding, which is consistent with previous studies in western Kenya (Olsen et al., 1998; Handzel et al., 2003), is probably due in part to the low intensity of hookworm infection in this study area, and highlights how different factors contribute to anaemia in different parasite transmission settings.

However, the difference in Hb between children infected with malaria and those uninfected, though significant, was small. There was not found any evidence of an increased risk of anaemia in children co-infected with multiple helminth species and/or malaria. This finding is in contradiction with a similar study from Leyte, in The Philippines (Ezeamama et al., 2005) which suggested that even low-intensity polyparasite infections were associated with increased odds of having anemia.

The results of the linear hierarchical approach applied here (Table 3.3), indicate that a high degree of variation remains to be explained and that there are other factors beyond what was measured in this study which still need to be considered. One advantage of a hierarchical approach over conventional statistical approaches is the partitioning of the unexplained variance into variability between clusters and individual level variation within clusters, which shows that most of the residual variation within the study population examined here was attributable to individual level variation occurring between children. More precisely, although dietary iron insufficiency is very likely to impact on Hb and anaemia (Olivares et al., 1999), no information was available on iron status of the children included in the study. Nutritional variables such as stunting and wasting, representing chronic and severe acute undernutrition, respectively, may not adequately capture moderate, current undernutrition which may also explain variation in mean Hb levels. Reported age is also often uncertain and may be inaccurate. This may have implications for the reliability of the derived HAZ and BMIZ scores. A further source of variation not measured in this study is the effect of menarche in adolescent girls (Leenstra et al.,

2004). When interaction terms of age and sex were fitted to the model these were not found to be significant, however as described above, reported age may be both unreliable and too crude a proxy for individual variation in the timing of onset of menarche. Similarly, the SES index used here may not fully capture socio-economic variability within the population as it was based on a small number of assets and relied on reporting by schoolchildren. A final source of individual variation which was not taken into account in this study was genetic traits such as sickle-cell and other haemoglobinopathies (Tolentino and Friedman, 2007).

In conclusion, the current study demonstrated that lower mean Hb levels were significantly associated with heavy intensity of *S. mansoni,* chronic undernutrition and malaria and that anaemia was associated with heavy intensity of *S. mansoni*. Such findings validate the results reported in Chapter 2 where it was concluded that anaemia is likely to represent a valuable marker for morbidity caused by heavy intensity of *S. mansoni* infection. Results from both Chapters 2 and 3 have important implications for the control of anaemia among African schoolchildren and can help guide the design of appropriate interventions. Integrated school health programmes which include deworming, micronutrients and potentially malaria control, will help alleviate the anaemia burden faced by the school-aged children of Africa. Further research is required to identify the optimal packages and to identify areas where different packages of interventions may be required.

Chapter 4: *Schistosoma haematobium* infection and morbidity before and after large-scale administration of praziquantel in Burkina Faso

Summary

In sub-Saharan Africa 112 million people are infected with *Schistosoma haematobium* with the most intense infections in children aged 5 to 15 years. In the current Chapter I describe a longitudinal epidemiological study that evaluated the relationship between *S. haematobium* infection and associated morbidity in children before and after large-scale administration of praziquantel for schistosomiasis and albendazole for soil-transmitted helminth infections. At baseline, higher intensities of *S. haematobium* infection were observed in children with anaemia and/or severe microhaematuria, but there was no apparent association between the risk of undernutrition and *S. haematobium* infection intensity. Significant reductions in the prevalence and intensity of *S. haematobium* infection one year post-treatment were, however, observed. Children who benefited the most from anthelminthic treatment in terms of increased haemoglobin counts were those who had anaemia at baseline and those with highly positive microhaematuria scores at baseline. In conclusion, this study suggests that even a single round of mass chemotherapy can have a substantial impact on *S. haematobium* infection and its associated morbidity in children.

Keywords: schistosomiasis, *Schistosoma haematobium*, morbidity, control programme, chemotherapy, hierarchical models, West Africa

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4.1 Introduction

Improving the health of school-aged children, particularly in developing countries, has emerged as a policy priority in international health (The World Bank, 1993; Stoltzfus et al., 1997). Over the past two decades significant progress has been made in improving child survival, resulting in more children reaching primary school age. However, human infections with one of the five parasitic helminths of the family *Schistosomatidae* still represent a significant segment of the global burden of illness with approximately 200 million people infected and with highest intensities in children aged 5 to 15 years old (Steinmann et al., 2006). Schistosomiasis causes granuloma formation, and both reversible and irreversible damage to the urinary and/or intestinal tracts, depending on the infecting species (Mahmoud, 2001). New estimates of schistosomiasis-related disability indicate the need to reassess priorities for treating this chronic infection in areas where it is endemic (King et al., 2005).

Praziquantel has been established in several controlled trials as a safe and effective drug for the treatment of infection with all human schistosome species (Kardaman et al., 1983; Nokes et al., 1999; King et al., 2002; Kabatereine et al., 2003). The dramatic reduction in its price since 1990 by over 90 %, from US$4 to treat a person to approximately US$0.30, has led to the resolution of many of the challenges surrounding large-scale chemotherapy campaigns (Lammie et al., 2006) and through the Schistosomiasis Control Initiative (SCI) over 20 million treatments were carried out in 2005/2006 in 6 sub-Saharan African countries (Garba et al., 2006; Kabatereine et al., 2006). As mentioned previously in Chapter 2 of this PhD thesis, one of the primary objectives of these SCI-supported control programs is to achieve, and hence also demonstrate, a quantifiable reduction in schistosome-associated morbidity as a consequence of chemotherapeutic intervention.

The aim of the present study was to evaluate the relationship between *Schistosoma haematobium* infection and associated morbidity in children before and after large-scale administration of praziquantel and albendazole (against soil-transmitted-helminths) by the national Burkinabé helminth control program. A secondary aim was to identify those individuals whom one may

predict to show the greatest improvements in nutritional status and haemoglobin (Hb) concentrations after chemotherapy.

4.2 Materials and methods

4.2.1 The control programme, study sites, sampling and cohort design. Both *Schistosoma mansoni* and *S. haematobium* are endemic throughout Burkina Faso (Poda et al., 2004). The SCI-supported schistosomiasis control program was implemented during 2004 and has treated 3.322.564 school age children in the 13 regions of the country through October 2006. Further details about the national Burkinabé helminth control program have been described elsewhere (Gabrielli et al., 2006).

For the present study, parasitological and morbidity data were collected on a cohort of 1727 Burkinabé children 6 to 14 years old, randomly sampled from 16 schools before and 1 year after chemotherapy (2004 and 2005 respectively). The schools included in these surveys were randomly selected from all schools in four Regional Health Directorates known a priori to be places where schistosomiasis is highly endemic. Details concerning the sample size calculations and cohort design have been described in Chapter 2 of this PhD thesis as well as in previous studies (Brooker et al., 2004; Koukounari et al., 2006).

4.2.2 Parasitological and morbidity measures. All children enrolled in the study were interviewed by appropriately trained personnel Ministry of Health. Ethical clearance was obtained from the Ministry of Health, Burkina Faso and Imperial College London.

Stool examination. A single stool sample was collected from each child and 41.7 mg processed to make duplicate Kato Katz slides for microscopic determination of intestinal helminths infections. Individual egg output was expressed as eggs per gram of faeces (epg) calculated as the arithmetic mean of the two individual slide counts whenever these were available.

Urine examination. One urine specimen was collected from each child to determine the prevalence and intensity of *S. haematobium* infection by the filtration method. The intensity of *S. haematobium* infection was expressed as the number of eggs per 10 ml of urine. To determine

the presence and severity of microhaematuria, all urine specimens were tested for presence of detectable blood with urine reagent strips (Bayer Hemastix ®). The results were recorded semi-quantitatively: negative; trace hemolysed; weakly positive (+), moderately positive (++) and highly positive (+++).

Nutritional assessment. Heights and weights were measured to determine Height-for-Age Z-scores (HAZ) and Body Mass Index Z-scores (BMIZ). All measures were taken with height poles and electronic balances in the morning and children were barefoot, wearing only light indoor clothing. A low BMIZ is the index of choice for the assessment of recent undernutrition, resulting in thinness or wasting, while a low HAZ represents long-term growth and nutritional status resulting in shortness or stunting. Z-scores for each nutritional index were calculated from Centre for Disease Control (National Center for Health Statistics) reference values using EpiInfo version 2000 (Centers for Disease Control and Prevention [CDC], Atlanta, GA) (Ogden et al., 2002).

Anaemia assessment. Blood samples for Hb concentrations were obtained from each individual by the finger prick method using a photometer Hemocue (Hemocue AB, Angelholm, Sweden) (Parker et al., 1997). Anaemia was defined according to WHO guidelines (UNICEF/United Nations University/World Health Organization(WHO), 2001).

4.2.3 Statistical analyses. Differences between drop-outs and children successfully followed-up were tested by univariate analysis using a Wilcoxon 2-sample test for means and a Chi^2 test, or a Fisher's exact test if there was a small expected value, for proportions. SAS software was used (version 8; SAS Institute Inc., Cary, NC, USA).

As mentioned in Chapters 2 and 3 of this PhD thesis, hierarchical models are often applicable to modeling of data from complex surveys of a population with a hierarchical structure used to explain relations between individual and 'supra-individual' determinants. A two-level linear hierarchical model assuming normally distributed errors was fitted to the logarithmically transformed baseline *S. haematobium* egg counts (ln(x+1)), using Gibbs sampling for all

parameters in order to quantify any associations with anaemia, measures of nutritional status and microhematuria while adjusting at the same time for demographic factors such as age and sex. In this way I tested whether children with pathology, potentially induced by *S. haematobium* infection, had higher *S. haematobium* egg counts before treatment. I used a similar model to quantify changes in *S. haematobium* egg counts from baseline to one-year follow-up. Mlwin software (version 2.01, Multilevel Models Project, Institute of Education, University of London, UK) was used. Boxplots of the *S. haematobium* egg counts at baseline were used for validation and comparison of the significant findings from the model described above.

Changes in Hb concentration and in HAZ and BMIZ-scores over time were evaluated using two-level linear hierarchical models of raw change scores between baseline and the 1-year follow-up time point. With this approach, I aimed to compare the average change in each of the studied outcomes over the studied period between different groups of children. All of the models presented were also adjusted for age and gender. P-values < 0.05 were considered to be significant. I have also plotted HAZ and BMIZ-scores at baseline versus HAZ and BMIZ-scores at 1-year follow-up time point to examine observed trends.

An additional three-level hierarchical linear modeling analysis was performed to determine any change in the children's Hb concentrations. With this model, I aimed to quantify the adjusted overall change of Hb from baseline to follow-up and to quantify average Hb concentrations of different groups of children at baseline as well as to examine whether the intensity of *S. haematobium* infection was associated with lower Hb concentrations.

Logistic random intercepts regression models were fitted in order to examine whether the intensity of *S. haematobium* infection was associated with an increased risk of thinness and shortness at baseline while adjusting for potential confounders.

4.3 Results

A total of 1,727 children from 16 schools were recruited at baseline. Of these, 1131 (65%) were successfully re-traced at 1-year follow-up time point and 321 new children were recruited into

the cohort during the second year of the study (data not shown). There were significant differences between the children who were successfully followed-up and the drop-outs, according to their demographic characteristics and nutritional status as defined by baseline thinness and shortness as well as by baseline Hb concentrations (Table 4.1). The children who dropped-out were of an older mean age than those successfully followed up, and boys proved most difficult to recruit into the cohort during the second year of the study. All children who dropped-out were wasted and stunted at baseline and had slightly higher mean Hb concentrations. No other baseline characteristic measured varied significantly between children followed up and those who dropped out.

Table 4.1 Baseline characteristics of Burkinabé schoolchildren followed-up for one year, and not followed-up, sd = standard deviation; e/10 ml = eggs per 10 ml liter; epg = eggs per gram faeces.

	Followed up for 1 year	**Drop outs**	***p*-values**
Demographic characteristics			
Mean age	9.8 (n=1131)	11.3 (n=686)	<0.001
% male	55.0 (n=1131)	62.0 (n=686)	0.004
Parasitology			
% infected with *S. haematobium*	53.9 (n=1124)	54.1 (n=690)	0.953
% infected with *S. mansoni*	6.2 (n=536)	5.8 (n=432)	0.810
% infected with hookworm	6.3 (n=556)	4.3 (n=418)	0.174
Mean (sd) *S. haematobium* intensity (e/10 ml)	83.6 (229.2)	94.2 (234.6)	0.728
Mean (sd) *S. mansoni* intensity (epg)	8.0 (73.9)	11.3 (76.1)	0.869
Mean (sd) hookworm intensity (epg)	12.5 (90.7)	3.3 (21.0)	0.158
Haematology			
% anaemic[a]	65.8 (n=1130)	66.4 (n=687)	0.390
Mean (sd) haemoglobin (g/dL)	11.0 (1.4)	11.1 (1.4)	0.036
Nutritional status			
% wasted	32.8 (1131)	100.0 (n=686)	<0.001
% stunted	13.3 (n=1131)	100.0 (n=686)	<0.001
Hemastix test	(n=1124)	(n=692)	0.460
% of negative	50.4	53.5	
% of trace	12.9	10.7	
% of children with '+'	6.8	5.6	
% of children with '++'	9.3	8.9	
% of children with '+++'	20.6	21.4	

[a]Anaemia was defined for all tables displayed (according to WHO guidelines), as Hb less than 11.5 g/dL for children from 5 to 11 years old and for children between 12 and 14 years old as Hb less than 12.0 g/dL.

Table 4.2 presents the health indicators of children surveyed at baseline and successfully followed up 1 year after treatment. During the 12 months between examinations, the overall prevalences of *S. haematobium*, *S. mansoni* and hookworm infections decreased significantly ($p<0.001$). For both years examined, *Ascaris lumbricoides* infection was absent and the prevalence of *Trichuris trichura* infection was estimated to be 1.1% at baseline and totally absent 1 year later. Because prevalences, and co-infections with *S. haematobium*, were so low for the intestinal helminth species at both time points such data were not analyzed further here.

A significant increase in mean Hb concentration ($p<0.001$) and a significant decrease in the prevalence of anaemia ($p=0.021$) were also observed between 2004 and 2005. Finally the unadjusted observed changes in both recent and chronic undernutrition from baseline to follow-up were not significant ($p=0.135$ and $p=0.093$ respectively).

Table 4.2 Health characteristics of children at baseline and post-treatment (2004-2005) successfully followed up for one year.

	2004	2005	p-values
Parasitology			
% infected with *S. haematobium* (n=1,124)[a]	53.9 (51.0 – 56.8)	5.8 (4.4-7.2)	<0.001
% infected with *S. mansoni* (n=536)	6.2 (4.1–8.2)	0.2 (0.0 – 0.6)	<0.001
% infected with hookworm (n=555)	6.3 (4.3 – 8.3)	1.6 (0.6 - 2.7)	<0.001
Mean *S. haematobium* intensity (e/10 ml)	83.6 (70.1 – 97.0)	0.9 (0.4 – 1.5)	<0.001
Mean *S. mansoni* intensity (epg)	8.0 (1.8 – 14.3)	0.0 (0.0 – 0.1)	<0.001
Mean hookworm intensity (epg)	12.5 (4.9 – 20.0)	0.8 (0.2 – 1.3)	<0.001
Haematology (n=1,131)			
Mean haemoglobin (g/dl)	11.0 (10.9 – 11.1)	11.3 (11.2 – 11.3)	<0.001
% anaemic	65.8 (63.0 – 68.5)	61.6 (58.8 – 64.4)	0.021
Microhaematuria as diagnosed by hemastix test (n=1,124)			<0.001
% of negative	50.4 (47.5-53.4)	89.5 (87.7-91.3)	
% of trace	12.9 (10.9-14.9)	4.9 (3.6-6.2)	
% with '+'	6.8 (5.3-8.2)	2.3 (1.4-3.2)	
% with '++'	9.3 (7.6-11.0)	1.1 (0.5-1.7)	
% with '+++'	20.6 (18.2-22.9)	2.2 (1.4-3.1)	
Nutritional status (n=1,131)			
% of thinness or wasting	32.8 (30.1-35.5)	35.1 (32.3-37.9)	0.135
% of shortness or stunting	13.3 (11.3-15.2)	11.9 (10.0-13.7)	0.093

[a] sample sizes are provided into parentheses for each examined outcome

Table 4.3 presents the results of the model of the change in *S. haematobium* egg counts for 1 year post treatment as well as differences in *S. haematobium* egg counts between different groups of children at baseline. This model indicated that compared with baseline counts, there was on average an overall significant decrease in the *S. haematobium* egg counts by 52%, 1 year after treatment ($p<0.001$). At baseline, only children aged 10 and 12 years old had significantly higher *S. haematobium* egg counts, compared to those who were 14 years old ($p=0.035$ and $p=0.007$ respectively) after controlling for sex, nutritional and anaemia status and microhaematuria test scores. Children with '+++', '++', '+' and trace microhaematuria scores had on average significantly higher *S. haematobium* egg counts than those of children with negative scores at baseline, by 4107%, 1470%, 462% and 242% respectively. Additionally, children with anaemia at baseline had significantly higher *S. haematobium* egg counts (by 11%) than children without anaemia ($p=0.026$). Boys also had significantly higher *S. haematobium* egg counts (by 11%) than girls at baseline ($p=0.020$).

Table 4.3 Estimates from two-level hierarchical model for *S. haematobium* egg counts among 1,130 Burkinabé schoolchildren at baseline and post-treatment (2004-2005).

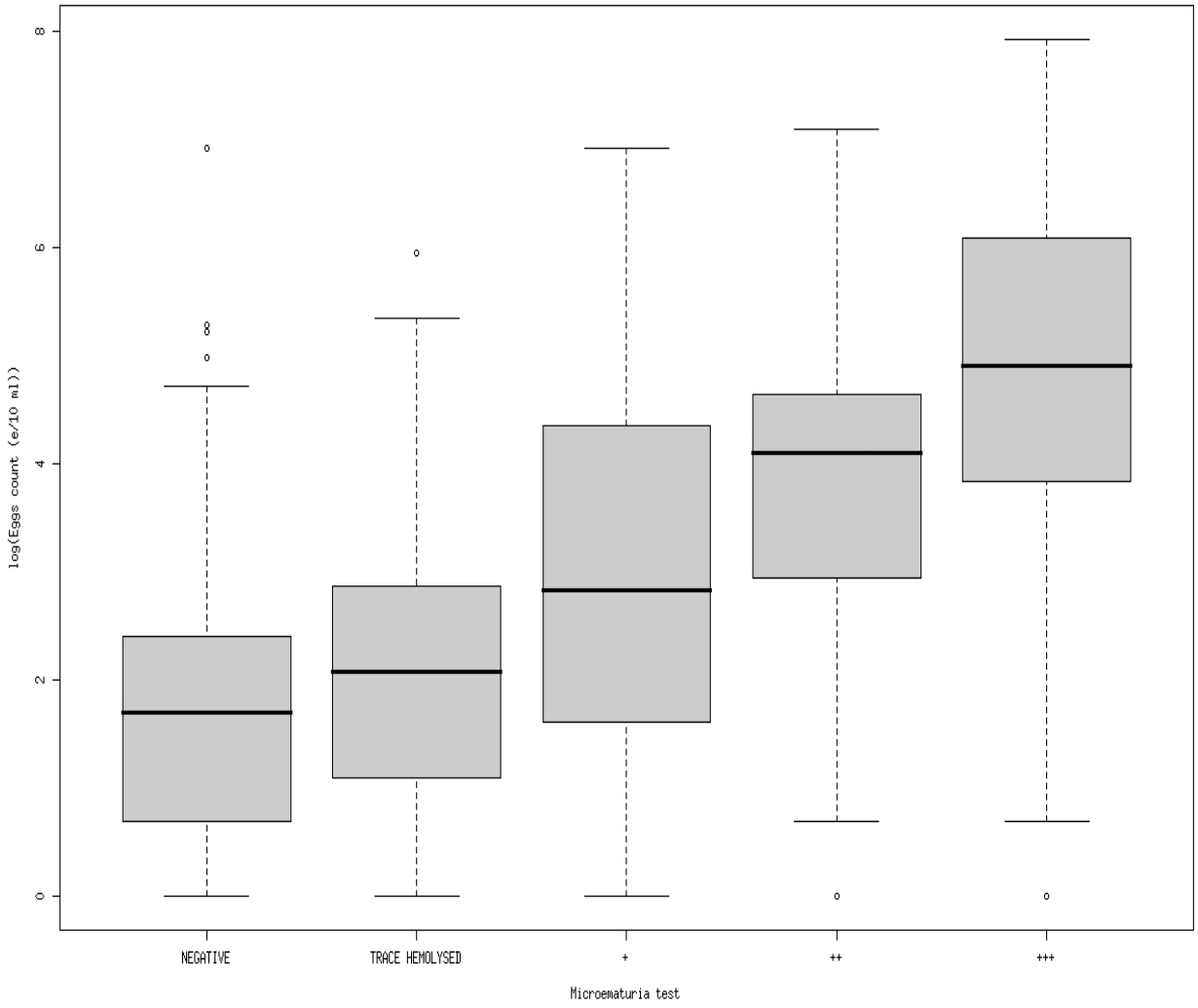
Fixed effects	**Coefficient (95% CIs)**	**p-values**
Effect of follow-up relative to baseline		
Follow-up year 1	-52% (-57% to -47%)	<0.001
Gender (Reference category 'female')		
Male	11% (2% to 21%)	0.020
Baseline Age (Reference category '14 years old')		
13 years old	9%(-19% to 45%)	0.572
12 years old	45% (11% to 90%)	0.007
11 years old	25% (-4% to 63%)	0.095
10 years old	32% (2% to 72%)	0.035
9 years old	24% (-4% to 61%)	0.106
8 years old	22% (-6% to 59%)	0.143
7 years old	2% (-24% to 37%)	0.906
6 years old	19% (-27% to 92%)	0.488
Baseline anaemia (Reference category 'non anaemic')		
Anaemic[a]	11% (1% to 21%)	0.026
Baseline haematuria (Reference category 'negative')		
trace	242% (191% to 302%)	<0.001
'+'	462% (357% to 592%)	<0.001
'++'	1470% (1176% to 1833%)	<0.001
'+++'	4107% (3493% to 4827%)	<0.001
Baseline thinness or wasting (Reference category 'non wasted')		
wasted[b]	4% (-6% to 14%)	0.463
Baseline shortness or stunting (Reference category 'non stunted')		
stunted[c]	-13% (-26% to 2%)	0.089
Random effects	**Variance components (S.E)**	
Level-2 (i.e. between schools) variance	0.008 (0.004)	
Level-1 (i.e. measurement occasions within a child) variance	0.190 (0.006)	

[a]anaemia is defined for all tables displayed (according to WHO guidelines), as Hb less than 11.5 g/dL for children from 5 to 11 years old and for children between 12 and 14 years old as Hb less than 12.0 g/dL, [b]Wasting denotes reduced body weight for height and is defined as body mass index Z-score<-2 for all tables displayed, [c]Stunting denotes reduced body length in relation to a reference standard and is defined as height for age Z-score<-2 for all tables displayed.

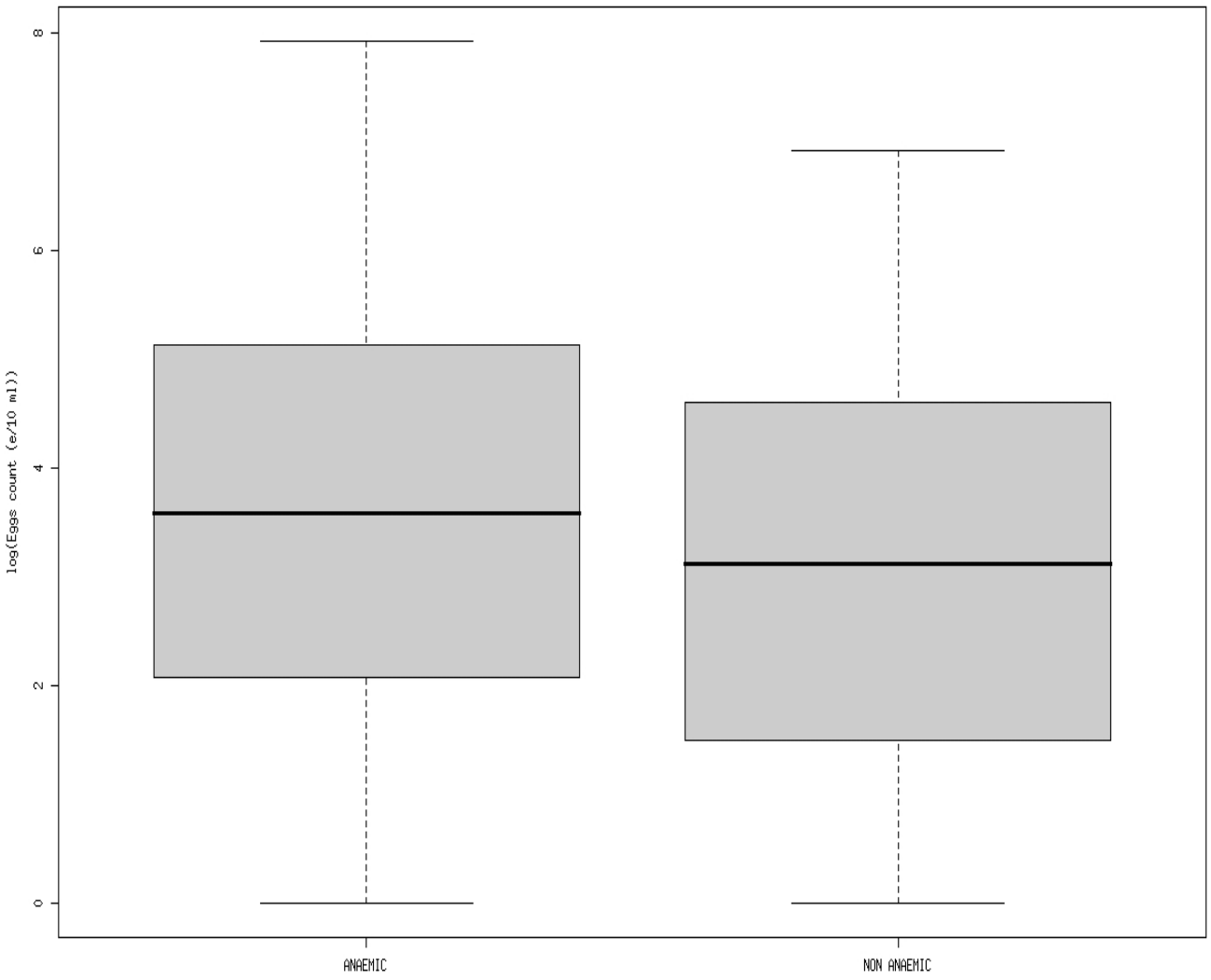
Figure 4.1a shows that children with the most severe microhaematuria scores harboured higher intensities of *S. haematobium* infection than children who were negative for microhaematuria at baseline. Children with anaemia at baseline also harboured slightly higher *S. haematobium* intensities than those without anaemia (Figure 4.1b).

Figure 4.1 Box plots on the logarithmically transformed (base e) *S. haematobium* egg counts of only positive subjects n=613 Burkinabé schoolchildren 2004. (a) Box plot for log *S. haematobium* egg counts with respect to different microhematuria test scores at baseline. (b) Box plots for log *S. haematobium* egg counts with respect to anaemia status at baseline

(a)



(b)



Results from the two-level logistic regression model for the probability of being wasted did not suggest associations with any baseline characteristics examined other than age. In particular, there was a trend toward younger children to be less likely to be wasted, although none of the other odds ratios (ORs), except for those for 10-year-old children, were significantly different from the ORs for the 14-year-old children (p=0.022). Furthermore, the two-level logistic regression model of the probability of being stunted at baseline suggested a trend toward younger

children being less likely to be stunted; the OR for 6 to 12-year-old children was significantly different from that for the 14-year-olds. In addition, children with anaemia were almost 1.5 times more likely than those without anaemia to be stunted at baseline (p=0.034; data not shown).

Table 4.4 contains estimates from the two-level linear multilevel models for changes in BMIZ and HAZ during the period studied. The effect for the 14-year-old comparison group was a decrease in BMIZ of 0.519 units, which was of borderline significance (p=0.053). This suggests that there was no dramatic change over the period studied in this group. However, children who were 7, 8 and 10 years old at baseline had a greater decrease in BMIZ, compared with 14-year-olds. The coefficients of the continuous variables that refer to the baseline BMIZ and HAZ-scores indicated that BMIZ increased for wasted children, whereas, BMIZ decreased for stunted children more than that of children with no nutritional problems. These results can be obtained if one multiplies the coefficients of BMIZ and HAZ by -2 (i.e. cut-off score that defines wasting and stunting respectively).

The effect for the 14-year-old comparison group was a non-significant (p=0.214) increase in HAZ-score of 0.143 units. Compared with 14-year-olds, 6-year-olds had a significantly greater increase, while the 12-year-olds had a decrease in HAZ (significantly different from that of 14-year-olds). Boys had a significantly smaller increase in HAZ than girls. Likelihood ratio tests indicated a better fit in both models mentioned above when I included BMIZ and HAZ as explanatory continuous variables and not the relevant categorical ones that would denote wasting and stunting if BMIZ or HAZ was, respectively, less than 2 SD below the CDC median.

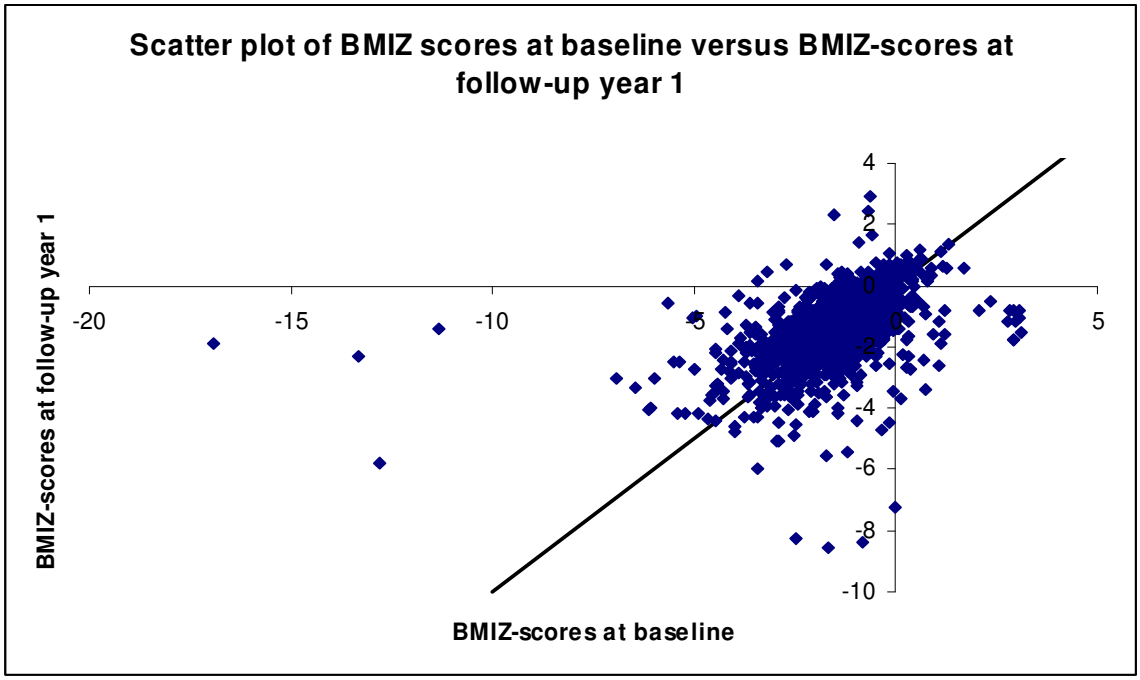
Table 4.4 Estimates from two-level hierarchical model for the change of body mass index (BMIZ) and Height for Age (HAZ) Z-scores (n = 1,130)

Fixed effects	**Coefficient (95 % CIs) for the change of BMIZ**	**p-values**	**Coefficient (95 % CIs) for the change of HAZ**	**p-values**
Intercept	-0.519 (-1.003 to -0.035)	0.053	0.143 (-0.073 to 0.359)	0.214
Baseline intensity of *S.haematobium* infection (Reference category' uninfected')				
lightly infected	0.060 (-0.133 to 0.253)	0.543	0.032 (-0.059 to 0.124)	0.490
heavily infected	0.218 (-0.030 to 0.465)	0.085	0.078 (-0.040 to 0.196)	0.193
Gender (Reference category' female')				
Male	0.011 (-0.100 to 0.122)	0.850	-0.066 (-0.119 to -0.013)	0.015
Baseline Age (Reference category' 14 years old')				
13 years old	-0.120 (-0.649 to 0.410)	0.658	-0.134 (-0.388 to 0.119)	0.300
12 years old	-0.375 (-0.816 to 0.067)	0.097	-0.236 (-0.447 to -0.025)	0.029
11 years old	-0.396 (-0.835 to 0.043)	0.077	-0.177 (-0.386 to 0.033)	0.099
10 years old	-0.483 (-0.915 to -0.051)	0.029	-0.008 (-0.215 to 0.198)	0.936
9 years old	-0.347 (-0.778 to -0.084)	0.115	0.000 (-0.206 to 0.206))	0.999
8 years old	-0.448 (-0.879 to -0.017)	0.042	0.051 (-0.155 to 0.257)	0.625
7 years old	-0.642 (-1.083 to -0.201)	0.004	-0.033 (-0.244 to 0.178)	0.757
6 years old	-0.483 (-1.051 to 0.085)	0.096	0.428 (0.157 to 0.699)	0.002
Baseline anaemia(Reference category 'non anaemic')	0.005 (-0.114 to 0.123)	0.939	-0.041 (-0.098 to 0.015)	0.153
Baseline haematuria (Reference category 'negative')				
trace	-0.166 (-0.389 to 0.057)	0.144	-0.063 (-0.169 to 0.043)	0.243
'+'	-0.147 (-0.416 to 0.123)	0.286	-0.014 (-0.142 to 0.114)	0.829
'++'	0.054 (-0.208 to 0.317)	0.685	-0.027 (-0.152 to 0.097)	0.669
'+++'	-0.110 (-0.356 to 0.136)	0.383	-0.058 (-0.174 to 0.059)	0.334
Baseline BMIZ-score	-0.588 (-0.628 to -0.548)	<0.001	0.054 (0.035 to 0.073)	<0.001
Baseline HAZ-score	0.184 (0.135 to 0.232)	<0.001	-0.138 (-0.161 to -0.115)	<0.001
Random effects	**Variance components (S.E)**		**Variance components (S.E)**	
Level-2 (i.e. between schools) variance	0.155 (0.065)		0.009 (0.004)	
Level-1 (i.e. between children within a school) variance	0.846 (0.036)		0.194 (0.008)	

The standard errors (and confidence intervals) obtained from the present analysis (i.e. estimates from models presented in Table 4.4) provide details on the level of precision achieved with the observed data. This relationship (albeit without the benefits of adjustments for demographic factors) can also be seen from plots of baseline BMIZ/HAZ versus follow-up BMIZ/HAZ (Figure 4.2a and Figure 4.2b).

Figure 4.2 Scatter plots of anthropometric z-scores at baseline versus anthropometric z-scores at follow-up year 1

(a)



(b)

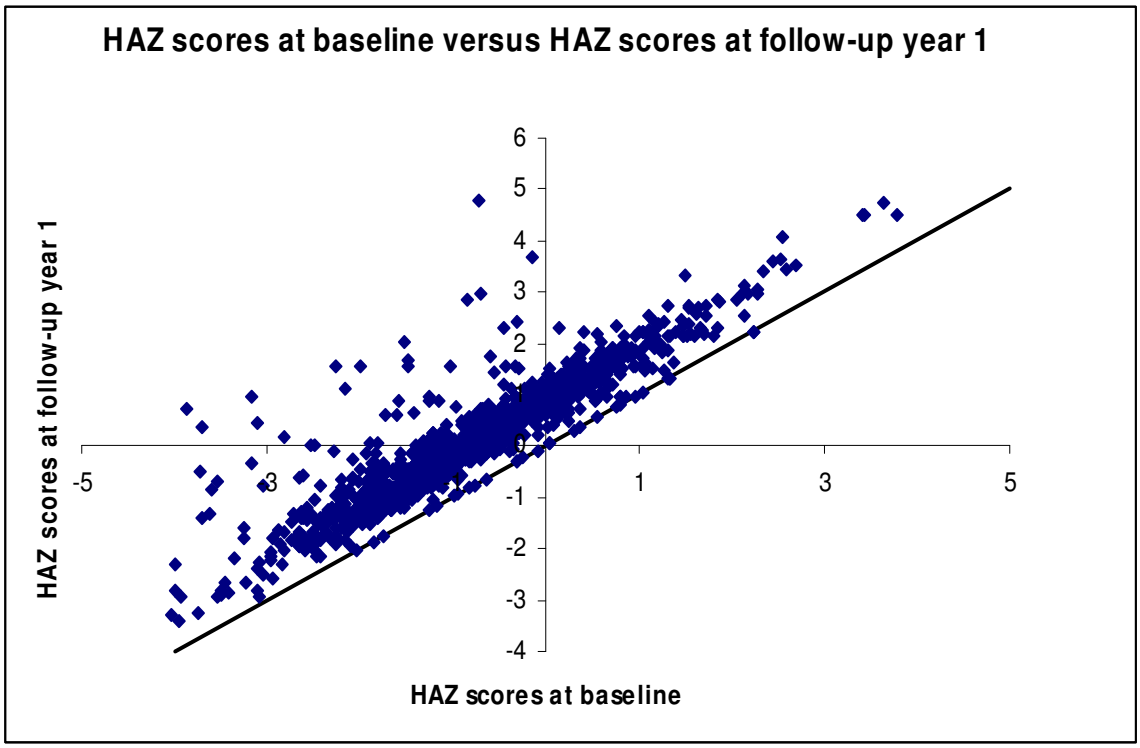


Table 4.5 contains the estimates of two three-level hierarchical models for Hb concentrations before and after chemotherapeutic treatment, taking into account adjustment and non-adjustment for microhaematuria scores. From the former model it was estimated that an overall increase of 0.092 g/dl in Hb concentration after treatment was not significant (p=0.146). Children with '+++' microhaematuria scores had significantly lower Hb concentrations (0.318 g/dL; p=0.016) than microhaematuria negative children at baseline. Children who were 6, 7, 8 and 10 years old at baseline had significantly lower Hb concentrations, (p=0.024, p<0.001, p=0.019 and p=0.008 respectively) than those who were 14 years old after controlling for intensity of *S. haematobium* infection, sex, haematuria, wasting and thinness. From the alternative model, which did not adjust for microhaematuria scores, estimates of the parameters mentioned previously remain approximately the same but the effect of *S. haematobium* infection intensity became significant, such that children who were heavily infected with *S. haematobium* at baseline had significantly lower Hb concentrations (0.220 g/dL; p=0.024) than uninfected children. This suggests that because the variable of microhaematuria scores is related both to Hb concentration and the intensity of *S. haematobium* infection, two different causal effects of *S. haematobium* infection on Hb concentrations are indicated. Two-way interaction terms were also tested but because they were not significant they were finally omitted from the models presented. Also, variances in all three levels of the second model were higher than the corresponding ones in the first model which implies that microhaematuria scores variable explains some of the variability in the studied outcome across all three levels of the models presented.

