Assessment, mapping and prediction of the spatial distribution of parasitic infections in western Côte d'Ivoire and implications for integrated control

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Summary

Almost half of the world's population are at risk of malaria infection, and the disease kills more than one million people each year, mainly children under the age of five years living in sub-Saharan Africa. More than one-quarter of the world's population are affected by schistosomiasis and soil-transmitted helminthiasis and approximately 300 million people suffer from associated morbidity. Recent estimates suggest that each year schistosomiasis alone causes more than 200,000 deaths due to kidney dysfunction and haematemesis in sub-Saharan Africa. Amoebiasis, a disease caused by the intestinal protozoan parasite *Entamoeba histolytica*, kills 40,000-100,000 people each year. An estimated 200 million people are currently infected with *Giardia duodenalis,* another intestinal protozoan infection, which can cause severe disease especially in children. The above-mentioned diseases are particularly prevalent in developing countries, especially in the poorest segments of rural communities. Underlying risk factors are lack of access to clean water and improved sanitation, inadequate hygiene behaviour, and lack of access to sound preventive measures and effective treatment. Because several of these parasitic infections are often prevalent in the same regions, it follows that people can harbour multiple parasite species infections concurrently. Individuals with multiple parasite infections are at an elevated risk of morbidity, hence the appraisal of the extent of this phenomenon and the underlying risk factors of single and multiple infections is important for the design and implementation of control strategies.

The overarching goal of this thesis was to enhance our understanding of demographic, ecological, environmental and socio-economic factors that influence disease distribution in space in the region of Man, western Côte d'Ivoire. This knowledge base will facilitate the creation of risk maps and predictions of parasitic infections. For this purpose two different studies, namely (i) a community-based study in a single village with participants of all age groups and (ii) a regional school-based study with more than 4,000 schoolchildren, were carried out.

The community-based survey was conducted in May-June 2002 in the village of Zouatta II. There, we first conducted a demographic survey among 561 individuals of 75 randomly selected households. Name, age and sex of household members were recorded, as well as the geographical coordinates of their houses. Then, we carried out a rigorous parasitological survey. From each study participant, stool samples were collected on three consecutive days. On the third day, finger prick blood samples were collected from each participant and thin and thick blood films were prepared. Stool and blood samples were analysed with standardized

quality-controlled, methods for diagnosis of parasitic infections. The stool samples were processed with the Kato-Katz technique for the identification of *Schistosoma mansoni* and soil-transmitted helminth eggs (hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*). The formalin concentration method was employed for the identification of intestinal protozoa cysts or trophozoites, including *E. histolytica/E. dispar* and *G. duodenalis*. Thin and thick blood films were stained with Giemsa and analysed by light microscopy for *Plasmodium* infections. In parallel, a questionnaire survey for the appraisal of perceived morbidity indicators was carried out among the same household members.

In the regional school-based study, demographic data, i.e. age and sex of all schoolchildren attending grades 3-5, were obtained from official class lists in the school year of 2001/2002. First, a cross-sectional parasitological survey was carried out among more than 4,000 schoolchildren from 57 rural schools. From each child single stool and blood samples were collected and processed according to the same standardized, quality-controlled, methods mentioned above. Subsequently, a questionnaire survey was carried out for appraisal of self-reported morbidity indicators and schoolchildren's socio-economic status. Questionnaires included 17 morbidity indicators and 12 household assets. Finally, a comprehensive geographical information system for the region of Man, including environmental data obtained from satellite imagery and digitised maps, was established.

The results of the community-based parasitological survey confirmed that several parasitic diseases were common among rural dwellers of western Côte d'Ivoire, with all age groups concerned. The prevalences of *P. falciparum*, hookworm, *E. histolytica/E. dispar* and *S. mansoni* were 76.4%, 45.0%, 42.2% and 39.8%, respectively, and polyparasitism was very common. In fact, more than three-quarters of the population harboured three or more parasites concurrently. Several parasitic infections showed associations with age and sex. Furthermore, multivariate models revealed significant associations between several parasites and morbidity indicators.

The administration of a single oral dose of praziquantel at 40 mg/kg against *S. mansoni* infections was efficacious, since 60.9% of the *S. mansoni*-positive study participants were cured. The egg reduction rate was 61.4%. Cure rates were strongly associated to the infection intensity pre-treatment, age of study participants, as well as the sampling effort.

The school-based parasitological survey revealed that several parasites were common among schoolchildren. Laboratory examinations showed that the pathogens *P. falciparum*, *S. mansoni*, hookworm, *G. duodenalis* and *E. histolytica/E. dispar* were found in 64.0%,

38.7%, 30.5%, 17.4% and 11.0% of the schoolchildren, respectively. Strikingly, approximately 90% of the children were suffering from a polyparasitic infection and four out of five children harboured at least three parasite infections concurrently. Associations of different parasites with schoolchildren's age and sex were found. The questionnaire survey revealed that in general, children did not perceive themselves as being healthy, as they responded to suffer, on average, from 5-6 different morbidity indicators concurrently. At present, only 10.4% of the schoolchildren reported to sleep under a bednet. While 22.4% of the least poor reported to have the opportunity to sleep under a bednet, none of the poorest schoolchildren gave a positive answer. Relationships to socio-economic status were further identified with parasitic infections and self-reported morbidity, as well as physical access to formal health care delivery services.

In the case of *S. mansoni* infections, Bayesian geostatistical models revealed that age, sex, socio-economic status, rainfall and elevation were explaining part of the geographical distribution of this parasite in the region of Man. Boys, schoolchildren aged 11-16 years, and poorer children were more likely to be infected with *S. mansoni* than their respective counterparts. Further, schoolchildren living at elevations above 400 m were at an increased risk of having an *S. mansoni* infection. Interestingly, results showed that demographic factors and socio-economic status had stronger influence on the model fit than environmental factors.

The results call for concerted efforts to reach the most disadvantaged segments of populations in this rural part of Côte d'Ivoire. This should include improved access to preventive and curative medicine, clean water and improved sanitation, coupled with sound hygiene behaviour education. The findings of the present investigations contribute to the planning of integrated control strategies of several human parasitoses and in particular to schistosomiasis, soil-transmitted helminthiasis and malaria control by providing risk maps that can guide decision makers in the region of Man, western Côte d'Ivoire.

Zusammenfassung

Mehr als die Hälfte der Weltbevölkerung ist dem Malariarisiko ausgesetzt und über eine Million Menschen sterben jedes Jahr an dieser Krankheit. Vor allem Kinder unter 5 Jahren im sub-saharischen Afrika sind davon betroffen. Weiter wird geschätzt, dass mehr als ein Viertel der Weltbevölkerung mit *Schistosoma* spp. (Erreger der Bilharziose), Spulwurm, Peitschenwurm und Hakenwurm infiziert sind, davon leiden 300 Millionen Menschen an den daraus resultierenden Krankheiten. In Afrika südlich der Sahara verursacht allein die Bilharziose jährlich 200'000 Todesfälle, als Folge von Nierenversagen und Hämatemesis. Amöbenruhr, welche durch den darmpathogenen Einzeller *Entamoeba histolytica* verursacht wird, tötet 40'000-100'000 Menschen jedes Jahr. Zudem, zeigen Schätzungen, dass 200 Millionen Menschen mit *Giardia duodenalis*, dem Erreger der Giardiose (Lamblienruhr), infiziert sind. Vor allem Kinder leiden an Giardiose-Erkrankungen.

Die oben erwähnten Krankheiten sind in Entwicklungsländern vorherrschend, und darin wiederum in den ärmsten Teilen ländlicher Gebiete. Risikofaktoren für diese parasitären Krankheiten sind das Fehlen von sauberem Wasser und sanitären Einrichtungen, inadäquates Hygieneverhalten, fehlende Präventivmassnahmen und wirksame Behandlung. Weil diese Parasiten oft in den gleichen Regionen der Welt vorkommen, kann man annehmen, dass dessen Bewohner an mehreren parasitären Infektionen gleichzeitig leiden. Da Menschen mit multiplen parasitären Infektionen einem höheren Morbiditätsrisiko ausgesetzt sind, ist es wichtig, das Ausmass dieses Phänomens und die dazugehörigen Risikofaktoren zu verstehen. Erst dann können Kontrollstrategien sinnvoll geplant und implementiert werden.

Ziel dieser Arbeit war es, demographische, ökologische, umweltbedingte und sozioökonomische Faktoren zu verstehen, welche die räumliche Verteilung von Krankheiten in der Region Man im Westen der Côte d'Ivoire (Westafrika) beeinflussen. Dieses Wissen vereinfacht die Herstellung von Risikokarten und Prediktion parasitärer Infektionen. Zu diesem Zweck wurden zwei verschiedene Studien durchgeführt. Die erste Studie konzentriert sich auf ein einziges Dorf mit Studienteilnehmern aus allen Altersklassen. Die zweite Studie ist regional angelegt und untersucht mehr als 4,000 Schulkinder.

Die Studie auf Dorfschaftsebene wurde zwischen Mai und Juni 2002 in Zouatta II durchgeführt. Als erstes wurden in 75 zufällig ausgewählten Haushalten demographische Daten erhoben, wobei Namen, Alter, Geschlecht und geographische Koordinaten von 561 Haushaltsmitglieder notiert werden konnten. In einem nächsten Schritt wurde eine parasitologische Untersuchung durchgeführt. Während drei aufeinanderfolgenden Tagen

wurden Stuhlproben von jedem Studienteilnehmer gesammelt. Am dritten Tag wurden zudem jedem Studienmitglied zwei Bluttropfen zur Herstellung von dünnen und dicken Blutausstrichen entnommen. Stuhl- und Blutproben wurden unter Anwendung standardisierter und qualitätskontrollierter Methoden nach Infektionen mit Darmparasiten (in Stuhlproben) und *Plasmodium* spp. (in Blutproben) untersucht. Die Kato-Katz Methode wurde für die Identifizierung von *Schistosoma mansoni*, Hakenwürmern, *Ascaris lumbricoides* (Spulwurm) und *Trichuris trichiura* (Peitschenwurm) angewendet. Zusätzlich wurde eine Formalin-Konzentrationsmethode für den Nachweis von Darmprotozoen (z.B. *E. histolytica/E. dispar* und *G. duodenalis*) im Stuhl benutzt. Giemsa gefärbte Blutausstriche wurden unter dem Lichtmikroskop auf *Plasmodium* Parasiten untersucht. Parallel dazu wurde eine Umfrage durchgeführt, wobei Studienteilnehmer anhand einer Liste von Morbiditätsindikatoren befragt wurden.

Für die regionale Schuluntersuchung wurden demographische Daten betreffend Alter und Geschlecht der Schulkinder durch die Schulinspektion zur Verfügung gestellt. Zuerst wurde eine parasitologische Untersuchung in 57 ländlichen Schulen mit mehr als 4'000 Primarschulkindern der 3. bis 5. Klasse durchgeführt. Von jedem Kind wurden eine Stuhl- und eine Blutprobe eingesammelt und gemäss den oben erwähnten Prozeduren weiterverarbeitet. Danach wurde eine Umfrage mit denselben Kindern zur Erfassung von Morbiditätsindikatoren und sozioökonomischem Status durchgeführt. Dazu wurden Fragebogen mit einer Liste von 17 Morbiditätsindikatoren und 12 Wertgegenstände des Haushalts verwendet. Schliesslich wurde für die Region Man anhand von Umweltdaten aus Satellitenbildern und digitalisierten Karten ein geographisches Informationssystem etabliert.

Die Resultate der parasitologischen Untersuchung auf Dorfschaftsebene bestätigten, dass mehrere der untersuchten parasitären Infektionen bei den Studienteilnehmer aller Altersgruppen häufig waren. Die Prävalenz von *P. falciparum* lag bei 76.4%, die von Hakenwürmern bei 45.0%, die von *E. histolytica/E. dispar* bei 42.2% und die von *S. mansoni* bei 39.8%. Polyparasitismus war häufig anzutreffen. Bei mehr als drei Vierteln der Studienpopulation wurden Infektionen mit drei oder noch mehr Parasiten gleichzeitig diagnostiziert. Verschiedene parasitäre Infektionen waren mit dem Alter und Geschlecht der Studienteilnehmer assoziiert. Einzelnen Parasiten zeigten Assoziationen mit anderen Parasiten und verschiedenen Morbiditätsindikatoren.