Finally, Table 4.6 contains estimates from the two-level linear multilevel model for the change in Hb concentration over the course of the period studied. This model suggested that the change varied significantly as a function of the following baseline characteristics: anaemic status, '+++' microhaematuria score and age. The effect for the comparison group (baseline uninfected, male, 14 years old, not anaemic, negative microhaematuria score, not wasted, and not stunted) was a decrease in Hb concentrations of 0.128 g/dL which was not significant (p=0.747). Significant increases in Hb concentration during the period studied were observed among children with anaemia at baseline (increase by 1.360 g/dL-[that is 1.488-0.128 g/dL]; p<0.001) and among children with '+++' microhaematuria scores at baseline (increase by 0.233 g/dL [that is 0.361-

0.128 g/dL]; p=0.039). Marginally significant decreases in Hb concentration during the same period were observed in children who were 12 years old at baseline; these children had a greater decrease by 0.733 g/dL, (that is -0.605-0.128 g/dL; p=0.055) in Hb concentration than 14- year-old children. In addition, 9-year-old had a significantly greater decrease in Hb concentration by 0.847 g/dL, (that is -0.719-0.128 g/dL; p=0.019).

Table 4.5 Estimates from three-level hierarchical models for haemoglobin concentration before and after treatment taking into account adjustment and non adjustment for microhaematuria scores (n = 1,124)

Fixed effects	**Coefficients (95 % CIs) with adjustment for Microhaematuria**	**p-values**	**Coefficients (95 % CIs) without adjustment for Microhaematuria**	**p-values**
Intercept	11.473 (11.067 to 11.879)	<0.001	11.480 (11.074 to 11.886)	<0.001
Effect of follow-up relative to baseline				
follow-up year 1	0.092 (-0.031 to 0.215)	0.146	0.093 (-0.030 to 0.216)	0.139
Baseline intensity of *S.haematobium* infection (Reference category 'uninfected')				
lightly infected	-0.107 (-0.311 to 0.097)	0.303	-0.079 (-0.238 to 0.080)	0.332
heavily infected	-0.084 (-0.354 to 0.186)	0.542	-0.220 (-0.410 to -0.030)	0.024
Gender (Reference category female)				
Male	-0.035 (-0.158 to 0.088)	0.577	-0.039 (-0.162 to 0.084)	0.538
Baseline Age (Reference category 14 years old)				
13 years old	-0.026 (-0.383 to 0.331)	0.888	-0.037 (-0.396 to 0.322)	0.838
12 years old	-0.136 (-0.489 to 0.217)	0.452	-0.138 (-0.493 to 0.217)	0.445
11 years old	-0.334 (-0.683 to 0.015)	0.061	-0.353 (-0.704 to -0.002)	0.048
10 years old	-0.468 (-0.813 to -0.123)	0.008	-0.471 (-0.818 to -0.124)	0.008
9 years old	-0.296 (-0.643 to 0.051)	0.095	-0.290 (-0.639 to 0.059)	0.103
8 years old	-0.425 (-0.780 to -0.070)	0.019	-0.415 (-0.772 to -0.058)	0.022
7 years old	-0.817 (-1.205 to -0.429)	<0.001	-0.816 (-1.206 to -0.426)	<0.001
6 years old	-0.693 (-1.297 to -0.089)	0.024	-0.685 (-1.289 to -0.081)	0.026
Baseline haematuria (Reference category 'negative')				
trace	0.108 (-0.115 to 0.331)	0.344		
'+'	0.135 (-0.149 to 0.419)	0.352		
'++'	0.229 (-0.063 to 0.521)	0.124		
'+++'	-0.318 (-0.577 to -0.059)	0.016		
Baseline thinness or wasting (Reference category 'non wasted')				
Wasted	-0.042 (-0.162 to 0.078)	0.488	-0.042 (-0.162 to 0.078)	0.491
Baseline shortness or stunting (Reference category 'non stunted')				
Stunted	-0.149 (-0.351 to 0.053)	0.148	-0.155 (-0.359 to 0.049)	0.135
Random effects	**Variance components (S.E) from model with adjustment for Microhaematuria**		**Variance components (S.E) from model without adjustment for Microhaematuria**	
Level-3 (i.e. between schools) variance	0.136 (0.056)		0.140 (0.057)	
Level-2 (i.e. between children within a school) variance	0.514 (0.051)		0.519 (0.051)	
Level-1 (i.e. measurement occasions within a child) variance	1.106 (0.047)		1.116 (0.047)	

Table 4.6 Estimates from two-level hierarchical model for the change of haemoglobin counts (n = 1,130)

Fixed effects	**Coefficient (95 % CIs)**	**p-values**
Intercept	-0.128 (-0.888 to 0.633)	0.747
Baseline intensity of *S.haematobium* infection (Reference category' uninfected')		
lightly infected	-0.081 (-0.350 to 0.187)	0.553
heavily infected	-0.258 (-0.602 to 0.086)	0.141
Gender (Reference category female)		
Male	0.050 (-0.105 to 0.205)	0.529
Baseline Age (Reference category 14 years old)		
13 years old	-0.625 (-1.364 to 0.114)	0.097
12 years old	-0.605 (-1.222 to 0.013)	0.055
11 years old	-0.435 (-1.049 to 0.179)	0.165
10 years old	-0.464 (-1.067 to 0.140)	0.132
9 years old	-0.719 (-1.321 to -0.117)	0.019
8 years old	-0.284 (-0.886 to 0.318)	0.355
7 years old	-0.139 (-0.753 to 0.475)	0.657
6 years old	-0.788 (-1.575 to -0.002)	0.050
Baseline anaemia(Reference category 'non anaemic')		
Anaemic	1.488 (1.323 to 1.653)	<0.001
Baseline haematuria (Reference category 'negative')		
Trace	0.106 (-0.204 to 0.415)	0.504
'+'	-0.029 (-0.404 to 0.345)	0.878
'++'	0.010 (-0.354 to 0.375)	0.955
'+++'	0.361 (0.019 to 0.702)	0.039
Baseline thinness or wasting (Reference category 'non wasted')		
Wasted	-0.057 (-0.226 to 0.112)	0.506
Baseline shortness or stunting (Reference category 'non stunted')		
Stunted	0.004 (-0.225 to 0.234)	0.971
Random effects	**Variance components (S.E)**	
Level-2 (i.e. between schools) variance	0.123 (0.062)	
Level-1 (i.e. between children within a school) variance	1.647 (0.070)	

4.4 Discussion

Among the different schistosomes infecting humans, *S. haematobium* is responsible for the largest number of infections. It has been estimated that in sub-Saharan Africa 112 million people are infected with *S. haematobium*, compared with 54 million infected with *S. mansoni* (van der Werf et al., 2003). However, *S. haematobium* remains largely unstudied, particularly in

comparison to *S. mansoni*, primarily due to the more demanding conditions for laboratory maintenance of the former. This is also reflected in the paucity of research examining the potential effectiveness of praziquantel against *S. haematobium* under various experimental and clinical circumstances (Botros et al., 2005). To my knowledge, the data presented in this Chapter represent the first longitudinal study in Africa that reports on the relationship between *S. haematobium* infection and its associated morbidity as a whole, by use of a uniquely detailed large dataset from 16 randomly selected schools across the entire national territory of Burkina Faso. Moreover, these data have the potential to provide important evidence characterizing urinary schistosomiasis-associated morbidity, particularly in a population such as that of Burkinabé, where the prevalence of hookworms and other soil-transmitted helminthiasis is estimated to be very low. Although previous studies have tended to focus on the impact of large-scale control programmes on intense transmission of *S. haematobium* infection solely with regard to Hb concentrations and anaemia (Guyatt et al., 2001; Nagi, 2005) or reference to parasitological measures only (Magnussen et al., 2001; Saathoff et al., 2004), or to *S. haematobium* morbidity indicators before and after treatment (Prual et al., 1992), the present study examines all these issues together and also adjusted for potential differences in demographic characteristics as well as potential confounders.

In this Chapter I have demonstrated that children with anaemia, or children with more severe microhaematuria scores at baseline, had higher *S. haematobium* infection intensities (Table 4.3, Figure 4.1), which suggests that heavy intensities of *S. haematobium* infection can be associated with anaemia and haematuria. I also provide evidence that heavy infections of urinary schistosomiasis are associated with lowered Hb concentrations and, as a consequence, with potential anaemia, given that the models in Table 4.5 indicate that *S. haematobium* infection might be associated with anaemia in 2 different ways. More precisely, haematuria which, as demonstrated previously is associated to *S. haematobium* infection, can be an important cause of blood and iron loss which also may lead to anaemia (Beasley et al., 1999; Nagi, 2005). The data used in the current study suggest significant reductions in the prevalence and, more importantly, intensity of infection of *S. haematobium* as well as in percentages of children with positive microhematuria scores 1 year after treatment (Tables 4.2 & 4.3).

The children who most benefited from anthelminthic treatment in terms of increased Hb concentration were those with anaemia at baseline and those who had '+++' microhaematuria scores at baseline (Table 4.6), which confirms similar findings in other endemic settings (Stephenson et al., 1985) and presented previously in Chapter 2 of this PhD thesis where chidlren's data from Uganda were examined. The mechanisms by which treatment for *S. haematobium* infection allows Hb concentrations to increase in children with anemia may be the decrease in blood in urine that results from reduction in the intensity of *S. haematobium* infection (Parraga et al., 1996).

Growth and nutritional status have been proposed to represent the most sensitive indicators of health in children (Stephenson, 1993). Furthermore, one of the factors emphasized in the World Development Report 1993 is the relationship between parasitic infections and malnutrition (World Bank, 1993). In this Chapter I also examined whether greater *S. haematobium* egg counts were associated with increased risks of wasting or stunting at baseline, adjusting for demographic characteristics and other potential predictors such as microhaematuria and anaemia status. The results of the current study did not suggest any significant association between the risk of undernutrition and intensity of *S. haematobium* infection, with only age being revealed as a significant factor. Younger children tended to be less likely to be wasted or stunted than the 14-year-old children, which could imply prior malnutrition in these older children as has been reported elsewhere (Stoltzfus et al., 1997). In the aforementioned study, the authors also reported that in Zanzibari boys, the association between microhaematuria and poor growth was only marginally significant (Stoltzfus et al., 1997) which is in line with the findings reported in the current Chapter that, in the Burkinabé children, changes in the BMIZ depended only on age, whereas changes in the HAZ scores depended on age and sex (Table 4.4). A more plausible explanation for the lack of statistical association between the intensity of *S. haematobium* infection and stunting may relate to dropout bias towards stunted children (Table 4.1); this means that it is difficult to make a definitive conclusion regarding the relationship between urinary schistosomiasis and stunting in the population examined here (Stephenson, 1993).

Nevertheless, it must be considered, as it has been mentioned previously in Chapter 2 of this PhD thesis that essential methodological constraints inherent in the present study design - in particular the lack of a control group, which was necessary for ethical reasons, could result in some potential bias towards the estimation of the absolute impact of the treatment, thereby allowing only the relative impact of the treatment in different groups of children to be calculated. However, even though these data were obtained from a large-scale control programme and studies such as the one presented here are generally difficult to execute in terms of design, methodology and sampling, I believe that these results will be of substantial value in estimating the total benefit that control of schistosomiasis can provide to communities (Kabatereine et al., 2007).

This study shows that praziquantel can have a substantial impact on *S. haematobium* infection and associated disease when delivered as part of a large-scale control programme in a country such as Burkina Faso, which was indeed the first country in the WHO African Region to achieve nationwide coverage with anthelminthic drugs against three major neglected tropical diseases: lymphatic filariasis, schistosomiasis and soil-transmitted helminthiasis (WHO Regional Office for Africa-press release). The findings presented in this Chapter suggest a dramatic reduction in the prevalence and intensity of *S. haematobium* infection, an improvement in Hb concentration in certain groups of children, and reductions in schistosome-related morbidity in a cohort of Burkinabé schoolchildren which demonstrate that mass chemotherapy can offer a practical strategy for the control of *S. haematobium* infection and its associated morbidity.

Chapter 5: Assessment of ultrasound morbidity indicators for schistosomiasis in the context of large-scale programmes, illustrated with experiences from Malian children

Summary

In the current Chapter, morbidity indicators for both *Schistosoma haematobium* and *Schistosoma mansoni* infections were assessed; the evaluation of the appropriateness of the WHO guidelines for ultrasound in schistosomiasis in the context of large-scale control interventions was another topic of this Chapter. Abdominal and urinary tract ultrasonography (US) was performed on 2,247 and 2,822 school children respectively from 29 randomly-selected schools in Mali, before the implementation of mass anthelminthic drug administration. Using two-level logistic regression models, associations of potential factors with the risk of having positive ultrasound global score (morbidity indicative of *S. haematobium* infection), abnormal image pattern scores, dilatation of the portal vein and/or enlarged liver (morbidity indicative of *S. mansoni* infection) were examined. The WHO protocol was found useful for detection of *S. haematobium* pathology but overestimated the risk of portal vein dilatation and left liver lobe enlargement associated with *S. mansoni* infection. In conclusion, I would recommend ultrasonography to be included in large-scale control interventions, where logistics allow, but cautiously.

Keywords: hierarchical models, ultrasound, Mali, *Schistosoma mansoni*, *Schistosoma haematobium*

Note: A modified version of this chapter has been published as: Koukounari A, Sacko M, Keita AD, Gabrielli AF, Landouré A, Dembelé R, Clements AC, Whawell S, Donnelly CA, Fenwick A, Traoré M, Webster JP. (2006) *Am J Trop Med* 75, 1042-52. See publications by the candidate (Appendix).

5.1 Introduction

As discussed in previous chapters of this PhD thesis, schistosomiasis remains one of the most prevalent parasitic diseases in developing countries and has significant economic and public health consequences (Chitsulo et al., 2000). Recently, it has been estimated that the urinary type, resulting from infection with *Schistosoma haematobium*, causes haematuria in 70 million people and major bladder wall pathology in 18 million people in sub-Saharan Africa (van der Werf et al., 2004). *Schistosoma mansoni*, one of the etiological agents of the intestinal type of schistosomiasis, is responsible for bloody diarrhea in an estimated 4.4 million individuals and 8.5 million people are estimated to have hepatomegaly due to the infection. The death rate due to *S. mansoni*-related haematemesis could be up to 130,000 per year in sub-Saharan Africa (van der Werf et al., 2004). King and others have argued convincingly that additional subtle morbidities (i.e. symptoms such as anaemia, chronic pain, diarrhea, exercise intolerance, growth stunting and/or nutritional and cognitive impairment which have been difficult so far to quantify and are based on observed association rather than established causality) should be added to these estimates of disease burden (King et al., 2005).

Since 1984 the WHO Expert Committee on the Control of Schistosomiasis has recommended a strategy for morbidity control, which is now feasible because of the availability of effective, affordable and safe single-dose drugs (World Health Organisation, 1985). As a consequence, since 2003, the Schistosomiasis Control Initiative (SCI) has assisted six sub-Saharan African countries to develop and implement schistosomiasis morbidity control programmes, through the provision of health education and mass treatment, using praziquantel for schistosomiasis and co-administering, where appropriate, albendazole for soil-transmitted helminthiasis. The primary objective of these SCI-supported control programmes - as already discussed in previous chapters of this PhD thesis - is to achieve, and hence also demonstrate such an achievement, a quantifiable reduction in schistosome-associated morbidity as a consequence of such chemotherapeutic intervention. Inherent within such an objective, it is therefore imperative to both define and characterize pre-treatment baseline morbidity levels within the *risk* populations, so that any subsequent changes in morbidity due to the intervention can be accurately determined (Gryseels & Polderman, 1991). Furthermore, identification of sensitive and specific indicators of

schistosome-associated morbidity, that may be practically implemented within such large scale-control programmes, as distinct from the individual clinical or research-based setting, should also prove invaluable in assisting identification of target populations for ongoing and future intervention (Kariuki et al., 2001). Campagne and others also emphasized the need to validate indirect morbidity indicators in order to know the development of their predictive value during different stages of a schistosomiasis control programme (Campagne et al., 2001).

Ultrasonography is currently the diagnostic tool of choice for detecting pathologic conditions associated with schistosomiasis, such as dilatation of the renal pelvis, bladder wall lesions, liver fibrosis and enlargement, and dilatation of the portal vein (Richter et al., 2003; Kabatereine et al., 2004). For detection of infection from *S. haematobium,* ultrasonography is an established method for detecting urinary tract pathology not only in the hospital setting (Abdel-Wahab et al., 1978; Mongy et al., 1978; Browning et al., 1984; Devidas et al., 1989) but also in field-based studies (Hatz et al., 1992), with the advantage of being non-invasive, relatively simple to perform and very well accepted by communities, as well as of providing a direct image of the pathological changes (Hatz et al., 1990). Additionally, ultrasonography gives sensitive and precise measurements of *S. mansoni*-associated pathologic changes (Richter et al., 2001; King et al., 2003). In the attempt to objectively define and categorize the pathological changes associated with schistosomiasis, and to standardize the different scoring systems used in the past in different endemic areas (Richter et al., 2000; Hatz, 2001), successive "ultrasound consensus" meetings were held in Niamey, Niger in 1996 and Belo Horizonte, Brazil in 1997, leading to the revision of standardized scoring protocols and the development of the WHO-recommended US protocol (Niamey-Belo Horizonte protocol) (Richter et al., 2000). Nevertheless, the prognostic features of individual ultrasonography findings in different disease-endemic situations (King et al., 2003), as well as whether ultrasonography can be incorporated into a mass chemotherapy programme in order to monitor morbidity, are still to be confirmed.

The aim of this study was to assess indicators of US-detectable morbidity due to infection with both *S. haematobium* and *S. mansoni* in the context of large-scale control interventions targeting school-age children in Mali, prior to large-scale administration of praziquantel by the National Schistosomiasis Control Programme with support from SCI. In Mali, both *S. haematobium* and

S. mansoni pose serious public health problems (Coulibaly et al., 2004). Fishing, market gardening and rice cultivation all expose the population to the risk of occupational transmission, while children are regularly exposed through bathing and playing in ponds, streams and rivers. *S. haematobium* transmission is more widespread, occurring along river and streams as well as around ponds and in irrigation schemes (Vercruysse et al., 1994; Traoré et al., 1998). The geographic distribution of *S. mansoni* infections is more restricted, mainly occurring in irrigation schemes, such as Office du Niger, Bandiagara, Sélingue and Baguinéda-Koulikoro (Vercruysse et al., 1994; Keita et al., 2005).

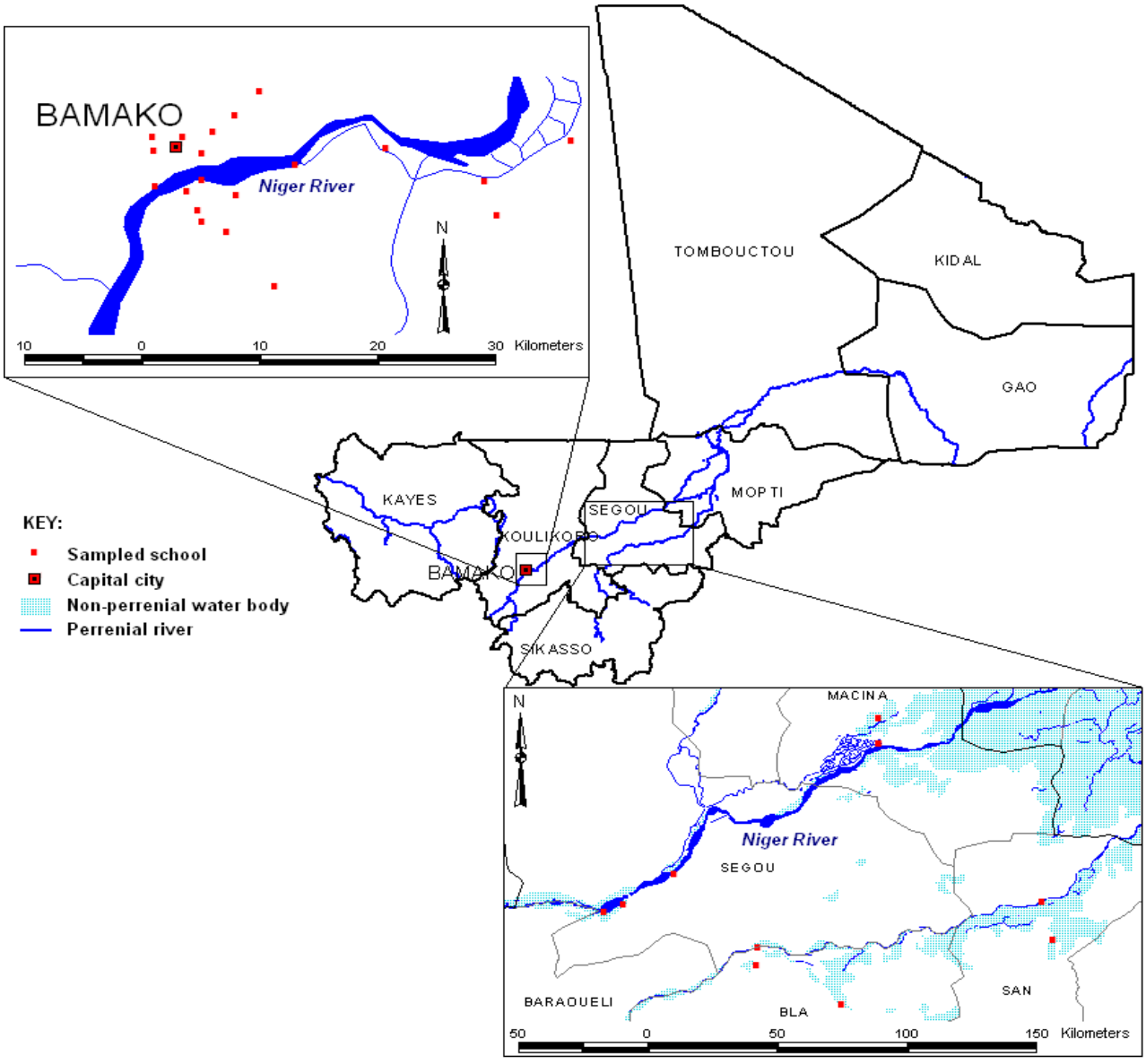
The results obtained here should contribute to evaluate the appropriateness and the role of the full WHO protocol in the context of large-scale schistosomiasis control programmes. Another aim of this study was to determine whether the numeric WHO cut-offs, originally derived from a healthy Senegalese population in a non-endemic *S. mansoni* area, contain bias in the estimation of the risk of dilatation of portal vein and enlargement of left liver lobe in a Malian setting. This will be achieved here by comparison with local height-indexing of portal vein diameter (PVD) scores and longitudinal parasternal line scores (PSL) respectively, obtained from all children who had normal image patterns as assessed by US following recommendations of King and others (King et al., 2003).

5.2 Materials and methods

5.2.1 Study sites, sampling and design. The Malian Ministry of Health, through its National Institute of Research in Public Health (*Institut National de Recherche en Santé Publique (INRSP)*) and its Disease Prevention and Control Division (*Direction Nationale de la Santé*), was charged with planning and implementing data collection with assistance from SCI. Baseline data collection was initiated in the Ségou region in late March 2004 and was completed in Bamako in the month of June 2004. Ultrasonographic examination based on the WHO protocol was performed on children aged 7 to 14 (2,841 for *S. haematobium* and 2,820 for *S. mansoni*) from 29 schools. These schools were randomly-selected from all schools located in three areas highly endemic for schistosomiasis: Bamako, Ségou and Baguinéda-Koulikoro (Figure 5.1). All children enrolled in the study were interviewed by appropriately trained

Ministry of Health staff. Ethical clearance was obtained from the Ministry of Health, Mali and Imperial College, London.

Figure 5.1 Location of sampled schools in Mali. Schools in Bamako and Koulikoro are in the upper box and schools in Ségou are in the lower box.



In schools from areas known to be endemic only for *S. haematobium*, the estimated sample size was calculated to be 180 individuals per sampling unit (usually a school) with 80 % power to detect a 70 % reduction in *S. haematobium* mean intensity over a two-year period (two annual treatments). The expected reductions of 70 % in *S. haematobium* and 60 % in *S. mansoni* mean intensities over 2 annual treatments were calculated using the EpiSchisto software tool (http://www.schoolsandhealth.org/epidynamics.htm). The figure of 180 was selected based on prevalence/intensity data from schools in various African countries with similar age ranges.

Where both *S. haematobium* and *S. mansoni* infections were known *a priori* to be prevalent, the same methodology as for *S. haematobium* only was followed, although the number of surveyed children was increased to 300 per school with 80 % power because EpiSchisto simulations showed an expected reduction in mean *S. mansoni* intensity of 60 %. No schools were included in the present study in which *S. mansoni* infection only was prevalent.

Whenever it was difficult to recruit the required number of children in any one school (usually because of the small size of the school), data from two (or more) adjacent schools were combined, provided that they appeared to be ecologically similar (e.g. with the same relative proximity to the nearest supposed focus of transmission). For ethical reasons, it was not appropriate to include any untreated control groups. Further technical details concerning the sample size calculations can be found elsewhere (Brooker et al., 2004).

5.2.2 Parasitologic examination. From each child, two urine specimens were collected on two consecutive days to determine the intensity of *S. haematobium* infection using filtration method. 10 ml of urine were passed through a Whatman (Brentford, United Kingdom) filter Paper (Ø=25mm) using a Millipore (Billerica, MA) Swinnex® filter support. Filters were stained with 3 % ninhydrine and microscopically examined for eggs. The intensity of *S. haematobium* infection was expressed as number of eggs per 10 ml of urine and the mean intensity of infection was the arithmetic mean of egg counts in the two urine samples. To determine the presence and extent of microhaematuria (invisible haematuria), all urine specimens were tested for presence of detectable blood with urine reagent strips (Bayer Hemastix®). The results were recorded semi-quantitatively: negative, +, ++ and +++. Additionally, two faecal

specimens of 41.7 mg each were screened for *S. mansoni* by the Kato-Katz technique (Kato & Miura, 1954; Katz et al., 1972; Feldmeier & Poggensee, 1993). Individual egg output was expressed as eggs per gram of faeces (epg), calculated as the arithmetic mean of the two individual slide counts.

5.2.3 Ultrasound examinations. Ultrasonographic assessments were performed with a portable ultrasonography device (Aloka SSD-500®). A convex 3.5 Mhz transducer was used to detect pathological changes associated with both *S. haematobium* and *S. mansoni* infection. All examinations were performed by the same clinician Prof Adama Keita, who was blind to schistosome infections status of the individual children. US examinations were performed according to current WHO guidelines (Richter et al., 2000).

Pathologic changes caused by *S. haematobium* was assessed by recording the shape of the urinary bladder, defining any lesions detected on the bladder wall and measuring the degree of dilation of the ureters and renal pelvis. The exact coding of each of the above characteristics was made according to the recommendations of Richter and others (Richter et al., 2000). Further categorization of pathological changes was performed by calculating the "global score" which serves as an index of severity of morbidity and lesions. Children were provided with water and asked to drink abundantly before undertaking US examination, which took place only when the bladder was filled. In case of detection of dilatation of the renal pelvis, which is suggestive of hydronephrosis, the child was re-examined after urination in order to rule out the possibility that such dilatation was due to bladder and urethral repletion.

To characterize morbidity caused by *S. mansoni*, the size of the left liver lobe was measured in PSL. Measurements of PVD were also recorded. Liver patterns were graded from A to F, in order of the severity of the pathology they indicate. B0, B1 and B2 are most often grouped together, as are C1 and C2. It should be noted, however, that the SCI protocol did not include periportal thickening measurements, due to concerns about both the reproducibility of measurements (Richter et al., 2001) and the time-consuming component of the examination. Therefore, interpretation of the final score for morbidity due to *S. mansoni* infection was based on assessment of image patterns and portal hypertension only. Presence of ascites and

portosystemic collaterals was also recorded. Detection of pathologic changes not caused by schistosomiasis was also recorded but is not discussed in the present study. Individuals in need of health care were directed to the nearest medical facility.

5.2.4. Ultrasonography protocol definitions. The WHO protocol states that measurements of organ size and vein diameter should be height-adjusted, using standard reference measurements for healthy members of the same population group (Richter et al., 2000). King and others, found that the numeric WHO cut-offs derived from a healthy Senegalese population in an area not endemic for *S. mansoni*, seriously overestimated the risk of portal vein enlargement in Kenyan and Egyptian patients infected with *S. mansoni* (King et al., 2003). In the current Chapter, I have also investigated this issue in a Malian setting as the Niamey workshop members anticipated the refinement of the guidelines through continued use and evaluation, by using alternative height-indexing of portal vein diameter scores obtained from all children that had normal image patterns as assessed by ultrasonography (n=2719). With reference to the PSL measurement, the liver was considered enlarged, or much enlarged, if the height-adjusted value exceeded 2 or 4 SD in relation to the 'normogram' produced for a Senegalese population respectively (Yazdanpanah et al., 1997). In addition, I also calculated local cut-off scores for liver left lobe enlargement and verified if the overestimation also applied to this parameter.

At the end of all examinations, each child enrolled in the survey was treated with the WHO-recommended dose of praziquantel (40mg/kg) for schistosomiasis and with albendazole 400 mg against intestinal helminths. Side-effects were monitored, and adverse reactions following drug administration were infrequent: when present, these were minimal and transient, and no severe adverse experiences were observed.

5.2.5. Statistical analysis. Data from children with incomplete parasitologic or ultrasonographic records were excluded from analysis presented here and no replacements were made for missing subjects under the assumption that data were missing at random (Carpenter & Kenward, 2005). Descriptive statistics for subject characteristics and outcomes were calculated using SAS version 8 (SAS Institute Inc., Cary, NC, USA).

To examine *S. haematobium* morbidity, I modelled the probability of a child having a positive individual global score using hierarchical multi-variable logistic regression. Potential predictors included *S. haematobium* infection intensity category [light (defined as < 50 schistosome eggs per 10 ml of urine); or heavy (≥ 50 eggs/10 ml) (WHO Expert Committee on the Control of Schistosomiasis, 2002), microhaematuria, gender, and age. The model structure was a two-level random intercept logistic regression model with level-one defined as the individual child and level-two the school, allowing for assessment of the extent of between-school variation in individual global scores. The model had the form:

$$\log[\pi_{ij} / (1 - \pi_{ij})] = x_{ij}a + w_j b + u_j + e_{ij}$$

where π_{ij} is the probability that child i in school j has a positive individual global score; x_{ij} and w_j are vectors of individual- and school-level characteristics respectively; a and b are vectors of estimated parameter coefficients; $u_j (\sim Normal(0, \sigma_u^2))$ is an error term at the school level; and $e_{ij} (\sim Normal(0, \sigma_e^2))$ is an error term at individual level.

To study the morbidity of *S. mansoni*, I used three hierarchical multi-variable logistic regression models where I aimed to model: (1) the probability of having abnormal image pattern scores; (2) the probability of having dilatation or marked dilatation as assessed by the PVD based on the Malian cut-off scores; and (3) the probability of having enlarged liver as assessed by the PSL measurements based on the Malian cut-off scores. Potential predictors included *S. mansoni* infection intensity category [light (defined as 1 to 99 eggs per gram (epg) of faeces); moderate (100 to 399 epg), or heavy (≥ 400 epg) (WHO Expert Committee on the Control of Schistosomiasis, 2002), gender, and age. The structure and form of the model used to assess each of these *S. mansoni* morbidity indicators, were identical to those used for evaluation of *S. haematobium*.

All four models were constructed using the Mlwin software (version 2.01, Multilevel Models Project, Institute of Education, University of London, UK). The method of estimation was the

second order penalized quasi-likelihood procedure (Goldstein & Rasbash, 1996), and first order marginal quasi-likelihood (MQL) estimates were used to provide the starting values for the estimation procedure, the stability of the algorithm and convergence criteria (Rasbash et al., 2004). The model structure was selected because of the hierarchical nature of the dataset, i.e. children were clustered in schools and observations from individuals within the same school were therefore not independent. As discussed in previous chapters of this PhD thesis, multilevel models account for this dependence by partitioning the total variance in the data into variation between and variation within the higher-level units (Goldstein et al., 2002). While partitioning of variance is straightforward in models with a continuous dependent variable and with a normally-distributed error at each level of the hierarchy, their extension to models with binary responses is more problematic. For the school effect in each model I calculated the median odds ratio (MOR) to quantify the variation between schools (Larsen et al., 2000; Larsen & Merlo, 2005). The MOR is always ≥ 1 and directly comparable with fixed-effects odds ratios. More precisely, if the MOR is = 1, there is no variation between clusters (no second-level variation). If there is considerable between cluster variation, the MOR will be large.

This quantification of the heterogeneity of the schools is not simply of technical value; the apportioned variances are of substantive interest in much of biomedical research as they give important insights to the level 'at which the action lies' (Browne et al., 2005) and for epidemiologic reasons (in this case quantification of the importance of the schools for understanding individual health) (Larsen & Merlo, 2005). The percentage of total variation in the ultrasonographic global scores as well as in the liver image patterns, the PVD and the PSL, which are explained by each of the corresponding models presented here, was estimated using an R^2 measure developed by Snidjers and Boskers (Snidjers & Bosker, 1999).

5.3 Results

Schistosoma haematobium was prevalent in all the 29 schools surveyed, and *S. mansoni* was prevalent in 25 of these schools.

5.3.1 *Schistosoma haematobium*. Ultrasonographic examination was performed on 2,841 children. Of these, parasitological data were obtained from 2,822 children. In 136 (4.8 %) of 2,822 there was no second examination of urine and prevalence and mean intensity calculations were based on one urine filtration result for these children. Overall, *S. haematobium* prevalence of infection was 59% and the arithmetic mean intensity was 43.0 eggs/10 ml of urine. At school-level, prevalence of infection ranged from 10.8% to 100.0% and arithmetic mean intensity from 0.7 to 202.5 eggs/10 ml of urine. Girls accounted for 53% of children in the survey and approximately equal numbers of children were recruited in each year group from 7 to 14 years of age. Bladder wall thickening and irregularities, bladder masses and pseudo-polyps were found in 6.0% of the children. The prevalence of upper urinary tract (kidney) pathology was estimated to be 3.7%. The prevalence of positive global scores was estimated to be 10.1%, while at the school-level this ranged from 1.0% to 61.4%.

The odds ratios (ORs) from two-level logistic regression analysis for the probability of having a positive ultrasonographic global score are shown in Table 5.1. Children with either light or heavy *S. haematobium* infection intensities were more likely to have a positive ultrasonographic global score than uninfected children (light: OR=3.0, p=0.004 and heavy: OR=6.9, p<0.001). Children with '+', '++' and '+++' microhematuria scores were significantly more likely to have positive schistosomiasis ultrasonographic global scores compared to microhematuria-negative children (OR=2.5, p=0.003; OR=3.1, p<0.001 and OR=5.0, p<0.001 respectively). Boys showed significantly higher ultrasonographic morbidity global scores than girls (OR = 2.0, p<0.001). Age was not a statistically significant indicator of *S. haematobium* morbidity, although there was a trend for older children to be more likely to have a positive schistosomiasis ultrasonographic global score. This table also shows that a relatively high MOR (2.6) is associated with between-school variation. Of the total variation in the global US score, 13.7% remained unexplained at the school-level and 45.7 % remained unexplained at the child-level.

5.3.2 *Schistosoma mansoni*. Both ultrasonographic and parasitologic data were obtained from 2,247 (79.7%) of 2,820 children. Overall prevalence of infection was 27% and the overall arithmetic mean intensity was 119.5 epg. Calculations were based on two faecal smear examinations from all but four children, for whom the second measurement was missing. School-level prevalence of infection ranged from 0.0% to 96.0% and arithmetic mean intensity from 0.0 to 814.9 epg.

A total of 2,820 children were examined by ultrasonography for *S. mansoni*-related pathologic changes. Of these children, 96% had normal liver as assessed by liver image patterns. Of the children who had abnormal liver image patterns, 84% had grade B patterns and 16% had grade C patterns. Figure 5.2 shows that using the current WHO cut-off, 85 % of children had a 0 PVD score. In contrast, 96% children had a 0 PVD score using the cut-off derived from the data from the Malian children with normal liver image patterns was used. The difference between these two proportions was significant ($p<0.001$). Statistically significant differences were also found between the proportions of children allocated positive PVD scores of four and six using the two different cut-offs (both $p<0.001$).

Table 5.1 Odds ratios (OR) with 95% CI estimates and percentage of variation explained for two-level logistic model of prevalence of positive global score as measured by ultrasound using data set of children with complete parasitologic and ultrasound data on *S. haematobium* infection (n = 2,822)

Fixed effects				
Variables	**Categories**	**Odds ratios**	**95% CI**	**p-value**
Age	7 years old	1		
	8 years old	1.200	0.632 - 2.277	0.578
	9 years old	0.932	0.481 - 1.808	0.836
	10 years old	1.369	0.743 - 2.523	0.314
	11 years old	1.537	0.836 - 2.828	0.168
	12 years old	1.231	0.659 - 2.301	0.515
	13 years old	1.226	0.659 - 2.283	0.520
	14 years old	1.626	0.865 - 3.056	0.130
S. haematobium intensity infection	Not Infected	1		
	Lightly Infected	3.004	1.424 – 6.339	0.004
	Heavily Infected	6.862	3.036 - 15.508	< 0.001
Gender	Female	1		
	Male	2.026	1.489 - 2.756	< 0.001
Results of Hemastix test	Negative	1		
	Trace Hemolysed	1.600	0.781 - 3.278	0.199
	'+'	2.479	1.361 - 4.517	0.003
	'++'	3.068	1.751 - 5.374	< 0.001
	'+++'	5.078	3.093 - 8.338	< 0.001
Random effects				
School		**Median odds ratio**		
		2.572		
Variation	%			
Total variance explained	40.63			
Total variance unexplained				
at school-level	13.66			
at child level	45.71			

In 99.9% of the children, no collateral vessels were detected and no free fluid was detected in abdomen. Figure 5.3 shows that 50% of the children in the ultrasonographic cohort had left lobe hepatomegaly as assessed by PSL using the current WHO cut-off value. A total of 41% of the children had an enlarged liver and 8% had a greatly enlarged liver. Conversely, 99% did not have an enlarged liver when the cut-off value derived from the Malian children with normal liver image patterns was used. Statistically significant differences were found between all proportions of children allocated null or positive PSL scores of one and two using the two different cut-off values ($p<0.001$).