Die Behandlung mit einer einmaligen oralen Dosis von 40 mg/kg Praziquantel gegen *S. mansoni* war wirkungsvoll, da 60.9% der *S. mansoni*-positiven Personen erfolgreich behandelt werden konnten. Die Reduktionsrate der ausgeschiedenen Wurmeier lag bei 61.4%.

Die Erfolgsrate der Behandlung war stark mit der Infektionsintensität vor der Behandlung, dem Alter des Patienten und der Anzahl untersuchter Stuhlproben/Individuum assoziiert.

Die parasitologische Untersuchung auf Schulebene erwies, dass gewisse Parasiten bei Schulkindern häufig waren. Die Pathogene *P. falciparum*, *S. mansoni*, Hakenwürmer, *G. duodenalis* und *E. histolytica/E. dispar* wiesen entsprechende Prävalenzen von 64.0%, 38.7%, 30.5%, 17.4% und 11.0% auf. Fast 90% der Kinder litten an einer multiplen Infektion, wobei bei vier von fünf Kindern gleichzeitig drei oder mehr Infektionen nachgewiesen werden konnten. Infektionen durch gewisse Parasiten hingen mit dem Alter und Geschlecht der Schulkinder zusammen. Die Analyse der Fragebögen ergab, dass Kinder sich nicht als gesund einstuften. Sie gaben durchschnittlich vier bis fünf Morbiditätsindikatoren gleichzeitig an. Weiter ergab die Analyse, dass nur 10.4% der Schulkinder unter einem Moskitonetz schliefen, davon gehörten 22.4% der reichsten sozioökonomischen Gruppe an. Kein einziges Schulkind aus der ärmsten Gruppe berichtete, unter einem Moskitonetz zu schlafen. Es wurden weitere Zusammenhänge zwischen sozioökonomischem Status eines Kindes und parasitärer Infektionen, Krankseins-Wahrnehmung und physischer Zugang zu öffentlichen Behandlungsstrukturen gefunden.

Im Falle von *S. mansoni* Infektionen zeigten geostatistische Modelle nach Bayes auf, dass Alter, Geschlecht, sozioökonomischer Status, Niederschlag und Höhenlage der Schule einen Teil der räumlichen Verteilung dieses Parasiten in der Region Man erklärten. Bei Knaben, Kindern im Alter von 11 bis 16 Jahren, und ärmeren Kindern war die Wahrscheinlichkeit höher, dass diese an einer *S. mansoni* Infektion litten. Kinder, die in Höhen unter 400 Meter über Meer lebten, waren einem höheren Risiko einer *S. mansoni* Infektion ausgesetzt. Die Resultate der Modelle zeigten ebenfalls, dass demographische Faktoren und sozioökonomischer Status im Vergleich zu den untersuchten Umweltfaktoren einen grösseren Einfluss auf die räumliche Verteilung hatten.

Diese Resultate zeigen, dass weitere Anstrengungen nötig sind, um die benachteiligsten Segmente der Bevölkerung in ländlichen Gebieten der Côte d'Ivoire zu erreichen. Dies kann zum Beispiel durch Verbesserung des Zugangs zu Präventivmassnahmen und behandelnder Schulmedizin, sauberem Wasser, sanitären Einrichtungen und Einführung von Hygieneinformation geschehen. Die Resultate dieser Analysen tragen zur Planung integrierter Kontrollstrategien gegen Human-Parasitosen und speziell zur Bilharziose-, Geohelminthen- und Malariakontrolle bei, indem Risikokarten für die Region Man im Westen der Côte d'Ivoire politischen Entscheidungsträgern zur Verfügung gestellt werden.

1. Introduction

The purpose of this introduction is to provide succinct summaries of the key pathogens investigated in the present thesis. Of course, the complexity of each parasite and the cross-talk with the environment are immense, hence not all aspects can be discussed here. Individually, emphasis is placed on the current geographical distribution, life-cycle and the burden caused (section 1.1), the epidemiology (section 1.2), diagnosis and treatment (section 1.3), and control (section 1.4). In addition to single parasite-single host systems, I introduce the phenomenon of polyparasitism, which is the norm rather than the exception in areas where different parasitic infections are common. Section 1.5 discusses the use of geographical information system and remote sensing in public health. The issue of inequities in health is briefly introduced in section 1.6.

1.1 Global burden, geographical distribution and life-cycle of malaria and intestinal parasitic infections

1.1.1 Malaria

The estimated global malaria burden is 46.5 million disability adjusted life years (DALYs) lost (WHO, 2004). Malaria kills between one and three million people each year, mainly children under five years of age, causing a global daily loss of more than 2,000 young lives. In other words, every 40 seconds a child dies of malaria (Breman, 2001; Sachs & Malaney, 2002; Breman *et al.*, 2004). Another 300 to 500 million people manifest clinical malaria every year. Malaria has been described already in the antiquity as seasonal periodic fever. Until the early decades of the 20th century it was found in marshy areas in Europe, North America and the former Soviet Republic. Since then, it has been eliminated in those areas, while in the Middle East, China and India, the number of cases have decreased (White, 2003). In the tropics, however, there has been a resurgence of malaria, mainly due to insecticide and antimalarial drug resistance and environmental alterations (WHO, 2000). Today, malaria is defined as a disease of the global poor, as it is most prevalent within the tropical and subtropical geographical boundaries, where the average gross domestic product (GDP) is low (Gallup & Sachs, 2001).

The term "malaria" originates from the Italian and means "bad air", because people thought that the emanated vapours of Tiberian marshes were causing this disease (Bruce-

Chwatt, 1988). In fact, it is a vector-borne disease; the red blood cell parasites from the genus *Plasmodium* (*P. falciparum*, *P. malariae*, *P. ovale, P. vivax*) are transmitted by infected blood-feeding female anopheline mosquito. When the mosquito feeds on a gametocyte-carrying human, the parasite is ingested and undergoes sporogony in the mosquito's salivary glands. The plasmodial sporozoites are then transmitted to humans by the next blood meal causing the infection. In the human, sporozoites reproduce asexually in the hepatocytes, developing to merozoites that are then released by rupture of the hepatic schizont into the host blood stream, where they invade red blood cells. After undergoing several asexual stages, which is always associated with reproduction of the parasite and the rupture of the invaded red blood cell, the merozoites can develop into sexual forms, i.e. gametocytes (White, 2003).
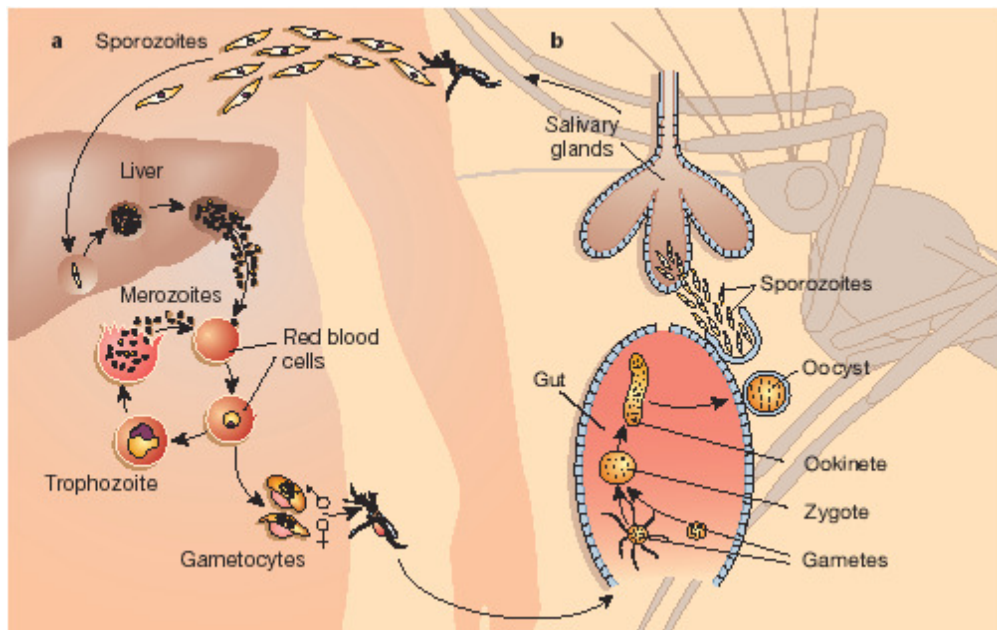
Figure 1 Life-cycle of *Plasmodium falciparum.* Parasite stages within the host (a), and within the mosquito (b) (Wirth, 2002).

The clinical features of uncomplicated malaria are mild symptoms such as headache, muscular ache, vague abdominal pain and fever, and are caused by all four malaria species that parasitize humans. Severe malaria, however, is mainly due to *P. falciparum*. Clinical

manifestations include severe anaemia, convulsions, kidney and renal failure, acute pulmonary oedema, metabolic acidosis and coma, and can potentially be lethal.

P. falciparum is predominant in sub-Saharan Africa, Papua New Guinea and Haiti, whereas *P. vivax* is the predominant species found in Central and parts of South America, North Africa, the Middle East and the Indian subcontinent. Both species are equally prevalent in other parts of South America, East Asia and Oceania. *P. vivax* is rare in sub-Saharan Africa, while *P. ovale* is common in West Africa. *P. malariae* is found in most areas but less commonly so in Africa. *P. vivax* is also found in China and adjacent countries (White, 2003).

1.1.2 Intestinal parasitic infections

Schistosomiasis

Schistosomiasis is a parasitic disease, which involves the gastrointestinal and urinary tracts of the human host. Five trematode species are known to cause schistosomiasis in humans, namely *S. haematobium, S. intercalatum, S. japonicum*, *S. mansoni*, and *S. mekongi* (WHO, 2002a; Utzinger & Keiser, 2004). Interestingly, a recent study analysing mitochondrial genes of geographically isolated *S. intercalatum* strains, suggested that phylogeneticly these strains can be considered as two separate species (Kane *et al.*, 2003). Schistosomiasis remains of significant public health and economic importance, especially in sub-Saharan Africa, where millions of people suffer from morbidity and 200,000 people die every year as a consequence of the chronic nature of infection (van der Werf *et al.*, 2003). The global burden of schistosomiasis may be as high as 4.5 million DALYs lost (WHO, 2002a). Recent estimates for sub-Saharan Africa suggest that 112 million people are infected with *S. haematobium* and 54 million people with *S. mansoni* (van der Werf *et al.*, 2003). School-age children are at a particular risk of schistosomiasis; infections left untreated can result in growth retardation and impairment of physical and cognitive functioning (Jukes *et al.*, 2002). Chronic morbidity has major impact from a public health point of view, as it causes severe disease manifestations, e.g. obstructive uropathy, bladder calcification and renal failure in *S. haematobium* infections, and periportal hepatic fibrosis in *S. mansoni*, *S. japonicum* and *S. mekongi* infections (Ross *et al.*, 2001; Davis, 2003).

Schistosomiasis is currently endemic in 76 countries and is documented as a disease with focal distribution. While *S. mansoni* infections are found mainly in South America, the African continent, Madagascar and the Arabian peninsula, *S. haematobium* infections are

endemic in the Middle East, the African continent and some islands of the Indian Ocean (Mauritius, Madagascar, Zanzibar and Pemba). Schistosomiasis due to *S. japonicum* infections is found in China, Indonesia and the Philippines, and was eliminated in Japan three decades ago. *S. intercalatum* is endemic in 10 countries in Central and West Africa, while *S. mekongi* is found only on Khong Island, Lao People's Democratic Republic and Cambodia (Davis, 2003; Utzinger & Keiser, 2004).
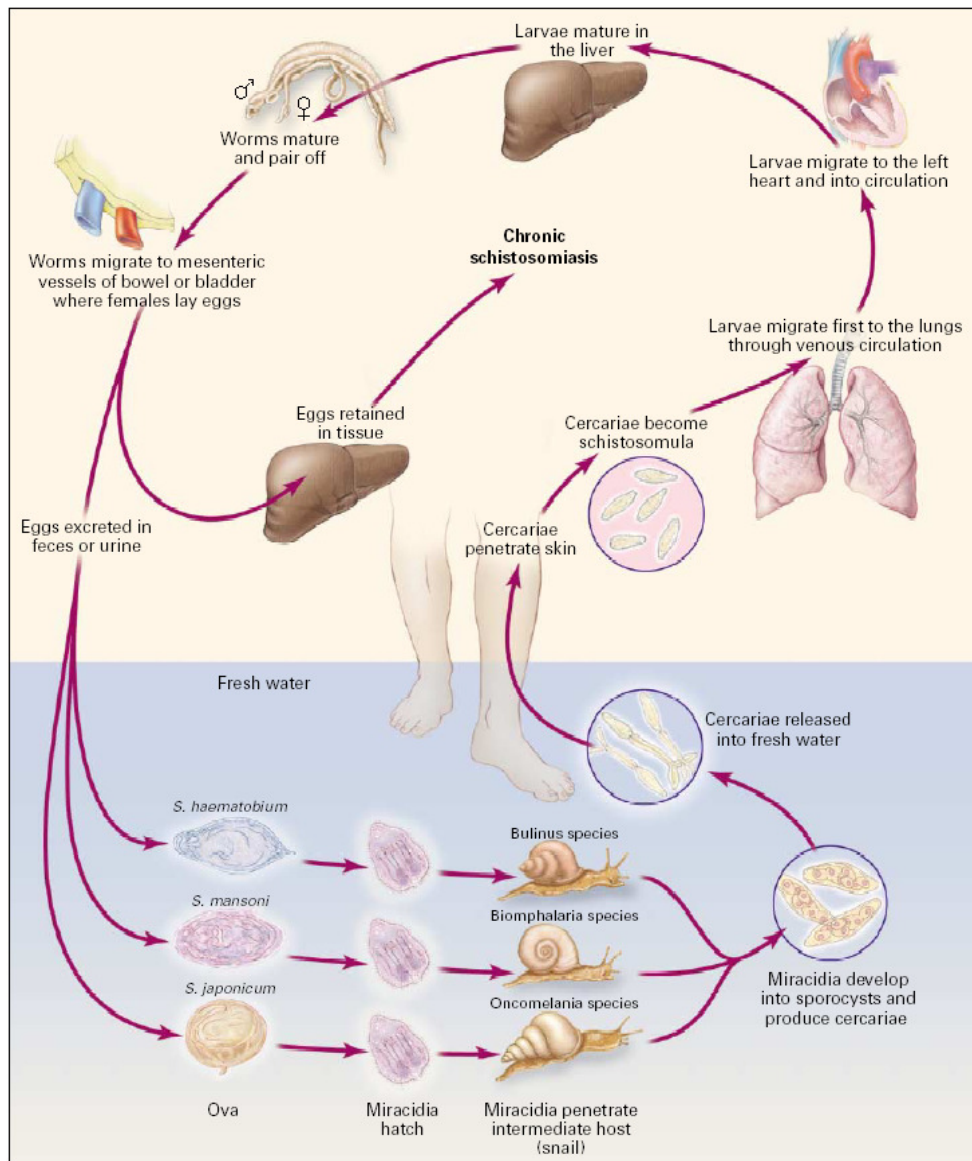
Figure 2 Life-cycle of *Schistosoma mansoni* (Ross *et al.*, 2002)

Aquatic snails from the genus *Bulinus* act as intermediate hosts for *S. haematobium* and *S. intercalatum*, while those from the genus *Biomphalaria* are intermediate hosts for *S. mansoni*. *Tricula aperta* is the aquatic intermediate host for *S. mekongi*. The amphibious snail from the genus *Ocomelania* is the intermediate host for *S. japonicum*. Both aquatic and amphibious snails have the capacity to aestivate, therefore enabling them to carry infections from one wet season to the next. Transmission occurs in suitable water bodies or marshlands, in which the snails release tiny cercariae that can penetrate the skin of the human host. In the host the parasites develop into schistosomula and migrate to the final location in the hepatic portal system where they pair and mature. These adult worm pairs then migrate to the mesenteric venules of the intestine (*S. intercalatum, S. japonicum, S. mansoni* and *S. mekongi*) or the vesical plexus and veins that drain the ureters (*S. haematobium*), where they mate and produce eggs. A part of the eggs is trapped in the tissues of organs causing inflammation and severe morbidity in the chronic stages of the disease. Approximately half of the eggs produced are released into the environment through host excreta. In the water the eggs hatch into miracidia, which can invade the intermediate host.

Soil-transmitted helminthiasis

Soil-transmitted helminthiasis in humans is caused by nematodes that inhabit the gastrointestinal tract of more than 25% of the world's population (de Silva *et al.*, 2003). This disease accounts for 2.95-39.0 million disability adjusted life years (DALY) lost and costs 12,000-135,000 lives each year (WHO, 2002a, 2004; Utzinger & Keiser, 2004). The most common species responsible for soil-transmitted helminthiasis are the roundworm *Ascaris lumbricoides*, the hookworms (*Ancylostoma duodenale* and *Necator americanus*) and the whipworm (*Trichuris trichiura*). It is currently estimated that 1,200 million people are infected with *A. lumbricoides* worldwide, 795 million people are infected with *T. trichiura* and 740 million people are infected with hookworms (de Silva *et al.*, 2003; Brooker *et al.*, 2004; Hotez *et al.*, 2004). Less severe infections are often asymptomatic, while heavy infection can lead to severe anaemia, adverse effects on pregnancy, impairment of children's growth and development, as well as worker productivity.

Although the infections occur worldwide, most of the burden is concentrated in the tropics and subtropics. The highest prevalences of *A. lumbricoides* occur in China (39%), East Asia and the Pacific islands (36%), South Asia (27%) and sub-Saharan Africa (25%). Hookworm infections are most prevalent in sub-Saharan Africa (29%), East Asia and the

Pacific islands (26%), while *T. trichiura* is most prevalent in East Asia and the Pacific islands (28%), sub-Saharan Africa (24%), and South Asia (20%) (de Silva *et al.*, 2003).
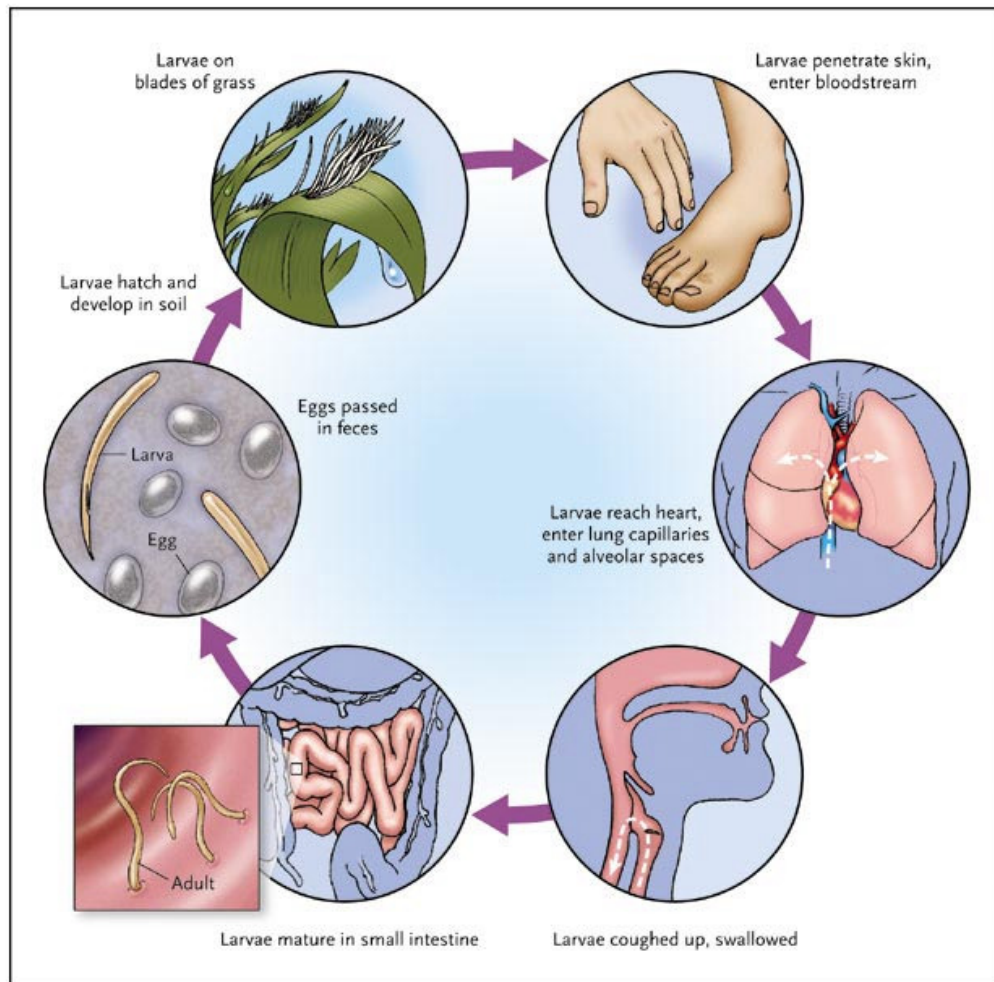
Figure 3 Life-cycle of hookworm (Hotez *et al.*, 2004)

In the transmission of soil-transmitted helminth infections, it is common that part of the development takes place outside the body, notably in the soil. Hookworms mature and mate in the small intestine of the host. The female of *A. duodenale* produces 25,000-35,000 eggs each day and some 18-54 million eggs during its lifetime. The eggs are elliptical, with a transparent shell that contain blastomers, and have dimensions of 50-60 µm x 35-40 µm. Daily, *N. americanus* females lay 6,000-20,000 eggs, which are slightly larger than those of

A. duodenale (Gilles, 2003). The eggs are passed with the faeces and hatch in the environment provided that adequate moisture, shade and warm soil are found. The newly hatched larvae develop into the infective filariform larvae, which can invade the human host through the skin. The larvae enter the circulation and penetrate the lungs. They move up the respiratory tract, enter the oesophagus and reach the small intestine, where they mature into adult. The life-span varies from 4-5 years in the case of *N. americanus* and 6-8 years in *A. duodenale* (Gilles, 2003).

A. lumbricoides and *T. trichiura* can infect the host via direct ingestion of contaminated raw food – i.e. fruit and vegetables – and fingers. The adult male and female worms of *T. trichiura* are 30-45 mm and 30-35 mm long, respectively. The eggs (50 x 22 μm) are brown with a characteristic band shape and a single cell with a plug at each end. Eggs cannot withstand desiccation. After being swallowed, the eggs hatch in the intestine, where the shell is digested by intestinal juices, and the larva emerges in the small intestine. From there they pass the caecum and colorectum where they attach to the mucosa and mature into adults. In contrast to *T. trichiura,* which has a direct life-cycle, *A. lumbricoides* eggs have to undergo a period of development in the soil before being ingested (modified direct life-cycle). In the stomach, larvae are released, which penetrate the mucous membrane entering the circulation to reach the lungs. From there they pass up the respiratory tract to enter the oesophagus, reaching the intestine where they mature into adults (Gilles, 2003). *A. lumbricoides* females are 3-6 mm thin and 20-25 cm long, while males are 2-4 mm thin and 15-31 cm long.

Intestinal protozoa infections

Human intestinal protozoan infections are found worldwide. The highest infection prevalences are documented from the developing world, particularly among the poorest of the poor. Some of the intestinal protozoa live as commensales in the intestine of humans, without causing any harm. Others are pathogens infecting the intestine of humans, e.g. *Giardia duodenalis* and *Entamoeba histolytica*, which can cause severe morbidity. Emerging pathogens like *Cryptosporidium parvum* and *Isospora belli* are experiencing an increase in morbidity due to acquired immunodeficiency states caused by HIV/AIDS infections and cancer chemotherapy. Other 'new' pathogens like microsporidia and *Cyclospora cayetanensis* are increasingly recognized as a result of improved detection methods that have been developed over the past several years. It is expected that more pathogens will be discovered alongside the development of novel diagnostic techniques. Two pathogenic

infections, which are important from a public health point of view, are discussed in this section: *E. histolytica* that causes amoebiasis and *G. duodenalis* that causes giardiasis.

E. histolytica is the causative agent of amoebic colitis, which is characterised by cramping abdominal pain, weight loss, and watery or bloody diarrhoea. Further, it is responsible for extraintestinal amoebiasis like amoebic liver abscess. For example, the annual incidence of amoebic liver abscess in Hue, Viet Nam, was reported to be 21 cases per 100,000 inhabitants, while in Mexico, serological studies indicate that 8% of the population have had amoebiasis (Caballero-Salcedo *et al.*, 1994; Pham *et al.*, 1996). Worldwide, an estimated 480 million people are affected and 40,000-100,000 people die yearly from amoebiasis (WHO, 1997).