Figure 5.2 Percentages of children with Portal Vein Scores based on different cut-off values

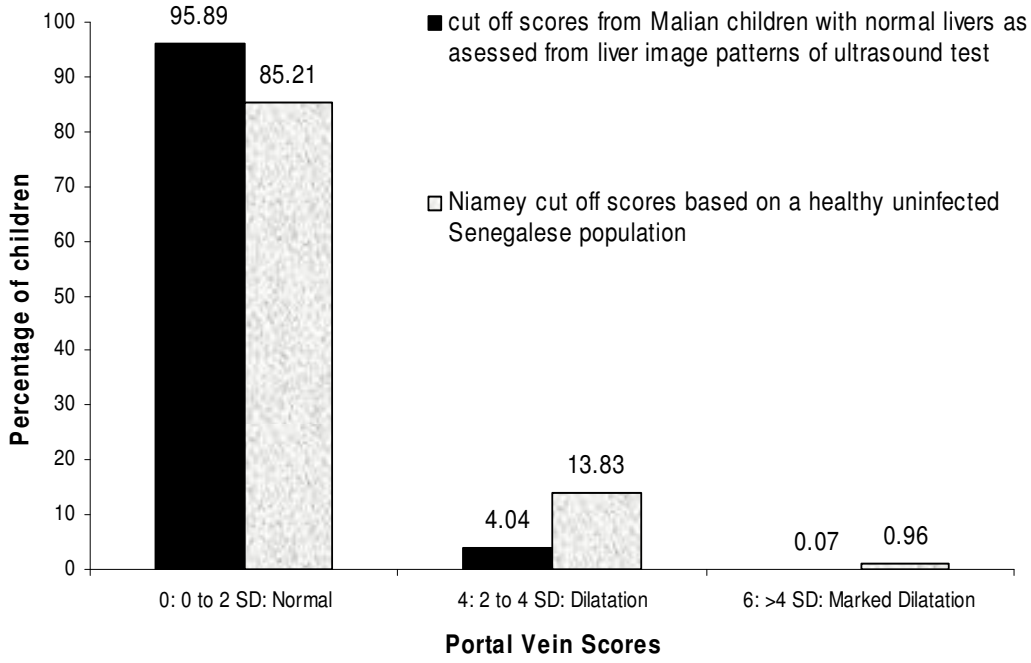


Table 5.2 shows the ORs from two-level logistic regression analysis for the probability of having abnormal image pattern scores as assessed from ultrasonography for *S. mansoni* infection. Children with light, moderate or heavy *S. mansoni* infection intensities were more likely to have an abnormal liver image pattern than uninfected children (light: OR=2.6, p=0.023; moderate: OR= 1.3; p=0.62 and heavy: OR=3.1, p=0.036). There was a trend for older children to be less likely to have an abnormal liver image pattern, but these differences were only significant for 11-, 12- and 14-year-olds, relative to 7-year-old children (OR=0.3, p=0.027; OR=0.3, p=0.036 and OR=0.2, p=0.016 respectively). Table 5.2 also shows that a high MOR (13.4) was associated with between-school variation. Of the total variation in the liver image pattern, 67.0% remained unexplained at the school level and 30.0% remained unexplained at the child level.

Figure 5.3 Percentages of children with Longitudinal Parasternal Scores based on different cut-off values

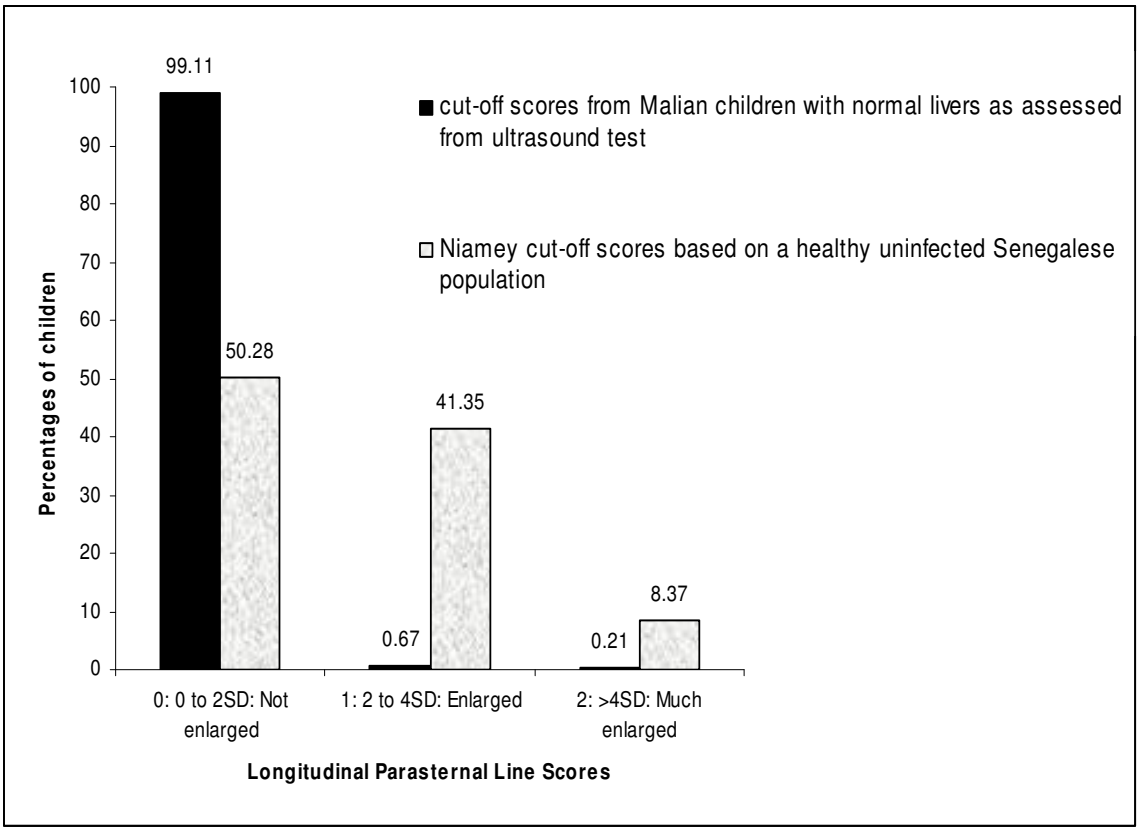


Table 5.2 Odds ratios (OR) with 95% CI estimates and percentage of variation explained for two-level logistic model of prevalence of abnormal liver image patterns as measured by ultrasound using data set of children with complete parasitologic and ultrasound data on *S. mansoni* infection (n = 2,247)

Fixed effects				
Variables	**Categories**	**Odds ratios**	**95% CI**	**p-value**
Age	7 years old	1		
	8 years old	0.550	0.209 - 1.450	0.226
	9 years old	0.412	0.152 - 1.120	0.082
	10 years old	0.523	0.202 - 1.353	0.182
	11 years old	0.305	0.107 - 0.875	0.027
	12 years old	0.323	0.112 - 0.926	0.036
	13 years old	0.516	0.189 - 1.404	0.196
	14 years old	0.224	0.066 - 0.755	0.016
S. mansoni intensity infection	Not Infected	1		
	Lightly Infected	2.622	1.144 - 6.008	0.023
	Moderately Infected	1.336	0.423 - 4.223	0.621
	Heavily Infected	3.099	1.080 -8.895	0.036
Gender	Female	1		
	Male	1.520	0.873 - 2.648	0.138
Random effects				
School		**Median odds ratio**		
		13.444		
Variation	%			
Total variance explained	3.388			
Total variance unexplained				
at school-level	66.981			
at child level	29.631			

Table 5.3 Odds ratios (OR) with 95% CI estimates and percentage of variation explained for two-level logistic model of prevalence of having dilatation or marked dilatation as assessed by the ultrasound PVD score based on the Malian cut-off scores by using data set of children with complete parasitological and ultrasound data on *S. mansoni* infection (n=2,247)

Fixed effects				
Variables	**Categories**	**Odds ratios**	**95 % CI**	**p-value**
Age	7 years old	1		
	8 years old	1.567	0.444 - 5.525	0.484
	9 years old	3.640	1.159 - 11.435	0.027
	10 years old	3.245	1.035 - 10.172	0.043
	11 years old	2.835	0.887 - 9.064	0.079
	12 years old	1.980	0.586 - 6.687	0.271
	13 years old	3.647	1.134 - 11.730	0.030
	14 years old	4.655	1.462 – 14.826	0.009
S. mansoni intensity infection	Not Infected	1		
	Lightly Infected	1.402	0.619 - 3.175	0.418
	Moderately Infected	1.408	0.547 - 3.621	0.478
	Heavily Infected	0.774	0.303 -1.979	0.593
Gender	Female	1		
	Male	2.250	1.373 - 3.687	0.001
Random effects				
School		**Median odds ratio**		
		4.364		
Variation	%			
Total variance explained	6.480			
Total variance unexplained				
at school-level	39.360			
at child level	54.159			

Table 5.3 shows the ORs from two-level logistic regression analysis for the probability of having dilatation or marked dilatation as assessed by ultrasonography for *S. mansoni* infection PVD height-adjusted measurements based on the Malian cut-off value. The ORs of having dilatation or marked dilatation as assessed by ultrasonographic PVD measurements for children with light, moderate or heavy *S. mansoni* infection intensities were not significantly different compared to uninfected children (P=0.418, 0.478 and 0.593 respectively). There was a trend for older children to be more likely to have increased PVD, but these differences were only significant for 9-, 10-, 13- and 14-year-old children, compared with 7-year-old children. (OR=3.7, p=0.027; OR=3.3, p=0.043; OR=3.7, p=0.030 and OR=4.7, p=0.009 respectively). The MOR to have an increased PVD was 4.4, which is still high OR and is associated with between-school variation. Of the total variation in the

PVD, 39.0% remained unexplained at the school level and 54.2% remained unexplained at the child level.

Relative to modeling of the probability of having an enlarged liver as assessed by the PSL measurements based on the Malian cut-off scores, the algorithm did not converge. Therefore, I was unable to provide any estimates and establish any associations for this measure.

5.4 Discussion

Ultrasonography has become an invaluable extension of the clinical investigation of patients with schistosomiasis and has provided direct evidence of the pathological changes associated with this infection (Hatz, 2001;Brouwer et al., 2004). This evidence has been well validated in the individual patient clinical setting (Bahakim et al., 1986; Abdel-Wahab & Strickland, 1993; Lougue-Sorgho et al., 2002; de Celis et al., 2003) and the relatively small-scale research setting (Friis et al., 1996; Leutscher et al., 2000; van der Werf et al., 2004; Vennervald et al., 2005). However, the overall aim of this study was to test the suitability of the full WHO-recommended ultrasonography protocol in the context of large-scale schistosomiasis control programmes. There is a requirement to elucidate whether ultrasonography could, and indeed should, be incorporated into a mass chemotherapy programme in order to monitor morbidity associated with *S. haematobium* infection and, perhaps in particular, morbidity associated with the often more difficult to characterize *S. mansoni* infections in all but the most severe cases (Lengeler et al., 1991; Utzinger et al., 1998).

The current study complements and furthermore expands upon previous ultrasonography-based studies within Africa on a number of aspects. First, although previous published surveys (Kardorff et al., 1994; Kardorff et al., 1996; Kardorff et al., 1997; Vester et al., 1997; Frenzel et al., 1999; Boisier et al., 2001; King et al., 2003) have used ultrasonography to measure schistosomiasis-associated morbidity both in children and in adults, indicative of long-term chronic infections, in this Chapter I assess ultrasonography in monitoring schistosomiasis morbidity for control programmes focused on children. Although I recognize that measuring only children might be a limitation, if one considers the overall aim of this study, the results of the current Chapter still contribute to assessing the suitability of ultrasonography for more recent infections and targeting age groups for future

disease control programmes. This study should also provide a unique opportunity to clarify the relationship of early lesions to later ones through a subsequent comparison of the baseline findings presented here, particularly where there are identifiable cohorts, over extended period of time. Moreover, in terms of size of cohorts, a much larger number of individuals than typical of previous research or clinical-based studies is followed and used here. Finally, the current study has methodological advantages, particularly since I account for the interdependence of observations by partitioning the total variance into different components of variation due to various cluster levels in the data.

Children are probably the most important age group for ultrasound detectable morbidity due to *S. haematobium* and the results obtained from Mali confirm that the current WHO protocol (Niamey-Belo Horizonte protocol) is a suitable and valid public health tool because its scoring criteria performed acceptably well in defining ultrasound pathology due to urinary schistosomiasis. Sophisticated statistical models yielded significant associations between the global ultrasonography scores from the WHO protocol and several other morbidity predictors. A significant association between the degree of morbidity as defined by ultrasonography global scores and *S. haematobium* infection intensity and microhematuria was demonstrated. Boys had a higher prevalence of morbidity than girls and this has also been observed in the studies from Heurtier and others and Keita and others (Heurtier et al., 1986; Keita et al., 2001). The results reported in this Chapter also indicated that there was considerable variation between schools in the prevalence of positive global scores, thus showing the focal clustering of morbidity due to urinary schistosomiasis also in areas of overall intense transmission. In conclusion, ultrasonography global scores and microhematuria scores are likely to be valuable markers in children for morbidity caused by both light and heavy infections with *S. haematobium*. The inclusion of ultrasonographic examinations in the routine monitoring and evaluation activities of control programmes against urinary schistosomiasis whenever resources are available, as in the case of middle-income countries (i.e. North and South Africa and potentially also some Middle-Eastern countries such as Iraq), is therefore recommended. In sub-Saharan Africa, such a recommendation should be weighted against the additional costs that a subsequent decision would bring (equipment, personnel, training) and the available national or external funds of the control programs.

With regards to intestinal schistosomiasis, the significant associations observed between liver image patterns with *S. mansoni* intensity of infection confirms that liver image patterns are likely to be valuable markers for morbidity caused by light or heavy infections with *S. mansoni,* as suggested by King and others (King et al., 2003). However although findings reported in this Chapter suggest that the current scoring criteria perform acceptably well in defining disease caused by *S. haematobium* infection, they also show in accordance with those of King and others (King et al., 2003), that the current WHO cut-offs can lead to serious overestimation of the risk of portal vein enlargement in patients infected with *S. mansoni*. In addition, data used in this Chapter show that also the risk of left liver lobe enlargement may be overestimated by the WHO cut-offs. Therefore, cut-offs norms should be recalculated at least in each endemic country from a subset of local individuals with 'pattern A' prior to the implementation of the WHO protocol which also fulfills the recommendations included in the Niamey-Belo Horizonte protocol guidelines. Further studies to confirm appropriate cut-off scores for these measurements are therefore required.

Nevertheless, the observation that much of the variability in the liver image pattern remained unexplained, as well as the very high MOR, suggests that other variables might be needed to explain the between school heterogeneity. Another explanation for this very high MOR and unexplained variation might be that among children with abnormal liver image patterns, 84 % were found to have grade B (coding for the earliest pathologic changes in the liver) which may not be schistosomiasis specific. This same fact might also explain the unexpected finding here that older children have less likelihood of abnormal patterns than younger ones compared with other studies of schistosomiasis morbidity. Therefore, liver image patterns of grade B may have represented a confounding factor in the analysis presented in the current Chapter. Further studies are therefore needed to fully elucidate the relationship between liver fibrosis and schistosomiasis, with particular reference to the degree of association between pattern B and *S. mansoni* infection and to the role played by other factors in determining such fibrosis. Likewise, in the case of hepatomegaly, I was unable to distinguish clear associations, which may have been due to the fact that these observed morbidities were likely to have been multifactorial, with *S. mansoni* infection being only one of a number of potential causes (Kabatereine et al., 2004). There are often many factors (genetic and possibly most importantly malaria which is transmitted throughout the year in the whole of Mali) other than *S. mansoni* that can

determine liver enlargement, and their role and interaction with *S. mansoni* infection also requires further clarification.

Although these data are on children and as such were expected to be less likely to demonstrate ultrasonography-detectable morbidity due to *S.mansoni* infection because of the amount of time of exposure associated with the time taken for fibrosis to build up, in contrast, they do show morbidity for this type of infection, suggesting that in these communities children may become infected early in life (Odogwu et al., 2006). I predict that, in adults, the dynamics of exposure, treatment and host immunity would show even more ultrasonography-detectable *S.mansoni* morbidity than observed in this study. It might also be important to include the periportal thickening measurement in the ultrasonographic examinations when the adult population is examined to evaluate the performance of the whole protocol. I predict that in adults infected with *S .mansoni*, the comparison between Malian and WHO cut-off values (derived in a similar way as previously described), would show significant differences in the estimations of risk of PVD and left liver lobe enlargement, since King and others (King et al., 2003) also observe the same pattern irrespective of age.

For *S.haematobium*, as in high transmission areas such as those under study here, successive episodes of infection would result in recrudescence of urinary tract abnormalities detected by ultrasonography (Hatz et al., 1998), and I would expect to observe more severe pathology due to urinary schistosomiasis in young adults because of continuing reinfection. However, ultrasonography may not be the most appropriate tool to detect and define late-stage morbidity due to *S. haematobium* infection in older adults, because of decreased rates of reinfection in this age group, which leads to decreased development of new inflammatory bladder wall lesions pathognomonic of urinary schistosomiasis. Thus, it would be interesting to conduct a survey on adults from the same communities of children described in this Chapter, and to investigate up to which age group ultrasonography is a suitable tool to monitor morbidity due to urinary schistosomiasis in a field setting.

Thus for both intestinal and urinary schistosomiasis, it will be necessary to obtain longitudinal data to fully elucidate the natural history of morbidity related to infection, with the aim of formulating recommendations for treatment and retreatment based on such natural history and especially on its

evolution after large-scale administration of anthelminthic drugs. Such work has been recently completed by INRSP, SCI and the National Schistosomiasis Control Programme (PNLSH) and analysis is underway and hopefully it will help the planning and evaluation of sustainable morbidity control.

Conversely, if only parasitological measurements were incorporated into the monitoring process of schistosomiasis morbidity of a mass chemotherapy programme, the following three issues should be taken into consideration (Hatz et al., 1990): 1) Diagnosis of infected individuals might be missed because of substantial day-to-day variation of egg output more importantly in *S. mansoni* and then in *S. haematobium* infections; some of the subjects could lack shed eggs at the time of the stool or the urine examination or simply eggs could be missed; 2) Signs of disease could still be present even in the true absence of egg excretion. Eggs could be trapped in lesions, especially in long standing infections. Just after treatment, eggs could also be absent, but lesions would still be present. In this case ultrasonography would still provide detection of irreversible lesions long time after treatment; 3) Confounding causes other than schistosomiasis on observed pathologic signs could be excluded by ultrasonography. Epidemiological importance of confounding causes of uropathy in areas where *S. haematobium* is endemic appears to be small, but reference to *S. mansoni*, information on this point is still lacking.

In conclusion, the results of this study suggest that the current WHO protocol (Niamey-Belo Horizonte protocol) is a suitable and valid public health tool for urinary schistosomiasis for morbidity control programmes focused on children. In the case of morbidity detection of intestinal schistosomiasis in large scale control interventions this same protocol is a useful tool provided that local cut-off values are used to define abnormal values and that results are interpreted with caution.

Chapter 6: Sensitivities and specificities of diagnostic tests and infection prevalence of *Schistosoma haematobium* estimated from data on adults in villages northwest of Accra in Ghana.

Summary

Substantial uncertainties surround the sensitivities and specificities of diagnostic techniques for urinary schistosomiasis. I used latent class (LC) modeling to address this problem. In this study 220 adults in three villages northwest of Accra region in Ghana were examined using five *Schistosoma haematobium* diagnostic measures: microscopic examination of urine for detection of *S. haematobium* eggs, dipsticks for detection of haematuria, tests for circulating antigens, serological antibody tests and ultrasound scans of the urinary system. Testing of the LC model indicated non-invariance of the performance of the diagnostic tests across different age groups while measurement invariance held for males and females and for the three villages. I therefore recommend the use of LC models for comparison between, and the identification of, the most accurate schistosomiasis diagnostic tests. Furthermore, microscopy and haematuria dipsticks were indicated as the most appropriate techniques for detection of *S. haematobium* infection.

Keywords: latent class models, diagnostic tests, Ghana, *Schistosoma haematobium*

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6.1 Introduction

In spite of the prolific generation of new knowledge in the area of urinary schistosomiasis, such as that of global burden, treatment and associated morbidity (King, 2006; Koukounari et al., 2007; Midzi et al., 2008; Rudge et al., 2008), there remains the unsolved practical issue associated with the basic diagnosis of this important parasitic disease. This relates to both the direct (i.e. microscopical examination of filters of urine for detection of *S. haematobium* eggs) as well as with the indirect (i.e. detection of haematuria, detection of schistosome-specific antibodies, detection of circulating egg antigens and ultrasound scans of the urinary system) diagnostic methods of this schistosome infection. There are several reasons for the limitations in the diagnosis of urinary schistosomiasis infections, such as, for example daily variation in egg excretion levels and/or duration of infection influencing the potential accuracy of determining the correct current infection status (Hatz et al., 1990).

Haematuria (blood in urine) alone has been proposed as a valid indication of current infection in schistosomiasis endemic populations (van der Werf & de Vlas, 2004). Microhaematuria can be detected by reagent strips (dipsticks) which recognize blood and protein. However, for the distinction of an active from a previous infection, particularly after treatment, in many populations and individuals, the circulating schistosome antigen has been proposed as the most reliable test (Van Lieshout et al., 1992; Attallah et al., 1999). In addition, although the serological diagnosis of schistosomiasis is generally accurate (el Missiry et al., 1990), it can also produce false negatives, particularly in patients with longstanding infections while elevated antibody levels can be still detectable many years after treatment (van Lieshout et al., 2000). Ultrasound is currently the diagnostic tool of choice for detecting pathological conditions associated with urinary schistosomiasis, such as dilatation of the renal pelvis and bladder wall lesions, although its usefulness has been questioned, particularly in low transmission areas, because of its lack of specificity (Hatz, 2001; Ruiz et al., 2002). In addition, large variations of sensitivity and specificity estimates have been observed among different endemic zones, age groups and sexes for all the aforementioned diagnostic methods of urinary schistosomiasis in several studies (Amis et al., 1982; Degremont et al., 1985; Taylor et al., 1990; Webb, 1990; Etard, 2004).

One explanation for the inconsistencies between all these diagnostic tests relates to the current lack of a definitive 'gold standard' reference test for urinary schistosomiasis. Consequently, the diagnosis of schistosomiasis as well as the control of this disease becomes problematic. Diagnostic assays with low sensitivities are unsuitable for evaluation of schistosomiasis control programmes, such as those aimed at morbidity reduction through mass human chemotherapy (Bosompem et al., 2004). Indeed, methods that allow infections to be correctly diagnosed are a prerequisite for effective disease control (Doenhoff et al., 2004). One solution may therefore relate to the need for more sophisticated statistical models to be developed and utilized in order to obtain more reliable empirical estimates of sensitivities and specificities of diagnostic tests (Begg, 1987; Formann, 1994).

In the present study I assessed the performance of five diagnostic tests for *S. haematobium* infection and estimated the prevalence of this infection in different age and sex groups in three villages northwest of Accra in Ghana. Specifically I used five different diagnostic tests for the prevalence of urinary schistosomiasis infection: that of the urine antigen detection test, performed on membranes or in ELISA plates, the serology anti-IgG test, an ultrasound assessment by recording the shape and state of the urinary bladder, the dipstick for haematuria using urine reagent strips on all urine specimens for presence of detectable blood, and finally detection of *S. haematobium* eggs by microscopy. Through the application of a latent class (LC) model to all of these five tests, the sensitivity and specificity of each test can be determined, and the overall urinary schistosomiasis prevalence levels within the different population groups estimated.

6.2 Materials and methods

6.2.1 Study sites and subjects. Three Ghanaian villages northwest of Accra, Ayiki Doblo, Chento and Ntoaso were visited and consenting adults over 19 years of age formed a convenience sample of passers by. However, in general, as regards to the demography in Greater Accra's region, the age structure is still a youthful one, characterized by a somewhat high fertility which has begun to show signs of a steep downward trend (Oheneba-Sakyi & Heaton, 1993). The general public in the three aforementioned villages are familiar with the work of the Noguchi Memorial Institute for

Medical Research and its personnel. Through discussions with local authorities the public was alerted, and people were approached and asked to participate. These volunteers were then interviewed and requested to provide specimens of urine, stool and blood for examination. Praziquantel (at 40mg per kg) was offered and taken following diagnosis of all infected cases of schistosomiasis. At subsequent visits, bladder ultrasound scans were performed on the majority of participants. All examinations were performed at the village clinics. Participants responded to a questionnaire, the majority of which were reported to be peasant farmers and persons involved in agriculture. Others responded as traders or vendors, but most reported regular water contact in the nearby river system. Although there was municipal water available in the village of Ntoaso, many residents do not have access to clean running water, and through their daily activities were thereby potentially exposed to risks of schistosome transmission. A total of 220 individuals consented to participate, had complete data on the variables examined here and were included in the analysis of the present study. The age and sex structure as well as the village location of all the sampled individuals is given in Table 6.1, which illustrates a significantly lower proportion of individuals who consented to participate and had complete data were below 39 years old and from villages Ayiki Doblo and Chento.

Table 6.1 Participation by age class, sex and village

Variable	***Number of individuals who consented to participate and had complete data (%)***	***Number of individuals who dropped out or did not have complete data (%)***	***p-value****
Age class			
19--29 years old	51 (29.8)	120 (70.2)	<0.001
30--39 years old	57 (42.5)	77 (57.5)	
40--49 years old	56 (57.1)	42 (42.9)	
50--59 years old	33 (52.4)	30 (47.6)	
≥60 years old	23 (37.7)	38 (62.3)	
Sex			
Female	117 (40.8)	170 (59.2)	0.618
Male	103 (42.9)	137 (57.1)	
Village location			
Ayiki Doblo	102 (49.0)	106 (51.0)	<0.001
Chento	39 (26.2)	110 (73.8)	
Ntoaso	79 (46.5)	91 (53.5)	
Total n	***220***	***307***	

*p-value for chi-square test.

6.2.2 Urine-antigen detection test. Detection of schistosome antigen in urine was performed after the method of Bosompem and colleagues (Bosompem et al., 1996) which has shown that *S. haematobium* antigen complexed with complement C3 can be isolated from the urine of infected people using a mouse monoclonal antibody. The authors demonstrated that goat-antihuman C3 would also detect schistosome antigen/complement complex in the urine of infected people, but not in non-infected people as case controls, and subsequently developed a monoclonal antibody dipstick test based on these findings (Bosompem et al., 1998). Briefly, methanol treated polyvinylidene difluoride (PVDF) membrane strips were incubated in test urine for 30min at room temperature (21-25^{o}C), rinsed with Tris-buffered saline (TBS) (50 mM Tris and 200 mM NaCl, pH 7.4) and then blocked for 15 min in 5% skimmed milk in TBS. The strips were then incubated in a reagent mixture of *S. haematobium* species-specific MoAb (1:100) and goat anti-mouse-immunoglobulins conjugated to horseradish peroxidase (1:10) in 0.1% skimmed milk in TBS for 1 h.

The strips were washed three times each by 10 min incubation in TBS and then incubated in substrate solution 0.05% (w/v) (3,3-diaminobenzidine), 0.15% (v/v) H2O2 and 5 mM Co (NO3)2.6H2O in TBS for 1 min. A bluish-black reaction represented positive results while negative results remained colourless.

6.2.3 Serology anti-IgG test. Detection of anti-schistosome IgG in serum was performed on serum eluted from dried blood spots on Whatman No1 filter paper. Blood spots filled a 1 cm diameter circle were taken at the time of examination, desiccated and kept dry until analysis. These were eluted in 1 ml PBS, diluted 1:100, and tested in ELISA plates (Immunolon-2) in triplicate. Analyses were repeated if there was more than 10% discrepancy. Plates were sensitized with SWAP antigen (6.44 mg/ml) prepared from *S. mansoni* adult worms provided by Biomedical Research Institute, Rockville MD. Antigen dilution was optimized against sera from known positive *S. haematobium* infections and known schistosome negative sera. Optical densities were read from a Vmax kinetic microplate reader (Molecular Devices. USA). Results were scored positive when the OD exceeded 2 x SD of the negative controls.

6.2.4 Ultrasound examination. A portable ultrasound apparatus, Aloka SSD-500 portable ultrasound with 3.5 MHz curvilinear probe (Aloka, Tokyo, Japan) was used for ultrasound examination, with the diagnoses made by a medically qualified person with prior training in ultrasound examination and interpretation. Examinations were performed using a curvilinear probe and recorded photographically. Diagnosis of pathological lesions was made *in situ*, and later confirmed by review of the ultraradiograph. For the purpose of this study, lesions were classified as positive or negative. Positive cases were registered when any two of the following situations were evident: epithelium enlarged more than 5 mm, evidence of polyps in the bladder wall, calcification of the epithelium, evidence of hydronephrosis.

6.2.5 Parasitological examination. Classic parasitological methods usually used by field clinicians were employed and evaluated in this study. Microscopy was performed on the product of a single measure of filtration of 10 ml urine taken from a specimen passed between 10:00 and 14:00, the time of optimum egg passage (Weber et al., 1967). Urine specimens were kept cool in an insulated ice box and processed in the laboratory within 4 hours of passing. The presence of any *S.*

haematobium eggs was recorded as positive. Haematuria was detected by the use of standard "hemastix", with any positive reaction being designated positive for urinary schistosomiasis (Multistix, Bayer Diagnostics).

6.2.6 Statistical analysis. By considering the true *S. haematobium* infection status of a sample of Ghanaian adults as a latent variable with two categories: 'infected' and 'non-infected', I validated the five diagnostic tests. In other words, I considered the observed data of the five diagnostic tests (urine antigen detection, serology anti-IgG test, ultrasound, dipstick for haematuria and microscopy) as indicators of an underlying, not directly observable variable (i.e. *S. haematobium* infection). Results of the five diagnostic tests are directly observed and are known as *manifest* variables while the *S. haematobium* infection is the unobservable latent variable (Bartholomew & Knott, 1999).

Given a sample of individuals with unknown infection status, for whom results from several diagnostic tests are available, latent class analysis can model the probability of each combination of tests results conditional on latent class (i.e. infection status). The manifest binary variables ($x_{1j,}$ x_{2j}, x_{3j}, x_{4j} and x_{5j}) were defined such that $x_{ij}=0$ represents a negative result for test i and $x_{ij}=1$ represents a positive test result for test i on individual j. I tested whether correlations between these manifest variables could be accounted for by a single latent dichotomous variable *Y* (i.e. the absence *Y*=0 or presence *Y*=1 of *S. haematobium* infection) and I defined $\eta = P(Y=1)$ the probability of being in the infected latent class. In other words, I divided the studied population into two classes (i.e. non infected and infected) assuming that the x_{ij}'s were mutually independent within each class (i.e. true infection status). It is expected that the x_{ij}'s are correlated as they are attempting to measure the presence of the same infection; the model assumes that these correlations are negligibly small only once one has accounted for an individual's true infection status (i.e. latent class membership). This assumption results in a more parsimonious model compared to one in which residual correlations are estimated, and one that is often adequate for the data. In the unlikely case that there are substantial residual correlations between the x_{ij}'s, additional latent classes would likely be required for an adequate fit to the data.

The likelihood function of the LC model was

$$L(X) = \prod_{j=1}^{N} \left(\eta \prod_{i=1}^{d} \pi_{i1}^{x_{ij}} \left(1-\pi_{i1}\right)^{1-x_{ij}} + \left(1-\eta\right) \prod_{i=1}^{d} \pi_{i0}^{x_{ij}} \left(1-\pi_{i0}\right)^{1-x_{ij}} \right) \quad (1)$$

Such a model has two types of parameters. First, there is the unconditional probability η that a person is in the infected latent class.

The second type of parameters are the conditional probabilities π_{i1} and π_{i0} that an individual in a particular latent class has a specified value of each of the manifest variables (Rindskopf & Rindskopf, 1986). In fact, π_{i1} represents the sensitivity and is the conditional probability $P(x_i=1|y_j=1)$ while $(1-\pi_{i0})$ represents the specificity and is the conditional probability $P(x_i=0|y_j=0)$. The LC model hence produces an estimate of disease prevalence as η is the proportion of individuals in the population of which our sample is expected to be in infection class $Y=1$. It also provides direct estimates of sensitivity and specificity for all the diagnostic tests (Formann & Kohlmann, 1996).

A natural way to extend the LC model (1) is to include stratification or grouping variables and examine group differences of measurement invariance. In this study such group differences were examined for males/females, different village locations and age groups. Likelihood ratio tests between less and more restrictive models were used to examine differences in infection prevalence and measurement invariance between groups. A significant measurement invariance tests suggests that specificities and sensitivities of the diagnostic tests vary by group and should be estimated for each group. Such an approach is referred to in the literature as multigroup latent class analysis (LCA) and comparisons of this sort are useful for at least two purposes: (a) to test whether the distribution of the latent variable is the same in each group and (b) to test whether the manifest observed variables are equally reliable indicators of the latent variable in each group (Clogg & Goodman, 1984).

Expectation-maximization (EM) algorithm was applied to produce maximum likelihood estimates for all parameters in the model using PROC LCA in SAS Version 9.1 (SAS Institute, Cary, NC,

USA). Identifiability of maximum likelihood parameter estimates was checked by using several different seed values.

6.3 Results

Table 6.2 represents the observed positive results expressed as percentages of *S. haematobium* infection for the five diagnostic tests. Different diagnostic tests gave different proportions of positive results.

Table 6.2 Positive results expressed as percentages by each of the five diagnostic tests among 220 Ghanaian adults studied

Diagnostic tests	***Positive results expressed as % with (95% CI)****
Urine-antigen detection	68.6 (62.5-74.8)
Serology anti-IgG	44.1 (37.5 50.7)
Ultrasound	31.8 (25.7-38.0)
Haematuria	21.8 (16.4-27.3)
Microscopy	15.5 (10.7 -20.2)

* CIs are based on normal approximation methods

Table 6.3 presents the results of one latent class model as it was dictated by likelihood ratio tests. Specifically, this model and denoted in table as 'LC Model 1' is a latent class model where measurement invariance was found to hold among males and females. Because of the measurement invariance found here, we obtain a common set of specificities and sensitivities for both males and females. The best diagnostic test for the detection of the prevalence of *S. haematobium* infection among the five diagnostic tests examined here was microscopy with a specificity estimated as 98% and a sensitivity estimated as 93%. In addition, 'LC Model 1' yielded quite high specificities and sensitivities also for haematuria and ultrasound. From this same model estimates of prevalence of *S. haematobium* infection by sex were also obtained. It is estimated that the prevalence of *S. haematobium* infection was highest among males (21%) compared to females (10%).

Table 6.3 Accuracy of diagnostic tests performance as estimated from latent class model 1 when measurement invariance was imposed across males and females

LC Model 1		***Diagnostic tests***				
	S. haematobium prevalence (%)	Urine-antigen detection Specificity/Sensitivity (%)	Serology anti-IgG Specificity/Sensitivity (%)	Ultrasound Specificity/Sensitivity (%)	Haematuria Specificity/Sensitivity (%)	Microscopy Specificity/Sensitivity (%)
Sex						
Male	21	36/98	57/48	74/65	87/73	98/93
Female	10					

'LC Model 2' in Table 6.4 is a latent class analysis model where measurement invariance was found to hold among different village locations and this is again the reason why it is obtained only a set of specificities and sensitivities for this group of sampled subjects. Results of this model agree with results of LC Model 1 in Table 6.3. The best diagnostic test for the detection of the prevalence of *S. haematobium* infection was again microscopy with specificity estimated as 95 % and sensitivity as 100%. In addition, 'LC Model 2' yielded quite high specificities and sensitivities also for haematuria and ultrasound. Furthermore, 'LC Model 2' also indicated Chento village as the one with the highest prevalence of *S. haematobium* infection (39%) among the three examined villages here.

Table 6.4 Accuracy of diagnostic tests performance as estimated from latent class model 2 when measurement invariance was imposed across different village locations

LC Model 2		***Diagnostic tests***				
	S. haematobium prevalence (%)	Urine antigen detection Specificity/Sensitivity (%)	Serology anti-IgG Specificity/Sensitivity (%)	Ultrasound Specificity/Sensitivity (%)	Haematuria Specificity/Sensitivity (%)	Microscopy Specificity/Sensitivity (%)
Village location						
Ayiki Doblo	7	35/100	56/47	73/70	86/91	95/100
Chento	39					
Ntoaso	2					

Finally, 'LC Model 3' in Table 6.5 is a latent class model where measurement non-invariance was found for different age groups and this is the reason why different specificities and sensitivities were calculated for each of these groups. Using this model, diagnostic tests which could be characterized as acceptable for the detection of the prevalence of *S. haematobium* infection were those of ultrasound, haematuria and microscopy in the age groups of 19 to 29 and 40 to 49 years old; haematuria and microscopy were indicated as good diagnostic tests in the age group of 30 to 39 years as they both gave quite high specificities and sensitivities at the same time. Finally, in the age group of ≥60 years old the estimates of specificity and sensitivity were sufficiently high (94% and 100% respectively) only for haematuria, whilst for the age group of 50 to 59 years old, when taking into consideration both estimates of specificity and sensitivity, none of the diagnostic tests examined here was indicated as appropriate. From this same model estimates of prevalence of *S. haematobium* infection by age group were also obtained. 'LC model 3' shows that the highest prevalence of active, i.e. by egg passage *S. haematobium* infection was determined among the youngest age group of the sampled individuals of this study (30%).

Table 6.5 Accuracy of diagnostic tests performance as estimated from latent class model 3 when measurement invariance was not imposed across different age groups

LC Model 3		***Diagnostic tests***				
	S. haematobium prevalence (%)	Urine antigen detection Specificity/Sensitivity (%)	Serology anti-IgG Specificity/Sensitivity (%)	Ultrasound Specificity/Sensitivity (%)	Haematuria Specificity/Sensitivity (%)	Microscopy Specificity/Sensitivity (%)
Age group (in years)						
19 to 29	30	36/100	43/82	89/74	88/84	93/82
30 to 39	9	31/81	50/0	77/41	86/100	94/99
40 to 49	14	31/100	58/13	73/75	88/75	100/100
50 to 59	20	61/100	78/20	72/53	100/45	100/45
>=60	11	39/100	60/71	46/0	93/100	100/0

6.4 Discussion

Current estimates of the prevalence of schistosomiasis depend on the use of well-established, but imperfect, diagnostic tests (Wilson et al., 2006). Appropriate schistosomiasis diagnosis becomes increasingly important for several reasons. For example, clinical diagnosis might lose its value because of lack of specificity and mass treatment might only remain cost-effective through the use of appropriate diagnostic tools to only target further drug treatment to those groups of people actually infected (van Lieshout et al., 2000). The purpose of the epidemiological survey reported here was to assess the performance of five diagnostic tests for *S. haematobium* infection and examine if the prevalence of this infection varied across different age and sex groups of sampled individuals from three villages northwest of Accra in Ghana where there has been reported previously medium *S. haematobium* prevalence (Bosompem et al., 2004). I have addressed this specific problem by taking into account the absence of a gold standard diagnostic test for *S. haematobium* infection and by fitting LC models with a frequentist approach to these data obtained from adults northwest of Accra in Ghana. Although LC models have been used extensively in epidemiological literature of several infectious diseases (Alvord et al., 1988; Engels et al., 2000; Langhi et al., 2002; Kudel et al., 2006; Strauss et al., 2006; Hebert et al., 2007), they have rarely been used in parasite epidemiology and particularly in the area of schistosomiasis. More precisely, to my knowledge, only two previously published studies, both based within Côte d'Ivoire, have used LC models through a Bayesian approach in order to assess performance of the Kato-Katz technique in diagnosing *S. mansoni* and hookworm co-infections as well as to estimate reduction of prevalence and intensity for hookworm infection in humans post-praziquantel treatment (Utzinger et al., 2002; Booth et al., 2003), while only one study in the Philippines has provided estimates of sensitivity and specificity of a faecal examination method for *Schistosoma japonicum* infection in mammals, using also a LC modeling approach within a Bayesian framework (Carabin et al., 2005).