G. duodenalis also occurs worldwide and infects an estimated 200 million people epidemically. In the United States it has been identified as the main cause of water-borne outbreaks. Prevalences in industrialized countries range from 2-5%, while in developing countries, prevalences can reach 20-30% (Mineno & Avery, 2003). Infections can be asymptomatic. Clinical manifestations include nausea, anorexia, abdominal bloating, flatulence, eructation, and self-limited diarrhoea, which can further develop to chronic diarrhoea accompanied by malabsorption in some cases. Giardiasis might cause poor cognitive functioning in early childhood, as a result of malnutrition caused by malabsorption of nutrients (Berkman *et al.*, 2002).

E. histolytica has a simple life-cycle and exists either as infectious cyst or amoeboid trophozoite. Infection begins with the ingestion of the cysts present in food or water that has been contaminated with human faeces. The round cysts, 10-15 μm in diameter, have a refractive wall and survive the passing of the acid stomach. They travel through the small intestine and, when having arrived in the terminal ileum or colon, they excyst to form the trophozoite stage. The trophozoites are motile, shaped from 10-50 μm, feed on bacteria and food particles and reproduce by fission. They encyst to infectious cysts within the colon, completing thus the life-cycle when they are excreted into the environment by faeces. In less than 1% of the cases, trophozoites invade the intestinal mucosa causing amoebic colitis. Further, they can disseminate extraintestinally causing severe disease (Farthing *et al.*, 2003). Similarly, *G. duodenalis* exists as infective ovoid or ellipsoid cyst (8-12 μm x 6-10 μm) and pear-shaped bilaterally symmetrical trophozoite with four pairs of flagella (9-21 μm in length, 5-15 μm in width, and 2-4 μm in thickness). The life-cycle is similar to the one of

E. histolytica. This disease is highly contagious, and often family members who are in close contact, infect each other by person-to-person contact (Farthing *et al.*, 2003).
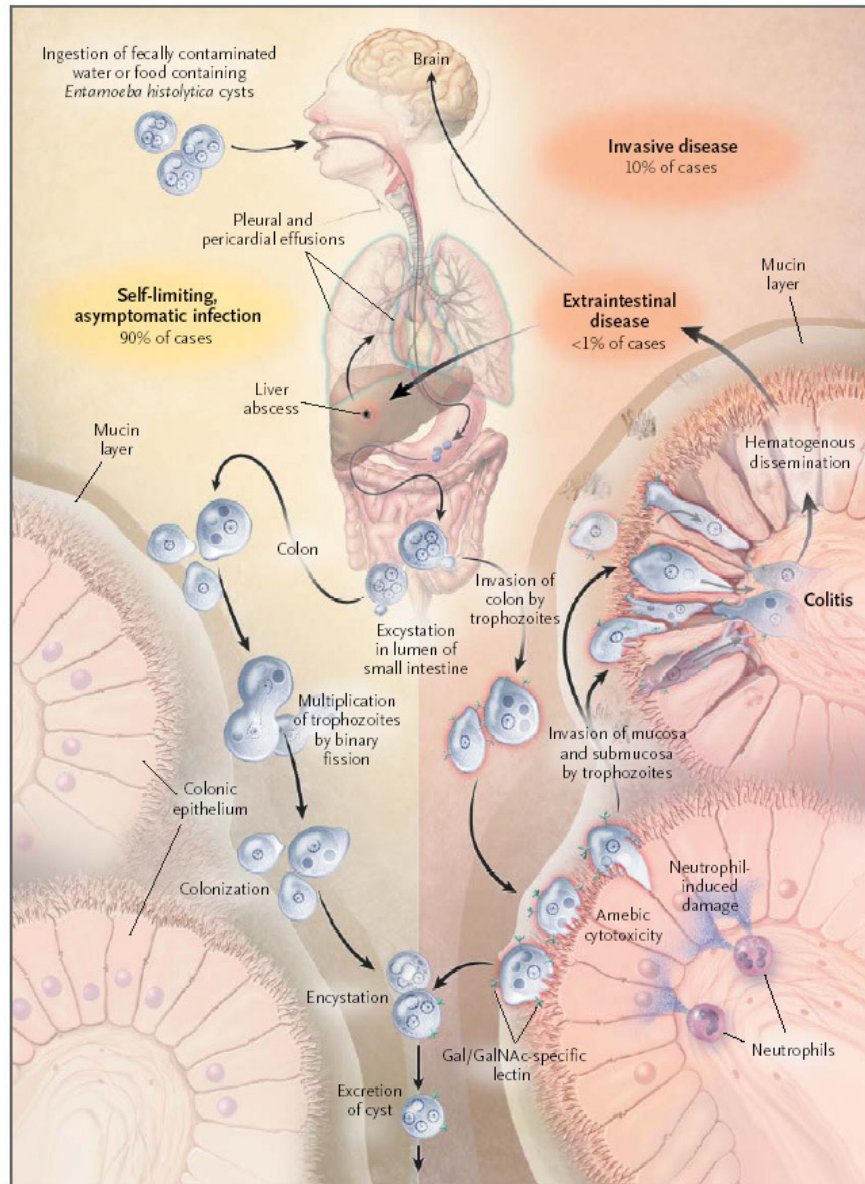
Figure 4   Life-cycle of *Entamoeba histolytica* (Haque *et al.*, 2003).

1.2 Epidemiology of malaria, intestinal parasitic infections and multiple species parasitic infections

1.2.1 Malaria

The epidemiology of malaria involves several dozen species of the anopheline mosquito as vector and humans as definitive host, to whom the parasite is transmitted. Of the 400 anopheline species, approximately 80 can transmit malaria, and 66 are considered natural vectors. Vectors differ in their natural abundance, feeding and resting behaviours, breeding sites, flight ranges, choice of blood source and vulnerability to environmental conditions and insecticides. In total, 45 anopheline species are considered to represent important vectors of malaria (Gillies, 1988; Molineaux *et al.*, 1988). The effectiveness of a malaria vector is characterized by its longevity, abundance and human biting frequency. The most efficient vectors belong to the *Anopheles gambiae* complex (White, 2003).

Longevity of the vectors is one of the most important features in the epidemiology of malaria. As sporogony takes more than a week, the mosquito has to live longer than this period, after feeding on a gametocyte-carrying human, so that the transmission cycle can be completed (MacDonald, 1957; Gillies, 1988). Optimal conditions for transmission are ambient temperatures between 20°C and 30°C and high humidity. Hence the tropics represent the most suitable environment. This is explained by the parasite undergoing sporogony in the mosquito only if the temperatures are above 16°C or below 33°C. These temperature ranges are normally not found at altitudes above 2,000 m. Higher mosquito survival rates occur where the humidity is high. Rainy seasons provide water for vector breeding sites and increase the abundance of mosquitoes, which coincides with the seasonal character of malaria transmission in many epidemiological settings. However, heavy rainfall may have a negative effect for the vector species, as the mosquito larvae and pupae may be washed away (Gillies, 1988).

In areas of high transmission, infants and young children under five years of age are particularly susceptible to severe malaria, because in this age group immunity has yet to be developed. Over the first years of life, children are repeatedly inoculated with sporozoites and, if they survive, a state of semi-immunity is acquired, protecting them from severe malaria. If non-immune adults from a non-malaria endemic area enter an endemic area, they will acquire semi-immunity more rapidly than children. In areas of less intense, seasonal or

focal transmission of malaria, semi-immunity is often not acquired, causing symptomatic disease and severe malaria in all age groups alike (White, 2003).

1.2.2 Intestinal parasitic infections

Schistosomiasis

As already mentioned in the previous section, the epidemiology of schistosomiasis involves humans as definitive host and aquatic or amphibious snails that act as intermediate hosts. Transmission occurs only in freshwater. In the absence of appropriate sanitary facilities and lack of hygiene behaviour, freshwater environments are contaminated with human excreta. Humans acquire the infection through contact of infested water. The frequency, duration and surface of body exposed to infested water during occupational and recreational activities in such freshwater are key features for the intensity of infection in the human host (Davis, 2003).

An important epidemiological feature of schistosomiasis is its focal distribution, which is a result of the complex interrelationship between the density of infected persons and the contaminated environment, the distance between infected persons and the contaminated freshwater, and the frequency, duration and body parts exposed during water contact with the infested environment (Davis, 2003).

The distribution of intermediate host snails determines to a large extent the observed variability in schistosome infections, characterised by seasonal transmission patterns in most endemic areas (Babiker *et al.*, 1985; Woolhouse & Chandiwana, 1989). Key determinants for the intermediate host snail abundance, like water flow velocity and temperature that vary over time and show a seasonal pattern, are therefore important factors in explaining the heterogeneity of epidemiological patterns in time (Appleton, 1978).

Aggregation of schistosome infections are commonly found in school-age children, adolescents and young adults, who show highest prevalence and intensity peaks of schistosomiasis infection (Woolhouse, 1998). This is explained by their frequent exposure to schistosome-infested water. The schistosome pairs are unevenly distributed among the infected human population, resulting in a great variation in excreted schistosome eggs. A small proportion of people are responsible for the bulk of egg excretion, while the majority of infected people excrete only few eggs (Bradley, 1972; Polderman, 1979; Anderson & May, 1985). There is also important individual day-to-day variation in schistosome egg output and

an intra-stool variation in the number of eggs (Engels *et al.*, 1997; Utzinger *et al.*, 2001a; Booth *et al.*, 2003).

Ecological transformations, e.g. caused by the construction of dams for hydroelectric power production and irrigation schemes for intensive agriculture, can cause an outbreak of schistosomiasis or spread the disease to previously non-endemic areas (Hunter *et al.*, 1993; Gryseels *et al.*, 1994; Chitsulo *et al.*, 2000). In these areas preventive action is required, such as health impact assessment and compliance with its recommendations, which consist largely of environmental engineering and management measures (WHO, 2002a).

Soil-transmitted helminthiasis

Common to all human soil-transmitted helminth species is that transmission occurs in areas of humid contaminated soils coupled with poor sanitation. Moist and warm soils are required to complete the life-cycle of hookworms, hence transmission can occur all over the year in tropical and subtropical countries, whilst in cooler or drier climates, transmission takes place only in the warmer or wet seasons. The eggs of *A. lumbricoides* develop best in shady, damp soils and are resistant to cold and to disinfectants. Direct sunlight and temperatures above 45°C can kill the eggs. The embryo develop at 36-40°C, within a period of 2-4 months; at an optimum temperature of 25°C the developmental period is reduced to 3 weeks. Similarly, *T. trichiura* is primary a human infection and is common in areas of high rainfall, high humidity and dense shade (Gilles, 2003).

The distribution among individuals infected with soil-transmitted helminths is highly aggregated. Most individuals harbour few parasites, while only a few harbour a high parasitic load. Expressed in percentages, 10% of the infected population carry approximately 70% of the worms (Anderson & Schad, 1985; Bundy, 1995). Interestingly, the worm fecundity is decreased when the worm load in an individual is high (Schad & Anderson, 1985; Bradley *et al.*, 1992). *Ascaris* and *Trichuris* infection intensities vary among age groups, with an increase in childhood and a decline in adulthood. The heaviest burden with *T. trichiura* is found in school-age children, who may pollute the soil around their house, thus the disease can be transmitted when playing (Gilles, 2003). In contrast, hookworm infection intensities increase steadily with age (Bradley *et al.*, 1992). Behavioural, social, nutritional and genetic factors influence the predisposition of heavily infected individual within a community (Chan *et al.*, 1992; Chan *et al.*, 1994; Brooker *et al.*, 2004). For example, cultural or agricultural practices, such as the use of faeces for fertilizer are risk factors for infection.

In areas where *Ascaris* infections are common, three distinct trends have been described. First, high prevalences of over 60% in the whole population over 2 years, with lower infection in adults, where the population is constantly exposed to *Ascaris* eggs by contaminated hands or food. Second, moderate prevalence below 50% with a peak at young school-age children and low prevalence in adults, where family or household transmission is dominant. Third, the overall prevalence is below 10%, which is related to a focal distribution due to inadequate housing and sanitary conditions or agricultural and behavioural practices (Crompton & Pawlowski, 1985).

Intestinal protozoa infections: amoebiasis and giardiasis

Before modern diagnostic methods made it possible to distinguish between the non-pathogenic strain *E. dispar* and the morphological identical pathogen *E. histolytica,* it was estimated that 12% of the world's population was infected with *E. histolytica* (Farthing *et al.*, 2003). In fact, most of the asymptomatic infections reported in industrialised countries were due to *E. dispar* infection. In endemic areas, studies have shown that the ratio between *E. dispar* and *E. histolytica* is often in the order of 10:1 (Li & Stanley, 1996; Farthing *et al.*, 2003). Today, high prevalences of *E. histolytica* are reported mainly in countries of low socio-economic status, while low prevalences are found in industrialized countries. High prevalence of infection with *E. histolytica/E. dispar* have been reported from the Indian subcontinent and Indonesia, sub-Saharan Africa and Central and South America (Li & Stanley, 1996; Heckendorn *et al.*, 2002).

Cyst carriers are the main reservoir of *E. histolytica* infection. Infection occurs via the faecal-oral route by ingesting faecal contaminated water or food. Food-borne outbreaks are caused by unsanitary handling of food, and during the preparation of food by infected persons. Agricultural practices such as the use of raw domestic sewage for vegetable cultivation can increase the risk of amoebiasis. It has recently been shown in the suburbs of Asmara, Eritrea, that farming people were significantly more infected with pathogenic intestinal protozoa and bacteria, due to the intake of raw vegetables grown on raw sewage (Srikanth & Naik, 2004). Epidemics with *E. histolytica* can also occur when raw sewage comes into contact with water supplies and contaminates the drinking water (Farthing *et al.*, 2003).

The major reservoirs of *Giardia* cysts are humans and contaminated surface water. *Giardia* cysts are able to survive for long periods outside the host and they are often found in

surface water. Chlorination of contaminated water alone cannot inactivate the *Giardia* cysts and defects in municipal water supplies have been shown to account for many giardiasis water-borne outbreaks (Craun, 1984; Jephcott *et al.*, 1986). Contaminated food is also a probable route of transmission, although it is thought to be relatively uncommon. Further, person-to-person spread by faecal-oral transmission is described in residential institutions, schools, and day care centres. *Giardia* spp., which is genotypically indistinguishable from human *Giardia* isolates, is found in wild and domestic animals, and higher *Giardia* prevalence especially in children that are in close contact to animals has been found (Mineno & Avery, 2003). Although, direct evidence that animal-to-human transmission occurs has not yet been proven (Farthing *et al.*, 2003).

Giardiasis age-prevalence rises throughout childhood and declines in adolescence. Undernutrition may increase the susceptibility to infection. In Gambian children with chronic diarrhoea and malnutrition, 45% were diagnosed as suffering from giardiasis, while only 12% of the healthy comparison group were harbouring a giardiasis infection (Jones *et al.*, 1975).

1.2.3 Multiple species parasitic infections

Although the phenomenon of polyparasitism has been recognized for decades, and although it is the norm rather than the exception in the developing world (Buck *et al.*, 1978; Tanner *et al.*, 1987), there are only few in-depth studies currently available. Recent cross-sectional surveys conducted in sub-Saharan Africa, Southeast Asia and South America confirmed that polyparasitism is very common in developing countries (Utzinger *et al.*, 1999; Guignard *et al.*, 2000; Keiser *et al.*, 2002a; Keiser *et al.*, 2002b; Waikagul *et al.*, 2002; Tchuem Tchuenté *et al.*, 2003). Interestingly, among the few existing studies that have investigated the issue of polyparasitism, most of them focussed on a narrow age range, e.g. school-age children. The few studies performed in entire communities found that polyparasitism increases with age, reaching a plateau in adolescent and young adults and decreases in older age groups (Keiser *et al.*, 2002b). The epidemiology of multiple species parasitic infections is complex and most research groups have, traditionally, concentrated on single parasite-single host interactions (Cox, 2001).

A myriad of factors contribute to polyparasitism, including lack of access to clean water and improved sanitary facilities, as well as low hygiene conditions (Asaolu & Ofoezie, 2003; Utzinger *et al.*, 2003a). Historically, polyparasitism was also common in temperate zones. In

fact, many human parasite species were widely distributed across Europe and the United States (Brothwell & Sandison, 1967; Cockburn *et al.*, 1998; Gonçalves *et al.*, 2003). However, social and economic advance, going hand-in-hand with improved sanitation and hygiene behaviour, and better access to chemotherapy and preventive measures have decreased the burden of polyparasitism in these parts of the world.

1.3 Diagnosis and treatment

1.3.1 Malaria

The most commonly employed method for malaria diagnosis in endemic areas remains microscopic examination of Giemsa-stained thin and thick blood smears (Hira & Behbehani, 1984). Simple and rapid antigen detection methods with high sensitivity and high specificity, as well as increasingly affordable dipstick or card tests have been developed in recent years (White & Silamut, 1989; Humar *et al.*, 1997; Proux *et al.*, 2001; Moody, 2002). However some of these rapid tests are based on antigens, which are detectable in blood up to one month after treatment, particularly if parasitaemia was high; hence they are disadvantageous in areas where transmission is high (Mayxay *et al.*, 2001). Other techniques exist, which rely on fluorescent dye of the parasite DNA and RNA and visualisation by ultraviolet light microscopy (Keiser *et al.*, 2002c; White, 2003).

In the cross-sectional epidemiological surveys presented in the current thesis we have used Giemsa-stained thin and thick blood smears for detection of *Plasmodium*-positive study participants. Hence, this technique is briefly discussed here.

Giemsa-stained blood smears

Two drops of blood are placed at one end of a microscope slide. A thin film is immediately made by placing the smooth leading edge of a second slide in the central drop of blood, and adjusting the angle, whilst holding the edges of the slide, smearing the blood with a swift and steady sweep along the surface to the other end. Following, the thick film is prepared from the second drop of blood. It is stirred in a circular motion with the corner of the second slide until clotting takes place (White, 2003). The blood smears are dried thoroughly and thin blood films are fixed in methanol. Subsequently, the thin and thick blood smears are stained in Giemsa for 30 min. Thereafter, the stain of the slides is gently washed off and the slides are allowed to dry. Slides are microscopically examined under oil immersion at a magnification of x 1,000. The thick film is used to estimate parasitaemia, as it is

approximately 30 times more sensitive than thin films, while the thin film is used for speciation of malaria at the trophozoite stage and for parasite counting (Moody, 2002). The number of parasites are counted per 200 white blood cells (WBC). If less than 10 parasites are found reading is continued up to 500 WBC. The count is then converted to the number of parasites per µl blood, assuming for a standard WBC of 8,000/µl of blood. The sensitivity and specificity of this method depends on the quality of the blood film, the amount of blood volume (number of microscopic fields) examined, and to some extent also on the experience of the microscopist (Moody, 2002).

There are three main groups of antimalarial drugs, namely (i) aryl aminoalcohols (quinoline-related or quinoline-like such as quinine, quinidine, chloroquine, mephloquine, halophanitrine, prima-quine) compounds, (ii) antifols (pyrimethamine, proguanil, chlorproguanil, trimethoprim), and (iii) artemisinin compounds (artemisinin, dihydroartemisinin, artemether, artesunate) (Ridley, 2002). Very promising results have just been pubished, exhibiting excellent antimalarial properties of OZ277, a semi-synthetic peroxide (Vennerstrom *et al.*, 2004). This compound has now entered pre-clinical testing and might become a new antimalarial drug class. Several antibacterial drugs such as sulphonamides and sulphones, tetracyclines and chloramphenicol also have antiplasmodial activities, however their action is slow compared to the currently used antimalarial drugs and a high level of resistance has been reported to sulphonamids.

One of the reasons why the global burden of malaria continues to rise is attributed to the development and rapid spread of drug resistance. With the exception of the artemisinins, *P. falciparum* has developed resistance to all existing drug classes (Simon *et al.*, 1988; White, 1992, 1999b; Trape, 2001). To prevent or delay emergence and spread of resistance, combination therapy, employing two compounds with unrelated mechanisms of action, is increasingly promoted (Peters, 1990; White, 1999a; Hastings *et al.*, 2002).

1.3.2 Schistosomiasis

The most widely used approach for diagnosis in endemic settings is the detection of schistosome eggs in either stool or urine specimens by light microscopy. The methods are relatively rapid and inexpensive. In the case of *S. mansoni,* the Kato-Katz technique is the most frequently employed method in research settings (Katz *et al.*, 1972). Direct faecal smear and more sophisticated formalin-based techniques for sedimentation and concentration are

also used (Kato & Miura, 1954; Marti & Escher, 1990). Immuno diagnostic techniques for the detection of adult worm antigen have been developed over the past decade (van Lieshout *et al.*, 1995a; van Lieshout *et al.*, 1995b; van Etten *et al.*, 1997). They usually have a high sensitivity and a high specificity (Doenhoff *et al.*, 2004). Other methods exist for the indirect diagnosis, which rely on perceived symptoms, clinical examinations, and biochemical or immunological disease markers. Nuclear magnetic resonance (NMR)-based metabonomics is currently being investigated as a novel approach for biomarker identification, which in turn could be utilized for new diagnostic tools (Wang *et al.*, 2004).

The use of the Kato-Katz technique for *S. mansoni*, self-reported morbidity indicators for S. *haematobium* and *S. mansoni*, and the use of reagent strips for the detection of microhaematuria (biochemical marker for *S. haematobium*) were used in the present study. These approaches are discussed below.

Kato-Katz thick smear

The technique described by Katz and colleagues in the early 1970s has become the most widely used method in epidemiological surveys for the diagnosis of *S. mansoni*. It is often considered the 'gold' standard for diagnosis, especially when repeated stool samples are examined. In brief, a small portion of fresh stool is sieved through a fine screen and filled into the hole of a reusable plastic template that is placed on a microscope slide. The templates used in our epidemiological studies correspond to 42 mg of stool analysed; hence multiplication of the number of eggs counted by a factor of 24 provides the number of eggs per gram of stool (epg). After removing the template, the faecal material is covered with a strip of glycerine-malachite green soaked cellophane. After clearing the slides for at least 30 min, they are examined under a light microscope at low magnification. An important aspect of the Kato-Katz method is that it facilitates quantification of egg counts. Infection intensity is usually stratified as follows: (i) light infections (1-100 epg), (ii) moderate infection (100-400 epg), and (iii) heavy infections (>400 epg) (WHO, 2002a). The Kato-Katz technique permits concurrent soil-transmitted helminth infections to be examined and quantified, i.e. hookworm, *A. lumbricoides* and *T. trichiura*.

Unfortunately, the Kato-Katz technique has several limitations. First, the collection of stool specimens can be tedious and may not be well accepted in all socio-cultural settings. Second, its diagnostic performance depends on the overall endemicity. Third, in view of important day-to-day and intra-specimen variation of egg-output, multiple stool examinations

are mandatory to enhance sensitivity and specificity (Engels *et al.*, 1996b; Engels *et al.*, 1997; Utzinger *et al.*, 2001a; Booth *et al.*, 2003; Berhe *et al.*, 2004): Examination of several stool specimen, however, might decrease study compliance (Engels *et al.*, 1996a; WHO, 1999).

Self-reported morbidity indicators

Over the past 15 years, a simple schoolchildren questionnaire has been developed and extensively validated for rapid identification of high risk communities and individuals for *S. haematobium* (Lengeler *et al.*, 1991; Group, 1995; Lengeler *et al.*, 2002a). Subsequently, the diagnostic value of symptoms, i.e. diarrhoea, bloody diarrhoea, blood in stool and abdominal pain, has been assessed as a means of rapid diagnosis for *S. mansoni* infections. Studies have been carried out in different epidemiological settings in sub-Saharan Africa (Lengeler *et al.*, 2002a, 2002b). The most promising results were found for reported blood in stool in studies carried out in Côte d'Ivoire, Democratic Republic of the Congo, Ethiopia and Tanzania (Hailu *et al.*, 1995; Booth *et al.*, 1998; Lengeler *et al.*, 2000; Utzinger *et al.*, 2000b). However, questionnaires for *S. mansoni* have not achieved the same diagnostic performance as in the case of *S. haematobium* and further validation in other settings is required (Lengeler *et al.*, 2002a).