The current study therefore provides the first, to my knowledge, evaluation of the performance of multiple diagnostic criteria and estimation of the prevalence of *S. haematobium* infection in Africa which raises important implications to consider with reference to reliable tests for the diagnosis of urinary schistosomiasis. Such findings should be also of direct relevance and application to current mass chemotherapeutic control programmes. Nevertheless, as the current dataset focuses on adults, I

would recommend additional similar studies aimed to assess the application of such LC models on data from school-aged children across varying schistosomiasis endemic countries within sub-Saharan Africa since school children form the major target age group of current mass chemotherapeutic control in human helminthiasis (WHO, 2006).

In this Chapter it was clearly demonstrated that in adults the microscopic detection of the parasite's eggs in the urine is the best currently available diagnostic tool for *S. haematobium* infection whenever this is of course performed properly (results in Tables 6.3, 6.4 and 6.5 support this argument) with the exception for the age group of ≥50 years old where very low specificities were estimated (Table 6.5). Standard errors of the estimates were larger for the older age groups compared to the young age groups due to the smaller sample sizes here (Table 6.1) and therefore such results should be interpreted cautiously. Based on these findings, I would thus recommend the inclusion of microscopic examination in the monitoring process of human mass chemotherapy programmes whenever financial resources allow for this option, mainly because of its relatively low operational cost compared to other urinary schistosomiasis diagnostic techniques and its feasibility under most conditions. Furthermore, as microscopic examination can quantify the intensity of the *S. haematobium* infection, it enables evaluation of important indicators in the control planning, such as possible risk factors, presence of severe clinical forms, degree of transmission and reinfection in the area, as well as intervals for necessary re-treatments.

In addition, this study confirms that haematuria dipsticks can be sufficiently sensitive and specific indicators (results in Tables 6.3, 6.4 and 6.5 support this argument with the exception of results in Table 6.5 where for the age group of 50 to 59 years old haematuria dipsticks yielded a very low sensitivity (45%)) for detection of *S. haematobium* infection in endemic areas, and therefore I would also recommend their inclusion in the monitoring process of human mass chemotherapy programmes. Indeed, in Chapters 4 and 5 of this PhD Thesis I have also found that semiquantitative reading of dipsticks correlates well with intensity of *S. haematobium* infection and ultrasound pathology.

On the other hand, whilst the urine antigen detection test showed similar sensitivity to microscopy (results in Tables 6.3, 6.4 and 6.5 support this argument), it was also suggested that false-positive urine antigen detection tests may be more common than previously reported (Bosompem et al.,

1998). One potential explanation for the low specificity of this test might be that potentially cross-reactive parasites are more prevalent in the age group studied here and polyparasitism is of course common in these areas. Indeed, Dunyo and colleagues (1996) found filarial infections in the towns or in the villages east of Accra in a similar age group and I would thus recommend further studies to define both the prevalence of such parasites in this same endemic area and examine any potential cross-reactivity between helminth species within the urine antigen detection test. Results from the current study suggest that the urine-antigen detection tests I evaluated should perhaps not be used for the identification of high-risk groups which, due to the possibility of false positive reactions produced by such tests, could artificially inflate the actual numbers of people targeted for mass chemotherapy. Furthermore, estimates from all LC models presented here yielded low sensitivities and specificities for the serology anti-IgG tests. The observation here that antibody detection lacks specificity is consistent with findings of other epidemiological studies which reported that antibody is often found without concomitant parasitological evidence of infection (Doenhoff et al., 1993; Xue et al., 1993).

Furthermore, antigen detection methods are generally more expensive than antibody ones (Hamilton et al., 1998). On the other hand, microscopy and haematuria dipsticks require relatively unsophisticated equipment and, in areas of high endemicity, personnel with only basic training. These two latter diagnostic tests could therefore constitute the lowest cost option when technical assistance is plentiful. Thus the current findings, if combined with consideration of costs involved, which is a critical issue in the economically developing countries, leads me to the conclusion that antibody and antigen detection tests should not be used in the determination of the prevalence of long term urinary schistosomiasis infections.

With reference to the detection of urinary schistosomiasis infection through ultrasound examination, the results of this study indicated that the performance of this diagnostic tool was quite acceptable in all age groups except in those of 30 to 39 years old and $\geq$50 years old. An explanation for the variability in these results among different age groups might be that successive episodes of infection would result in recrudescence of urinary tract abnormalities and more severe pathology caused by urinary schistosomiasis would be expected to be observed in young adults because of continuing reinfection. Thus, I would conclude that ultrasound examination is not a reasonable substitute for microscopy or dipsticks in regards to determining the prevalence of *S. haematobium* infection.

Nevertheless, I would still support the argument that the best currently available diagnostic tool for morbidity assessment in *S. haematobium* infections is the visualization of urinary tract pathology through ultrasound examination (Koukounari et al., 2006)

Finally, with statistical analysis alone, one can never be certain about the validity of a dependence model as it is not known from the observed data how each of the examined diagnostic tests relates to the others conditional on disease status (Albert & Dodd, 2004). Consequently, I certainly recognize that the results of this study depend upon the assumption of conditional independence assumed by the models fitted here. In addition, LC models based on current assumptions may not be appropriate for some similar alternative datasets as very large correlations (if these are present after accounting for latent class membership i.e. the true infection status) could potentially bias parameter estimates and result in an underestimation of the error rates of the examined tests (Vacek, 1985). Finally the SAS procedure for latent class analysis 'PROC LCA' applied here which has been recently developed by The Methodology Center at Penn State, does not yet supply standard errors for the parameter estimates; thus a limitation in this study is that the magnitude of the standard error and consequently of the confidence interval of the acquired predictions is not provided.

To conclude, in this Chapter I assessed the prevalence of *S. haematobium* infection through the use of LC models, because accurate sensitive and specific measures for this indicator are imperative, particularly at later stages of successful mass chemotherapy control programmes. I demonstrate that LC models proved a useful tool for validation research in the absence of a perfect gold-standard diagnostic technique. These models have suggested microscopy and haematuria dipsticks as sensitive and specific indicators of prevalence of *S. haematobium* infection in Ghanaian adults. In addition, they have provided estimated prevalences of *S. haematobium* infection that fit well with those previously obtained by those such as Nsowah-Nuamah and colleagues (2001) in Southern Ghana, and Amankwa and colleagues (1994) in upper-east region of Ghana as well as the focality of this infection even within small areas of the same country. However, it must be also considered that in the general context of chemotherapy programmes, if monitoring and evaluation results are based exclusively on determining infection prevalence, the impact data obtained may be inaccurate in terms of validity reflecting the success of any programme. Therefore, it is fundamental to also monitor the impact of such control programmes on the intensity of the infection in the treated

population, particularly as modern day chemotherapy programmes are aimed at reducing morbidity and hence intensity and further research in this area is thereby warranted.

Chapter 7: General discussion

In this Chapter I discuss the motives behind this thesis, drawing together the various findings and emphasizing how these relate to one another. Finally, based on the findings of this thesis I also attempt to identify implications for both future schistosomiasis research and control.

Over the past 50 years, the challenge of schistosomiasis control has persisted as a complex dilemma for health policy makers (Gryseels, 1989), while past studies on the effects of chemotherapy on morbidity induced by schistosomiasis have produced somewhat controversial results leaving fragmentary knowledge on the impact of chemotherapy against schistosomiasis (Richter, 2003). Despite the long and rich history of research in the field of schistosomiasis with major scientific accomplishments, the majority of past studies have often been statistically underpowered to examine each parasite's particular role in the causation of morbidities (King et al., 2005), resulting in schistosomiasis to be underappreciated as a serious health problem (Ouma et al., 2001). However, in recent years international interest and political commitment for control of helminthic diseases in sub-Saharan Africa has grown substantially with schistosomiasis on the international health agenda (Utzinger et al., 2003; Stothard & Gabrielli, 2007) and major shifts in global health policy towards the implementation of mass chemotherapeutic control programmes at the national scale in sub-Saharan Africa (Fenwick & Webster, 2006). Evaluation of such mass human chemotherapy programmes was clearly an area that deserved more careful investigation at the time this PhD thesis started as it could serve the need of each national schistosomiasis control programme to define national policy and to prioritize areas where intervention is most needed and producing the greatest benefit.

The fact that there is a lack of large-scale studies and the need for proper evaluation of mass human chemotherapy programmes,inspired the studies presented in Chapters 2, 3, 4 and 5 of this thesis, using appropriate biostatistical methods for the analysis of large cohorts sampled as part of these newly established national schistosomiasis control programmes in various geographical endemic African settings, examined where possible the independent and interactive effects of schistosomiasis with other parasitic diseases on different health outcomes. The aim of these studies was to elaborate more clearly the significant morbidity caused by schistosomiasis (Chapters 2, 3, 4 and 5) and to

refine the understanding of the specific benefits of mass treatment in various schistosomiasis-endemic settings (Chapters 2 and 4). King and Dangerfield-Cha (2008) have recently stressed the necessity for such studies, as most people living in the developing world are vulnerable to a great variety of chronic diseases throughout the course of their lives. I also believe that the findings presented in these chapters have made significant contributions to the current state of knowledge because of the limited large scale community screening of morbidity and rare geographic comparisons (Balen et al., 2006).

More precisely, in Chapters 2 and 4, I used uniquely detailed data arising from the Ugandan and Burkinabé National Schistosomiasis Control Programmes, before and one year following praziquantel and albendazole chemotherapy, with the aim to evaluate the potential relationship between *S. mansoni* or *S. haematobium* infections, anti-helminthic chemotherapy, and anaemia as well as to assess the extent to which schistosomiasis might be a cause of anaemia. Within different schistosome species, it is possible that different biological interactions exist by age and gender while complexities in the ecological context and varying clinical presentations make quantifying the magnitude of the relationship between schistosomiasis and anaemia challenging (Friedman et al., 2005). Both these SCI datasets are on school-aged children (6 to 14 years old) and cover areas of different endemicity for *S. mansoni*, *S. haematobium* and other soil transmitted helminths; in Uganda *S. haematobium* is known not to be endemic in the areas where the surveys took place (Stothard et al., 2006; Kolaczinski et al., 2007), while in Burkina Faso, prevalence of *S. mansoni*, hookworms and other soil-transmitted helminthiasis was estimated to be very low. Despite the different burden of parasitic diseases and schistosome species in the two countries, in both these studies heavy infections of intestinal or urinary schistosomiasis were demonstrated to be associated with lower Hb counts and consequently with potential anaemia. It was also shown that children most benefiting from anthelminthic treatment in terms of increased Hb counts were those who were anaemic at baseline. Therefore, both these studies imply that anaemia is a likely morbidity indicator of schistosomiasis and it can be reduced following chemotherapy.

Additional data from older school-aged children (10-21 years old) with *S. mansoni* infections were analyzed through Bayesian hierarchical modeling from Western Kenya with the aim to examine further the issue of lowered Hb levels and potentially anaemia risk identified in Chapter 3. The

surveyed location in western Kenya is another well known endemic schistosomiasis area (Brooker et al., 2000; Thiong'o et al., 2001) and different from those surveyed areas in Uganda in the sense that in this specific dataset there were also observed medium prevalences of *T. trichiura* and *A. lumbricoides* infections as well as a high prevalence of hookworm infection. It should be noted that all of these aforementioned parasitic infections have the potential to cause iron deficiency by extra-corporeal blood loss. In addition, this Kenyan specific dataset is a richer one if compared to those from Uganda and Burkina Faso in the sense that it contained malaria measurements and socio-economic status-both potential important risk factors of anaemia- and which could add important information into the statistical analyses. Results from this Chapter also suggest that heavy intensity of *S. mansoni* infection, being stunted and malaria parasitaemia were significantly associated with lower mean Hb although only heavy intensity of *S. mansoni* infection was significantly associated with the risk of anaemia among schoolchildren over 10 years of age. The fact that hookworm infection was not associated either with lower Hb or with anemia in the present study is in contrast with the findings of Chapter 2 where I found statistically significant associations of moderate and heavy intensities of hookworm infections with the examined health outcomes of interest here. This is probably due in part to the low intensity of hookworm infection in this Kenyan study area, and highlights how different factors contribute to anaemia in different parasite transmission settings.

I believe that all the above findings provide strong evidence that anaemia represents a significant part of chronic disease burden associated with schistosomiasis. The mechanisms by which schistosome infections lead to anemia have been discussed in Chapters 2, 3 and 4, while a very analytical description of the pathophysiology that underlies each mechanism is provided by Friedman et al. (2005) and hence I will not discuss this issue further here. Based on the current findings, I conclude that anaemia should continue being used as a measurable target in programmes to reduce morbidity due to schistosomiasis and helminthiasis as it reflects the aim of the programmes to reduce disease morbidity rather than transmission indicators (Bates et al., 2007).

In Chapter 4, analyses were expanded to examine also associations of urinary schistosomiasis with undernutrition and pathologic effects on the human organism such as microhematuria pre- and post-treatment. Children with more severe microhaematuria scores at baseline were demonstrated to have higher *S. haematobium* infection intensities which suggests that positive microhaematuria scores are

likely to be valuable markers in children for morbidity caused by *S. haematobium* infections. It should also be noted that such morbidity was significantly decreased one year post chemotherapeutic treatment. On the other hand, neither the associations between the risk of wasting or stunting at baseline with the intensity of *S. haematobium* infection were found, nor the percentages of wasted and stunted children one year post treatment were significantly decreased. Nevertheless, as the study design included neither a random allocation nor a placebo group it is very difficult to demonstrate cause and effect of urinary schistosomiasis on malnutrition and generalize further these results. This is clearly an area that needs and deserves more careful investigation and thus it would be interesting to apply and expand the biostatistical analyses presented in this thesis in other endemic countries and SCI datasets such as those from Tanzania and Zambia.

From a disease-impact perspective, both Chapters 2 and 4 also served to confirm that praziquantel can have a substantial effect on schistosomiasis infection and associated morbidity when delivered as part of a large-scale control programmes. In Burkina Faso there was observed a decrease of approximately 90% in the schistosomiasis prevalence at 1st follow-up compared to baseline, while in Uganda this was observed to be approximately 50%. One of the factors likely to have contributed to the great impact demonstrated in Burkina Faso is the high nationwide treatment coverage (over 90%) achieved in a relatively short space of time by the control programme. Another factor is that 2004 was a very dry year and treatment was delivered in the dry season. These two important factors together may have helped to reduce the prevalence and, therefore, should be considered when implementing a national control programme in other sub-Saharan countries to maximize the treatment impact. It must be considered, nevertheless, as briefly mentioned above, that essential methodological constraints inherent in both these present study designs, in particular that of the necessary, due to ethical reasons, lack of the inclusion of an untreated control group, could result in some potential bias to the estimation of the absolute impact of the treatment, thereby allowing only the relative impact of the treatment in different groups of children to be calculated.

From a research standpoint, two recent articles stress the necessity for better diagnostics in the field of schistosomiasis (Colley & Secor, 2007; King & Dangerfield-Cha, 2008). In Chapters 5 and 6 I have attempted to validate and evaluate field applicable diagnostic tools of schistosomiasis infection. More precisely, in Chapter 5 I initially tested the suitability of the full WHO-recommended ultrasound protocol in the context of large-scale schistosomiasis control programmes using

hierarchical modeling with a frequentist approach for the statistical analysis of SCI baseline data on school-aged children (7 to 14 years old) from Mali. Findings from this chapter suggested that ultrasound is a suitable and valid public health tool for urinary schistosomiasis morbidity control programs focused on children while in the case of intestinal schistosomiasis morbidity detection in large scale control interventions, this same protocol is a useful tool provided that local cut-offs are used to define abnormal values and that results are interpreted with cautiousness.

As mentioned in several sections of Chapters 2, 3, 4 and 5 I explored and took hierarchical modeling approaches for the analysis of all these datasets because the children were clustered within schools and schools were clustered within districts. In addition, in Chapters 2 and 4 there were repeated measurements for each child. Ignoring this correlation structure would have resulted in underestimation of variability and thus to narrower associated confidence intervals of the relevant regression coefficients. The fact that I have used similar statistical modeling for the analysis of these data could facilitate later meta-analysis of these studies. Recent advances in hierarchical models have, I believe, been invaluable to the scientific field of parasite epidemiology. However, in contrast to the richness of this statistical methodology, I feel that these models have not yet been fully utilized in the analysis of schistosomiasis infection and therefore I hope that the studies presented here will contribute to initiate such applications for the statistical analysis of relevant data.

Finally, in Chapter 6 I expanded on the theme of schistosomiasis diagnosis by comparing diagnostic measures of *S. haematobium* infection prevalence through the use of latent class (LC) models with a frequentist approach on data from Ghanaian adults (19-86 years old). I used such models in order to estimate in the absence of a perfect gold-standard diagnostic technique, sensitivities and specificities for five diagnostic tests: microscopic examination of urine for detection of *S. haematobium* eggs, dipsticks for detection of haematuria, tests for circulating antigens, serology tests and ultrasound scans of the urinary system. The dataset I used in Chapter 6 also has the potential to provide a good comparison reference to the results reported in Chapter 5 as in the northwest of Accra in Ghana it was estimated that the *S. haematobium* prevalence was low (15.5 %) among adults while in Mali the prevalence of *S. haematobium* infection was found to be high among children (59 %)-as both detected by microscopic examination.

Results from LC models in Chapter 6 have suggested microscopy and haematuria dipsticks as sensitive and specific indicators of prevalence of *S. haematobium* infection in Ghanaian adults. These models also provided evidence that the urine-antigen detection test I examined in Chapter 6 should perhaps not be used for the identification of high risk groups which would be probably unnecessarily included for treatment in human mass chemotherapy programs as they were found to be non-specific. Furthermore, estimates from all models presented in this Chapter yielded low sensitivities and specificities for the serology anti-IgG tests. These findings, if combined with cost-analyses, the latter being a key influential factor with respect to public health in economically developing countries such as those examined in this PhD thesis, leads me to the conclusion that currently available antibody and antigen detection tests should not be used in the determination of the prevalence of latest stages of urinary schistosomiasis infection. In order to make recommendations about their inclusion in the monitoring and evaluation process of schistosomiasis control, I suggest that similar studies should be repeated on children using the combination of such diagnostic tests together with latent class analysis.

As regards to ultrasonographic examination, based on the findings of Chapters 5 and 6, I believe that ultrasound global scores are likely to be valuable markers in children for morbidity caused by both light and heavy infections with *S. haematobium* but that it is not a reasonable substitute for microscopy or dipsticks in regards to determining the prevalence of this infection. Furthermore, based on findings from Chapter 6 where I found a very poor performance of ultrasound in older age groups and which might be explained by decreased rates of reinfection in older adults, this diagnostic tool might not be the most appropriate one to detect late stage morbidity caused by urinary schistosomiasis. I would agree with observations from colleagues in Venezuela that the specificity of the method must be improved, especially for the recognition of precocious pathology (Ruiz et al., 2002).

In addition, it must be also considered that in the general context of chemotherapy-based programmes, if based exclusively in determining the prevalence, the evaluation of the results reached with the control measurements introduced in an endemic region may be inaccurate and thus future work emerging from Chapter's 6 findings is compulsory. It is fundamental to also observe the impacts of treatment on the intensity of the infection in the treated population particularly as modern

day chemotherapy programs are aimed at reducing morbidity and hence intensity. I believe that use of latent trait models (Uebersax & Grove, 1993; Agresti, 1999), which regard intensity and not only prevalence of infection as a latent variable pre and post treatment, constitutes a very interesting field in the research of schistosomiasis' diagnostics because particularly in communities where schistosomiasis transmission levels are decreasing, appropriate diagnosis becomes increasingly important for the mass treatment to remain cost effective (van Lieshout et al., 2000).

In conclusion, results from this PhD thesis established several schistosomiasis morbidity indicators while it was validated that mass human chemotherapeutic control for schistosomiasis in sub-Saharan African countries has significant impact at reducing the intensity of schistosomiasis and associated morbidity. As King and Dangerfield-Cha (2008) recently propose such drug-based anti-schistosome control programmes will need to continue for long intervals, i.e. time-spans of more than a single generation, in order to eliminate the true burden of schistosomiasis in endemic countries.

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Appendix: Publications by the candidate

Morbidity indicators of *Schistosoma mansoni*: relationship between infection and anemia in Ugandan schoolchildren before and after praziquantel and albendazole chemotherapy.

ARTEMIS KOUKOUNARI, ALAN FENWICK, SARAH WHAWELL, NARCIS B. KABATEREINE, FRANCIS KAZIBWE, EDRIDAH M. TUKAHEBWA, RUSSELL STOTHARD, CHRISTL A. DONNELLY, JOANNE P. WEBSTER

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MORBIDITY INDICATORS OF *SCHISTOSOMA MANSONI*: RELATIONSHIP BETWEEN INFECTION AND ANAEMIA IN UGANDAN SCHOOLCHILDREN BEFORE AND AFTER PRAZIQUANTEL AND ALBENDAZOLE CHEMOTHERAPY

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Abstract. The potential relationship between *Schistosoma mansoni* and anemia was examined using data obtained by the Schistosomiasis Control Initiative (SCI) before (baseline) and 1 year after (follow-up) a chemotherapeutic treatment program in Uganda. Changes in hemoglobin (Hb) levels in 2,788 children in relation to their schistosomiasis and/or hookworm infection intensity category and baseline anemia status were analyzed. At baseline, significant predictors of childhood anemia were intensities of *S. mansoni* and hookworm infection. At follow-up, moderate or heavy hookworm as well as heavy *S. mansoni* infections were important. Children heavily infected with *S. mansoni* or hookworm had significantly lower Hb counts at baseline compared with those not infected. Among anemic children at the baseline survey, a significant increase in Hb counts of 0.834 g/dL after treatment was found. Our results suggest that anemia is associated with schistosomiasis and hookworm in Ugandan children and that such anemia shows a significant improvement after chemotherapy.

INTRODUCTION

Anemia is a common problem throughout the world and of enormous public health concern in developing countries, but its etiology is very complex, making the effect of any one factor difficult to assess. Malaria plays a key causative role for anemia among young African children, although HIV infection, hemoglobinopathies, intestinal helminths, in particular that of hookworm infection, poor nutritional status, and micronutrient deficiencies are also likely to make important additional contributions.[1] Schistosomiasis is a parasitic disease of profound medical and veterinary importance, second only to malaria in terms of parasite-induced human morbidity and mortality, with some 600 million people exposed and 200 million infected at any time throughout the tropical world.[2] The relative role of schistosomiasis as a causative agent for anemia, however, particularly compared with that known for hookworm infection, remains controversial.[3–7] Preston and Dargie,[8] for instance, provided convincing evidence that schistosomiasis causes anemia in experimental animals; however, although Foy and Nelson[9] did acknowledge that anemia was generally associated with heavy schistosome infections, they doubted that early or light infections were of importance. There is, nevertheless, a general consensus for the need for further epidemiologic research into the potential role of schistosomiasis as a causative agent for anemia.[9,10]

Since 2003, the Schistosomiasis Control Initiative (SCI) has assisted six sub-Saharan African countries with the objective to develop sustainable national schistosomiasis morbidity control programs and reach at least 75% of school-aged children and other high-risk group through mass deworming using praziquantel for schistosomiasis and albendazole for intestinal helminths. Such control programs thereby provide a unique opportunity to assess the potential role of schistosomiasis and/or other intestinal helminths as causative agents of anemia morbidity, together with the potential ameliorative impact of chemotherapy on a large scale. This may be particularly pertinent in terms of identifying and evaluating morbidity associated with *S. mansoni* (intestinal schistosomiasis), which is frequently very difficult to assess and quantify precisely except in the most severe or late chronic cases.[11–14] Moreover, because demonstration of successful morbidity control is feasible only if there are reliable morbidity markers capable of showing reversion within the time frame of disease surveillance, it is vital to identify *S. mansoni* morbidity indicators for sustainable disease control and for evaluating the success of intervention.[15,16]

Using uniquely detailed data arising from the Ugandan National Schistosomiasis Control Program, before and 1 year after praziquantel and albendazole chemotherapy, we aimed to evaluate the potential relationship between *S. mansoni* infection, anti-helminthic chemotherapy, and anemia and to assess the extent to which schistosomiasis is a cause of anemia.

MATERIALS AND METHODS

Study sites, sampling, and cohort design. Parasitological and morbidity data were collected on a cohort of 4,351 Ugandan children, 6–14 years old, randomly sampled from 37 schools situated in eight districts just before and one year after implementation (2003 and 2004, respectively), as part of ongoing monitoring and evaluations of the SCI program in Uganda. The eight districts were selected to represent the Albert Nile (Nebbi, Arua, and Moyo), Lake Victoria (Bugiri, Busia, and Mayuge) and Lake Albert basins (Masindi and Hoima). Further details on these districts and their inhabitants are provided elsewhere.[17,18] Schools were chosen on the basis of existing *S. mansoni* data for schoolchildren from Uganda (SCI-National Survey of Bilharziasis) and parasitological stratification with different categories of infection prevalence, classified as low (< 10%), medium (11–50%), and high prevalence (> 50%) within each district, which also al-

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lowed pooling to reach sample sizes technically detailed in Brooker and others.[19] Fixed cohort structure was recruited at each school to allow comparisons to be made across schools and districts. Required sample sizes were calculated using the EpiSchisto software tool (http://www.schoolsandhealth.org/epidynamics.htm) using an expected reduction in mean intensity of 60% (*S. mansoni*) after chemotherapy to achieve 80% statistical power and a significance level of 5%. The value of 60% was chosen as a conservative estimate of the expected reduction over a 2-year period (two annual treatments). An overall drop-out rate of 40% over the course of the monitoring period was also allowed.

As it was not logistically possible to survey all schools within the same month, surveys were staggered. Children were identified at follow-up using a named roll call as well as retrieval of SCI individual treatment cards with unique code identifier given to the children the previous year as well as hardcopy of a group cohort photograph to ensure that children remember to which group they were reassigned. Before the 1-year follow-up visit a pre-survey team re-registered the children and also sensitized the children to ensure that as many children as possible in the cohorts were at school the day of survey. All children enrolled into this study were interviewed and examined by appropriately trained Ministry of Health field workers. Administration of praziquantel and albendazole was according to WHO guidelines (praziquantel 40 mg/kg and single 400 mg albendazole tablet). Ethical clearance was obtained from the Ugandan National Council of Science and Technology and Imperial College London. For ethical reasons, it was not appropriate to include any untreated control groups in the study design.

Parasitological and anemia morbidity data. A single stool sample was collected from each individual and 41.7 mg was processed to make duplicate Kato-Katz slides for microscopic determination of schistosome and/or hookworm infection and, where applicable, egg per gram counts. For comparative reasons between the two successive years of study, where the second measurement of the Kato-Katz smear was missing, *S mansoni* prevalence and individual intensities were calculated using a single thick Kato-Katz smear, although we are aware that we miss a certain proportion of infections for assessment of individuals' infection status, by this way. Anemia was defined (according to WHO guidelines), as a dichotomous variable taking the value 1 for children from 5 to 11 years old with Hb < 11.5 g/dL and for children between 12 and 14 years old with Hb < 12.0 g/dL. Further details for other indicators included and methodology followed in the SCI questionnaires are given elsewhere.[19] All Kato-Katz smears were read within 1 hour of preparation so that hookworm eggs could be easily seen.

S. haematobium is known not to be endemic in these areas of Uganda.[20] However, for absolute confirmation, urine from all children sampled was also tested for micro-hematuria using hemastix dipsticks and all urine sampled from children from the 11-year-old cohort was pooled for concentration of schistosome eggs using a Pitchford and Visser funnel and no *S. haematobium* egg was observed. We are therefore fully confident that *S. haematobium* was absent within this cohort. The prevalence of *Trichuris trichura* and *Ascaris lumbricoides* is unevenly distributed in the country with prevalence greatest in southwestern Uganda and such infections were also examined from the feces of all children sampled.[21]

Nutritional assessment data (i.e., weight and height) and blood samples for hemoglobin levels were obtained from each individual by the finger prick method, because this provides a sufficient sample for accurate Hb measurement using a Hemocue photometer.[22]

Statistical analyses. Because of clustered data with a natural hierarchical structure, we chose to use linear hierarchical models to model changes in Hb levels from baseline to follow-up and hierarchical logistic regression models to model the risk of being anemic. Additionally, because schistosomiasis occurs focally, heterogeneity in the degree of endemicity among schools has to be accounted for.[23] In such circumstances, ordinary least squares regression can overestimate the precision associated with an analysis yielding spuriously statistically significant results (i.e., a type 1 error). Hierarchical models permit combination of exposures to group and individual factors that is important in epidemiologic analyses of infectious disease data.[24,25]

The hierarchical logistic regression models were fitted to data coming from children with complete records at each time-point of interest. Because of variable cohort recovery success, attributable to frequent population movements within Uganda, the remainder of the statistical analyses were restricted to children with complete records from both time-points of interest, and no replacements were used for missing subjects. Summary reportings, figures, and analyses are based on data from all available subjects. A comparison between the baseline characteristics of children successfully followed-up and those that dropped out in the second year of the study was performed through χ^2 tests with regard to all the parameters examined in this study. Frequency tables were obtained using SAS V8 (SAS Institute, Cary, NC). A forward selection procedure was used for the evaluation of the remaining variables in the final models.

An association of anemia with parasitic infections (schistosome and hookworm) was studied after simultaneously adjusting for potential confounders in the statistical models used. Indices of the anthropometric status of the studied children based on the 1978 CDC/WHO growth reference curves were computed using the Nutstat program within Epi Info V 3.3. Body mass index is considered an indicator of acute under-nutrition (thinness or wasting) and is generally associated with failure to gain weight or a loss of weight.[26–28] The Z-score cut-off point recommended by WHO, CDC, and others to classify low anthropometric levels is 2 SD units below the reference median for this specific index. Cut-off of –2 body mass index Z-scores (BMIZ) were calculated to classify underweight children and finally this categorical variable was incorporated in all models as a potential predictor. Initially we examined the impact of parasitic infections, sex, and nutritional status as defined from Z-scores at baseline and follow-up using two-level multivariate logistic regression models with level 1 (the children) and level 2 (the schools). We also tested three-level multivariate logistic regression models with level 3 (the districts), but it was proved that the districts were rather homogeneous in the risk of being anemic, and therefore this random effect was not finally included in the models. Nutritional status was included in the baseline hierarchical logistic regression model as generally malnutrition in children is the consequence of a range of factors that are often related to poor food quality, insufficient food intake, and severe and repeated infectious diseases, or frequently some combina-

tions of the three. The same variable (BMI Z-score at baseline) was included in the hierarchical logistic regression model referring to 1-year post-praziquantel and albendazole treatment, because in this study, the duration of this period did not produce a nutritional impact. Finally, in both hierarchical logistic regression models the BMI Z-score at baseline was not significant, and therefore it was omitted from these models. Age was included in both hierarchical logistic regression models (baseline and follow-up) as a categorical variable.

Two separate hierarchical linear modeling analyses were carried out to determine any change in the children's Hb levels in relation to their schistosomiasis and/or hookworm infection intensity category, controlling at the same time for age and sex and anemia status (as defined above). Children's Hb levels at baseline and 1-year post-treatment were modeled through three-level hierarchical models where level 1 (two periods of interest), level 2 (the children), and level 3 (the schools). The validity of the distributional assumptions of this model was examined using plots of level 1, level 2, and level 3 residuals against their normal scores. To take into account the paired data structure, a dummy variable corresponding to the second year of study was included as a covariate in the model. Through this model we aimed to quantify the adjusted overall change of Hb from baseline to follow-up and to quantify average Hb counts of different groups of children at baseline.

Changes in Hb levels in relation to their baseline schistosomiasis and/or hookworm infection intensity category and anemia status from baseline to 1-year follow-up were modeled through two-level hierarchical models with level 1 (the children) and level 2 (the schools). Through this approach we aimed to compare the average change in Hb counts over the two examined periods between different groups of children. Baseline anemia status was also included in the explanatory part of the model to be able to examine rises in Hb levels in anemic and not anemic subjects as earlier recommended.[10] We made an effort to control for the fact that by adjusting for baseline anemia the effect of moderate or heavy infection for *S. mansoni* or hookworm may be underestimated if the type of anemia in the uninfected group is predominantly mild and self-limiting. We therefore also tested the two-way interaction terms of intensity of *S. mansoni* and of hookworm infections with anemia status, and because none were significant, these were omitted from the model. To test the statistical significance of the fixed effects, Wald tests were used, whereas likelihood ratio tests were performed for the random effects. All of the hierarchical models presented are random intercepts models with multiple independent variables and were obtained using Mlwin (Multilevel Models Project, Institute of Education, London, UK).

RESULTS

Because of drop-outs during the second year of the study, baseline and follow-up data concerning the Hb counts were available from 2,788 children, 6–14 years old, from 36 of the 37 schools initially visited at baseline, over the period 2003 and 2004 inclusive. In school Tonya in the Hoima district, Hb counts were measured neither at baseline nor at follow-up so this specific school was not included in this analysis. Table 1 presents the health indicators of children surveyed during this period. Over the 12 months between examinations, overall prevalence significantly decreased for both *S. mansoni* and hookworm infections as well as for anemia. For both years of the study the prevalences of severe anemia were negligible and therefore these cases were not examined further here. Similarly, overall point prevalences, at both time-points, for *T. trichura* and *A. lumbricoides* were very low. The arithmetic mean intensities for all subjects (negatives and positives) for *S. mansoni* and hookworm infections decreased significantly 1-year post-praziquantel and albendazole treatment. A significant increase in hemoglobin concentration was also observed during the study period.

Table 2 shows that there were significant differences between the children that were successfully followed up and the drop-outs with reference to their age, sex, home district, and schistosomiasis infection. Children of 6 and 11 years old or above as well as children living in Arua and Mayuge proved most difficult to recruit into the cohort in the second year of the study. The largest recovery cohort failure was observed in children heavily infected with *S. mansoni* at baseline.

The results of the hierarchical multivariate logistic regression model for the probability of a child being anemic at baseline are presented in Table 3. This model included intensities of *S. mansoni* and hookworm infections and the analysis controlled for age and sex, allowing also for assessment of the extent of between-school variation in anemia prevalence. At follow-up, there were very few children who were heavily infected with hookworm and therefore the convergence of the

TABLE 1
Health characteristics of children in 2003–2004 successfully followed up for 1 year

	2003	2004
Parasitology (n = 2,682)*		
Percent infected with *S. mansoni*	43.91 (42.01–45.81)‡	28.78 (27.06–30.52)
Percent infected with hookworm	52.13 (50.21–54.04)	24.04 (22.40–25.67)
Percent infected with *T. trichura*	2.34 (1.75–2.91)	2.29 (1.72–2.87)
Percent infected with *A. lumbricoides*	2.41 (1.82–3.00)	1.45 (1.00–1.91
Mean *S. mansoni* intensity (epg)	239.28 (215.82–262.73)	75.66 (64.58–86.74)
Mean hookworm intensity (epg)	307.19 (253.80–360.58)	79.36 (66.48–92.25)
Hematology (n = 2,788)		
Mean haemoglobin (g/dL)	11.43 (11.37–11.48)	11.67 (11.61–11.72)
Percent anemic	49.96 (48.11–51.82)	45.80 (43.95–47.65)
Percent severely anemic†	0.29 (0.09–0.49)	0.18 (0.02–0.34)

* Sample sizes are provided into parentheses for each examined outcome.
† Severe anemia: Hb < 7 g/dL.
‡ Values in parens are 95% CIs.

TABLE 2
Comparison of children successfully followd-up and drop-outs

Variable	Categories	Drop-outs	Followed up	*P***
Intensity of schistosomiasis infection	Uninfected	786 (50.29%)	1512 (54.23%)††	0.034
	Lightly infected*	245 (1.67%)	426 (15.28%)	
	Moderately infected†	171 (10.94%)	314 (11.26%)	
	Heavily infected‡	295 (18.87%)	440 (15.78%)	
	Missing	66 (4.22%)	96 (3.44%)	
Intensity of hookworm infection	Uninfected	736 (47.09%)	1289 (46.23%)	0.527
	Lightly infected§	724 (46.32%)	1330 (47.70%)	
	Moderately/heavily infected¶	37 (2.37%)	72 (2.58%)	
	Missing	66 (4.22%)	97 (3.48%)	
Age	≥ 11 years old	422 (27.00%)	707 (25.36%)	< 0.001
	8 years old	360 (23.03%)	739 (26.51%)	
	7 years old	352 (22.52%)	683 (24.50%)	
	6 years old	401 (25.66%)	654 (23.46%)	
	Missing	28 (1.79%)	5 (0.18%)	
Sex	Male	723 (46.26%)	1406 (50.43%)	< 0.001
	Female	702 (44.92%)	1380 (49.50%)	
	Missing	138 (8.83%)	2 (0.07%)	
Anemia	Not anemic	754 (48.24%)	1395 (50.04%)	0.260
	Anemic	809 (51.76%)	1393 (49.96%)	
	Missing	–	–	
District	Nebbi	148 (9.47%)	414 (14.85%)	< 0.001
	Arua	74 (4.73%)	44 (1.58%)	
	Bugiri	218 (13.95%)	380 (13.63%)	
	Busia	188 (12.03%)	440 (15.78%)	
	Hoima	233 (14.91%)	322 (11.55%)	
	Masindi	212 (13.56%)	366 (13.13%)	
	Mayuge	261 (16.70%)	332 (11.91%)	
	Moyo	229 (14.65%)	490 (17.58%)	
	Missing	–	–	

* Lightly infected with *S. mansoni:* 1–99 epg.
† Moderately infected with *S. mansoni:* 100–399 epg.
‡ Heavily infected with *S. mansoni:* ≥ 400 epg.
§ Lightly infected with hookworm: 1–1999 epg.
¶ Moderately/heavily infected with hookworm: ≥ 2000 epg.
** *P* value for χ^2 test.
†† Column percentages are given in the parentheses.

fitting algorithm in some of the models led us to merge the heavy and moderate hookworm intensities into one category. Moreover, there were very few children that were 9 and 10 years old (only two children were 9 and none were 10 years old) within the cohort with these ages who caused problems to some of the models fits and again we decided to exclude them from all analyses. This was performed in all models presented in this study so that they can be comparable. Also for a similar reason, we do not specifically examine co-infections of schistosomiasis and hookworm, because overall,

TABLE 3
Adjusted odds ratios from hierarchical multivariate logistic regression model for baseline prevalence of anemia (n = 2,682)

Variable	Categories	Adjusted odds ratio (95% CI)	*P*
Fixed effects			
Intensity of schistosomiasis infection at baseline	Uninfected	1	
	Lightly infected*	0.958 (0.753–1.219)	0.727
	Moderately infected†	1.274 (0.962–1.686)	0.091
	Heavily infected‡	1.309 (0.993–1.725)	0.056
Intensity of hookworm infection at baseline	Uninfected	1	
	Lightly infected§	1.142 (0.965–1.352)	0.123
	Moderately/heavily infected¶	2.219 (1.312–3.752)	0.003
Age at baseline	≥ 11 years old	1	
	8 years old	1.464 (1.175–1.823)	< 0.001
	7 years old	1.824 (1.453–2.290)	< 0.001
	6 years old	2.212 (1.755–2.788)	< 0.001
Sex	Male	1	
	Female	0.908 (0.774–1.064)	0.230
Random effects	σ^2	SE	
School-level variance	0.291	0.083	

* Lightly infected with *S. mansoni* at baseline: 1–99 epg.
† Moderately infected with *S. mansoni* at baseline: 100–399 epg.
‡ Heavily infected with *S. mansoni* at baseline: ≥ 400 epg.
§ Lightly infected with hookworm at baseline: 1–1999 epg.
¶ Moderately/heavily infected with hookworm: at baseline ≥ 2,000 epg.

there were too few co-infected children in our cohort to support model fitting.

Table 3 shows that children moderately or heavily infected with *S. mansoni* were marginally significantly more likely to be anemic than those uninfected with *S. mansoni*. Children moderately or heavily infected with hookworm were significantly more likely to be anemic than those uninfected with hookworm. The school-level variance at the bottom of the same table shows that schools differed significantly in the baseline prevalence of anemia observed. We also tested whether the effects of the intensities of schistosomiasis and hookworm infections varied by age, sex, or state of weight. For this reason we incorporated the relevant two-way interaction terms in the model, and because none of these were significant, these estimates are not presented here.

Results from the hierarchical logistic regression model for the probability of a child being anemic at 1-year follow-up are presented in Table 4. Children heavily infected with *S. mansoni* at follow-up were significantly more likely than uninfected children to be anemic. Age, sex, and moderate or heavy hookworm infections were also significant predictors. Finally, schools varied in the prevalence of anemia observed at follow-up.

Table 5 contains the estimates of the three-level hierarchical model for Hb counts before and after praziquantel and albendazole treatment. Normal plots of the different levels residuals appeared fairly linear, which suggested that the assumption of normality and the selection of a linear hierarchical model were reasonable. An overall increase of 0.061 g/dL in the Hb level after chemotherapeutic treatment was not significant ($P = 0.115$). Children heavily infected with *S. mansoni* had significantly lower Hb counts (0.322 g/dL; $P < 0.001$) compared with uninfected children at baseline. Children with moderate or heavy hookworm infection at baseline had significantly lower Hb counts (0.595 g/dL; $P < 0.001$) than those not infected after controlling for *S. mansoni* intensity, age, sex, and anemia status. The random effects variance components indicate that much of the variation is between children within a school, but there was also statistically significant variation between schools. The total variance is 1.758, the sum of the three variance components [$\mathrm{Var}(v_{0k})$, $\mathrm{Var}(u_{0jk})$, and $\mathrm{Var}(\varepsilon_{0ijk})$]. Of the total variability in Hb counts, 0.122/1.758 = 6.94% was situated at the school level, whereas 0.551/1.758 = 31.34% arose between children within a school.

Figure 1 shows 36 level 3 residuals plotted one for each school with the schools ordered according to the rank of their posterior mean. It indicates that most of them have approximately average Hb counts that cannot be distinguished statistically. The school with the lowest intercept residual (at the bottom left) and mean Hb count was Kibiro school in Hoima district. At baseline the *S. mansoni* and hookworm point prevalences in this school were 94.87% and 10.26%, whereas at follow-up these decreased to 87.18% and 2.56%, respectively. The school with the highest intercept and mean Hb count was Arua Public in the Arua district. The raw data for this school showed that *S. mansoni* and hookworm prevalence at baseline were 22.86% and 25.71% and decreased to 14.29% and 2.86%, respectively, at follow-up.

Finally, Table 6 contains the estimates of the two-level hierarchical model for the change of Hb counts between the 2 years of the study. It indicates that most risk groups had a relatively slowed reduction or actual increase in Hb counts after praziquantel and albendazole chemotherapy compared with the reference group (baseline uninfected, non-anemic, ≥ 11 years old). More precisely, children heavily infected with *S. mansoni* at baseline had a smaller decrease by 0.229 g/dL in their Hb counts in comparison with the uninfected with *S. mansoni*. Among children found to be anemic at the baseline survey, Hb counts increased significantly by 0.834 g/dL (i.e., 1.240–0.406 g/dL) after treatment.

DISCUSSION

Friedman and others[29] recommended that future research aimed to quantify a relationship between schistosomiasis and anemia should include an adequate follow-up period and include individuals with a range of infection intensities. To our knowledge, our study represents the first longitudinal examination into the occurrence of anemia in a cohort of children

TABLE 4

Adjusted odds ratios from hierarchical multivariate logistic regression model for follow-up prevalence of anemia ($n = 2,696$)

Variable	Categories		Adjusted odds ratio (95% CI)	*P*
Fixed effects				
Intensity of schistosomiasis infection at follow-up	Uninfected		1	
	Lightly infected*		1.158 (0.905–1.483)	0.244
	Moderately infected†		1.203 (08.66–1.672)	0.272
	Heavily infected‡		1.567 (1.038–2.365)	0.032
Intensity of hookworm infection at follow-up	Uninfected		1	
	Lightly infected§		1.037 (0.842–1.276)	0.732
	Moderately/heavily infected¶		4.289 (1.468–12.530)	0.008
Age at follow-up	≥ 12 years old		1	
	9 years old		0.697 (0.560–0.868)	0.001
	8 years old		0.760 (0.607–0.950)	0.015
	7 years old		1.184 (0.941–1.489)	0.147
Sex	Male		1	
	Female		0.837 (0.713–0.983)	0.029
Random effects	σ^2	SE		
School-level variance	0.367	0.100		

* Lightly infected with *S. mansoni* at follow-up: 1–99 epg.
† Moderately infected with *S. mansoni* at follow-up: 100–399 epg.
‡ Heavily infected with *S. mansoni* at follow-up: ≥ 400 epg.
§ Lightly infected with hookworm at follow-up: 1–1999 epg.
¶ Moderately/heavily infected with hookworm at follow-up: ≥ 2,000 epg.

TABLE 5
Estimates from three-level hierarchical model for hemoglobin counts before and after chemotherapeutic treatment (n = 2,682)

Fixed effects	Parameter	Coefficient (SE)	Wald test statistics (P)
Intercept	γ_{00}	11.854 (0.083)	< 0.001
Baseline intensity of schistosomiasis infection (reference category uninfected)	γ_{01}	−0.073 (0.055)	0.183
Moderate†	γ_{02}	−0.153 (0.068)	0.024
Heavy‡	γ_{03}	−0.322 (0.072)	< 0.001
Baseline intensity of hookworm infection (reference category uninfected)			
Light§	γ_{04}	−0.046 (0.041)	0.262
Moderate/heavy¶	γ_{05}	−0.595 (0.139)	< 0.001
Sex (reference category male)			
Female	γ_{06}	0.097 (0.042)	0.115
Baseline age (reference category ≥ 11 years old)			
8 years old	γ_{07}	−0.319 (0.052)	< 0.001
7 years old	γ_{08}	−0.509 (0.053)	< 0.001
6 years old	γ_{09}	−0.615 (0.069)	< 0.001
Effect of follow-up (relative to baseline)—linear coefficient of time	γ_{10}	0.061 (0.039)	0.115
Random effects			
Level-3 (i.e., between schools) variance			
$\sigma_v^2 = \mathrm{Var}(v_{0k})$		0.122 (0.032)	
Level-2 (i.e., between children within a school) variance			
$\sigma_u^2 = \mathrm{Var}(u_{0\,jk})$		0.551 (0.038)	
Level-1 (i.e., measurement occasions within a child) variance			
$\sigma_\varepsilon^2 = \mathrm{Var}(\varepsilon_{0ijk})$		1.085 (0.034)	
Deviance		15,398.82	

* Lightly infected with *S. mansoni* at baseline: 1–99 epg.
† Moderately infected with *S. mansoni* at baseline: 100–399 epg.
‡ Heavily infected with *S. mansoni* at baseline: ≥ 400 epg.
§ Lightly infected with hookworm at baseline: 1–1999 epg.
¶ Moderately/heavily infected with hookworm at baseline: ≥ 2,000 epg.

infected with *S. mansoni* and hookworm in Uganda using a uniquely detailed large dataset arising from the National Control Schistosomiasis Program within the country. We examined the relationship of *S. mansoni* and hookworm infection to anemia and Hb levels and evaluated the effectiveness of practical interventions to control or reduce the severity of such anemia.

Helminth infections at a young age may induce, among other factors, pro-inflammatory mediators that are detrimental to protein metabolism, appetite, and erythropoiesis and the WHO therefore recommends the use of anti-schistosome treatment.[1] In Uganda, it has been proven that drug distribution in schools is excellent and community-directed treatment is a feasible health approach for mass drug distribution in poor remote communities.[30] Our study provides further convincing evidence to show that young children may benefit from deworming in terms of increased Hb and consequently reduced anemia levels. In particular, while our data clearly show there was association between anemia and both schistosomiasis and hookworm infection at baseline, by the follow-up data, the probability of anemia was only associated with heavy *S. mansoni* and moderate or heavy hookworm infections (Tables 3 and 4).

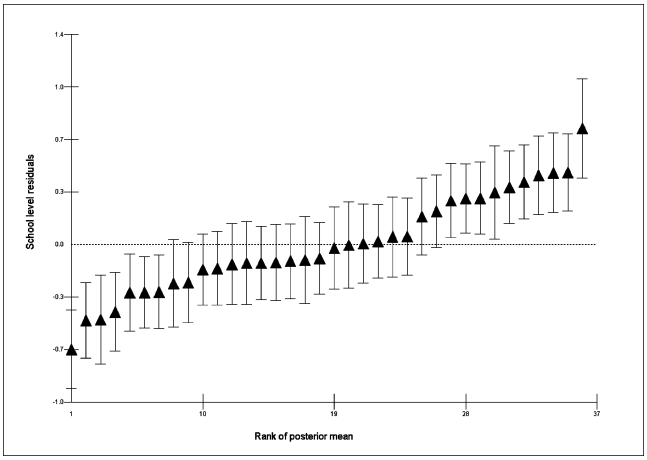

FIGURE 1. Caterpillar plot: 36 level 3 residuals from model presented in Table 5 (bars represent 95% CIs and the triangles level 3 residuals).

However, whereas the first hierarchical model of Hb counts (Table 5) showed that treatment arrests the drop in Hb, it suggests that without iron replacement, Hb levels probably will not rise. Additionally, the second hierarchical model (Table 6) revealed a drop in Hb counts in the comparison group of baseline uninfected, non-anemic, ≥ 11-year-old children. One could speculate that this could reflect, for example, an increased malaria infection rate at follow-up and/or a bad harvest during the second year of this study, perhaps explained from the fact that there were > 1.6 million internally displaced persons in Uganda's north and east where malnutrition is one of the most pressing health concerns as a result of fighting between government forces and rebels from the Lord's Resistance Army.[31] It is likely that inadequate dietary iron was another factor contributing to this observed decline. These results confirm the findings of Taylor and others[32] in South Africa where through a randomized controlled trial provided two different anthelmintic treatment regimens twice at 6-month intervals combined with iron supplementation for 1 year in groups of 428 primary school pupils. These authors observed a significant decrease in Hb levels of pupils receiving triple dose of albendazole and praziquantel only and a significant increase in Hb levels of pupils receiving three doses of these drugs and iron supplementation. Such combined results thereby strengthen the argument of Stoltzfus

TABLE 6
Estimates from two-level hierarchical model for the change of hemoglobin counts (n = 2,682)

Fixed effects	Parameter	Coefficient (SE)	Wald test statistics (P)
Intercept	γ_{00}	–0.406 (0.103)	< 0.001
Baseline intensity of schistosomiasis infection (reference category uninfected)			
Light*	γ_{01}	–0.061 (0.076)	0.428
Moderate†	γ_{02}	–0.004 (0.090)	0.963
Heavy‡	γ_{03}	0.229 (0.089)	0.010
Baseline intensity of hookworm infection (reference category uninfected)			
Light§	γ_{04}	0.021 (0.054)	0.694
Moderate/heavy¶	γ_{05}	0.274 (0.160)	0.087
Sex (reference category male)			
Female	γ_{06}	0.030 (0.050)	0.552
Baseline age (reference category ≥ 11 years old)			
8 years old	γ_{07}	0.015 (0.070)	0.824
7 years old	γ_{08}	0.028 (0.071)	0.694
6 years old	γ_{09}	–0.161 (0.073)	0.028
Baseline anemia (reference category nonanemic children)			
Anemic	γ_{10}	1.240 (0.052)	< 0.001
Random effects		Variance components	
Level-2 (i.e., between schools) variance			
$\sigma_u^2 = \mathrm{Var}(u_{0\,jk})$		0.180 (0.048)	
Level-1 (i.e., between children within a school) variance			
$\sigma_\varepsilon^2 = \mathrm{Var}(\varepsilon_{0ijk})$		1.660 (0.046)	
Deviance		9,076.5	

* Lightly infected with *S. mansoni* at baseline: 1–99 epg.
† Moderately infected with *S. mansoni* at baseline: 100–399 epg.
‡ Heavily infected with *S. mansoni* at baseline: ≥ 400 epg.
§ Lightly infected with hookworm at baseline: 1–1999 epg.
¶ Moderately/heavily infected with hookworm at baseline: ≥ 2,000 epg.

and others[33] that deworming programs should be combined with increased iron intake through supplementation, fortification, or improved diet to reduce the incidence of anemia substantially.

Moreover, data from Beasley and others[34] in rural Tanga, Tanzania, and Friis and others[35] in western Kenya suggest that children most benefited from anthelmintic treatment in terms of increased Hb levels were those who were anemic at baseline. From our second hierarchical model of Hb counts (Table 6), we also found a significant increase in the change of Hb counts and a stronger effect for anemic children compared with non-anemic. This same model also showed a slower reduction in Hb counts for heavily infected children with *S. mansoni* and moderately or heavily infected children with hookworm compared with uninfected after treatment, which suggests that the effect of anthelmintic treatment on Hb was mediated by reductions in intensities of *S.mansoni* and hookworm infections.

Ethical reasons as well as the operational reality of the national control program did not permit the inclusion of a control (i.e., untreated) group here. Consequently, the study design did not allow estimation of the absolute impact of treatment only the relative impact in different groups. Therefore, to provide substantial support for the plausibility of the impact of the intervention, the epidemiologic findings from the models presented in this study should be further validated with quantitative predictions arising from mathematical models and this work is underway. The high percentage of drop outs in the group of heavily infected children with *S. mansoni* (Table 1) because of frequent population movements within Uganda, might also add some bias to the epidemiologic findings of this study. Moreover, although malaria is a well-known cause of anemia and *Plasmodium falciparum* is almost holoendemic in our study population,[36] this factor as well as dietary iron intake were not examined, but we highly recommend that this information should be included in future evaluation follow-ups.[29] Indeed the variance components of the linear hierarchical models (Tables 5 and 6) have shown that there remains substantial variability that is not explained by the models, and this might well be caused by malaria and iron deficiency not being taken into account here. In addition to these, variables referring to mosquito control at the school/village level were not available, and future studies may benefit from their incorporation. The hierarchical model did, however, show that Kibiro school in Hoima district, with significantly lower mean Hb count compared with the overall mean Hb count, had also very high *S. mansoni* prevalence, possibly because of its proximity to Lake Albert, the major source of transmission. On the other hand, the school with significantly higher mean Hb count compared with the overall mean Hb count was situated in the northern part of the country, where *S. mansoni* and hookworm prevalences were moderately low. These results agree with previous work on the distribution of *S. mansoni* in Uganda, which indicates that highest schistosomiasis prevalences are found close to the eastern shores of Lakes Albert and Victoria, whereas areas of low or zero prevalence are found in the northeast of the country.[37]

To conclude, our results, from a large scale national control program suggest that *S. mansoni* and hookworm infections may be related to anemia in specific districts of Uganda and chemotherapy with praziquantel and albendazole may reduce anemia. Anemia is therefore likely to represent a valuable marker for morbidity caused by heavy infection with *S. mansoni*, provided that other likely causes, such as hookworm, dietary iron intake, and malaria, are taken into consideration.

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Relationships between anaemia and parasitic infections in Kenyan schoolchildren: A Bayesian hierarchical modelling approach.

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Relationships between anaemia and parasitic infections in Kenyan schoolchildren: A Bayesian hierarchical modelling approach

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Abstract

Anaemia is multi-factorial in origin and disentangling its aetiology remains problematic, with surprisingly few studies investigating the relative contribution of different parasitic infections to anaemia amongst schoolchildren. We report cross-sectional data on haemoglobin, malaria parasitaemia, helminth infection and undernutrition among 1523 schoolchildren enrolled in classes 5 and 6 (aged 10–21 years) in 30 primary schools in western Kenya. Bayesian hierarchical modelling was used to investigate putative relationships. Children infected with *Plasmodium falciparum* or with a heavy *Schistosoma mansoni* infection, stunted children and girls were found to have lower haemoglobin concentrations. Children heavily infected with *S. mansoni* were also more likely to be anaemic compared with uninfected children. This study further highlights the importance of malaria and intestinal schistosomiasis as contributors to reduced haemoglobin levels among schoolchildren and helps guide the implementation of integrated school health programmes in areas of differing parasite transmission.

Keywords: Anaemia; Haemoglobin concentration; Malaria; Helminth infections; Bayesian hierarchical models; School children; Kenya

1. Introduction

Anaemia remains one of the most intractable public health problems in Africa, contributing to a quarter of Africa's nutrition-related Disability Adjusted Life Years (DALYs) lost (World Health Organization, 2002). Several studies have highlighted the contribution of parasitic diseases to childhood anaemia. Recent meta-analyses of malaria intervention trials among African children, for example, provide compelling evidence that both symptomatic and asymptomatic malaria contributes to anaemia (Geerligs et al., 2003; Korenromp et al., 2004). The effect of hookworm infection is also well documented, with risk of anaemia correlated with intensity of infection (Stephenson, 1993; Olsen et al., 1998; Brooker et al., 1999); in contrast, the contributory role of schistosomiasis remains unclear (Desai et al., 2005b; Friedman et al., 2005a). However, there are surprisingly few published studies describing the relative contribution of these different parasitic infections in populations of school-aged children (Olsen et al.,

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1998; Tatala et al., 1998; Friis et al., 2003; Leenstra et al., 2003; Leenstra et al., 2004; Desai et al., 2005a; Friedman et al., 2005b). Investigation of this issue is particularly relevant for the design of integrated control strategies aimed at reducing anaemia, including anthelmintic treatment programmes, micronutrient supplementation and malaria control measures, through school health programmes. In particular, the same suite of school-based interventions will not be relevant everywhere, and the selection of intervention options will need to be guided by an informed understanding of the epidemiology of parasite-related anaemia (Crawley, 2004), as well as of the geography of infection (Brooker et al., 2007).

The aim of the present study was to examine the relationships of haemoglobin (Hb) concentration and anaemia with common parasitic infections, including malaria, hookworm, *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma mansoni* in school children in western Kenya through Bayesian hierarchical modelling. Adjustment for nutritional and socioeconomic status (SES) has also been taken into account here as they might influence anaemia risk (Ong'echa et al., 2006). By employing a Bayesian approach for the statistical modelling of the Hb counts and of anaemia prevalence, our model specification via Markov chain Monte Carlo (MCMC) algorithms offers flexibility in fitting complex models and enables estimates for the whole distribution of the unknown parameters, including point and interval estimates, to be derived. This approach is in contrast to the frequentist approach which often only gives estimates and crude standard errors based on asymptotic results.

2. Materials and methods

2.1. Study area

The study was conducted between February and March 2005 in 30 primary schools in Bondo district in western Kenya. Malaria transmission is intense and perennial (Beier et al., 1994), with two seasonal peaks, March–May and November–December, following the long and short rainy seasons, respectively. Previous studies in western Kenya have reported a high prevalence of hookworm and *T. trichiura* infections and a medium prevalence of *S. mansoni* and *A. lumbricoides* infections (Brooker et al., 2000; Thiong'o et al., 2001).

2.2. Study design

This study used cross-sectional, baseline data from a stratified, cluster-randomised placebo-controlled trial of the impact of antimalarial intermittent preventive treatment (IPT) among schoolchildren. The trial design and protocol are described elsewhere (Clarke et al., 2008). Briefly, sample size was estimated on the basis of the expected impact of IPT on anaemia, using the methods for cluster-randomised trial design proposed by Hayes and Bennett (1999). The 30 study schools were randomly selected from primary schools in Usigu and Maranda Divisions with $\geqslant$150 pupils with >15 pupils per class and located more than 5 km from the shores of Lake Victoria, so as to minimise the effect of *S. mansoni* which is generally only prevalent along the shoreline (Brooker et al., 2001; Handzel et al., 2003). No stratification by intestinal nematode was undertaken because of their relatively homogeneous distribution (Handzel et al., 2003) but schools were stratified according to past school examination performance. We present data from the baseline survey on a sub-sample of children enrolled in classes 5 and 6 (age range 10–21 years) for whom complete data on anaemia, helminth infection, malaria parasitaemia, nutritional status and SES were available.

2.3. Procedures

Finger-prick blood samples were obtained from all children to assess Hb levels and malaria parasitaemia. Haemoglobin was measured in the field using a portable photometer (Haemocue, Angelholm, Sweden). Malaria parasite prevalence and parasite densities were estimated in Giemsa-stained thick blood films, assuming an average white blood cell count of 8,000 per μl, with species identification carried out on Giemsa-stained thin films. A slide was declared negative after examination of 100 high-powered fields. Stool samples provided by each child were examined microscopically using the semi-quantitative Kato-Katz technique and intensity of infection was expressed as eggs/gram of faeces. Height was measured to the nearest 0.1 cm using a Leicester portable fixed base stadiometer (Chasmors, UK) and weight was measured to the nearest 0.1 kg using an electronic balance. A simple questionnaire was administered to pupils to obtain data on key socio-economic variables including: structure of the house, type of overall light, ownership of bicycle, use of bednet as well as education of the child's guardian.

Ethical clearance for the study was obtained from the ethics committee of the Kenyatta National Hospital, Kenya and from the London School of Hygiene and Tropical Medicine, UK. Permissions were obtained from the Ministry of Education, and the district education and health authorities, and headteachers. Prior to the start of the study, a series of meetings were held in participating schools to explain the nature and purpose of the trial and to obtain individual informed parental consent from the parents or legal guardians of children enrolled in study schools.

2.4. Statistical analysis

An index of SES was constructed from asset and education variables using principal component analysis (Filmer and Pritchett, 2001). Data were available for 1453 (92%) of the 1577 children in 30 schools. Analysis was done using the PROC PRINCOMP command in SAS version 9.1

(SAS Institute Inc., Cary, NC). For the index of SES, the first principal component explained 28% of the variance in the asset and education variables with the greatest weight given to the presence of a permanent house structure (0.43), and the lowest weight to the presence of a traditional house structure (−0.39), respectively. Weights for each variable were derived from the first principal component and applied to each child to derive a SES index. We then assigned these children to a group on the basis of their value on the index. Following the approach of Filmer and Pritchett, we classified children into a 0–39 percentile, 40–79 percentile and upper 20 percentile, which we refer to as 'most poor', 'poor' and 'least poor', respectively. Anthropometric indices were calculated on the basis of the 2000 Centres for Disease Control and Prevention (CDC) Growth Charts, and analysed as binary variables. Children were classified as stunted if z-scores of height-for-age were less than 2 S.D. below the CDC median. Body mass index, which is weight (kg)/height (cm)^2, was also calculated and a cut-off of −2 Body Mass Index Z-scores (BMIZ) were calculated to classify underweight children.

Data management and bivariate relationships between mean Hb concentration and key predictors examined were obtained using SAS V 9.1 (SAS Institute Inc., Cary, NC, USA).

2.4.1. Bayesian modelling

Bayesian hierarchical modelling was used to assess associations between anaemia, Hb and parasitic infection, including children and schools as random effects drawn from some common prior distribution with unknown parameters. Children within each school as well as the results from different schools were treated as 'exchangeable' in the sense that their joint probability densities should be invariant to permutations of the indexes (Gelman et al., 2004).

In Bayesian analysis, the proposed model of the observed data is combined with the prior distribution of all the unknown model parameters to give the posterior distributions for all unknowns. MCMC methods (Gilks et al., 1996) are used to sample from the posterior distributions of the unknown parameters. Analysis was conducted using WinBUGS which employs the Gibbs sampler to form the posterior distribution for each unknown parameter by drawing samples from their full conditional distributions (Spiegelhalter et al., 2003) to fit models. An advantage of the Bayesian hierarchical approach is that prior information can be incorporated in the model in probabilistic form. However, in the absence of any prior knowledge about the model parameters, the choice of non-informative improper priors is dictated by pragmatic conditions (Diggle et al., 2002). Model convergence was evaluated on the basis of inspection of sample traces, which all showed a reasonable degree of convergence to a stationary distribution.

We fitted Bayesian normal hierarchical models on Hb because there is a two-level data structure applied to cross-sectional data. Individual subjects were classified at the lower level for older children with data on helminth infection and age range 10–21 years old was classified by cluster at the higher level. We assume that individual i (=1,...,n) – where $n = 1523$ can belong in any of j (=1,...,$n2$) – where $n2 = 30$ schools. Hb at a child level may be affected by those childrens' characteristics (age, sex, intensities of helminth infections, nutritional and socio-economic status) but may also vary according to which school these children are enrolled in. Specifically for Hb, we used a random intercepts normal model which can be written as:

$$Hb_{ij} \sim \text{normal}(\mu_{ij}, \tau),$$
$$\text{with } \mu_{ij} = X_{ij}\beta + u_j \text{ and } \tau \sim \text{gamma}(0.001, 0.001),$$
$$u_j \sim \text{normal}(0, \tau_{u_j}) \text{ and } \tau_{u_j} \sim \text{gamma}(0.001, 0.001).$$

Where μ_{ij} is given by the sum of the product of X_{ij} with β – this constitutes the fixed part of the model, and u_{j-} constitutes the random part of the model. More precisely, X_{ij} is a vector of individual-level characteristics, β is a vector of k estimated parameter coefficients and u_j is the error term at the school-level which represents each school's difference from the overall population mean as its mean is set to 0. τ represents the *precision* (1/variance) of the normal distribution of the response Hb whereas τ_{uj} represents the *precision* (1/variance) of the normal distribution of the u_j's. For both variance components, we follow the usual practice of specifying a gamma prior distribution to the corresponding precision parameters which is proper and close to being uniform on log (τ).

For the vector of the k estimated parameter coefficients we assumed:

$$p(\beta_k) \propto \quad 1$$

Where $p(\beta_k)$ symbolizes the prior distribution of the k estimated parameters and is proportional to 1.

In order to examine the prevalence of anaemia with associated covariate vectors, we chose the single outcome of the probability that a child is anaemic as estimated by a hierarchical Bayesian logistic regression model. We labeled the survey responses $anaemia_{ij}$ as 1 for children i being anaemic (if Hb < 11.0 g/dL) in school j and 0 otherwise, and model them independently with $\Pr(\text{anaemia}_{ij} = 1) = \text{logit}^{-1}((X\beta)_{ij})$. We present an analysis based on a prior distribution for β that is independent and locally uniform in the k parameters; that is $p(\beta_1, \ldots, \beta_k) \propto 1$. Specifically for the risk of being anaemic, we used a random intercepts logit link model which can be written as:

$$\text{anaemia}_{ij} \sim \text{binomial}(\pi_{ij}, n_{ij}),$$
$$\text{with } \mathit{logit}(\pi_{ij}) = X_{ij}\beta + u_j \text{ and } \tau \sim \text{gamma}(0.001, 0.001),$$
$$u_j \sim \mathit{normal}(0, \tau_{u_j}) \text{ and } \tau_{u_j} \sim \text{gamma}(0.001, 0.001)$$

Where n_{ij} is the total number of events (this is equal to 1523 children). The rest of the notation remains identical to the normal hierarchical Bayesian models which were employed for the analysis of the Hb levels. In these aforementioned

regression models we used the same predictors of anaemia prevalence as those in the models of Hb counts.

To compare model complexities and goodness of fit we also monitored the recently proposed deviance information criterion (DIC) of (Spiegelhalter et al., 2002).

3. Results

Data on Hb, malaria parasitaemia and helminth infection were available for 1523 children (aged 10–21 years) in the 30 schools (Table 1). Overall 13.5%, (95% Confidence Interval (CI): 11.8–15.2) of these children were anaemic at the time of survey and the mean Hb concentration was estimated to be 12.43 g/dL, (95% CI: 12.35–12.50).

A total of 34.7% of children were infected with *Plasmodium falciparum* with a further 0.3% having mixed infections with *P. falciparum* and *Plasmodium malariae*. Up to 76.9% of children were infected with at least one parasitic helminth infection. Hookworm was the most prevalent helminth infection (47.3%); 14.1% were infected with *S. mansoni*, 23.7% with *A. lumbricoides* and 12.9% with *T. trichiura.* The prevalences of *P. falciparum*, hookworm and *S. mansoni* were, respectively, 35.8%, 41.2%

Table 1
Mean Hb concentration and prevalence of anaemia according to infection status and other characteristics in 1523 schoolchildren aged 10–21 years (univariate analysis)

Variable	Children No (%)	Mean Hb level, g/dL (95% CI)	Anaemia prevalence (%) (95% CI)
Sex			
Male	786 (51.6)	12.51 (12.40–12.61)	13.2 (10.9–15.6)
Female	737 (48.4)	12.34 (12.23–12.45)	13.7 (11.2–16.2)
Age			
10–12 years old	573 (39.0)	12.29 (12.17–12.41)	13.1 (10.3–15.9)
13–15 years old	896 (61.099)	12.52 (12.42–12.62)	13.8 (11.6–16.1)
> = 16 years old	54 (3.7)	12.44 (12.08–12.80)	11.1 (2.7–19.5)
Classification of SES ($n = 1404$[b])			
Least poor	281 (20.01)	12.42 (12.24–12.61)	12.81 (8.90–16.72)
Poor	560 (39.89)	12.45 (12.34–12.58)	13.93 (11.06–16.79)
Most poor	563 (40.10)	12.40 (12.28–12.53)	13.14 (10.35–15.93)
Classification of BMIZ			
Not underweight	1430 (93.9)	12.44 (12.36–12.52)	13.2 (11.4–14.9)
Underweight	93 (6.1)	12.22 (11.92–12.52)	18.3 (10.4–26.1)
Classification of HAZ			
Not stunted	1323 (86.9)	12.48 (12.40–12.56)	12.8 (11.0–14.6)
Stunted	200 (13.1)	12.10 (11.88–12.31)	18.0 (12.7–23.3)
Intensity of hookworm infection[c]			
Not infected	803 (52.7)	12.42 (12.31–12.53)	14.0 (11.6–16.3)
Lightly infected	691 (45.4)	12.45 (12.35–12.56)	12.9 (10.4–15.4)
Moderately infected	15 (1.0)	12.21 (11.60–12.83)	13.3 (0.0–30.5)
Heavily infected	14 (0.9)	11.87 (11.34–12.40)	14.3 (0.0–32.6)
Intensity of *S. mansoni* infection[c]			
Not infected	1309 (86.0)	12.44 (12.36–12.53)	13.2 (11.4–15.1)
Lightly infected	91 (6.0)	12.41 (12.11–12.71)	11.0 (4.6–17.4)
Moderately infected	76 (5.0)	12.54 (12.21–12.87)	13.2 (5.6–20.8)
Heavily infected	47 (3.0)	11.79 (11.28 to 12.30)	25.5 (13.1–38.0)
Intensity of *T.trichiura* infection[c]			
Not infected	1326 (87.1)	12.41 (12.33–12.50)	13.4 (11.6–15.3)
Lightly infected	188 (12.3)	12.47 (12.26–12.69)	14.4 (9.4–19.4)
Moderately infected	8 (0.5)	13.26 (12.30–14.23)	0.0 NA[a]
Heavily infected	1 (0.1)	12.80 NA[a]	0.0 NA[a]
Intensity of *A. lumbricoides* infection[c]			
Not infected	1162 (76.3)	12.46 (12.37–12.55)	13.6 (11.6–15.6)
Lightly infected	236 (15.5)	12.34 (12.16–12.52)	13.1 (8.8–17.5)
Moderately infected	125 (8.2)	12.27 (12.04–12.50)	12.8 (6.9–18.7)
Heavily infected	0 (0.0)	0.0 NA[a]	0.0 NA[a]
Malaria spp. infection			
Not infected	989 (69.9)	12.49 (12.40–12.58)	12.8 (10.8–14.9)
Infected	534 (35.1)	12.31 (12.18–12.44)	14.6 (11.6–17.6)

BMIZ, Body Mass Index *Z*-score; HAZ, Height for Age *Z*-score; CI, confidence interval.

[a] NA, not available.

[b] Socio-economic status (SES) data were missing for 119 children.

[c] Intensity of helminth infection was classified in light, moderate and heavy according to WHO recommended thresholds: *Schistosoma mansoni* infection, 1–99, 100–399 and ⩾400 eggs per gram of faeces (epg); hookworm, 1–1999, 2000–3999 and ⩾4000 epg; *Trichuris trichiura*, 1–999, 1000–9999 and ⩾10,000 epg; and *Ascaris lumbricoides*, 1–4999, 5000–49,999 and ⩾50,000 epg.

and 12.4% in the 10–12 years old age group, 34.4%, 51.2% and 14.7% in the 13–15 years old age group and 25.9%, 46.3% and 20.4% in the older age group (i.e. > = 16 years old).

3.1. Bayesian hierarchical normal model of haemoglobin

Table 2 contains the posterior means and 95% credible intervals of the final two-level Bayesian normal hierarchical model for the Hb of children with complete data on all covariates. According to this model, the posterior mean for the overall mean Hb was estimated to be 12.52 g/dL (95% credible interval: 12.28–12.76). On average, girls had lower mean Hb compared with boys by 0.18 g/dL. Older children tended to have higher mean Hb than children aged 10–12 years. Stunted children compared with non-stunted children had lower mean Hb by 0.34 g/dL. From the 95% credible intervals of all the parasitic infections, only children heavily infected with *S. mansoni* had significantly lower mean Hb by an average of 0.51 g/dL, (95% credible interval: −0.94 to −0.10) and children infected with malaria had significantly lower mean Hb by an average of 0.16 g/dL, (95% credible interval: −0.32 to 0.01) compared with uninfected children, respectively. Although there was no evidence of a significant effect of the intensities of single hookworm, *T. trichiura* or *A. lumbricoides* infection, we still allowed adjustment for these and therefore they were finally included in the model. The two-way interaction terms of intensities of helminth infections mentioned before, were also included and tested in the models in order to check for the effect of helminth co-infections on the mean Hb. As none of these terms, as well as the SES, were found to be significantly associated with Hb levels and/or anaemia, they were omitted from the final model. The random effects variance components indicate that most of the variance is between children within a school: of the total variability in Hb, 9.9% (0.225/2.256) occurred at the school level while 90.0% (2.031/2.256) occurred between children within a school. This is also illustrated by Fig. 1 which presents a box plot of the level-2 residuals among older children for each school, and indicates that most schools had similar Hb levels that

Table 2
Estimated posterior mean differences in mean Hb concentration for the effects of selected explanatory variables from a final Bayesian hierarchical model ($n = 1523$)

Variable	Mean	95% credible interval[a]
Fixed part of the model		
Intercept	12.520	(12.280–12.760)
Sex (Reference category: 'Male') Female	−0.183	(−0.330 to −0.036)[b]
Age (Reference category: '10–12 years old')		
13–15 years old	0.222	(0.064–0.377)[b]
> = 16 years old	0.417	(0.012–0.832)[b]
Classification of BMIZ (Reference category:' Not wasted') Wasted	−0.244	(−0.544 to 0.062)
Classification of HAZ (Reference category:' Not stunted') Stunted	−0.347	(−0.564 to −0.128)[b]
Intensity of hookworm infection (Reference category: 'Not Infected')		
Lightly infected	0.050	(−0.102 to 0.197)
Moderately infected	−0.310	(−1.048 to 0.419)
Heavily infected	−0.516	(−1.277 to 0.233)
Intensity of *Schistosoma mansoni* infection (Reference category: 'Not Infected')		
Lightly infected	−0.113	(−0.417 to 0.189)
Moderately infected	0.068	(−0.268 to 0.414)
Heavily infected	−0.513	(−0.942 to −0.097)[b]
Intensity of *Trichuris trichiura* infection (Reference category: 'Not Infected')		
Lightly infected	0.105	(−0.121 to 0.336)
Moderately infected	0.834	(−0.195 to 1.857)
Heavily infected	0.110	(−2.649 to 2.890)
Intensity of *Ascaris lumbricoides* infection (Reference category: 'Not Infected')		
Lightly infected	−0.164	(−0.372 to 0.047)
Moderately infected	−0.206	(−0.480 to 0.073)
Malaria spp infection (Reference category: 'Not Infected') Infected	−0.159	(−0.315 to −0.009)[b]
Random part of the model		
Level-2 (i.e. between schools) variance	0.225	(0.114–0.413)
Level-1 (i.e. between children within a school) variance	2.031	(1.889–2.186)

BMIZ, Body Mass Index *Z*-score; HAZ, Height for Age *Z*-score.

[a] 95% Credible intervals (CIs) are different from classical 95% confidence intervals in various ways, some of which are: (i) in their interpretation: we say there is a 95% probability that the true parameter lies in a 95% credible interval where this is certainly not the interpretation of a 95% confidence interval. In a long series of 95% confidence intervals, 95% of those should contain the true parameter value – unlike the Bayesian interpretation we cannot give a probability for whether a particular confidence interval contains the true value; and (ii) credible intervals will generally be narrower due to the additional information provided by the prior (Spiegelhalter et al., 2004).

[b] These are significant differences compared with the reference category in the sense that the probability is at least 95% that these parameters lie within the credible interval, which is significant.