Reagent strips

Reagent strips can detect trace amounts of blood and protein in urine, which can be the result of lesions caused by *S. haematobium* eggs by passing through the bladder wall. Previous work has shown that the amount of blood and protein in urine correlates with the number of eggs excreted, thus with intensity of infection (Wilkins *et al.*, 1979). Reagent strips have been widely used as an indirect indicator for an infection with *S. haematobium* (Wilkins *et al.*, 1979; Mott *et al.*, 1985; Lengeler *et al.*, 1993; Mafe *et al.*, 2000). The diagnostic performance has been assessed in different epidemiological setting, including rural parts of Côte d'Ivoire (N'Goran *et al.*, 1998), and urban parts of Tanzania (Mtasiwa *et al.*, 1996).

The current drug of choice for treatment and morbidity control of schistosomiasis is praziquantel (WHO, 2002a; Utzinger & Keiser, 2004). It is efficacious against all five human schistosome species, is generally well tolerated, and can be administrated at a single oral dose. Importantly, praziquantel experienced a substantial price reduction over the past

several years; treatment of a schoolchild is now in the order of US$ 0.20 (Bergquist, 2002; Doenhoff *et al.*, 2002; Fenwick *et al.*, 2003).

There is considerable concern that resistance to praziquantel might develop or already exists. In Senegal, for example, low cure rates of only 18-39% have been observed, which, however, could be partially attributed to high transmission intensity and the drug's inefficacity to kill immature schistosome parasites (Gryseels *et al.*, 2001; Danso-Appiah & De Vlas, 2002). Schistosome strains with a significantly reduced susceptibility to praziquantel have been observed in Egypt (Cioli, 2000; William *et al.*, 2001). Importantly though, resistance to praziquantel is yet of no public health significance, but rigorous monitoring of drug efficacy in different epidemiological settings should become an integral part of control programmes (Renganathan & Cioli, 1998).

The current arsenal of antischistosomal drugs also includes oxamniquine, which is efficacious against *S. mansoni,* but not against any of the other schistosome species (Cioli, 2000; Utzinger & Keiser, 2004). This drug has been successfully employed in Brazil's national schistosomiasis control programme, but it is now being replaced by praziquantel (Beck *et al.*, 2001). Substantial progress has been made over the past 10-15 years with artemether and other artemisinin derivatives. These compounds exhibit stronger anti-schistosomal properties against the young developmental stages of the parasite (Utzinger *et al.*, 2001c). They are safe and reduced the incidence of new infections significantly in the first randomised controlled trials against *S. mansoni* and *S. haematobium* carried out in Côte d'Ivoire (Utzinger *et al.*, 2000a; N'Goran *et al.*, 2003). Combination chemotherapy with artemether and praziquantel has been proposed and already showed promising results (Utzinger *et al.*, 2003b).

1.3.3 Soil-transmitted helminthiasis

The diagnosis of soil-transmitted helminth infections, i.e. hookworm, *A. lumbricoides* and *T. trichiura,* is done by egg detection in the faeces, either using direct smears, Kato-Katz thick smears or concentration methods, and subsequent microscopic examination (Kato & Miura, 1954; Katz *et al.*, 1972; Marti & Escher, 1990). As mentioned before, an important feature of the Kato-Katz technique is that it allows quantification of eggs per gram of stool. The World Health Organization (WHO) recommends thresholds for different infection intensities. For hookworm, they are as follows: (i) light infection, 1-1,999 epg; (ii) moderate infection, 2,000-3,999 epg; and (iii) heavy infections, ≥4,000 epg. Corresponding thresholds

for *T. trichiura* are (i) 1-999 epg; (ii) 1,000-99,999 epg; and (iii) 99,999 epg. For *A. lumbricoides* corresponding thresholds are as follows: (i) 1-4,999 epg, (ii) 5,000-49,999 epg, and (iii) ≥50,000 epg (WHO, 2002a).

Soil-transmitted helminth infections are effectively treated with a single dose of albendazole (400 mg) or mebendazole (500 mg) (WHO, 2002a; Utzinger & Keiser, 2004). The other two drugs currently on WHO's model list of essential drugs are levamisole and pyrantel pamoate (WHO, 2002a; Utzinger & Keiser, 2004). For *T. trichiura* combination of albendazole (400 mg) with ivermectin (200 μg/kg bodyweight) is also effective. Interesting results have recently been presented from China; tribendimidine has been developed there as a new, and safe anthelmintic drug with a broad spectrum of activity (Utzinger & Keiser, 2004; Xiao *et al.*, 2005).

It is well documented that resistance to anthelmintic drugs occurs in animal nematodes. This issue raises concern that the widespread use of albendazole and mebendazole in chemotherapy-based control programmes against soil-transmitted helminthiasis may trigger resistance, hence vigilance and careful monitoring is warranted, so that the emergence of drug resistance in humans can be immediately identified and sound mitigating strategies implemented (Bennett & Guyatt, 2000; Geerts & Gryseels, 2000).

1.3.4 Intestinal protozoa infections

The diagnosis of *E. histolytica/E. dispar* or *G. duodenalis* is done by detection of the cysts or trophozoites in stool specimens by light microscopy. There are several concentration and staining techniques, employing either fresh or fixed stool specimens (Farthing *et al.*, 2003). In our epidemiological surveys, we have used the sodium acetate-acetic acid-formalin (SAF) concentration technique, which is discussed in the next paragraph. To distinguish between the morphologically identical *E. histolytica* and *E. dispar* in reference diagnostic centers, enzyme linked immuno sorbent assay (ELISA) or polymerase chain reaction (PCR) techniques are used if stools are reported positive (Haque *et al.*, 1995). However, the cost of these techniques limits their use, especially in the developing world. Sensitive and specific ELISA for *Giardia* antigens have also been developed (Farthing *et al.*, 2003).

SAF concentration method

Cysts and trophozoites of intestinal protozoa, as well as eggs of helminths, can be examined in stool samples by the SAF concentration method (Yang & Scholten, 1977; Marti & Escher, 1990). Approximately 1-2 g of fresh stool is placed in a plastic tube containing 10 ml of sodium acetate-acetic acid-formalin solution. It needs to be shaken vigorously. After a minimum fixation time of 30 min the fixed stool is again shortly shaken, passed through a gauze into a 15 ml centrifuge tube and 0.85% NaCl is added until the tube is almost full. The tubes are centrifuged for 10 min at 2,000 rpm. Thereafter, the supernatant fluid is decanted. A second wash is done by adding again saline until the tube is almost full and centrifuged for 10 min at 2,000 rpm. After decanting the supernatant fluid the sediment is re-suspended 7 ml 0.85% NaCl. 2-3 ml of ethyl acetate are added, tubes are stoppered, and shaken vigorously for at least 30 sec. The tubes are centrifuged for 10 min at 2,000 rpm. After centrifugation four layers will be visible, namely (i) sediment, (ii) saline, (iii) plug of faecal debris, and (iv) ethyl acetate. With an applicator stick the plug of debris is freed and the supernatant fluid decanted. One or two drops of saline can be added to the sediment. The entire sediment material is put on one or more slides and covered by a slip. Subsequently the slides are examined under a light microscope. The slides are systematically scanned first with a 10x magnification objective, which facilitates identification of helminth eggs. Subsequently, slides are scanned at a 10-times higher magnification (i.e. x100) for cyst and trophozoite detection.

One shortcoming of this method is the lack of sensitivity in the case of only a single SAF-fixed stool sample being examined. It has been shown that 3 consecutive stool sample examinations can result in a sensitivity above 90% of intestinal protozoa, and if two stool samples are taken on separate days it may allow to detect more than 80% of infections, because of intermittent shedding of cysts (Knight, 1974; Juniper, 1978; Marti & Koella, 1993).

When *E. histolytica* infection has been confirmed, two classes of drugs can be used for the treatment. Diloxanide furoate and iodoquinol act on organisms in the intestinal lumen and are not effective against parasites in the tissues. In the case of invasive amoebiasis, metronidazole, dehydroemetine and chloroquine are the drugs of choice. In cases of liver abscess, aspiration of the abscess may be necessary (Farthing *et al.*, 2003; Stanley, 2003).

Drugs for the treatment of *G. duodenalis* infections are metronidazole and tinidazole, mepacrine and furazolidone. Single oral dose treatment in adults is possible with metronidazole and tinidazole (Farthing, 1996; Farthing *et al.*, 2003).

1.4 Control of human parasitic infections

1.4.1 Malaria

Objectives of malaria control depend on the epidemiological situation of the setting, the availability of resources, and overall feasibility (White, 2003). Several new initiatives are currently underway with the overarching goal to reduce the intolerable burden of malaria. The Roll Back Malaria (RBM) initiative, launched in 1998, set a very ambitious goal, namely to halve the malaria burden by the year 2010 considering 1990 as the designated baseline (Nabarro & Tayler, 1998). The Medicine for Malaria Venture (MMV) aims to deliver a new antimalarial drug once every 5 years (Ridley, 2002). The development of OZ277 has been supported by MMV and this might indeed become a novel antimalarial drug (Vennerstrom *et al.*, 2004). The Global Fund to Fight AIDS, Tuberculosis and Malaria is another important initiative that supports malaria control programmes in many different settings (http://www.theglobalfund.org).

The cornerstone of malaria control is to provide improved access to prompt diagnosis and effective treatment, particularly for the most vulnerable groups (i.e. young children and pregnant women). Preventive measures such as the use of insecticide-treated nets (ITNs), and other vector control approaches are also needed. The evidence-base is compelling that ITNs significantly reduce overall mortality and morbidity across diverse ecological, epidemiological and socio-cultural settings (Lengeler, 2004).

Vector control by means of environmental management has been an important control strategy in the first decades of the 20th century. Environmental management includes application of larvicides and imagocides, vegetation clearance, modification of river boundaries and removal of manmade obstructions, drainage of flooded areas and swamps, oil application to water bodies and changes in house design, e.g. screening of window and doors (Utzinger *et al.*, 2001b). A re-analysis of a malaria control programme primarily based on environmental management, which was launched in 1929 and sustained for two decades at copper mining communities in Zambia, showed that the overall mortality and malaria

incidence rates were reduced by approximately 50% already one year after implementation of an integrated control package (Utzinger *et al.*, 2001b; Utzinger *et al.*, 2002).

Recently, application of geographical information system (GIS) and satellite imagery, have become important tools for early warning, and hence prevention of the development of malaria epidemics, particularly in highlands and other settings, which are prone to malaria epidemics (Rogers & Randolph, 2000; Hay *et al.*, 2001; Rogers *et al.*, 2002).

1.4.2 Schistosomiasis

The current mainstay of schistosomiasis control is praziquantel-based morbidity control. This approach diminishes the consequences of the infection, i.e. early morbidity and late stage chronic and irreversible sequelae, so that the public health burden of schistosomiasis is reduced (WHO, 2002a). This strategy has been endorsed by the 54th World Health Assembly resolution 54.19, set forth in May 2001. It has been embraced by the recently launched "Schistosomiasis Control Initiative" (SCI). This initiative is supported by a US$ 30 million grant from the Bill and Melinda Gates Foundation, and is currently supporting national schistosomiasis control programmes in 6 African countries, namely (i) Burkina Faso, (ii) Mali, (iii) Niger, (iv) Tanzania, (v) Uganda and (vi) Zambia.

Once a country has reached a low endemic level, WHO's strategy is to consolidate control efforts, aiming for elimination of schistosomiasis. However, issues of integration and sustainability have received insufficient attention, as improved access to clean water and sanitation and sound hygiene behaviour are rarely considered (Utzinger *et al.*, 2003a).

Before the advent of praziquantel and other efficacious antischistosomal drugs, transmission control was the key control strategy. The use of molluscicides for the control of the intermediate host snails, had been applied at large-scale in the 1950s and 1960s in Sudan and Egypt (Sharaf el Din & El Nagar, 1955; Chu, 1976). Although the incidence rates decreased significantly, these large-scale interventions were expensive and of limited duration and effectiveness, as snails re-populated their habitats shortly after interventions. Focal mollusciding in specific geographical settings is still important today (Davis, 2003). Furthermore, decreasing the frequency and duration of contacts with contaminated water is also an important issue to reduce transmission (Kloos *et al.*, 1997; Useh & Ejezie, 1999). Sound health education campaigns can achieve a change of human behaviour by increasing awareness in the population about the transmission mode and health consequences of schistosomiasis (Useh & Ejezie, 1999).