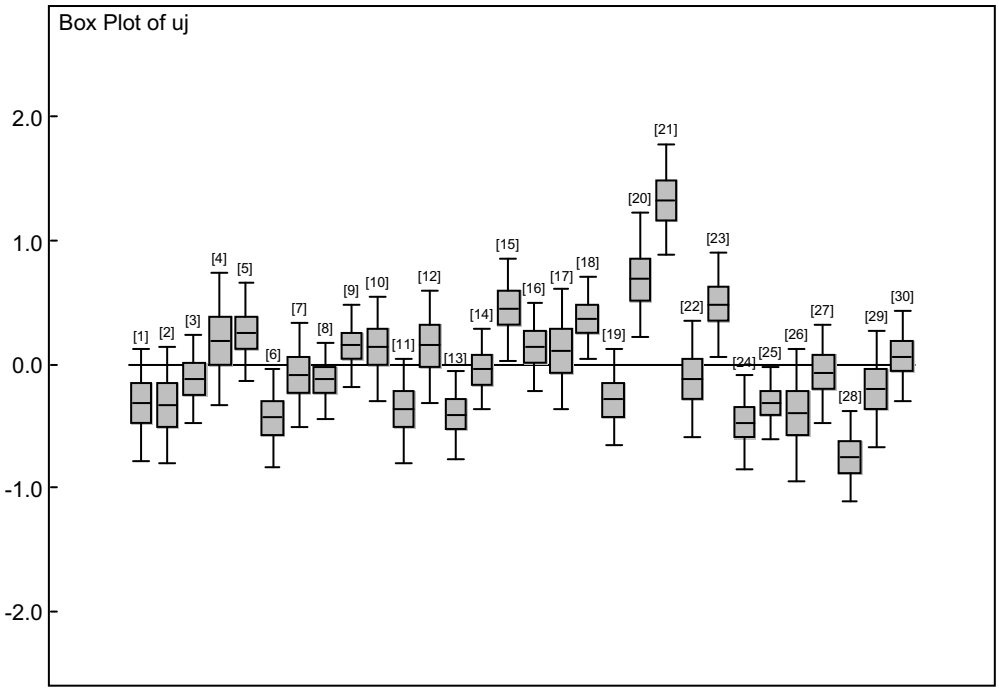

Fig. 1. Box plot of school-level residuals from a Bayesian hierarchical model for Hb counts among older children. Each box plot represents a school-level residual uj in our study. Numbers above each box plot are label identifiers for each school. This is a plot in which the posterior distributions of all ujs are summarised side by side. Boxes represent inter-quartile ranges and the solid black line at the (approximate) centre of each box is the mean of each specific uj; the arms of each box extend to cover the central 95 percent of the distribution–their ends correspond, therefore, to the 2.5% and 97.5% quantiles. The horizontal straight line in the middle of the graph represents the overall mean of the ujs which is set to 0.

could not be distinguished statistically, thereby confirming the suitability of assumptions for the chosen final model. Interestingly, mean Hb was substantially higher in one school (#21) relative to other schools.

3.2. Bayesian logistic regression model of anaemia

Table 3 presents the hierarchical logistic regression model of anaemia risk and shows that only children with

Table 3
Estimated posterior odds ratios for prevalence of anaemia (Hb < 110 g/L) for the effects of selected explanatory variables from final Bayesian hierarchical logistic regression model ($n = 1523$)

Variable	Odds ratio	95% credible interval
Fixed part of the model		
Main effects		
Sex (Reference category: 'Male')		
Female	1.073	(0.787 to 1.405)
Age (Reference category: '>=10–12 years old')		
13–15 years old	1.095	(0.804–1.462)
>= 16 years old	0.688	(0.218–1.547)
Intensity of hookworm infection (Reference category: 'Not Infected')		
Lightly infected	0.895	(0.645–1.208)
Moderately infected	1.156	(0.121–3.708)
Heavily infected	1.165	(0.132–3.861)
Intensity of *Schistosoma mansoni* infection (Reference category: 'Not Infected')		
Lightly infected	0.825	(0.360–1.442)
Moderately infected	1.004	(0.426–1.969)
Heavily infected	2.292	(1.070–4.258)[a]
Intensity of *Ascaris lumbricoides* infection (Reference category: 'Not Infected')		
Lightly infected	1.060	(0.680–1.584)
Moderately infected	1.057	(0.582–1.748)
Malaria spp infection (Reference category: 'Not Infected') infected	1.136	(0.821–1.540)
Random part of the model		
Level-2 (i.e. between schools) variance	0.288	(0.088–0.630)

[a] This is a significant odds ratio of heavily infected children with *S. mansoni* compared with uninfected children in the same sense as denoted in Table 2.

heavy *S. mansoni* intensities were more likely to be anaemic, defined as Hb < 11.0 g/dL, compared with uninfected children (Odds ratio: OR = 2.3, 95% credible interval: 1.1–4.3; Table 3). There was no evidence that other predictors were significantly associated with the risk of anaemia.

4. Discussion

Malaria, undernutrition and helminth infections have a large impact on the survival and quality of lives of school-aged children living in Africa. Understanding the direct and indirect consequences of these factors on lower Hb levels and anaemia is important, as findings may help guide the suite of school-based interventions in endemic areas where polyparasitism is the norm (Raso et al., 2004; Pullan and Brooker, 2008). Our analysis found evidence that malaria parasitaemia, heavy intensity of *S. mansoni* infection and being stunted were significantly associated with lower mean Hb, although only heavy intensity of *S. mansoni* infection was significantly associated with the risk of anaemia among schoolchildren over 10 years of age. Such results underscore current efforts to control helminths and malaria as part of integrated school health programmes (Bundy et al., 2006; Brooker et al., 2008).

Although our cross-sectional design hampers the interpretation of our finding, especially the direction of causality, the use of hierarchical Bayesian modelling allows the incorporation of both individual- and school-level factors, the omission of one or the other leading to biased estimates (Congdon, 2001). Furthermore, our results are consistent with previous studies which report similar associations (Stoltzfus et al., 1997b; Olsen et al., 1998; Leenstra et al., 2004; Desai et al., 2005a). However, the difference in Hb between children infected with malaria and those uninfected, though significant, was small. The anaemia of malaria is multifactorial, involving a complexity of mechanims including increased destruction of red blood cells (RBCs) through rupturing, phagocytosis and hypersplenism, and decreased RBC production through inflammation and dyserythropiesis (Menendez et al., 2000). The single time point of our cross-sectional design means that we were unable to capture information on clinical attacks of malaria and therefore separate haemolysis due to clinical malaria and the role of asymptomatic low grade infections on RBC concentrations and Hb. Neither can this design examine the effect of recently cleared infections on the process of haematological recovery and current Hb. However, clearer evidence of the contribution of malaria to anaemia in school-aged children is provided by the results of our intervention trial which showed that school-based intermittent preventive treatment with sulfadoxine-pyrimethamine and amodiaquine significantly reduced the prevalence of anaemia among the wider age range of schoolchildren included in the main trial (Clarke et al., 2008).

A surprising finding was the strong association between heavy intensity of *S. mansoni* infection, anaemia and Hb. Potential mechanisms through which *S. mansoni* may contribute to anaemia include: (i) blood loss caused by the rupture of blood vessels surrounding the intestine by the spined schistosome eggs; (ii) splenic sequestration; (iii) autoimmune hemolysis; and (iv) anaemia of inflammation which is typically characterised by decreased RBC production induced by pro-inflammatory cytokines (Friedman et al., 2005a; Tolentino and Friedman, 2007). In addition it is possible that the importance of *S. mansoni* infection is likely to be greater than estimated here since only schools located more than 5 km from the lake shore were sampled to purposively minimise confounding by *S. mansoni* in the intervention trial. By contrast, hookworm infection, a major attributable factor for anemia in schoolchildren in other areas of East Africa (Stoltzfus et al., 1997a; Lwambo et al., 2000), was not associated either with lower Hb or with anemia in the present study. This finding, which is consistent with previous studies in western Kenya (Olsen et al., 1998; Handzel et al., 2003), is probably due in part to the low intensity of hookworm infection in our study area, and highlights how different factors contribute to anaemia in different parasite transmission settings. We did not find any evidence of an increased risk of anaemia in children co-infected with multiple helminth species and/or malaria.

The results of our Bayesian linear hierarchical approach indicate that a high degree of variation remains to be explained and that there are other factors beyond what was measured in this study which still need to be considered. One advantage of a hierarchical approach over conventional statistical approaches is the partitioning of the unexplained variance into variability between clusters and individual level variation within clusters, which shows that most of the residual variation within our study population was attributable to individual level variation occurring between children. More precisely, although dietary iron insufficiency is very likely to impact on Hb and anaemia (Olivares et al., 1999), no information was available on iron status of the children included in the study. Nutritional variables which were measured such as stunting and wasting, representing chronic and severe acute undernutrition, respectively, may not adequately capture moderate, current undernutrition which may also explain variation in mean Hb levels. Reported age is also often uncertain and may be inaccurate. This may have implications for the reliability of the derived HAZ and BMIZ scores. A further source of variation not measured in this study is the effect of menarche in adolescent girls (Leenstra et al., 2004). When interaction terms of age and sex were fitted to the model these were not found to be significant, however as described above, reported age may be both unreliable and too crude a proxy for individual variation in the timing of onset of menarche. Similarly, the SES index used here may not fully capture socio-economic variability within the population as it was based on a small number of assets and relied on reporting by schoolchildren. A final source of individual variation which was not taken into account in this study was genetic traits such as sickle-cell and other haemoglobinopathies (Tolentino and Friedman, 2007).

In conclusion, this study demonstrated that lower mean Hb levels were significantly associated with malaria, chronic undernutrition and heavy intensity of *S. mansoni*, and that anaemia was associated with heavy intensity of *S. mansoni*. Such results have important implications for the control of anaemia among African schoolchildren and can help guide the design of appropriate interventions. Integrated school health programmes which include deworming, micronutrients and potentially malaria control, will help alleviate the anaemia burden faced by the school-aged children of Africa. Further research is required to identify the optimal packages and to identify areas where different packages of interventions may be required.

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Schistosoma haematobium infection and morbidity before and after large-scale administration of praziquantel in Burkina Faso

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MAJOR ARTICLE

Schistosoma haematobium Infection and Morbidity Before and After Large-Scale Administration of Praziquantel in Burkina Faso

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(See the editorial commentary by King, on pages 653–5.)

Background. In sub-Saharan Africa, 112 million people are infected with *Schistosoma haematobium*, with the most intense infections in children 5–15 years old.

Methods. We describe a longitudinal epidemiological study that evaluates the relationship between *S. haematobium* infection and associated morbidity in children before and after the large-scale administration of praziquantel for schistosomiasis and albendazole for soil-transmitted helminths.

Results. At baseline, higher intensities of *S. haematobium* infection were observed in children with anemia and/or severe microhematuria, but there was no apparent association between the risk of undernutrition and intensity of *S. haematobium* infection. Significant reductions in the prevalence and intensity of *S. haematobium* infection 1 year after treatment were, however, observed. Children who benefited the most from anthelmintic treatment in terms of increased hemoglobin concentrations were those who had anemia at baseline and those with highly positive microhematuria scores at baseline.

Conclusions. This study suggests that even a single round of mass chemotherapy can have a substantial impact on *S. haematobium* infection and its associated morbidity in children.

Improving the health of school-aged children, particularly in developing countries, has emerged as a policy priority in international health [1, 2]. Over the past 2 decades, significant progress has been made in improving child survival, resulting in more children reaching primary school age. However, human infections with 1 of the 5 parasitic helminths of the family Schistosomatidae still represent a significant segment of the global burden of illness, with ~200 million people infected and with the highest intensities in children 5–15 years old [3]. Schistosomiasis causes granuloma formation and both reversible and irreversible damage to the urinary and intestinal tracts [4]. Furthermore, new estimates of schistosomiasis-related disability have indicated the need to reassess priorities for treating this chronic infection in areas where it is endemic [5].

Praziquantel has been established in several controlled trials as a safe and effective drug for the treatment of infection with all human schistosome species [6–9]. The dramatic reduction in its price since 1990—by >90%, from US$4 to treat a person to approximately US$0.30—has led to the resolution of many of the challenges surrounding large-scale chemotherapy campaigns [10]; recently, through the Schistosomiasis Control Initiative (SCI), >20 million treatments were administered in 2005–2006 in 6 sub-Saharan African countries [11, 12]. One of the primary objectives of

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Table 1. Baseline characteristics of Burkinabé schoolchildren who were followed up for 1 year or dropped out.

Characteristic	Followed up for 1 year	Dropped out	*P*
Demographic			
Age, mean, years	9.8 (*n* = 1131)	11.3 (*n* = 686)	<.001
Male sex, %	55.0 (*n* = 1131)	62.0 (*n* = 686)	.004
Parasitologic			
Infected with *Schistosoma haematobium,* %	53.9 (*n* = 1124)	54.1 (*n* = 690)	.953
Infected with *S. mansoni,* %	6.2 (*n* = 536)	5.8 (*n* = 432)	.810
Infected with hookworm, %	6.3 (*n* = 556)	4.3 (*n* = 418)	.174
S. haematobium intensity, mean (SD), eggs/10 mL	83.6 (229.2)	94.2 (234.6)	.728
S. mansoni intensity, mean (SD), epg	8.0 (73.9)	11.3 (76.1)	.869
Hookworm intensity, mean (SD), epg	12.5 (90.7)	3.3 (21.0)	.158
Hematologic			
Anemia, %	65.8 (*n* = 1130)	66.4 (*n* = 687)	.390
Hemoglobin concentration, mean (SD), g/dL	11.0 (1.4)	11.1 (1.4)	.036
Nutritional status			
Wasted, %	32.8 (*n* = 1131)	100.0 (*n* = 686)	<.001
Stunted, %	13.3 (*n* = 1131)	100.0 (*n* = 686)	<.001
Hemastix test	(*n* = 1124)	(*n* = 692)	.460
Negative, %	50.4	53.5	
Trace, %	12.9	10.7	
+, %	6.8	5.6	
++, %	9.3	8.9	
+++, %	20.6	21.4	

NOTE. Anemia was defined (according to World Health Organization guidelines) as a hemoglobin concentration <11.5 g/dL for children 5–11 years old and <12.0 g/dL for children 12–14 years old. Wasting denotes reduced body weight for height, defined as a body-mass-index *z* score less than −2. Stunted denotes reduced body length in relation to a reference standard, defined as a height-for-age *z* score less than −2. +, weakly positive; ++, moderately positive; +++, highly positive; epg, eggs per gram of feces.

these SCI-supported control programs is to achieve, and hence also to demonstrate, a quantifiable reduction in schistosome-associated morbidity as a consequence of chemotherapeutic intervention.

The aim of the present study was to evaluate the relationship between *Schistosoma haematobium* infection and associated morbidity in children before and after the large-scale administration of praziquantel and albendazole (against soil-transmitted helminths) by the national Burkinabé helminth control program. A secondary aim was to identify those individuals whom one may predict to show the greatest improvements in nutritional status and hemoglobin (Hb) concentrations after chemotherapy.

SUBJECTS AND METHODS

Control program, study sites, sampling, and cohort design. Both *Schistosoma mansoni* and *S. haematobium* are endemic throughout Burkina Faso [13]. The SCI-supported schistosomiasis control program was implemented during 2004 and had treated 3,322,564 school-aged children in the 13 regions of the country through October 2006. Further details about the national Burkinabé helminth control program have been described elsewhere [14].

For the present study, parasitological and morbidity data were collected from a cohort of 1727 Burkinabé children 6–14 years old, randomly sampled from 16 schools before and 1 year after chemotherapy (2004 and 2005, respectively). The schools included in these surveys were randomly selected from all schools in 4 Regional Health Directorates known a priori to be places where schistosomiasis is highly endemic. Details concerning sample-size calculations and cohort design have been described elsewhere [15, 16].

Parasitological and morbidity measures. All children enrolled in the study were interviewed by appropriately trained personnel at the Ministry of Health, Burkina Faso. Ethical clearance was obtained from the Ministry of Health and Imperial College London.

Stool examination. A single stool sample was collected from each child, and 41.7 mg was processed to make duplicate Kato-Katz slides for microscopic determination of intestinal helminth infection. Individual egg output was expressed as eggs

Table 2. Health characteristics of children successfully followed up for 1 year (2004–2005), at baseline and after treatment.

Characteristic	2004	2005
Parasitologic		
Infected with *Schistosoma haematobium,* % (n = 1124)	53.91 (51.00–56.83)	5.78 (4.42–7.15)
Infected with *S. mansoni,* % (n = 536)	6.16 (4.12–8.19)	0.19 (0.00–0.55)
Infected with hookworm, % (n = 555)	6.31 (4.28–8.33)	1.62 (0.57–2.67)
S. haematobium intensity, mean, eggs/10 mL	83.55 (70.14–96.96)	0.94 (0.35–1.53)
S. mansoni intensity, mean, epg	8.04 (1.77–14.31)	0.02 (0.00–0.07)
Hookworm intensity, mean, epg	12.47 (4.92–20.03)	0.78 (0.22–1.34)
Hematologic (n = 1131)		
Hemoglobin concentration, mean, g/dL	10.97 (10.88–11.05)	11.25 (11.18–11.32)
Anemia	65.75 (62.99–68.52)	61.59 (58.76–64.43)
Microhematuria as diagnosed by Hemastix test (n = 1124)		
Negative, %	50.44 (47.52–53.37)	89.50 (87.71–91.29)
Trace, %	12.90 (10.94–14.86)	4.89 (3.63–6.15)
+, %	6.76 (5.29–8.23)	2.31 (1.43–3.19)
++, %	9.34 (7.64–11.04)	1.07 (0.47–1.67)
+++, %	20.55 (18.19–22.91)	2.22 (1.36–3.09)
Nutritional status (n = 1131)		
Thinness or wasting, %	32.80 (30.07–35.54)	35.10 (32.32–37.88)
Shortness or stunting, %	13.26 (11.29–15.24)	11.85 (9.96–13.73)

NOTE. Data are means or proportions (95% confidence interval) of children with characteristic, unless otherwise indicated. Sample sizes are provided in parentheses for each examined outcome in the left-hand column. Anemia was defined (according to World Health Organization guidelines) as a hemoglobin concentration <11.5 g/dL for children 5–11 years old and <12.0 g/dL for children 12–14 years old. Wasting denotes reduced body weight for height, defined as a body-mass-index z score less than −2. Stunted denotes reduced body length in relation to a reference standard, defined as a height-for-age z score less than −2. +, weakly positive; ++, moderately positive; +++, highly positive; epg, eggs per gram of feces.

per gram of feces, calculated as the arithmetic mean of the 2 individual slide counts whenever these were available.

Urine examination. One urine specimen was collected from each child to determine the prevalence and intensity of *S. haematobium* infection by the filtration method. The intensity of *S. haematobium* infection was expressed as the number of eggs per 10 mL of urine. To determine the presence and severity of microhematuria, all urine specimens were tested for presence of detectable blood using urine reagent strips (Bayer Hemastix). The results were recorded semiquantitatively: negative, trace hemolyzed, weakly positive (+), moderately positive (++), and highly positive (+++).

Nutritional assessment. Heights and weights were measured to determine height-for-age z scores (HAZ) and body-mass-index z scores (BMIZ). All measures were obtained using height poles and electronic balances in the morning, and children were barefoot, wearing only light indoor clothing. A low BMIZ is the index of choice for the assessment of recent undernutrition resulting in thinness or wasting, whereas a low HAZ represents long-term growth and nutritional status resulting in shortness or stunting [17, 18]. z scores for each nutritional index were calculated from Centre for Disease Control (National Center for Health Statistics; year 2000) reference values using EpiInfo (version 2000; US Centers for Disease Control and Prevention) [19].

Anemia assessment. Blood samples for Hb concentrations were obtained from each individual by the fingerprick method using a photometer (Hemocue) [20]. Anemia was defined according to World Health Organization (WHO) guidelines [21].

Statistical analyses. Differences between dropouts and children successfully followed up were tested by univariate analysis using a Wilcoxon 2-sample test for means and a χ^2 test or Fisher's exact test if there was a small value for proportions. SAS software was used (version 8; SAS Institute).

Hierarchical models are often applicable to modeling data from complex surveys of a population, with a hierarchical structure used to explain relations between individual and supraindividual determinants. A 2-level linear hierarchical model assuming normally distributed errors was fitted to the logarithmically transformed baseline *S. haematobium* egg counts ($\ln[\chi + 1]$), using Gibbs sampling for all parameters [22] to quantify any associations with anemia, measures of nutritional

Table 3. Estimates from 2-level hierarchical model for *Schistosoma haematobium* egg counts among 1130 Burkinabé schoolchildren at baseline and after treatment (2004–2005).

Model	Coefficient (95% CI)	*P*
Fixed effects		
Effect of year 1 follow-up relative to baseline	−52% (−57% to −47%)	<.001
Male sex (reference category: female)	11% (2% to 21%)	.020
Baseline age (reference category: 14 years)		
13 years	9% (−19% to 45%)	.572
12 years	45% (11% to 90%)	.007
11 years	25% (−4% to 63%)	.095
10 years	32% (2% to 72%)	.035
9 years	24% (−4% to 61%)	.106
8 years	22% (−6% to 59%)	.143
7 years	2% (−24% to 37%)	.906
6 years	19% (−27% to 92%)	.488
Baseline anemia (reference category: not anemic)	11% (1% to 21%)	.026
Baseline hematuria (reference category: negative)		
Trace	242% (191% to 302%)	<.001
+	462% (357% to 592%)	<.001
++	1470% (1176% to 1833%)	<.001
+++	4107% (3493% to 4827%)	<.001
Baseline thinness or wasting (reference category: not wasted)	4% (−6% to 14%)	.463
Baseline shortness or stunting (reference category: not stunted)	−13% (−26% to 2%)	.089
	Variance components (SE)	
Random effects		
Level 2 variance (between schools)	0.008 (0.004)	
Level 1 variance (measurement occasions within a child)	0.190 (0.006)	

NOTE. Anemia was defined (according to World Health Organization guidelines) as a hemoglobin concentration <11.5 g/dL for children 5–11 years old and <12.0 g/dL for children 12–14 years old. Wasting denotes reduced body weight for height, defined as a body-mass-index *z* score less than −2. Stunting denotes reduced body length in relation to a reference standard, defined as a height-for-age *z* score less than −2. +, weakly positive; ++, moderately positive; +++, highly positive; CI, confidence interval.

status, and microhematuria while adjusting at the same time for demographic factors such as age and sex. In this way, we tested whether children with pathology potentially induced by *S. haematobium* infection had higher *S. haematobium* egg counts before treatment. We used a similar model to quantify changes in *S. haematobium* egg counts from baseline to 1 year of follow-up. Mlwin software (version 2.01; Multilevel Models Project, Institute of Education, University of London) was used. Box plots of the *S. haematobium* egg counts at baseline were used for validation and comparison of the significant findings from the model described above.

Changes in Hb concentration and in HAZ and BMIZ scores over time were evaluated using 2-level linear hierarchical models of raw change scores between baseline and the 1-year follow-up time point. With this approach, we aimed to compare the average change in each of the studied outcomes over the studied period between different groups of children. All of the models presented were also adjusted for age and sex. $P < .05$ was considered to be significant.

An additional 3-level hierarchical linear modeling analysis was performed to determine any change in the children's Hb concentrations. With this model, we aimed to quantify the adjusted overall change in Hb concentration from baseline to follow-up and to quantify average Hb concentrations of different groups of children at baseline as well as to examine whether the intensity of *S. haematobium* infection was associated with lower Hb concentrations. Logistic random-intercepts regression models were fitted to examine whether the intensity of *S. haematobium* infection was associated with an increased risk of thinness and shortness at baseline while adjusting for potential confounders.

RESULTS

A total of 1727 children from 16 schools were recruited at baseline. Of these, 1131 (65%) were successfully retraced at the 1-year follow-up time point, and 321 new children were recruited into the cohort during the second year of the study

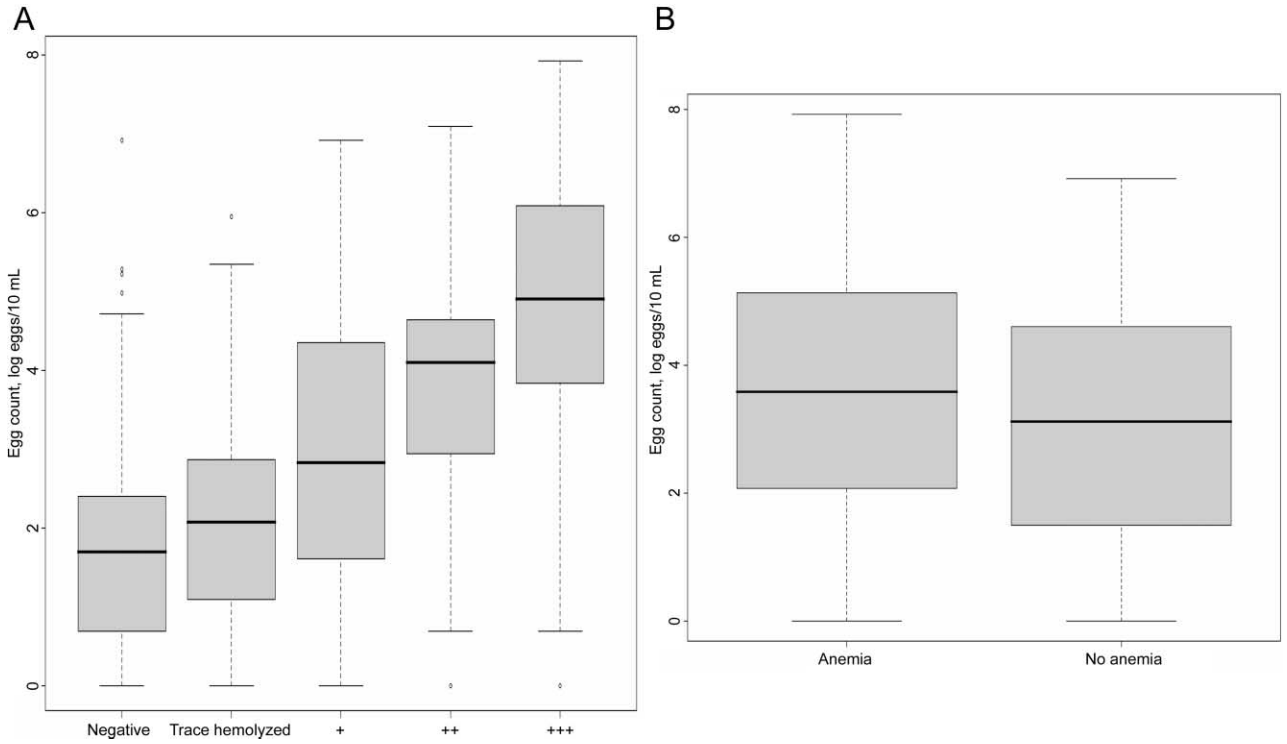

Figure 1. Box plots for the logarithmically transformed (base *e*) *Schistosoma haematobium* egg counts of positive subjects only ($n = 613$ Burkinabé schoolchildren, 2004). *A,* Box plot for log *S. haematobium* egg counts with respect to different microhematuria test scores at baseline. *B,* Box plots for log *S. haematobium* egg counts with respect to anemia status at baseline. Data are smallest observations, lower quartiles (Q1), medians, upper quartiles (Q3), and largest observations. +, weakly positive; ++, moderately positive; +++, highly positive.

(data not shown). There were significant differences between the children who were successfully followed up and the dropouts, according to their demographic characteristics and nutritional status as defined by baseline thinness and shortness as well as by baseline Hb concentrations (table 1). The children who dropped out were of an older mean age than those successfully followed up, and boys proved more difficult to recruit into the cohort during the second year of the study. All children who dropped out were wasted and stunted at baseline and had slightly higher mean Hb concentrations. No other baseline characteristic measured varied significantly between children followed up and those who dropped out.

Table 2 presents the health indicators of children surveyed at baseline and successfully followed up 1 year after treatment. During the 12 months between examinations, the overall prevalences of *S. haematobium, S. mansoni,* and hookworm infections decreased significantly ($P < .001$). For both years examined, *Ascaris lumbricoides* infection was absent, and the prevalence of *Trichuris trichura* infection was estimated to be 1.1% at baseline and totally absent 1 year later. Because prevalences and coinfections with *S. haematobium* were so low for the intestinal helminth species at both time points, such data were not analyzed further here.

A significant increase in mean Hb concentration ($P < .001$) and a significant decrease in the prevalence of anemia ($P = .021$) were also observed between 2004 and 2005. Finally, the unadjusted observed changes in both recent and chronic undernutrition from baseline to follow-up were not significant ($P = .135$ and $P = .093$, respectively).

Table 3 presents the results of the model of the change in *S. haematobium* egg counts for 1 year after treatment as well as differences in *S. haematobium* egg counts between different groups of children at baseline. This model indicated that, compared with baseline counts, there was on average an overall significant decrease in *S. haematobium* egg counts by 52% 1 year after treatment ($P < .001$). At baseline, only children 10 and 12 years old had significantly higher *S. haematobium* egg counts, compared with those who were 14 years old ($P = .035$ and $P = .007$, respectively) after controlling for sex, nutritional and anemia status, and microhematuria test scores. Children with +++, ++, +, and trace microhematuria scores had on average significantly higher *S. haematobium* egg counts than those of children with negative scores at baseline, by 4107%, 1470%, 462%, and 242%, respectively. Additionally, children with anemia at baseline had significantly higher *S. haematobium* egg counts (by 11%) than children without anemia ($P = .026$). Boys also had significantly higher *S. haematobium* egg counts (by 11%) than girls at baseline ($P = .020$).

Figure 1*A* shows that children with the most severe microhematuria scores harbored higher intensities of *S. haematobium* infection than children who were negative for microhematuria at baseline. Children with anemia at baseline also harbored slightly higher intensities of *S. haematobium* infection than those without anemia (figure 1*B*).

Results from the 2-level logistic regression model for the probability of being wasted did not suggest associations with any baseline characteristics examined other than age. In particular, there was a trend toward younger children being less likely to be wasted, although none of the other odds ratios (ORs), except for those for 10-year-old children, were significantly different from the ORs for 14-year-old children ($P = .022$). Furthermore, the 2-level logistic regression model of the probability of being stunted at baseline suggested a trend toward younger children being less likely to be stunted; the OR for 6–12-year-old children was significantly different from that for 14-year-olds. In addition, children with anemia were almost 1.5 times more likely than those without anemia to be stunted at baseline ($P = .034$; data not shown).

Table 4 contains estimates from the two 2-level linear multilevel models for changes in HAZ and BMIZ during the period studied. The effect for the 14-year-old comparison group was a decrease in BMIZ of 0.519 units, which was of borderline significance ($P = .053$). This suggests that there was no dramatic change over the period studied in this group. However, children who were 7, 8, and 10 years old at baseline had a greater decrease in BMIZ, compared with 14-year-olds. The coefficients of the continuous variables that refer to the baseline HAZ and BMIZ scores indicated that BMIZ increased for wasted children, whereas, BMIZ decreased for stunted children more than that of children with no nutritional problems. These results can be obtained if one multiplies the coefficients of HAZ and BMIZ by −2 (i.e., the cutoff score that defines wasting and stunting, respectively).

The effect for the 14-year-old comparison group was a non-

Table 4. Estimates from 2-level hierarchical model for the change of body-mass-index *z* score (BMIZ) and height-for-age *z* score (HAZ) ($n = 1130$).

Model	Coefficient (95% CI) for change in BMIZ	*P*	Coefficient (95% CI) for change in HAZ	*P*
Fixed effects				
Intercept	−0.519 (−1.003 to −0.035)	.053	0.143 (−0.073 to 0.359)	.214
Baseline intensity of *Schistosoma haematobium* infection (reference category: uninfected)				
Lightly infected	0.060 (−0.133 to 0.253)	.543	0.032 (−0.059 to 0.124)	.490
Heavily infected	0.218 (−0.030 to 0.465)	.085	0.078 (−0.040 to 0.196)	.193
Male sex (reference category: female)	0.011 (−0.100 to 0.122)	.850	−0.066 (−0.119 to −0.013)	.015
Baseline age (reference category: 14 years old)				
13 years	−0.120 (−0.649 to 0.410)	.658	−0.134 (−0.388 to 0.119)	.300
12 years	−0.375 (−0.816 to 0.067)	.097	−0.236 (−0.447 to −0.025)	.029
11 years	−0.396 (−0.835 to 0.043)	.077	−0.177 (−0.386 to 0.033)	.099
10 years	−0.483 (−0.915 to −0.051)	.029	−0.008 (−0.215 to 0.198)	.936
9 years	−0.347 (−0.778 to −0.084)	.115	0.000 (−0.206 to 0.206))	.999
8 years	−0.448 (−0.879 to −0.017)	.042	0.051 (−0.155 to 0.257)	.625
7 years	−0.642 (−1.083 to −0.201)	.004	−0.033 (−0.244 to 0.178)	.757
6 years	−0.483 (−1.051 to 0.085)	.096	0.428 (0.157 to 0.699)	.002
Baseline anemia (reference category: not anemic)	0.005 (−0.114 to 0.123)	.939	−0.041 (−0.098 to 0.015)	.153
Baseline hematuria (reference category: negative)				
Trace	−0.166 (−0.389 to 0.057)	.144	−0.063 (−0.169 to 0.043)	.243
+	−0.147 (−0.416 to 0.123)	.286	−0.014 (−0.142 to 0.114)	.829
++	0.054 (−0.208 to 0.317)	.685	−0.027 (−0.152 to 0.097)	.669
+++	−0.110 (−0.356 to 0.136)	.383	−0.058 (−0.174 to 0.059)	.334
Baseline BMIZ	−0.588 (−0.628 to −0.548)	<.001	0.054 (0.035 to 0.073)	<.001
Baseline HAZ	0.184 (0.135 to 0.232)	<.001	−0.138 (−0.161 to −0.115)	<.001
	Variance components (SE)			
Random effects				
Level 2 variance (between schools)	0.155 (0.065)		0.009 (0.004)	
Level 1 variance (between children within a school)	0.846 (0.036)		0.194 (0.008)	

NOTE. Anemia was defined (according to World Health Organization guidelines) as a hemoglobin concentration <11.5 g/dL for children 5–11 years old and <12.0 g/dL for children 12–14 years old. +, weakly positive; ++, moderately positive; +++, highly positive; CI, confidence interval.

significant ($P = .214$) increase in HAZ of 0.143 units. Compared with 14-year-olds, 6-year-olds had a significantly greater increase, whereas the 12-year-olds had a decrease in HAZ (significantly different from that of 14-year-olds). Boys had a significantly smaller increase in HAZ than girls. Likelihood ratio tests indicated a better fit in both models mentioned above when we included HAZ and BMIZ as explanatory continuous variables and not the relevant categorical ones that would denote wasting and stunting if HAZ or BMIZ was, respectively, less than −2 SD.

Table 5 contains the estimates of two 3-level hierarchical models for Hb concentrations before and after chemotherapeutic treatment, taking into account adjustment and nonadjustment for microhematuria scores. From the former model it was estimated that an overall increase of 0.092 g/dL in Hb concentration after treatment was not significant ($P = .146$). Children with +++ microhematuria scores had significantly lower Hb concentrations (0.318 g/dL; $P = .016$) than microhematuria-negative children at baseline. Children who were 6, 7, 8, and 10 years old at baseline had significantly lower Hb concentrations ($P = .024$, $P < .001$, $P = .019$, and $P = .008$, respectively) than those who were 14 years old, after controlling for intensity of *S. haematobium* infection, sex, hematuria, wasting, and thinness. From the alternative model, which did not

Table 5. Estimates from 3-level hierarchical models for hemoglobin concentration before and after treatment, taking into account adjustment and nonadjustment for microhematuria scores ($n = 1124$).

Model	Coefficient (95% CI) with adjustment for microhematuria	*P*	Coefficient (95% CI) without adjustment for microhematuria	*P*
Fixed effects				
Intercept	11.473 (11.067 to 11.879)	<.001	11.480 (11.074 to 11.886)	<.001
Effect of year 1 follow-up relative to baseline	0.092 (−0.031 to 0.215)	.146	0.093 (−0.030 to 0.216)	.139
Baseline intensity of *Schistosoma haematobium* infection (reference category: uninfected)				
Lightly infected	−0.107 (−0.311 to 0.097)	.303	−0.079 (−0.238 to 0.080)	.332
Heavily infected	−0.084 (−0.354 to 0.186)	.542	−0.220 (−0.410 to −0.030)	.024
Male sex (reference category: female)	−0.035 (−0.158 to 0.088)	.577	−0.039 (−0.162 to 0.084)	.538
Baseline age (reference category: 14 years old)				
13 years	−0.026 (−0.383 to 0.331)	.888	−0.037 (−0.396 to 0.322)	.838
12 years	−0.136 (−0.489 to 0.217)	.452	−0.138 (−0.493 to 0.217)	.445
11 years	−0.334 (−0.683 to 0.015)	.061	−0.353 (−0.704 to −0.002)	.048
10 years	−0.468 (−0.813 to −0.123)	.008	−0.471 (−0.818 to −0.124)	.008
9 years	−0.296 (−0.643 to 0.051)	.095	−0.290 (−0.639 to 0.059)	.103
8 years	−0.425 (−0.780 to −0.070)	.019	−0.415 (−0.772 to −0.058)	.022
7 years	−0.817 (−1.205 to −0.429)	<.001	−0.816 (−1.206 to −0.426)	<.001
6 years	−0.693 (−1.297 to −0.089)	.024	−0.685 (−1.289 to −0.081)	.026
Baseline hematuria (reference category: negative)				
Trace	0.108 (−0.115 to 0.331)	.344		
+	0.135 (−0.149 to 0.419)	.352		
++	0.229 (−0.063 to 0.521)	.124		
+++	−0.318 (−0.577 to −0.059)	.016		
Baseline thinness or wasting (reference category: not wasted)	−0.042 (−0.162 to 0.078)	.488	−0.042 (−0.162 to 0.078)	.491
Baseline shortness or stunting (reference category: not stunted)	−0.149 (−0.351 to 0.053)	.148	−0.155 (−0.359 to 0.049)	.135
	Variance components (SE) from the model with adjustment for microhematuria		Variance components (SE) from the model without adjustment for microhematuria	
Random effects				
Level 3 variance (between schools)	0.136 (0.056)		0.140 (0.057)	
Level 2 variance (between children within a school)	0.514 (0.051)		0.519 (0.051)	
Level 1 variance (measurement occasions within a child)	1.106 (0.047)		1.116 (0.047)	

NOTE. Wasting denotes reduced body weight for height, defined as a body-mass-index *z* score less than −2. Stunting denotes reduced body length in relation to a reference standard, defined as a height-for-age *z* score less than −2. +, weakly positive; ++, moderately positive; +++, highly positive; CI, confidence interval.

adjust for microhematuria scores, estimates of the parameters mentioned previously remained approximately the same, but the effect of *S. haematobium* infection intensity became significant, such that children who were heavily infected with *S. haematobium* at baseline had significantly lower Hb concentrations (0.220 g/dL; $P = .024$) than uninfected children. This suggests that because the variable of microhematuria scores is related to both Hb concentration and the intensity of *S. haematobium* infection, 2 different causal effects of *S. haematobium* infection on Hb concentrations are indicated. Two-way interaction terms were also tested, but because they were not significant, they were omitted from the models presented. Also, variances in all 3 levels of the second model were higher than the corresponding ones in the first model, which implies that the microhematuria scores explain some of the variability in the studied outcome across all 3 levels of the models presented.

Finally, table 6 contains estimates from the 2-level linear multilevel model for the change in Hb concentration over the course of the period studied. This model suggested that the change varied significantly as a function of the following baseline characteristics: anemic status, +++ microhematuria score, and age. The effect for the comparison group (baseline uninfected, male, 14 years old, not anemic, negative microhematuria score, not wasted, and not stunted) was a decrease in Hb concentration of 0.128 g/dL, which was not significant ($P = .747$). Significant increases in Hb concentration during the period studied were observed among children with anemia at baseline (increase by 1.360 g/dL [that is, 1.488–0.128 g/dL]; $P < .001$)

Table 6. Estimates from a 2-level hierarchical model for changes in hemoglobin concentration ($n = 1130$).

Model	Coefficient (95% CI)	*P*
Fixed effects		
Intercept	−0.128 (−0.888 to 0.633)	.747
Baseline intensity of *Schistosoma haematobium* infection (reference category: uninfected)		
Lightly infected	−0.081 (−0.350 to 0.187)	.553
Heavily infected	−0.258 (−0.602 to 0.086)	.141
Male sex (reference category: female)	0.050 (−0.105 to 0.205)	.529
Baseline age (reference category: 14 years old)		
13 years	−0.625 (−1.364 to 0.114)	.097
12 years	−0.605 (−1.222 to 0.013)	.055
11 years	−0.435 (−1.049 to 0.179)	.165
10 years	−0.464 (−1.067 to 0.140)	.132
9 years	−0.719 (−1.321 to −0.117)	.019
8 years	−0.284 (−0.886 to 0.318)	.355
7 years	−0.139 (−0.753 to 0.475)	.657
6 years	−0.788 (−1.575 to −0.002)	.050
Baseline anemia (reference category: not anemic)	1.488 (1.323 to 1.653)	<.001
Baseline hematuria (reference category: negative)		
Trace	0.106 (−0.204 to 0.415)	.504
+	−0.029 (−0.404 to 0.345)	.878
++	0.010 (−0.354 to 0.375)	.955
+++	0.361 (0.019 to 0.702)	.039
Baseline thinness or wasting (reference category: not wasted)	−0.057 (−0.226 to 0.112)	.506
Baseline shortness or stunting (reference category: not stunted)	0.004 (−0.225 to 0.234)	.971
	Variance components (SE)	
Random effects		
Level 2 variance (between schools)	0.123 (0.062)	
Level 1 variance (between children within a school)	1.647 (0.070)	

NOTE. Anemia was defined (according to World Health Organization guidelines) as a hemoglobin concentration <11.5 g/dL for children 5–11 years old and <12.0 g/dL for children 12–14 years old. Wasting denotes reduced body weight for height, defined as a body-mass-index *z* score less than −2. Stunting denotes reduced body length in relation to a reference standard, defined as a height-for-age *z* score less than −2. +, weakly positive; ++, moderately positive; +++, highly positive; CI, confidence interval.

and among children with +++ microhematuria scores at baseline (increase by 0.233 g/dL [that is, 0.361–0.128 g/dL]; $P = .039$). Marginally significant decreases in Hb concentration during the same period were observed in children who were 12 years old at baseline; these children had a greater decrease, by 0.733 g/dL (that is, −0.605–0.128 g/dL; $P = .055$), in Hb concentration than 14-year-old children. In addition, 9-year-old children had a significantly greater decrease in Hb concentration, by 0.847 g/dL (that is, −0.719–0.128 g/dL; $P = .019$).

DISCUSSION

Among the different schistosomes infecting humans, *S. haematobium* is responsible for the largest number of infections. It has been estimated that, in sub-Saharan Africa, 112 million people are infected with *S. haematobium,* compared with 54 million infected with *S. mansoni* [23]. However, *S. haematobium* remains largely unstudied, particularly in comparison to *S. mansoni,* primarily because of the more demanding conditions for its laboratory maintenance. This is also reflected in the paucity of research examining the potential effectiveness of praziquantel against *S. haematobium* under various experimental and clinical circumstances [24]. To our knowledge, the present study represents the first longitudinal study in Africa that reports on the relationship between *S. haematobium* infection and its associated morbidity as a whole, by use of a uniquely detailed large data set from 16 randomly selected schools across the entire national territory of Burkina Faso. Moreover, these data have the potential to provide important evidence characterizing urinary schistosomiasis-associated morbidity, particularly in a population such as that of Burkinabé, where the prevalence of hookworms and other soil-transmitted helminthiasis is estimated to be very low. Although previous studies have tended to focus on the impact of large-scale control programs on intense transmission of *S. haematobium* infection with regard to Hb concentrations and anemia only (Tohon Z, Boubacar Mainassara H, Elhaj Mahamane A, et al., submitted) [25], to parasitological measures only [26, 27], or to *S. haematobium* morbidity indicators before and after treatment [28], the present study examined all these issues together and also adjusted for potential differences in demographic characteristics as well as potential confounders.

We have demonstrated that children with anemia or children with more severe microhematuria scores at baseline had higher *S. haematobium* infection intensities (table 3 and figure 1), which suggests that heavy intensities of *S. haematobium* infection can be associated with anemia and hematuria. We also provide evidence that heavy infections of urinary schistosomiasis are associated with lower Hb concentrations and, as a consequence, with potential anemia, given that the models in table 5 indicate that *S. haematobium* infection might be associated with anemia in 2 different ways. More precisely, hematuria—which, as demonstrated previously (Tohon Z, Boubacar Mainassara H, Elhaj Mahamane A, et al., submitted) [29], is associated with *S. haematobium* infection—can be an important cause of blood and iron loss, which also may lead to anemia. Our data suggest significant reductions in the prevalence and, more importantly, intensity of infection of *S. haematobium* as well as in the percentages of children with positive microhematuria scores 1 year after treatment (tables 2 and 3).

The children who most benefited from anthelmintic treatment in terms of increased Hb concentration were those with anemia at baseline and those who had +++ microhematuria scores at baseline (table 6), which confirms similar findings presented elsewhere [30, 31]. The mechanisms by which treatment for *S. haematobium* infection allows Hb concentrations to increase in children with anemia may be the decrease in blood in urine that results from a reduction in the intensity of *S. haematobium* infection [32].

Growth and nutritional status have been proposed to represent the most sensitive indicators of health in children [33]. Furthermore, one of the factors emphasized in the World Development Report 1993 is the relationship between parasitic infections and malnutrition [2]. We examined whether greater *S. haematobium* egg counts were associated with increased risks of wasting or stunting at baseline, adjusting for demographic characteristics and other potential predictors such as microhematuria and anemia status. The results of this study did not suggest any significant association between the risk of undernutrition and intensity of *S. haematobium* infection, with only age being revealed as a significant factor. Younger children tended to be less likely to be wasted or stunted than 14-year-old children, which could imply prior malnutrition in these older children, as has been reported elsewhere [1]. In the aforementioned study, the authors also reported that, in Zanzibari boys, the association between microhematuria and poor growth was only marginally significant [1], which is in line with our findings that, in the Burkinabé children, changes in the BMIZ scores depended only on age, whereas changes in the HAZ scores depended on age and sex (table 4). A more plausible explanation for the lack of statistical association between the intensity of *S. haematobium* infection and stunting may relate to dropout bias toward stunted children (table 1); this means that it is difficult to make a definitive conclusion regarding the relationship between urinary schistosomiasis and stunting in our study population [34].

Nevertheless, it must be considered that essential methodological constraints inherent in the present study design—in particular the lack of a control group, which was necessary for ethical reasons, could result in some potential bias toward the estimation of the absolute impact of the treatment, thereby allowing only the relative impact of the treatment in different groups of children to be calculated. However, even though these

data were obtained from a large-scale control program and studies such as ours are generally difficult to execute in terms of design, methodology, and sampling, we believe that the results will be of substantial value in estimating the total benefit that control of schistosomiasis can provide to communities [35].

This study shows that praziquantel can have a substantial impact on *S. haematobium* infection and associated disease when delivered as part of a large-scale control program in a country such as Burkina Faso, which was the first country in the WHO African Region to achieve nationwide coverage with anthelminthic drugs against 3 major neglected tropical diseases: lymphatic filariasis, schistosomiasis, and soil-transmitted helminthiasis [36]. Our findings suggest a dramatic reduction in the prevalence and intensity of *S. haematobium* infection, an improvement in Hb concentration in certain groups of children, and reductions in schistosome-related morbidity in a cohort of Burkinabé schoolchildren, which demonstrate that mass chemotherapy can offer a practical strategy for the control of *S. haematobium* infection and its associated morbidity.

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Assessment of ultrasound morbidity indicators of schistosomiasis in the context of large-scale programs illustrated with experiences from Malian children.

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ASSESSMENT OF ULTRASOUND MORBIDITY INDICATORS OF SCHISTOSOMIASIS IN THE CONTEXT OF LARGE-SCALE PROGRAMS ILLUSTRATED WITH EXPERIENCES FROM MALIAN CHILDREN

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Abstract. We assessed morbidity indicators for both *Schistosoma haematobium* and *Schistosoma mansoni* infections and evaluated the appropriateness of the World Health Organization (WHO) guidelines for ultrasound in schistosomiasis in the context of large-scale control interventions. Abdominal and urinary tract ultrasonography was performed on 2,247 and 2,822 school children, respectively, from 29 randomly selected schools in Mali before the implementation of mass anthelminthic drug administration. Using two-level logistic regression models, we examined associations of potential factors with the risk of having a positive ultrasound global score (morbidity indicative of *S. haematobium* infection), abnormal image pattern scores, dilatation of the portal vein, and/or enlarged liver (morbidity indicative of *S. mansoni* infection). The WHO protocol was found useful for detection of *S. haematobium* pathology but overestimated the risk of portal vein dilatation and left liver lobe enlargement associated with *S. mansoni* infection. We conclude that ultrasonography should be included in large-scale control interventions, where logistics allow, but cautiously.

INTRODUCTION

Schistosomiasis remains one of the most prevalent parasitic diseases in developing countries and has significant economic and public health consequences.[1] Recently, it has been estimated that the urinary type of schistosomiasis, resulting from infection with *Schistosoma haematobium*, causes hematuria in 70 million people and major bladder wall pathologic effects in 18 million people in sub-Saharan Africa.[2] *Schistosoma mansoni*, one of the etiologic agents of the intestinal type of schistosomiasis, is responsible for bloody diarrhea in an estimated 4.4 million people, and an estimated 8.5 million people have hepatomegaly due to the infection. The death rate due to *S. mansoni*-related hematemesis may be up to 130,000 per year in sub-Saharan Africa.[2] King and others have argued convincingly that additional subtle morbidities (i.e. symptoms such as anemia, chronic pain, diarrhea, exercise intolerance, growth stunting, and nutritional and cognitive impairment, which have so far been difficult to quantify and are based on observed association rather than established causality) should be added to these estimates of disease burden.[3]

Since 1984 the World Health Organization (WHO) Expert Committee on the Control of Schistosomiasis has recommended a strategy for morbidity control that is now feasible because of the availability of effective, affordable, and safe single-dose drugs.[4] As a consequence, since 2003, the Schistosomiasis Control Initiative (SCI) has assisted six sub-Saharan African countries to develop and implement schistosomiasis morbidity control programs through the provision of health education and mass treatment, using praziquantel for schistosomiasis and co-administering, where appropriate, albendazole for soil-transmitted helminthiasis. The primary objective of these SCI-supported control programs is to achieve and demonstrate a quantifiable reduction in schistosome-associated morbidity as a consequence of such chemotherapeutic intervention. Inherent within such an objective, it is therefore imperative to both define and characterize pretreatment baseline morbidity levels within the risk populations so that any subsequent changes in morbidity caused by the intervention can be accurately determined.[5] Furthermore, identification of sensitive and specific indicators of schistosome-associated morbidity that may be practically implemented within such large scale-control programs, as distinct from the individual clinical or research-based setting, should also prove invaluable in assisting identification of target populations for ongoing and future intervention.[6] Campagne and others also emphasized the need to validate indirect morbidity indicators to know the development of their predictive value during different stages of a schistosomiasis control program.[7]

Ultrasonography is currently the diagnostic tool of choice for detecting pathologic conditions associated with schistosomiasis, such as dilatation of the renal pelvis, bladder wall lesions, liver fibrosis and enlargement, and dilatation of the portal vein.[8,9] For detection of infection with *S. haematobium*, ultrasonography is an established method for detecting urinary tract pathologic effects not only in the hospital setting,[10–13] but also in field-based studies,[14] with the advantage of being non-invasive, relatively simple to perform, well accepted by communities, and providing a direct image of the pathologic changes.[15] Additionally, ultrasonography provides sensitive and precise measurements of *S. mansoni*-associated pathologic changes[16,17] In the attempt to objectively define and categorize the pathologic changes associated with schistosomiasis and to standardize the different scoring systems used in the past in different disease-endemic areas,[18,19] successive ultra-

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sound consensus meetings were held in Niamey, Niger in 1996 and Belo Horizonte, Brazil in 1997. These meetings led to the revision of standardized scoring protocols and the development of the WHO-recommended ultrasonography protocol (Niamey-Belo Horizonte protocol).[19] Nevertheless, the prognostic features of individual ultrasonography findings in different disease-endemic situations,[17] as well as whether ultrasonography can be incorporated into a mass chemotherapy program to monitor morbidity, are still to be confirmed.

The aim of this study was to assess indicators of ultrasonography-detectable morbidity caused by infection with both *S. haematobium* and *S. mansoni* in the context of large-scale control interventions targeting school age children in Mali before large-scale administration of praziquantel by the National Schistosomiasis Control Program with support from SCI. In Mali, both *S. haematobium* and *S. mansoni* pose serious public health problems.[20] Fishing, market gardening, and rice cultivation all expose the population to the risk of occupational transmission, and children are regularly exposed through bathing and playing in ponds, streams, and rivers. *Schistosoma haematobium* transmission is more widespread, occurring along river and streams, as well as around ponds and in irrigation schemes.[21,22] The geographic distribution of *S. mansoni* infections is more restricted, mainly occurring in irrigation schemes, such as Office du Niger, Bandiagara, Sélingue, and Baguinéda-Koulikoro.[22,23]

The results obtained here should contribute to evaluate the appropriateness and the role of the full WHO protocol in the context of large-scale schistosomiasis control programs. We also aimed to determine whether the numeric WHO cut-off values, originally derived from a healthy Senegalese population in an area not endemic for *S. mansoni*, contain bias in the estimation of the risk of dilatation of portal vein and enlargement of left liver lobe in a Malian setting. This will be achieved here by comparison with local height-indexing of portal vein diameter (PVD) scores and longitudinal parasternal line scores (PSL), respectively, obtained from children who had normal image patterns as assessed by ultrasonography using the recommendations of King and others.[17]

MATERIALS AND METHODS

Preparation of the survey and selection of schools. The Malian Ministry of Health, through its National Institute of Research in Public Health (Institut National de Recherche en Santé Publique [INRSP]) and its Disease Prevention and Control Division (Direction Nationale de la Santé), was charged with planning and implementing data collection with assistance from the SCI. Baseline data collection was initiated in the Ségou region in late March 2004 and was completed in Bamako in June 2004. Ultrasonagraphic examination based on the WHO protocol was performed on children 7–14 years of age (2,841 for *S. haematobium* and 2,820 for *S. mansoni*) from 29 schools. These schools were randomly selected from all schools in three areas highly endemic for schistosomiasis: Bamako, Ségou, and Baguinéda-Koulikoro (Figure 1). All children enrolled in the study were interviewed by appropriately trained Ministry of Health staff. Ethical clearance was obtained from the Ministry of Health, Mali and Imperial College, London.

Sampling design. In schools from areas known to be endemic for only *S. haematobium*, the estimated sample size was calculated to be 180 persons per sampling unit (usually a school) with 80% power to detect a 70% reduction in *S. haematobium* mean intensity over a two-year period (two annual treatments). The expected reductions of 70% in *S. haematobium* and 60% in *S. mansoni* mean intensities over two annual treatments were calculated using the EpiSchisto software tool (http://www.schoolsandhealth.org/epidynamics.htm). The figure of 180 was selected based on prevalence/intensity data from schools in various African countries with similar age ranges (Table 1). Since the relationship between prevalence and intensity varied between countries, a different k value (an inverse measure of worm aggregation in the host population that was estimated by maximum likelihood estimation using the negative binomial relationship between prevalence and intensity) was estimated separately for each country. Consequently, a different sample size was calculated for each country in Table 1 and the value of 180 was selected because it was the maximum of all values. Calculations were made for the sample size of the entire school so that results were comparable between countries. A simple approach of a paired sample *t*-test with level of type I error = 0.05 for testing the difference between pre-treatment mean intensity and post-treatment mean intensity was followed. Finally, we allowed for an overall dropout rate of 40% over the course of the monitoring period.

Where both *S. haematobium* and *S. mansoni* infections were known *a priori* to be prevalent, the same methodology as for only *S. haematobium* was followed, although the number of surveyed children was increased to 300 per school with 80% power because EpiSchisto simulations showed an expected reduction in mean *S. mansoni* intensity of 60%. Since the difference of the two means became larger compared with that of *S. haematobium* infection, the magnitude of the non-centrality parameter increased the required sample size needed to achieve suitable statistical power. No schools were included in the present study in which only *S. mansoni* infection was prevalent.

Whenever it was difficult to recruit the required number of children in any one school (usually because of the small size of the school), we combined data from two or more adjacent schools provided that they appeared to be ecologically similar (e.g., with the same relative proximity to the nearest supposed focus of transmission). For ethical reasons, it was not appropriate to include any untreated control groups. Further technical details concerning the sample size calculations can be found elsewhere.[24]

Parasitologic examination. From each child, two urine specimens were collected on two consecutive days to determine the intensity of *S. haematobium* infection using filtration method. 10 mL of urine were passed through a Whatman (Brentford, United Kingdom) filter paper (Ø = 25 mm) using a Millipore (Billerica, MA) Swinnex® filter support. Filters were stained with 3% ninhydrin and microscopically examined for eggs. The intensity of *S. haematobium* infection was expressed as number of eggs per 10 mL of urine and the mean intensity of infection was the arithmetic mean of egg counts in the two urine samples. To determine the presence and extent of microhematuria (invisible hematuria), all urine specimens were tested for detectable blood with urine reagent strips (Hemastix®; Bayer, Tarrytown, NY). The results were recorded semi-quantitatively as –, +, ++, and +++. Additionally, two fecal specimens (each 41.7 mg) were screened for *S. man-*

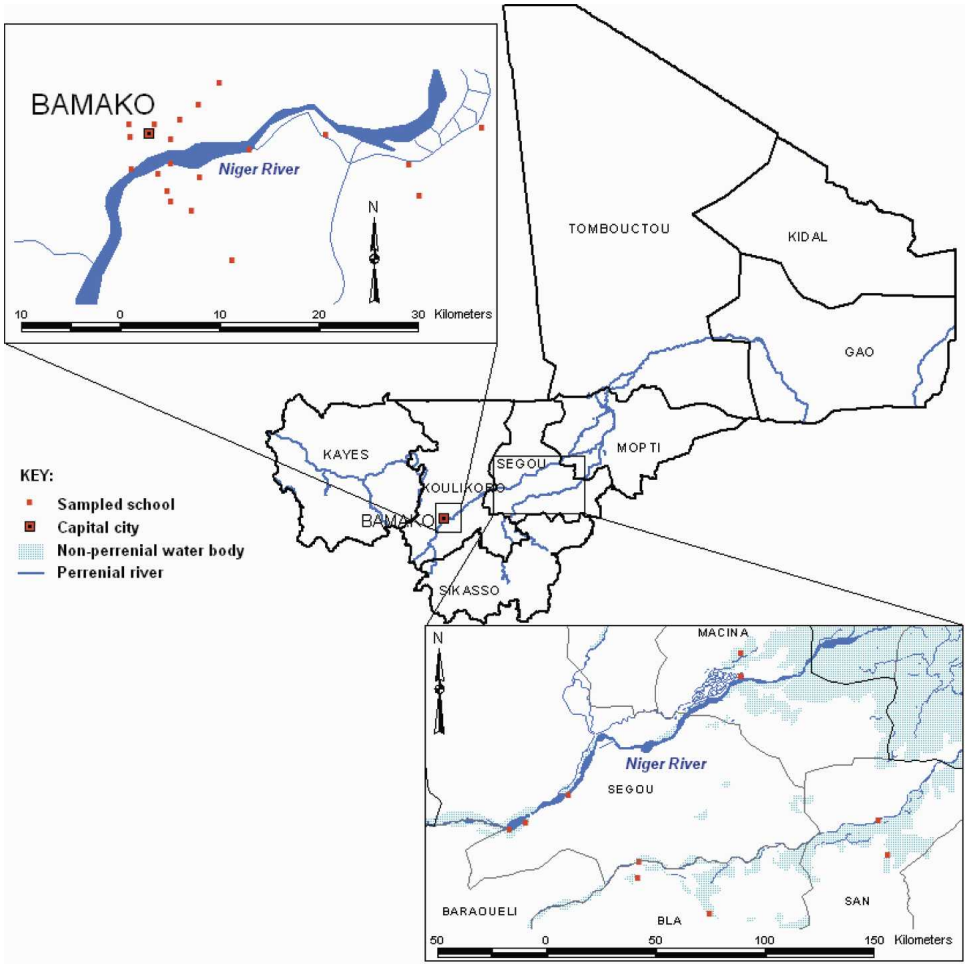

FIGURE 1. Location of sampled schools in Mali. Schools in Bamako and Koulikoro are in the upper box and schools in Ségou are in the lower box.

soni by the Kato-Katz technique.[25–27] Individual egg output was expressed as eggs per gram of faces (epg), which was calculated as the arithmetic mean of the two individual slide counts.

Ultrasound examinations. Utrasonographic assessments were performed with a portable ultrasonography device (SSD-500®; Aloka, Tokyo, Japan). A convex 3.5-Mhz transducer was used to detect pathologic changes associated with both *S. haematobium* and *S. mansoni* infection. All examinations were performed by the same clinician (A.D.K.), who was blind to schistosome infections status of the individual children. Ultrasonographic examinations were performed according to current WHO guidelines.[19]

Pathologic changes caused by *S. haematobium* were assessed by recording the shape of the urinary bladder, defining any lesions detected on the bladder wall, and measuring the degree of dilation of the ureters and renal pelvis. The exact coding of each of these characteristics was made according to the recommendations of Richter and others.[19] Further categorization of pathologic changes was performed by calculating the global score, which serves as an index of severity of morbidity and lesions. Children were provided with water and asked to drink abundantly before having an ultrasonographic examination, which took place only when the bladder was filled. In case of detection of dilatation of the renal pelvis, which is suggestive of hydronephrosis, the child was re-examined after urination to rule out the possibility that such dilatation was caused by bladder and urethral repletion.

To characterize morbidity caused by *S. mansoni*, the size of the left liver lobe was measured in PSL. Measurements of PVD were also performed. Liver patterns were graded from A to F, in order of the severity of the pathologic changes they indicate. B0, B1, and B2 are most often grouped together, as are C1 and C2. It should be noted, however, that the SCI protocol did not include periportal thickening measurements

TABLE 1
Sample data used in the study

Infection	Data used
S. mansoni	Uganda SCI survey, 11,844 children 5–12 years of age from 135 schools
S. mansoni	Uganda SCI baseline survey, 3,689 children 6–12 years of age from 32 sentinel schools in 7 districts
S. mansoni	Busia region, Kenya, 603 children 6–12 years of age from 25 schools
S. haematobium	Tanga region, Tanzania, 1,396 children 8–13 years of age from 41 schools
S. haematobium	Volta region, Ghana, 1,216 children 6–12 years of age from 57 schools

because of concerns about both the reproducibility of measurements[16] and the time-consuming component of the examination. Therefore, interpretation of the final score for morbidity caused by *S. mansoni* infection was based on assessment of image patterns and portal hypertension only. Presence of ascites and portosystemic collaterals was also recorded. Detection of pathologic changes not caused by schistosomiasis was also recorded but is not discussed in the present paper. Persons in need of health care were directed to the nearest medical facility.

Ultrasonography protocol definitions. The WHO protocol states that measurements of organ size and vein diameter should be height-adjusted, using standard reference measurements for healthy members of the same population group.[19] King and others found that the numeric WHO cut-off values derived from a healthy Senegalese population in an area not endemic for *S. mansoni* seriously overestimated the risk of portal vein enlargement in Kenyan and Egyptian patients infected with *S. mansoni*.[17] We have also investigated this issue in a Malian setting because the Niamey workshop members anticipated the refinement of the guidelines through continued use and evaluation, by using alternative height-indexing of PVD scores obtained from all children that had normal image patterns as assessed by ultrasonography (n = 2,719). With reference to the PSL measurement, the liver was considered enlarged, or much enlarged if the height-adjusted value exceeded two or four SD in relation to the normogram produced for a Senegalese population, respectively.[28] In addition, we also calculated local cut-off scores for liver left lobe enlargement and verified if the overestimation also applied to this parameter.

At the end of all examinations, each child enrolled in the survey was treated with the WHO-recommended dose of praziquantel (40 mg/kg) for schistosomiasis and with albendazole (400 mg) for intestinal helminths. Side effects were monitored, and adverse reactions after drug administration were infrequent; when present, these were minimal and transient, and no severe adverse experiences were observed.

Statistical analysis. Data from children with incomplete parasitologic or ultrasonographic records were excluded from our analysis and no replacements were made for missing subjects under the assumption that data were missing at random.[29] Descriptive statistics for subject characteristics and outcomes were calculated using SAS version 8 (SAS Institute Inc., Cary, NC).

To examine *S. haematobium* morbidity, we modeled the probability of a child having a positive individual global score using hierarchical multi-variable logistic regression. Potential predictors included *S. haematobium* infection intensity category (light [< 50 schistosome eggs per 10 mL of urine] or heavy [≥ 50 eggs/10 mL),[24] microhematuria, sex, school-level *S. haematobium* infection prevalence (classified as low [< 10%], medium [11–49%], and high [≥ 50%]), and age. The model structure was a two-level random intercept logistic regression model with level-1 defined as the individual child and level-2 as the school allowing for assessment of the extent of between-school variation in individual global scores. The model had the form

$$\log[\pi_{ij}/(1-\pi_{ij})] = x_{ij}a + w_j b + u_j + e_{ij}$$

where π_{ij} is the probability that child i in school j has a positive individual global score, x_{ij} and w_j are vectors of individual- and school-level characteristics respectively, a and b are vectors of estimated parameter coefficients, u_j (~Normal$(0, \sigma^2)$) is an error term at the school level, and e_{ij} (~Normal$(0, \sigma^2)$) is an error term at individual level.

To study the morbidity of *S. mansoni*, we used three hierarchical multi-variable logistic regression models where we aimed to model 1) the probability of having abnormal image pattern scores, 2) the probability of having dilatation or marked dilatation as assessed by the PVD based on the Malian cut-off scores, and 3) the probability of having enlarged liver as assessed by the PSL measurements based on the Malian cut-off scores. Potential predictors included *S. mansoni* infection intensity category (light [1–99 epg of feces, moderate [100–399 epg], or heavy (≥ 400 epg]),[30] sex, school-level *S. mansoni* prevalence (included in the relevant models as a categorical variable and classified as that of *S. haematobium*), and age. The structure and form of the model used to assess each of these *S. mansoni* morbidity indicators, were identical to those used for evaluation of *S. haematobium*.

All four models were constructed using the Mlwin software (version 2.01; Multilevel Models Project, Institute of Education, University of London, London, United Kingdom). The method of estimation was the second-order, penalized, quasi-likelihood procedure,[31] and first-order marginal quasi-likelihood estimates were used to provide the starting values for the estimation procedure, the stability of the algorithm, and convergence criteria.[32] The model structure was selected because of the hierarchical nature of the dataset, i.e., children were clustered in schools and observations from children within the same school were therefore not independent. Multilevel models account for this dependence by partitioning the total variance in the data into variation between and variation within the higher-level units.[33] Although partitioning of variance is straightforward in models with a continuous dependent variable and with a normally-distributed error at each level of the hierarchy, their extension to models with binary responses is more problematic. For the school effect in each model we calculated the median odds ratio (MOR) to quantify the variation between schools.[34,35] The MOR is always ≥ 1 and directly comparable with fixed-effects odds ratios. More precisely, if the MOR = 1, there is no variation between clusters (no second-level variation). If there is considerable between cluster variation, the MOR will be large.

This quantification of the heterogeneity of the schools is not simply of technical value; the apportioned variances are of substantive interest in much of biomedical research because they give important insights to the level at which the action lies[36] and for epidemiologic reasons (in this case quantification of the importance of the schools for understanding individual health).[35] The percentage of total variation in the ultrasonographic global scores as well as in the liver image patterns, the PVD and the PSL, which are explained by each of the corresponding models presented, was estimated using an R^2 measure developed by Snidjers and Boskers.[37]

RESULTS

Schistosoma haematobium was prevalent in all 29 schools surveyed, and *S. mansoni* was prevalent in 25 of these schools.

Schistosoma haematobium. Ultrasonographic examination was performed on 2,841 children. Of these, parasitologic data were obtained from 2,822 children. In 136 (4.8%) of

2,822, there was no second examination of urine and prevalence and mean intensity calculations were based on one urine filtration result for these children. Overall, *S. haematobium* prevalence of infection was 59% and the arithmetic mean intensity was 43.0 eggs/10 mL of urine. At school level, prevalence of infection ranged from 10.8% to 100.0% and arithmetic mean intensity ranged from 0.7 to 202.5 eggs/10 mL of urine. Girls accounted for 53% of children in the survey and approximately equal numbers of children were recruited in each year group from 7 to 14 years of age. Bladder wall thickening and irregularities, bladder masses, and pseudo-polyps, were found in 6.0% of the children. The prevalence of upper urinary tract (kidney) pathology was estimated to be 3.7%. The prevalence of positive global scores was estimated to be 10.1%, and this prevalence at the school-level ranged from 1.0% to 61.4%.

The odds ratios (ORs) from two-level logistic regression analysis for the probability of having a positive ultrasonographic global score are shown in Table 2. Children with either light or heavy *S. haematobium* infection intensities were more likely to have a positive ultrasonographic global score than uninfected children (light: OR = 2.6, $P = 0.013$ and heavy: OR = 5.7, $P < 0.001$). Children with +, ++, and +++ microhematuria scores were significantly more likely to have positive schistosomiasis ultrasonographic global scores than microhematuria-negative children (OR = 2.4, $P = 0.003$; OR = 3.0, $P < 0.001$; and OR = 5.0, $P < 0.001$, respectively). Boys showed significantly higher ultrasonographic morbidity global scores than girls (OR = 2.0, $P < 0.001$). Age was not a statistically significant indicator of *S. haematobium* morbidity, although there was a trend for older children to be more likely to have a positive schistosomiasis ultrasonographic global score. Schools with high *S. haematobium* prevalence were significantly more likely to have positive global scores than those with medium *S. haematobium* prevalence (OR = 1.7, $P < 0.001$). Since there were no schools with a low prevalence of *S. haematobium* included in the survey, this category does not appear in Table 2. This table also shows that a relatively high MOR (2.2) is associated with between-school variation. Of the total variation in the global ultrasonographic score, 9.4% remained unexplained at the school level and 48.0% remained unexplained at the child level.

Schistosoma mansoni. Both ultrasonographic and parasitologic data were obtained from 2,247 (79.7%) of 2,820 children. Overall prevalence of infection was 27% and the overall arithmetic mean intensity was 119.5 epg. Calculations were based on two fecal smear examinations from all but four children, for whom the second measurement was missing. School-level prevalence of infection ranged from 0.0% to 96.0% and arithmetic mean intensity ranged from 0.0 to 814.9 epg.

A total of 2,820 children were examined by ultrasonography for *S. mansoni*-related pathologic changes. Of these children, 96% had normal livers, as assessed by liver image patterns. Of the children that had abnormal liver image patterns, 84% had grade B patterns and 16% had grade C patterns. Figure 2 shows that using the current WHO cut-off value, 85% of the children had a 0 PVD score. In contrast, 96% of the children had a 0 PVD when the cut-off value derived from the data from the Malian children with normal liver image patterns was used. The difference between these two propor-

TABLE 2

Odds ratios (ORs) with 95% confidence interval (CI) estimates and percentage of variation explained for two-level logistic model of prevalence of positive global score as measured by ultrasound using data set of children with complete parasitologic and ultrasound data on *Schistosoma haematobium* infection (n = 2,822)

Fixed effects				
Variables	Categories	OR	95% CI	*P*
Age	7 years old	1		
	8 years old	1.206	0.636–2.284	0.565
	9 years old	0.928	0.480–1.792	0.825
	10 years old	1.380	0.750–2.538	0.300
	11 years old	1.567	0.853–2.877	0.148
	12 years old	1.252	0.671–2.336	0.480
	13 years old	1.267	0.682–2.355	0.454
	14 years old	1.665	0.888–3.124	0.112
S. haematobium intensity infection	Not infected	1		
	Lightly infected	2.578	1.219–5.451	0.013
	Heavily infected	5.709	2.521–12.927	≤ 0.001
Sex	Female	1		
	Male	2.018	1.483–2.745	< 0.001
Results of Hemastix text	Negative	1		
	Trace hemolyzed	1.578	0.772–3.227	0.212
	+	2.442	1.343–4.441	0.003
	++	3.010	1.722–5.262	< 0.001
	+++	4.973	3.035–8.149	< 0.001
S. haematobium school-level infection prevalence	Medium	1		
	High	4.051	1.824–8.995	< 0.001
Random effects				
School		Median OR		
		2.147		
Variation	%			
Total variance explained	42.640			
Total variance unexplained				
At school level	9.380			
At child level	47.980			

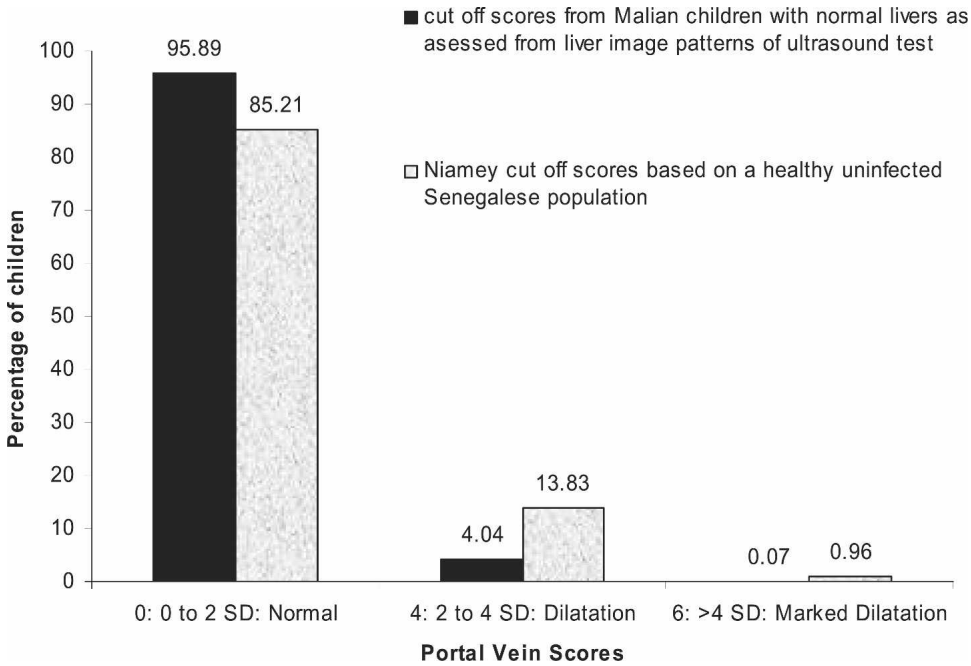

FIGURE 2. Percentages of children with portal veins scores based on different cut-off values.

tions was statistically significant ($P < 0.001$). Statistically significant differences were also found between the proportions of children allocated positive PVD scores of four and six using the two different cut-off values (both $P < 0.001$).

In 99.9% of the children, no collateral vessels were detected and no free fluid was detected in abdomen. Figure 3 shows that 50% of the children in the ultrasonographic cohort had left lobe hepatomegaly as assessed by PSL using the current WHO cut-off value. A total of 41% of the children had an enlarged liver and 8% had a greatly enlarged liver. Conversely, 99% did not have an enlarged liver when the cut-off value derived from the data from the Malian children with normal liver image patterns was used. Statistically significant differences were found between all proportions of children allocated null or positive PSL scores of one and two using the two different cut-off values ($P < 0.001$).

Table 3 shows the ORs from two-level logistic regression analysis for the probability of having abnormal image pattern scores as assessed from ultrasonography for *S. mansoni* infection. Children with light, moderate, or heavy *S. mansoni* infection intensities were more likely to have an abnormal liver image pattern than uninfected children (light: OR = 2.6, $P = 0.023$; moderate: OR = 1.3, $P = 0.62$; and heavy: OR = 3.1, $P = 0.036$). There was a trend for older children to be less

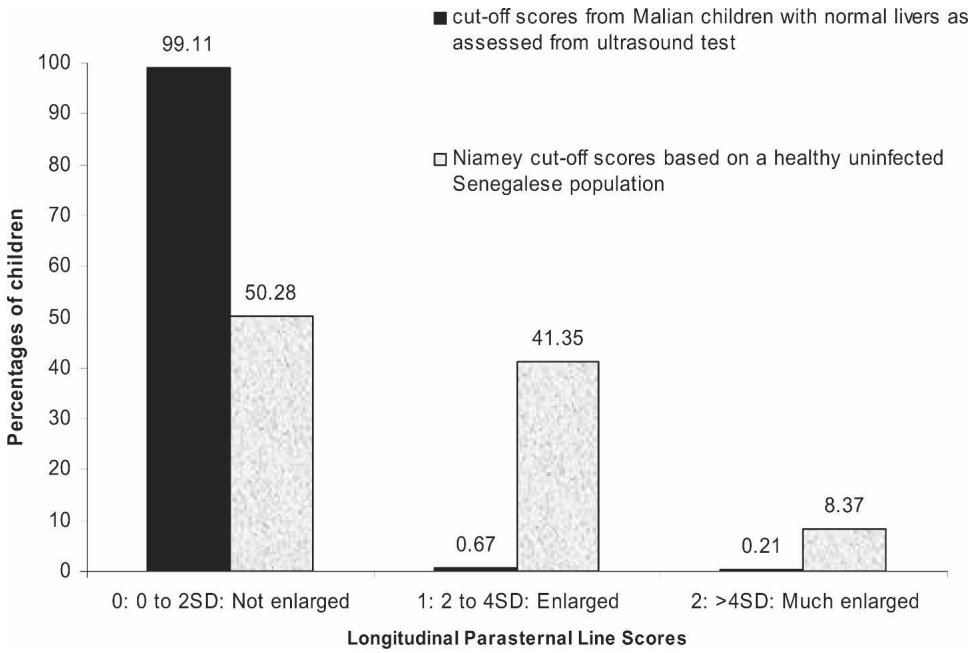

FIGURE 3. Percentages of children with longitudinal parasternal scores based on different cut-off values.