1.4.3 Soil-transmitted helminthiasis

Analogous to schistosomiasis, the recommended strategy for the control of soil-transmitted helminthiasis is morbidity control by chemotherapy, administering either albendazole (400 mg), or mebendazole (500 mg), or another efficacious anthelminthic drug. This approach proved successful, hence it has been endorsed in WHA 54.19 resolution (WHO, 2002b).

Transmission control through improved hygiene behaviour and sanitation is of pivotal importance to achieve long-term benefits (Arfaa *et al.*, 1977; Henry, 1981; Asaolu & Ofoezie, 2003). One of the limitations of installation of improved sanitation is that it is relatively expensive and the positive effects are often not sufficiently appreciated because they may only be seen after many years (Esrey *et al.*, 1991).

Sound information, education and communication campaigns, coupled with community participation are key features for sustainable control programmes. Results from an intervention, which was based on health education alone, showed a decrease of 26% in the prevalence of *A. lumbricoides*, while health education in combination with chemotherapy reduced the prevalence and intensity of infection by 42-75% and 73-85%, respectively (Albonico *et al.*, 1996; Hadidjaja *et al.*, 1998). The combined control approach also resulted in schoolchildren's improvements in cognition test results, learning ability and concentration, already five months after interventions commenced (Hadidjaja *et al.*, 1998).

1.4.4 Intestinal protozoa infections

The burden of disease due to intestinal protozoa infections could be reduced substantially by the implementation of adequate sanitary conditions such as water supply, adequate disposal of faeces, enhanced food safety, and sound health education. Individuals identified positive for an infection with a pathogenic intestinal protozoa should be treated, as they represent the main reservoirs for *E. histolytica* and partly also for *G. duodenalis* transmission (Farthing *et al.*, 2003).

However, it is unlikely that *G. duodenalis* will ever be eliminated from the environment, as cysts are able to survive outside the host for long periods, e.g. in surface water. Therefore, before drinking water enters the public water supply, it remains vital to ensure its appropriate treatment, as cysts of *E. histolytica* and *G. duodenalis* are resistant to simple chlorination (Farthing, 1996). In areas where adequate treatment of water is not attainable, boiling of water for at least 10 min is recommended.

1.4.5 Combined control

Multiple parasite infections can occur in different combinations, but some groupings are of particular significance from public health and control points of view. An example where combined control is applied is in the case of soil-transmitted helminthiasis, because hookworm, *A. lumbricoides* and *T. trichiura* respond to a single oral dose of albendazole or mebendazole, hence there is no need to determine which of the three parasites is responsible for the clinical manifestations in individuals or entire communities, where two or even three species coexist (Chiodini, 2001).

The demonstration that combined treatment of schistosomiasis and soil-transmitted helminthiasis with single oral doses of praziquantel and albendazole was safe and efficacious (Nokes *et al.*, 1999), triggered further interest for combined mass treatment. Schistosome and soil-transmitted helminth infections often coexist in the same epidemiological setting, which can be explained by conditions of poverty, poor hygiene, lack of clean water and improved sanitation (Brooker *et al.*, 1999). Hence WHO recommends a combined approach for the control of schistosomiasis and soil-transmitted helminthiasis (WHO, 2002a). The objective is to provide effective treatment (praziquantel and albendazole/mebendazole) to at least 75% of the individuals at high risk of infection, such as school-age children, by the year 2010 (WHO, 2001). If resources allow, these activities should be supported by health education and improved water supply and sanitation. The need for improving access to clean water and improved sanitation for sustainable control of schistosomiasis and soil-transmitted helminthiasis, and a host of other public health problems, cannot be emphasised enough (Utzinger *et al.*, 2003a).

1.5 Geographic information systems and remote sensing

A geographic information system (GIS) is a computer-based system for inputting, storing, accessing, analysing and presenting spatially referenced data from various sources in the form of maps (Nuttall *et al.*, 1995). Remote sensing (RS) is the process of acquiring information about an object, area or phenomenon from a distance, for instance through satellites or radar systems (Hay, 2000). GIS provides an integrated view for risk predictions in unsurveyed locations based on identification of moisture domains favourable to parasites, vectors and/or intermediate hosts. Consequently, GIS facilitates the monitoring and management of control programmes and can build effective bridges for intersectoral collaboration. Furthermore,

satellite imagery holds promise in the development of early-warning systems for disease epidemics such as in the case of malaria or dengue fever (Hay *et al.*, 2001; Rogers *et al.*, 2002; Tran *et al.*, 2004).

Application of GIS and RS has come a long way over the past 10-20 years. For example, these techniques have become important tools for assessment and prediction of the spatial and temporal distribution of disease risk areas, which in turn guide the design and implementation of sound control interventions (Singer & de Castro, 2001; Brooker *et al.*, 2002b; Yang *et al.*, 2005). A series of climate and environmental factors derived from satellite imagery have been used to predict the distribution of vectors and intermediate hosts responsible for the transmission of several parasitic infections such as malaria, sleeping sickness, dengue fever, lyme disease, filariasis, schistosomiasis and soil-transmitted helminthiasis (Dister *et al.*, 1997; Robinson *et al.*, 1997; Hassan *et al.*, 1998; Brooker *et al.*, 2000; Lindsay & Thomas, 2000; Brooker *et al.*, 2002a; Rogers *et al.*, 2002; Brooker *et al.*, 2003; Tran *et al.*, 2004). Other predictions have been made with estimates of entomological inoculation rate (EIR) linked to satellite data to measure malaria risk (Patz *et al.*, 1998).

Recently, GIS has also been proposed as a viable tool to measure access to health care as an integral part of health care planning. In fact, GIS can help develop a framework for equitable and effective resource allocation by simply mapping service providers and their location in relation to the population they should serve (Noor *et al.*, 2003; Noor *et al.*, 2004; Rosero-Bixby, 2004).

1.6 Health inequities among the poor

In the year 2000, a 29-fold higher mortality rate was observed among children living in sub-Saharan Africa when compared to industrialized countries. Of the estimated 10 million children under five years dying every year, more than 99% of the deaths occur in the developing world, 70% of which are caused by infectious diseases (Black *et al.*, 2003; WHO, 2004). Poorer children are more exposed to health risks through inadequate water and sanitation, indoor air pollution, crowding, poor housing conditions, and disease vectors than their richer counterparts. Furthermore, poor children are more susceptible to infectious diseases, because they are often undernourished or malnourished, and hence they have recurrent disease episodes (Victora *et al.*, 2003). New research suggests that these children are less likely to profit from health interventions (Jha *et al.*, 2002). An indication for this

'inverse care law' (Hart, 1971) is that despite the fact that effective vaccines against measles, pertussis and tetanus exist, each year 1.2 million children under five years are dying from these diseases (Shann & Steinhoff, 1999).

That the poor generally benefits less from public services is well documented, as well as several driving factors related to supply and demand (Hart, 1971; Castro-Leal *et al.*, 2000; Makinen *et al.*, 2000; Armstrong Schellenberg *et al.*, 2003; Ensor & Cooper, 2004). Although availability of good medical care from the supply side is essential, it has been recognized that more emphasis should be placed on minimizing effects that prevent patients from seeking care, whereas the costs of access, lack of information and cultural barriers are impeding factors for poorer segments of the population to benefit from health interventions (Castro-Leal *et al.*, 2000; Ensor & Cooper, 2004). Hence, it has been re-emphasized recently, that there is a need for innovative delivery strategies in health interventions to achieve and sustain high population coverage at sufficient quality, supported by better evaluation of the efficacy and costs-effectiveness of such delivery strategies (Ensor & Cooper, 2004; Victora *et al.*, 2004).

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2. Goal, objectives and study sites

2.1 Goal

Enhance the current understanding of demographic, ecologic, environmental and socio-economic factors that influence disease distribution in space in the region of Man, western Côte d'Ivoire, and produce risk maps and predictions of parasitic infections.

2.2 Objectives

- Assess the prevalence and intensity of *P. falciparum, S. mansoni*, *S. haematobium*, soil-transmitted helminths, and intestinal protozoan infections, as well as polyparasitic infections, and investigate the interrelationships of parasitic infections with self-reported morbidity and other parasite species among household members of all age groups in a rural community.

- Assess the efficacy and side-effects of a single oral dose of praziquantel at 40 mg/kg for the treatment of *S. mansoni* infections among study participants of all age groups in a single community.

- Assess the prevalence and intensity of single species infections with *P. falciparum*, *S. mansoni*, *S. haematobium*, soil-transmitted helminths and intestinal protozoan, as well as polyparasitism and self-reported morbidity among primary schoolchildren in the region of Man.

- Investigate relationships between socio-economic status and parasitic infections and perceived ill health, as well as physical access to formal health care structures among schoolchildren living in the region of Man.

- Establish a comprehensive GIS, based on data of cross-sectional parasitological and questionnaire surveys, and thoroughly validated environmental data derived from satellite imagery and digitised maps, facilitating the subsequent identification of environmental, climatic, socio-economic and demographic factors related to *S. mansoni* infection risk among schoolchildren from the region of Man.

- Map and predict the spatial distribution of *S. mansoni* among schoolchildren living in the region of Man.

2.3 Study sites

The surveys presented in this work were carried out in the region of Man, western Côte d'Ivoire. The town of Man is situated in the centre of the region. Community-based parasitological and questionnaire surveys were carried out in 75 randomly selected households in the village of Zouatta II, located 25 km east of Man. Cross-sectional parasitological survey and a questionnaire survey were carried out in 57 rural primary schools.
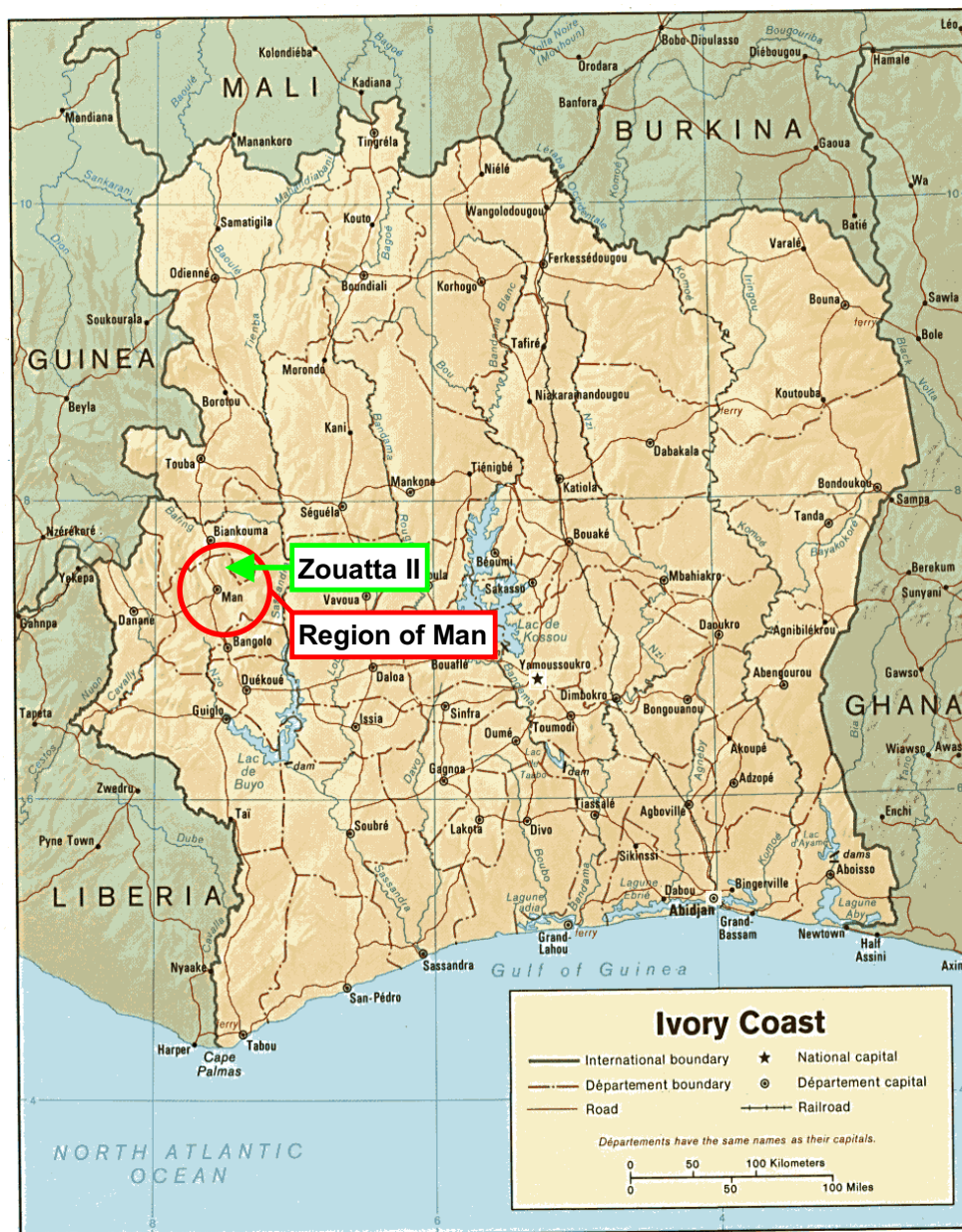
3. Multiple parasite infections and their relationship to self-reported morbidity in a community of rural Côte d'Ivoire

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3.1 Summary

Concomitant parasitic infections are common in the developing world, yet most studies focus on a single parasite in a narrow age group. We investigated the extent of polyparasitism and parasite associations, and related these findings to self-reported morbidity.