TABLE 3

Odds ratios (ORs) with 95% confidence interval (CI) estimates and percentage of variation explained for two-level logistic model of prevalence of abnormal liver image patterns as measured by ultrasound using data set of children with complete parasitologic and ultrasound data on *Schistosoma mansoni* infection (n = 2,247)

Fixed effects				
Variables	Categories	OR	95% CI	*P*
Age	7 years old	1		
	8 years old	0.550	0.209–1.450	0.226
	9 years old	0.412	0.152–1.120	0.082
	10 years old	0.523	0.202–1.353	0.182
	11 years old	0.305	0.107–0.875	0.027
	12 years old	0.323	0.112–0.926	0.036
	13 years old	0.516	0.189–1.404	0.196
	14 years old	0.224	0.066–0.755	0.016
S. mansoni intensity infection	Not infected	1		
	Lightly infected	2.622	1.144–6.008	0.023
	Moderately infected	1.336	0.423–4.223	0.621
	Heavily infected	3.099	1.080–8.895	0.036
Sex	Female	1		
	Male	1.520	0.873–2.648	0.138
Random effects				
School		Median OR		
		13.444		
Variation	%			
Total variance explained	3.388			
Total variance unexplained				
At school level	66.981			
At child level	29.631			

likely to have an abnormal liver image pattern, but these differences were only significant for 11-, 12-, 13-, and 14-year-old children relative to 7-year-old children (OR = 0.3, $P = 0.027$; OR = 0.3, $P = 0.036$; and OR = 0.2, $P = 0.016$, respectively). When we attempted to include the school-level categorical variable that was representing *S. mansoni* infection prevalence, classified as low, medium, and high, the algorithm did not converge; therefore, we were unable to include this variable in the final model. The same applies to the modeling of the probability of having dilatation or marked dilatation; therefore, this variable was also excluded from the model (Table 4).

Table 3 shows that a high MOR (13.4) was associated with between-school variation. Of the total variation in the liver image pattern, 67.0% remained unexplained at the school level and 30.0% remained unexplained at the child level.

Table 4 shows the ORs from two-level logistic regression analysis for the probability of having dilatation or marked

TABLE 4

Odds ratios (ORs) with 95% confidence interval (CI) estimates and percentage of variation explained for two-level logistic model of prevalence of having dilatation or marked dilatation as assessed by the ultrasound PVD score based on the Malian cut-off scores by using data set of children with complete parasitologic and ultrasound data on *Schistosoma mansoni* infection (n = 2,247)

Fixed effects				
Variables	Categories	OR	95% CI	*P*
Age	7 years old	1		
	8 years old	1.567	0.444–5.525	0.484
	9 years old	3.640	1.159–11.435	0.027
	10 years old	3.245	1.035–10.172	0.043
	11 years old	2.835	0.887–9.064	0.079
	12 years old	1.980	0.586–6.687	0.271
	13 years old	3.647	1.134–11.730	0.030
	14 years old	4.655	1.462–14.826	0.009
S. mansoni intensity infection	Not infected	1		
	Lightly infected	1.402	0.619–3.175	0.418
	Moderately infected	1.408	0.547–3.621	0.478
	Heavily infected	0.774	0.303–1.979	0.593
Sex	Female	1		
	Male	2.250	1.373–3.687	0.001
Random effects				
School		Median OR		
		4.364		
Variation	%			
Total variance explained	6.480			
Total variance unexplained				
At school level	39.360			
At child level	54.159			

dilatation as assessed by ultrasongraphy for *S. mansoni* infection PVD height-adjusted measurements based on the Malian cut-off value. The ORs of having dilatation or marked dilatation as assessed by ultrasonographic PVD measurements for children with light, moderate, or heavy *S. mansoni* infection intensities were not significantly different from those of uninfected children ($P = 0.418$, 0.478, and 0.593, respectively). There was a trend for older children to be more likely to have an increased PVD, but these differences were only significant for 9-, 10-, 13-, and 14-year-old children compared with 7-year-old children (OR = 3.7, $P = 0.027$; OR = 3.3, $P = 0.043$; OR = 3.7, $P = 0.030$; and OR = 4.7, $P = 0.009$, respectively). The MOR to have an increased PVD was 4.364, which has a high OR and is associated with between-school variation. Of the total variation in the PVD, 39.0% remained unexplained at the school level and 54.2% remained unexplained at the child level.

Relative to modeling of the probability of having an enlarged liver as assessed by PSL measurements based on the Malian cut-off scores, the algorithm did not converge. Therefore, we were unable to provide any estimates and establish any associations for this measure.

DISCUSSION

Ultrasonography has become an invaluable extension of the clinical investigation of patients with schistosomiasis and has provided direct evidence of the pathologic changes associated with this infection.[38] This evidence has been well validated in the individual patient clinical setting[39–42] and the relatively small-scale research setting.[43–46] However, the overall aim of this study was to test the suitability of the full WHO-recommended ultrasonography protocol in the context of large-scale schistosomiasis control programs. There is a requirement to elucidate whether ultrasonography could, and indeed should, be incorporated into a mass chemotherapy program to monitor morbidity associated with *S. haematobium* infection and, perhaps in particular, morbidity associated with the often more difficult to characterize *S. mansoni* infections in all but the most severe cases.[47,48]

The current study complements and expands previous ultrasonography-based studies within Africa on a number of issues. First, although previously published surveys[17,49–54] have used ultrasongraphy to measure schistosomiasis-associated morbidity both in children and in adults, which is indicative of long-term chronic infections, we assessed ultrasonography in monitoring schistosomiasis morbidity in control programs focused on children. Although we recognize that measuring only children might be a limitation, if one considers the overall aim of this study, our results still contribute to assessing the suitability of ultrasonography for more recent infections and targeting age groups for future disease control programs. This study should also provide a unique opportunity to clarify the relationship of early lesions to later ones through a subsequent comparison of the baseline findings presented here, particularly where there are identifiable cohorts, over extended periods of time. Moreover, in terms of cohort size, we followed a larger number of persons than previous research or clinical-based studies. Finally, our study has methodologic advantages, particularly since we account for the interdependence of observations by partitioning the total variance into different components of variation due to various cluster levels in the data.

Children are probably the most important age group for ultrasound-detectable morbidity caused by *S. haematobium*, and the results obtained from Mali confirm that the current WHO protocol (Niamey-Belo Horizonte protocol) is a suitable and valid public health tool because its scoring criteria performed acceptably well in defining ultrasound pathology caused by urinary schistosomiasis. Sophisticated statistical models yielded significant associations between global ultrasonography scores from the WHO protocol and several other morbidity predictors. A significant association between the degree of morbidity as defined by ultrasonography global scores and *S. haematobium* infection intensity and microhematuria was demonstrated. Boys had a higher prevalence of morbidity than girls and this has also been observed in studies of Heurtier and others[55] and Keita and others.[56] Our results also indicated that there was considerable variation between schools in the prevalence of positive global scores, thus showing the focal clustering of morbidity caused by urinary schistosomiasis in areas of overall intense transmission. We conclude that ultrasonography global scores and microhematuria scores are likely to be valuable markers in children for morbidity caused by both light and heavy infections with *S. haematobium*. We therefore recommend the inclusion of ultrasonographic examinations in the routine monitoring and evaluation activities of control programs against urinary schistosomiasis whenever resources are available, as in the case of middle-income countries (i.e., North and South Africa and potentially some Middle Eastern countries such as Iraq). In sub-Saharan Africa, such a recommendation should be weighted against additional costs that a subsequent decision would bring (equipment, personnel, training) and the available national or external funds of the control programs.

With regards to intestinal schistosomiasis, the significant associations observed between liver image patterns with *S. mansoni* intensity of infection confirms that these patterns are likely to be valuable markers for morbidity caused by light or heavy infections with *S. mansoni*, as suggested by King and others.[17] However, although our findings suggest that current scoring criteria perform well in defining disease caused by *S. haematobium* infection, they also show, in accordance with those of King and others,[17] that the current WHO cut-off values can lead to serious overestimation of the risk of PVD in patients infected with *S. mansoni*. In addition, our data show that the risk of left liver lobe enlargement may be overestimated by WHO cut-offs values. We therefore agree that cut-off norms should be recalculated at least in each disease-endemic country from a subset of local persons with pattern A prior to the implementation of the WHO protocol, which also fulfills the recommendations included in the Niamey-Belo Horizonte protocol guidelines. Further studies to confirm appropriate cut-off scores for these measurements are therefore required.

Nevertheless, the observation that much of the variability in the liver image pattern remained unexplained, as well as the high MOR, suggest that other variables might be needed to explain the between-school heterogeneity. Another explanation for this high MOR and unexplained variation might be that among children with abnormal liver image patterns, 84% were found to have grade B (coding for the earliest pathologic changes in the liver), which may not be schistosomiasis specific. This same fact might also explains the unexpected finding that older children have less likelihood of abnormal pat-

terns than younger ones compared with other studies of schistosomiasis morbidity. Therefore, liver image patterns of grade B may have represented a confounding factor in our analysis. Further studies are therefore needed to fully elucidate the relationship between liver fibrosis and schistosomiasis, with particular reference to the degree of association between pattern B and *S. mansoni* infection and to the role played by other factors in determining such fibrosis. Likewise, in the case of hepatomegaly, we were unable to distinguish clear associations, which may have been due to the fact that these observed morbidities were likely to have been multifactorial, with *S. mansoni* infection being only one of a number of potential causes.[57] There are often many factors (genetic and possibly most importantly malaria, which is transmitted throughout the year in Mali[58]) other than *S. mansoni* that can cause liver enlargement, and their role and interaction with *S. mansoni* infection also requires further clarification.

Although these data are on children and as such were expected to be less likely to demonstrate ultrasonagraphy-detected morbidity caused by *S. mansoni* infection because of the amount of time of exposure associated with the time taken for fibrosis to build up, in contrast, they show morbidity for this type of infection, suggesting that in these communities children may become infected early in life.[59] We expect that in adults the dynamics of exposure, treatment, and host immunity would show even more ultrasonography-detectable *S. mansoni* morbidity than observed in this study. It might also be important to include the periportal thickening measurement in the ultrasonography examinations when the adult population is examined to evaluate the performance of the protocol. We predict that in adults infected with *S. mansoni*, the comparison between Malian and WHO cut-off values (derived in a similar way as previously described) would show significant differences in the estimations of risk of PVD and left liver lobe enlargement because King and others[17] also observed the same pattern irrespective of age.

For *S. haematobium*, as in high transmission areas like those under study, successive episodes of infection would result in recrudescence of urinary tract abnormalities detected by ultrasonography,[60] and we would expect to observe more severe pathology caused by urinary schistosomiasis in young adults because of continuing reinfection. However, ultrasonography may not be the most appropriate tool to detect and define late-stage morbidity caused by *S. haematobium* infection in older adults because of decreased rates of reinfection in this age group, which leads to decreased development of new inflammatory bladder wall lesions pathognomonic of urinary schistosomiasis. Thus, it would be interesting to conduct a survey on adults from the same communities of children described in this report and investigate up to which age group ultrasonography is a suitable tool to monitor morbidity caused by urinary schistosomiasis in a field setting.

Thus, for both intestinal and urinary schistosomiasis, it will be necessary to obtain longitudinal data to fully elucidate the natural history of morbidity related to infection, with the aim of formulating recommendations for treatment and retreatment based on natural history and evolution of morbidity after large-scale administration of anthelminthic drugs. Such work is currently being conducted by INRSP, SCI, and the National Schistosomiasis Control Program and hopefully will help plan and evaluate sustainable morbidity control.

Conversely, if only parasitologic measurements were incorporated into monitoring of schistosomiasis morbidity of a mass chemotherapy program, the following three issues should be taken into consideration.[15] 1) Diagnosis of infected persons might be missed because of substantial day-to-day variation of egg output in *S. mansoni* infections and then in *S. haematobium* infections. Some persons might not shed eggs at the time of the stool or the urine examination or eggs could be missed. 2) Signs of disease could still be present even in the true absence of egg excretion. Eggs could be trapped in lesions, especially in long-standing infections. Just after treatment, eggs could also be absent, but lesions would still be present. In this case, ultrasonography would still provide detection of irreversible lesions long after treatment. 3) Confounding causes other than schistosomiasis of observed pathologic signs could be excluded by ultrasonography. The epidemiologic importance of confounding causes of uropathy in areas where *S. haematobium* is endemic appears to be small, but information for areas where *S. mansoni* is endemic is still lacking.

In conclusion, the results of this study suggest that the current WHO protocol (Niamey-Belo Horizonte protocol) is a suitable and valid public health tool for urinary schistosomiasis for morbidity control programs focused on children. In detection of morbidity of intestinal schistosomiasis in large-scale control interventions, this same protocol is a useful tool provided local cut-off values are used to define abnormal values and that results are interpreted with caution.

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Sensitivities and specificities of diagnostic tests and infection prevalence of *Schistosoma haematobium* estimated from data on adults in villages northwest of Accra in Ghana.

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Sensitivities and specificities of diagnostic tests and infection prevalence of *Schistosoma haematobium* estimated from data on adults in villages northwest of Accra in Ghana.

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Abstract. Substantial uncertainties surround the sensitivities and specificities of diagnostic techniques for urinary schistosomiasis. We used Latent Class (LC) modeling to address this problem. In this study 220 adults in three villages northwest of Accra in Ghana were examined using five *Schistosoma haematobium* diagnostic measures: microscopic examination of urine for detection of *S. haematobium* eggs, dipsticks for detection of haematuria, tests for circulating antigens, serological antibody tests and ultrasound scans of the urinary system. Testing of the LC model indicated non-invariance of the performance of the diagnostic tests across different age groups while measurement invariance held for males and females and for the three villages. We therefore recommend the use of LC models for comparison between and the identification of, the most accurate schistosomiasis diagnostic tests. Furthermore, microscopy and haematuria dipsticks were indicated through these models as the most appropriate techniques for detection of *S. haematobium* infection.

INTRODUCTION

In spite of the prolific generation of new knowledge in the area of urinary schistosomiasis, such as that of global burden, treatment and associated morbidity [1-4], there remains the unsolved practical issue associated with the basic diagnosis of this important parasitic disease. This relates to both the direct (i.e. microscopical examination of filters of urine for detection of *S. haematobium* eggs) as well as with the indirect (i.e. detection of haematuria, detection of schistosome-specific antibodies, detection of circulating egg antigens and ultrasound scans of the urinary system) diagnostic methods of this schistosome infection. There are several reasons for the limitations in the diagnosis of urinary schistosomiasis infections, such as, for example daily variation in egg excretion levels and/or duration of infection influencing the potential accuracy of determining the correct current infection status.[5] Haematuria (blood in urine) alone has been proposed as a valid indication of current infection in *S. haematobium* endemic populations.[6] Microhaematuria can be detected by reagent strips (dipsticks) which recognize blood and protein. However, for the distinction of an active from a previous infection, particularly after treatment, in many populations and individuals, the circulating schistosome antigen has been proposed as the most reliable test.[7,8] In addition, although the serological diagnosis of schistosomiasis is generally accurate[9], it can also produce false negatives, particularly in patients with longstanding infections while elevated antibody levels can be still detectable many years after treatment.[10] Ultrasound is currently the diagnostic tool of choice for detecting pathological conditions associated with urinary schistosomiasis, such as dilatation of the renal pelvis and bladder wall lesions, although its usefulness has been questioned, particularly in low transmission

areas, because of its lack of specificity.[11] In addition, large variations of sensitivity and specificity estimates have been observed among different endemic zones, age groups and sexes for all the aforementioned diagnostic methods of urinary schistosomiasis in several studies. [12-16]

One explanation for the inconsistencies between all these diagnostic tests relates to the current lack of a definitive 'gold standard' reference test for urinary schistosomiasis. Consequently, the diagnosis of schistosomiasis as well as the control of this disease becomes problematic. Diagnostic assays with low sensitivities are unsuitable for evaluation of schistosomiasis control programmes, such as those aimed at morbidity reduction through mass human chemotherapy.[17] Indeed, methods that allow infections to be correctly diagnosed are a prerequisite for effective disease control.[18] One solution may therefore relate to the need for more sophisticated statistical models to be developed and utilized in order to obtain more reliable empirical estimates of sensitivities and specificities of diagnostic tests.[19, 20]

In the present study we assessed the performance of five diagnostic tests for *S. haematobium* infection and estimated the prevalence of this infection in different age and sex groups in three villages of northwest of Accra in Ghana. Specifically we used five different diagnostic tests for the prevalence of urinary schistosomiasis infection: that of the urine antigen detection test, performed on membranes or in ELISA plates, the serology anti-IgG test, an ultrasound assessment by recording the shape and state of the urinary bladder, the dipstick for haematuria using urine reagent strips on all urine

specimens for presence of detectable blood, and finally detection of *S. haematobium* eggs by microscopy. Through the application of a latent class model to all of these five tests, the sensitivity and specificity of each test can be determined, and the overall urinary schistosomiasis prevalence levels within the different population groups estimated.

MATERIALS AND METHODS

Study sites and subjects. Three Ghanaian villages northwest of Accra, Ayiki Doblo, Chento and Ntoaso were visited and consenting adults over 19 years of age formed a convenience sample of passers by. However, in general, as regards to the demography in Greater Accra's region, the age structure is still a youthful one, characterized by a somewhat high fertility which has begun to show signs of a steep downward trend.[21] The general public in the three aforementioned villages are familiar with the work of the Noguchi Memorial Institute for Medical Research and its personnel. Through discussions with local authorities the public was alerted, and people were approached and asked to participate. These volunteers were then interviewed and requested to provide specimens of urine, stool and blood for examination. Praziquantel (at 40mg per kg) was offered and taken following diagnosis of all infected cases of schistosomiasis. At subsequent visits, bladder ultrasound scans were performed on the majority of participants. All examinations were performed at the village clinics. Participants responded to a questionnaire, the majority of which were reported to be peasant farmers and persons involved in agriculture. Others responded as traders or vendors, but most reported regular water contact in the nearby river system. Although there was municipal water available in the village of Ntoaso, many residents do not have access to clean running water, and through their daily activities were thereby potentially exposed to risks of

schistosome transmission. A total of 220 individuals consented to participate, had complete data on the variables examined here and were included in the analysis of the present study. The age and sex structure as well as the village location of all the sampled individuals is given in Table 1, which illustrates a lower proportion of individuals who consented to participate and had complete data were below 39 years old and from villages Ayiki Doblo and Chento.

Urine-antigen detection test. Detection of schistosome antigen in urine was performed after the method of Bosompem and colleagues[22] which has shown that *S. haematobium* antigen complexed with complement C3 can be isolated from the urine of infected people using a mouse monoclonal antibody. The authors demonstrated that goat-antihuman C3 would also detect schistosome antigen/complement complex in the urine of infected people, but not in non-infected people as case controls, and subsequently developed a monoclonal antibody dipstick test based on these findings.[23] Briefly, methanol treated polyvinylidene difluoride (PVDF) membrane strips were incubated in test urine for 30min at room temperature (21-25^{o}C), rinsed with Tris-buffered saline (TBS) (50mM Tris and 200 mM NaCl, pH7.4) and then blocked for 15 min in 5% skimmed milk in TBS. The strips were then incubated in a reagent mixture of *S. haematobium* species-specific MoAb (1:100) and goat anti-mouse-immunoglobulins conjugated to horseradish peroxidase (1:10) in 0.1% skimmed milk in TBS for 1 h. The strips were washed three times each by 10 min incubation in TBS and then incubated in substrate solution 0.05% (w/v) (3,3-diaminobenzidine), 0.15% (v/v) H2O2 and 5 mM Co (NO3)2.6H2O in TBS for 1 min. A bluish-black reaction represented positive results while negative results remained colourless.

Serology anti-IgG test. Detection of anti-schistosome IgG in serum was performed on serum eluted from dried blood spots on Whatman No1 filter paper. Blood spots filled a 1 cm diameter circle were taken at the time of examination, desiccated and kept dry until analysis. These were eluted in 1 ml PBS, diluted 1:100, and tested in ELISA plates (Immunolon-2) in triplicate. Analyses were repeated if there was more than 10% discrepancy. Plates were sensitized with SWAP antigen (6.44 mg/ml) prepared from *S. mansoni* adult worms provided by Biomedical Research Institute, Rockville MD. Antigen dilution was optimized against sera from known positive *S. haematobium* infections and known schistosome negative sera. Optical densities were read from a Vmax kinetic microplate reader (Molecular Devices. USA). Results were scored positive when the OD exceeded 2 x SD of the negative controls.

Ultrasound examination. A portable ultrasound apparatus, Aloka SSD-500 portable ultrasound with 3.5 MHz curvilinear probe (Aloka, Tokyo, Japan) was used for ultrasound examination, with the diagnoses made by a medically qualified person with prior training in ultrasound examination and interpretation. Examinations were performed using a curvilinear probe and recorded photographically. Diagnosis of pathological lesions was made *in situ*, and later confirmed by review of the ultraradiograph. For the purpose of this study, lesions were classified as positive or negative. Positive cases were registered when any two of the following situations were evident: epithelium enlarged more than 5 mm, evidence of polyps in the bladder wall, calcification of the epithelium, evidence of hydronephrosis.

Parasitological examination. Classic parasitological methods usually used by field clinicians were employed and evaluated in this study. Microscopy was performed on the product of a single measure of filtration of 10 ml urine taken from a specimen passed between 10:00 and 14:00, the time of optimum egg passage.[24] Urine specimens were kept cool in an insulated ice box and processed in the laboratory within 4 hours of passing. The presence of any *S. haematobium* eggs was recorded as positive. Haematuria was detected by the use of standard "hemastix", with any positive reaction being designated positive for urinary schistosomiasis (Multistix, Bayer Diagnostics).

Statistical analysis. By considering the true *S. haematobium* infection status of a sample of Ghanaian adults as a latent variable with two categories: 'infected' and 'non-infected', we validated the five diagnostic tests. In other words, we considered the observed data of the five diagnostic tests (urine antigen detection, serology anti-IgG test, ultrasound, dipstick for haematuria and microscopy) as indicators of an underlying, not directly observable variable (i.e. *S. haematobium* infection). Results of the five diagnostic tests are directly observed and are known as *manifest* variables while the *S. haematobium* infection is the unobservable latent variable.[25]

Given a sample of individuals with unknown infection status, for whom results from several diagnostic tests are available, latent class analysis can model the probability of each combination of tests results conditional on latent class (i.e. infection status). The manifest binary variables ($x_{1j,}$ x_{2j}, x_{3j}, x_{4j} and x_{5j}) were defined such that $x_{ij}=0$ represents a negative result for test i and $x_{ij}=1$ represents a positive test result for test i on individual j. We tested whether correlations between these manifest variables could be accounted for

by a single latent dichotomous variable *Y* (i.e. the absence *Y*=0 or presence *Y*=1 of *S. haematobium* infection) and we defined $\eta = P(Y=1)$ the probability of being in the infected latent class. In other words, we divided the studied population into two classes (i.e. non infected and infected) assuming that the x_{ij}'s were mutually independent within each class (i.e. true infection status). It is expected that the x_{ij}'s are correlated as they are attempting to measure the presence of the same infection; the model assumes that these correlations are negligibly small only once one has accounted for an individual's true infection status (i.e. latent class membership). This assumption results in a more parsimonious model compared to one in which residual correlations are estimated, and one that is often adequate for the data. In the unlikely case that there are substantial residual correlations between the x_{ij}'s, additional latent classes would likely be required for an adequate fit to the data.

The likelihood function of the latent class (LC) model was

$$L(X) = \prod_{j=1}^{N}\left(\eta \prod_{i=1}^{d} \pi_{i1}^{\,x_{ij}} \left(1-\pi_{i1}\right)^{1-x_{ij}} + \left(1-\eta\right)\prod_{i=1}^{d} \pi_{i0}^{\,x_{ij}} \left(1-\pi_{i0}\right)^{1-x_{ij}} \right) \quad (1)$$

Such a model has two types of parameters. First, there is the unconditional probability η that a person is in the infected latent class.

The second type of parameters are the conditional probabilities π_{i1} and π_{i0} that an individual in a particular latent class has a specified value of each of the manifest variables.[26] In fact, π_{i1} represents the sensitivity and is the conditional probability

$P(x_i=1|y_j=1)$ while $(1-\pi_{i0})$ represents the specificity and is the conditional probability $P(x_i=0|y_j=0)$. The LC model hence produces an estimate of disease prevalence as η is the proportion of individuals in the population of which our sample is expected to be in infection class $Y=1$. It also provides direct estimates of sensitivity and specificity for all the diagnostic tests.[27]

A natural way to extend the LC model (1) is to include stratification or grouping variables and examine group differences of measurement invariance. In this study such group differences are examined for males/females, different village locations and age groups. Likelihood ratio tests between less and more restrictive models were used to examine differences in infection prevalence and measurement invariance between groups. A significant measurement invariance tests suggests that specificities and sensitivities of the diagnostic tests vary by group and should be estimated for each group. Such an approach is referred to in the literature as multigroup latent class analysis (LCA) and comparisons of this sort are useful for at least two purposes: (a) to test whether the distribution of the latent variable is the same in each group and (b) to test whether the manifest observed variables are equally reliable indicators of the latent variable in each group.[28]

Expectation-maximization (EM) algorithm was applied to produce maximum likelihood estimates for all parameters in the model using PROC LCA in SAS Version 9.1 (SAS Institute, Cary, NC). Identifiability of maximum likelihood parameter estimates was checked by using several different seed values.

RESULTS

Table 2 represents the observed positive results expressed as percentages of *S. haematobium* infection for the five diagnostic tests. Different diagnostic tests gave different proportions of positive results.

Table 3 presents the results of one latent class model as it was dictated by likelihood ratio tests. Specifically, this model and denoted in table as 'LC Model 1' is a latent class model where measurement invariance was found to hold among males and females. Because of the measurement invariance found here, we obtain a common set of specificities and sensitivities for both males and females. The best diagnostic test for the detection of the prevalence of *S. haematobium* infection among the five diagnostic tests examined here was microscopy with a specificity estimated as 97.9 % and a sensitivity estimated as 92.5 %. In addition, 'LC Model 1' yielded quite high specificities and sensitivities for haematuria and ultrasound. From this same model estimates of prevalence of *S. haematobium* infection by sex were also obtained. It is estimated that the prevalence of *S. haematobium* infection was highest among males (20.6 %) compared to females (9.7 %).

'LC Model 2' in Table 4 is a latent class analysis model where measurement invariance was found to hold among different village locations as this is again the reason why we obtain only a set of specificities and sensitivities for this group of sampled subjects. Results of this model agree with results of LC Model 1 in Table 3. The best diagnostic test for the detection of the prevalence of *S. haematobium* infection was again microscopy with specificity estimated as 94.6 % and sensitivity as 100.0 %. In addition,

'LC Model 2' yielded quite high specificities and sensitivities also for haematuria and ultrasound. Furthermore, 'LC Model 2' also indicated Chento village as the one with the highest prevalence of *S. haematobium* infection (38.9 %) among the three examined villages here.

Finally, 'LC Model 3' in Table 5 is a latent class model where measurement non-invariance was found for different age groups and this is the reason why different specificities and sensitivities are calculated for each of these groups. Using this model, diagnostic tests which could be characterized as acceptable for the detection of the prevalence of *S. haematobium* infection were those of ultrasound, haematuria and microscopy in the age groups of 19--29 and 40--49 years old; haematuria and microscopy were indicated as good diagnostic tests in the age group of 30--39 years as they both gave quite high specificities and sensitivities at the same time. Finally, in the age group of $\geq$60 years old the estimates of specificity and sensitivity were sufficiently high (93.2 % and 100.0 % respectively) only for haematuria, whilst for the age group of 50--59 years old, when taking into consideration both estimates of specificity and sensitivity, none of the diagnostic tests examined here was indicated as appropriate. From this same model estimates of prevalence of *S. haematobium* infection by age group were also obtained. 'LC model 3' shows that the highest prevalence of active, i.e. by egg passage *S. haematobium* infection was determined among the youngest age group of the sampled individuals of this study (29.8 %).

DISCUSSION

Current estimates of the prevalence of schistosomiasis depend on the use of well-established, but imperfect, diagnostic tests.[29] Appropriate schistosomiasis diagnosis becomes increasingly important for several reasons. For example, clinical diagnosis might lose its value because of lack of specificity and mass treatment might only remain cost effective through the use of appropriate diagnostic tools to only target further drug treatment to those groups of people actually infected.[10] The purpose of the epidemiological survey reported here was to assess the performance of five diagnostic tests for *S. haematobium* infection and examine if the prevalence of this infection varied across different age and sex groups of sampled individuals from three villages northwest of Accra in Ghana where there has been reported previously medium *S. haematobium* prevalence.[30] We have addressed this specific problem by taking into account the absence of a gold standard diagnostic test for *S. haematobium* infection and by fitting latent class models with a frequentist approach to these data obtained from adults in northwest of Accra in Ghana. Although latent class models have been used extensively in the epidemiological literature of several infectious diseases,[31-36] they have rarely been used in parasite epidemiology and particularly in the area of schistosomiasis. More precisely, to our knowledge, only two previously published studies, both based within Côte d' Ivoire, have used latent class models through a Bayesian approach in order to assess performance of the Kato-Katz technique in diagnosing *S. mansoni* and hookworm co-infections as well as to estimate reduction of prevalence and intensity for hookworm infection in humans post-praziquantel treatment[37, 38], while only one study in the Philippines has provided estimates of sensitivity and specificity of a faecal examination

method for *Schistosoma japonicum* infection in mammals, using also a statistical modelling approach within a Bayesian framework.[39]

Our study therefore provides the first, to our knowledge, evaluation of the performance of multiple diagnostic criteria and estimation of the prevalence of *S. haematobium* infection in Africa which raises important implications to consider with reference to reliable tests for the diagnosis of urinary schistosomiasis. Such findings should be also of direct relevance and application to current mass chemotherapeutic control programmes. Nevertheless, as the current dataset focuses on adults, we would recommend additional similar studies aimed to assess the application of such latent class models on data from school-aged children across varying schistosomiasis endemic regions within sub-Saharan Africa since school children form the major target age group of current mass chemotherapeutic control in human helminthiasis[40]

The results of this study clearly demonstrate that in adults the microscopic detection of the parasite's eggs in the urine is the best currently available diagnostic tool for *S. haematobium* infection (results in Tables 3, 4 and 5 support this argument) with the exception for the age group of $\geq$50 years old where very low specificities were estimated (Table 5). Standard errors of the estimates were larger for the older age groups compared to the young age groups due to the smaller sample sizes here (Table 1) and therefore such results should be interpreted cautiously. Based on these findings, we would thus recommend the inclusion of microscopic examination in the monitoring process of human

mass chemotherapy programs whenever financial resources allow for this option, mainly because of its relatively low operational cost compared to other urinary schistosomiasis diagnostic techniques and its feasibility under most conditions. Furthermore, as microscopic examination can quantify the intensity of the *S. haematobium* infection, it enables evaluation of important indicators in the control planning, such as possible risk factors, presence of severe clinical forms, degree of transmission and reinfection in the area, and intervals for necessary re-treatments.

In addition, this study confirms that haematuria dipsticks can be sufficiently sensitive and specific indicators (results in Tables 3, 4 and 5 support this argument with the exception of results in Table 5 where for the age group of 50--59 years old haematuria dipsticks yielded a very low sensitivity (45%)) for detection of *S. haematobium* infection in endemic areas, and therefore we would also recommend their inclusion in the monitoring process of human mass chemotherapy program. Indeed, in recent studies we have also found that semiquantitative reading of dipsticks correlates well with intensity of *S. haematobium* infection and ultrasound pathology.[2, 41]

On the other hand, whilst the urine antigen detection test showed similar sensitivity to microscopy (results in Tables 3, 4 and 5 support this argument), it was also suggested that false-positive urine antigen detection tests may be more common than previously reported.[23] One potential explanation for the low specificity of this test might be that potentially cross-reactive parasites are more prevalent in the age group studied here and polyparasitism is of course common in these areas. Indeed, Dunyo et al. 1996 [42] found filarial infections in the towns or in the villages east of Accra in a similar age group and

we would thus recommend further studies to define both the prevalence of such parasites in this same endemic area and examine any potential cross-reactivity between helminth species within the urine antigen detection test. Results from the current study suggests that the urine-antigen detection tests we evaluated should perhaps not be used for the identification of high risk groups which, due to the possibility of false positive reactions produced by such tests, could artificially inflate the actual numbers of people targeted for mass chemotherapy. Furthermore, estimates from all latent class models presented here yielded low sensitivities and specificities for the serology anti-IgG tests. The observation here that antibody detection lacks specificity is consistent with findings of other epidemiological studies which reported that antibody is often found without concomitant parasitological evidence of infection.[43, 44]

Furthermore, antigen detection methods are generally more expensive than antibody ones.[45] On the other hand, microscopy and haematuria dipsticks require relatively unsophisticated equipment and, in areas of high endemicity, personnel with only basic training. These two latter diagnostic tests could therefore constitute the lowest cost option when technical assistance is plentiful. Thus the current findings, if combined with consideration of costs involved, which is a critical issue in the economically developing countries, leads us to the conclusion that antibody and antigen detection tests should not be used in the determination of the prevalence of long term urinary schistosomiasis infections.

With reference to the detection of urinary schistosomiasis infection through ultrasound examination, the results of this study indicated that the performance of this diagnostic

tool was quite acceptable in all age groups except in those of 30--39 years old and ≥50 years old. An explanation for the variability in these results among different age groups might be that successive episodes of infection would result in recrudescence of urinary tract abnormalities and more severe pathology caused by urinary schistosomiasis would be expected to be observed because of continuing reinfection. Thus, we would conclude that ultrasound examination is not a reasonable substitute for microscopy or dipsticks in regards to determining the prevalence of *S. haematobium* infection. Nevertheless, we would still support the argument that the best currently available diagnostic tool for morbidity assessment in *S. haematobium* infections is the visualization of urinary tract pathology through ultrasound examination .

Finally, with statistical analysis alone, one can never be certain about the validity of a dependence model as it is not known from the observed data how each of the examined diagnostic tests relates to the others conditional on disease status.[46] Consequently, we recognize that the results of this study depend upon the assumption of conditional independence assumed by the models fitted here. In addition, latent class models based on current assumptions may not be appropriate for some similar alternative datasets as very large correlations (if these are present after accounting for latent class membership i.e. the true infection status) could potentially bias parameter estimates and result in an underestimation of the error rates of the examined tests.[47]

Through the use of latent class models we assessed the prevalence of *S. haematobium* infection, because accurate sensitive and specific measures for this indicator are

imperative, particularly at later stages of successful mass chemotherapy control programmes. We demonstrate that latent class models proved a useful tool for validation research in the absence of a perfect gold-standard diagnostic technique. These models have suggested microscopy and haematuria dipsticks as sensitive and specific indicators of prevalence of *S. haematobium* infection in Ghanaian adults. In addition, they have provided estimated prevalences of *S. haematobium* infection that fit well with those previously obtained by those such as Nsowah-Nuamah and colleagues[48] in Southern Ghana, and Amankwa and colleagues[49] in upper-east region of Ghana as well as the focality of this infection even within small areas of the same country. However, it must be also considered that in the general context of chemotherapy programs, if monitoring and evaluation results are based exclusively on determining infection prevalence, the impact data obtained may inaccurately reflect the success of any programme. Therefore, it is fundamental to also monitor the impact of such control programmes on the intensity of the infection and morbidity changes in the treated population, particularly as modern day chemotherapy programs are aimed at reducing morbidity and hence intensity and further research in this area is thereby warranted.

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TABLE 1

Participation by age class, sex and village

Variable	***Number of individuals who consented to participate and had complete data (%)***	***Number of individuals who dropped out or did not have complete data (%)***	***p-value****
Age class			
19--29 years old	51 (29.8)	120 (70.2)	<0.001
30--39 years old	57 (42.5)	77 (57.5)	
40--49 years old	56 (57.1)	42 (42.9)	
50--59 years old	33 (52.4)	30 (47.6)	
≥60 years old	23 (37.7)	38 (62.3)	
Sex			
Female	117 (40.8)	170 (59.2)	0.618
Male	103 (42.9)	137 (57.1)	
Village location			
Ayiki Doblo	102 (49.0)	106 (51.0)	<0.001
Chento	39 (26.2)	110 (73.8)	
Ntoaso	79 (46.5)	91 (53.5)	
Total n	***220***	***307***	

*p-value for chi-square test.

TABLE 2

Positive results expressed as percentages by each of the five diagnostic tests among the 220 Ghanaian adults studied

Diagnostic tests	***Positive results expressed as % with (95 % CI)****
Urine-antigen detection	68.6 (62.5-74.8)
Serology anti-IgG	44.1 (37.5 50.7)
Ultrasound	31.8 (25.7-38.0)
Haematuria	21.8 (16.4-27.3)
Microscopy	15.5 (10.7 -20.2)

* CIs are based on normal approximation methods

TABLE 3

Sensitivity and specificity of diagnostic tests as estimated from latent class model 1 when measurement invariance was imposed across males and females

LC Model 1		***Diagnostic tests***									
	S. haematobium prevalence (%)	Urine-antigen detection		Serology anti-IgG		Ultrasound		Haematuria		Microscopy	
		Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)
Sex											
Male	21	36	98	57	48	74	65	87	73	98	93
Female	10										

TABLE 4

Sensitivity and specificity of diagnostic tests as estimated from latent class model 2 when measurement invariance was imposed across different village locations

LC Model 2		***Diagnostic tests***									
	S. haematobium prevalence (%)	Urine antigen detection		Serology anti-IgG		Ultrasound		Haematuria		Microscopy	
Village location		Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)
Ayiki Doblo	7	35	100	56	47	73	70	86	91	95	100
Chento	39										
Ntoaso	2										

TABLE 5

Sensitivity and specificity of diagnostic tests as estimated from latent class model 3 when measurement invariance was not imposed across different age groups

LC Model 3		***Diagnostic tests***									
	S. haematobium prevalence (%)	Urine antigen detection		Serology anti-IgG		Ultrasound		Haematuria		Microscopy	
Age group (in years)		Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)
19--29	30	36	100	43	82	89	74	88	84	93	82
30--39	9	31	81	50	0	77	41	86	100	94	99
40--49	14	31	100	58	13	73	75	88	75	100	100
50--59	20	61	100	78	20	72	53	100	45	100	45
>=60	11	39	100	60	71	46	0	93	100	100	0