Inhabitants of 75 randomly selected households from a single village in western Côte d'Ivoire provided multiple faecal specimens and a single finger prick blood sample. The Kato-Katz technique and a formol-ether concentration method were employed to screen faecal samples for *Schistosoma mansoni*, soil-transmitted helminths and intestinal protozoa. Giemsa-stained blood smears were analysed for malaria parasites. A questionnaire was administered for collection of demographic information and self-reported morbidity indicators.

Complete parasitological data were obtained for 500/561 (89.1%) participants, similarly distributed among sex, with an age range from 5 days to 91 years. The prevalences of *Plasmodium falciparum,* hookworms, *Entamoeba histolytica/E. dispar* and *S. mansoni* were 76.4%, 45.0%, 42.2% and 39.8%, respectively. Three-quarters of the population harboured three or more parasites concurrently. Multivariate analysis revealed significant associations between several pairs of parasites. Some parasitic infections and the total number of parasites were significantly associated with self-reported morbidity indicators.

Our data confirm that polyparasitism is very common in rural Côte d'Ivoire and that people have clear perceptions about the morbidity caused by some of these parasitic infections. Our findings can be used for the design and implementation of sound intervention strategies to mitigate morbidity and co-morbidity.

Keywords

3.2 Introduction

Malaria accounts for about half a billion clinical attacks each year, and kills >1 million people, mainly children under the age of five years living in sub-Saharan Africa.[1] In view of the rapid spread of antimalarial drug resistance, this situation is likely to worsen.[2] An estimated two billion people are affected by schistosomiasis and soil-transmitted helminthiasis, about 300 million of whom are concerned with associated morbidity.[3,4] In children, these parasitic infections can have adverse effects on physical growth and cognitive development.[5,6] Recent analyses suggest that >200,000 people living in sub-Saharan Africa

die each year due to kidney dysfunction and haematemesis, which are consequential to sustained schistosome infections.[7] Amoebiasis is caused by the protozoan *Entamoeba histolytica*. The clinical manifestations include diarrhoea, dysentery, amoebid colitis and liver abscess. It is estimated that the disease kills 40,000-100,000 each year.[8] Giardiasis is a disease that is caused by the protozoan parasite *Giardia duodenalis*, with ~200 million people currently infected. The severe cases manifest acute and persistent diarrhoea, malabsorption of nutrients and impairment of children's growth and development.[9]

A common feature of the above-mentioned parasitic infections is that they are most prevalent in the developing world, particularly among the poorest segments of rural communities.[10-15] It follows that multiple parasite infections are widespread across diverse ecosystems in the tropics and subtropics. Recent cross-sectional surveys carried out in sub-Saharan Africa consistently confirmed these observations.[16-19] Lack of access to clean water and improved sanitation facilities, and inadequate personal hygiene are important underlying risk factors.[20,21]

Since individuals with multiple parasite infections are often at an elevated risk of morbidity,[22] appraisal of the extent of polyparasitism is a key measure of disease burden, and an important guide for sound control strategies. It should be noted, however, that the number of good quality studies pertaining to parasite communities in entire populations is small compared to the magnitude of this phenomenon and its public health significance. This is explained on several grounds. First, most field workers and research groups have focussed on single parasite-single host interactions.[23] Second, interactions between different parasite species are complex, hence challenging to elucidate.[23] Third, there is no readily available diagnostic tool that can be applied on a single bio-fluid or tissue, thereby facilitating simultaneous accurate identification of multiple species parasitic infections. Finally, most previous studies have focused on a narrow age range (e.g. school-age children) rather than on entire populations.[15,24]

Our own cross-sectional surveys carried out in western Côte d'Ivoire confirmed that polyparasitism is very common in this part of the tropics.[18,24,25] For example, in a population sample of 260 individuals screened for *Schistosoma mansoni*, soil-transmitted helminths and intestinal protozoa, two-thirds were found to harbour at least three parasites concurrently. Here we extend our previous work and provide an in-depth analysis of polyparasitism in a single village, with special reference to interactions between parasites and the relationship to self-reported morbidity. Study participants were screened over several days for the

identification of *S. mansoni*, soil-transmitted helminths, intestinal protozoa and *Plasmodia* infections, and individually interviewed with a questionnaire.

3.3 Materials and methods

Study area and population

Details of the study area and the population surveyed have been described elsewhere.[26] In brief, the study was carried out in May-July 2002 in the village of Zouatta II, located 25 km east of the town of Man in western Côte d'Ivoire. Here, we focus on the cross-sectional baseline survey for assessment of the parasite community, and self-reported morbidity indicators derived from a questionnaire survey carried out simultaneously.

Cross-sectional survey and laboratory procedures

The field procedures have been described previously.[26] In summary, following the village authorities' consent to the study, a demographic survey was carried out in 75 randomly selected households, employing the Expanded Programme on Immunization (EPI) survey approach.[27] Small plastic containers were distributed in the evening, and participants were invited to collect small portions of their faeces the next morning. Faecal collection was repeated over three consecutive days. On the last day, finger prick blood samples were also obtained and thick and thin blood films were prepared on microscope slides.

Faecal and blood specimens were transferred to the laboratory in the town of Man. From each faecal specimen a small amount, weighing 1-2 g, was placed in a plastic tube containing 10 ml of sodium acetate-acetic acid-formalin (SAF).[28] In addition, a single 42 mg Kato-Katz thick smear was prepared according to a standard method.[29] After clearing the slides for 30-45 minutes, they were examined under a light microscope by one of four experienced laboratory technicians for the presence of ova of *S. mansoni* and soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*). Thick and thin blood smears were stained with Giemsa. They were transferred to a reference laboratory in Abidjan, Côte d'Ivoire, and analysed within four weeks. Species-specific densities of *Plasmodia* were estimated under a light microscope at high magnification by counting the number of parasites per 200 white blood cells (WBC). If <10 parasites were found the reading was continued up to 500 WBC. These counts were converted to the number of parasites per µl of blood, assuming for a standard WBC count of 8,000/µl.

The SAF-conserved faecal samples were transferred to the Swiss Tropical Institute (Basel, Switzerland). They were processed with a formol-ether concentration method,[30] and examined by experienced technicians under a light microscope at high magnification. The presence of helminth ova and intestinal protozoa were recorded, including *Blastocystis hominis, Chilomastix mesnili, Entamoeba coli, Entamoeba hartmanni, Entamoeba histolytica/ E. dispar, Endolimax nana, G. duodenalis* and *Iodamoeba bütschlii*.

Questionnaire survey

A questionnaire was developed and adapted to the current epidemiological setting after discussions with local field assistants who were designated by the village chief. The assistants were trained on how to interview household members and to fill in the questionnaire. It included items on the interviewee's identity and characteristics (i.e. name, age and sex), common preventive measures against endemic diseases (e.g. sleeping under an insecticide-treated net (ITN) for malaria prevention), self-reported water contact patterns (e.g. swimming in the rivers in the vicinity of the village) and self-reported morbidity indicators. The latter included eight diseases (chicken-pox, diarrhoea, dysentery, malaria, respiratory infections, schistosomiasis, skin disease and worm infections) and eight symptoms (abdominal pain, blood in stool, convulsions, headache, hot body, lethargy, muscle aches and vomiting). Participants were asked whether they encountered any of these diseases or symptoms over the past 2-4 weeks.

After pre-testing, the questionnaire was administered and participants were interviewed individually. For children of age five years or below, their mothers or legal guardians were interviewed. The present article focuses on the self-reported morbidity indicators, excluding children aged ≤5 years.

Treatment

At the end of the epidemiological and questionnaire surveys, participants infected with *S. mansoni* were treated with praziquantel at a single oral dose of 40 mg/kg.[6] Among the remaining individuals, those who had an infection with soil-transmitted helminths were treated with a single dose of albendazole (400 mg), while the others were given poly-vitamins. Participants who complained of malaria-related symptoms and had an axillary temperature above 37.5°C were administered Nivaquine® and paracetamol, according to current national public health guidelines of Côte d'Ivoire. Our treatment protocols were

approved by the internal review boards of the Swiss Tropical Institute (Basel, Switzerland) and the Centre Suisse de Recherches Scientifiques (Abidjan, Côte d'Ivoire), and received ethical clearance from the Ministry of Public Health in Côte d'Ivoire.

Analysis

Double data entry and validation was performed in EpiInfo version 6.04 (Centers for Disease Control and Prevention, Atlanta, USA), and statistical analyses were done with STATA version 7.0 (Stata Corporation, College Station, USA). Only those participants who had complete parasitological data records, namely results derived from at least two Kato-Katz thick smears, one SAF-conserved faecal sample, and one blood smear, were retained for the final analyses.

For each individual, the arithmetic mean egg count of *S. mansoni* was calculated from the Kato-Katz thick smear readings. According to WHO (2002)[6], *S. mansoni*-positive individuals were stratified into three categories: (i) light infections (1-100 eggs/g of faeces; epg), (ii) moderate infections (101-400 epg), and (iii) heavy infections (>400 epg). An infection with *A. lumbricoides*, hookworm or *T. trichiura* was defined as the presence of one or more eggs detected in the Kato-Katz thick smears and/or the formol-ether processed faecal sample. Infections with intestinal protozoa were defined by their presence in the formol-ether processed faecal sample. *Plasmodium falciparum*, *P. malariae* and *P. ovale* infections were specified on the basis of blood smear examinations. Infection intensities of *P. falciparum* were stratified into four categories: (i) 1-50, (ii) 51-500, (iii) 501-5,000, and (iv) >5,000 parasites/µl of blood.

Six age groups were considered: (i) <5, (ii) 5-9, (iii) 10-14, (iv) 15-24, (v) 25-39, and (vi) ≥40 years. To compare single parasite infections by sex and age groups, χ^2-test or Fisher's exact test were used as appropriate. Infection intensity categories of *P. falciparum* were compared by sex and age, using a χ^2-test. The frequency of polyparasitism was assessed and stratified by sex and age groups. The relationships between different infection intensity categories of *S. mansoni* and an infection with hookworm or an infection with *E. histolytica/E. dispar* were examined using a χ^2-test. Parasite associations were investigated by fitting logistic regression models for each parasite investigated with all remaining parasites employed as covariates. These models were adjusted for age and sex. A stepwise approach with backward elimination of non-significant covariates was adopted to identify the parasites significantly related to the outcome (parasite under investigation). Covariates were included at

a significance level of 0.2. Adjusted odds ratios, including 95% confidence intervals (CI), were computed for those associations that resulted in *P*-values below 0.05. Finally, the same logistic regression modelling approach was used to investigate associations between a particular parasite and self-reported morbidity indicators.

3.4 Results

Compliance and operational results

Complete parasitological data were obtained from 500 of the 561 individuals, owing to a compliance of 89.1%.

Figure 1 shows that those 61 individuals who were excluded for further analyses had only one Kato-Katz thick smear reading (n = 16), lacked a SAF-conserved faecal sample for microscopic examination of soil-transmitted helminths and intestinal protozoa (n = 16), or gave no finger prick blood sample for investigation of malaria parasites (n = 29).

The final study cohort had 249 males and 251 females. The youngest participant was a newborn of 5 days and the oldest individual had an age of 91 years. Age distribution was as follows: <5 years, n = 88 (17.6%); 5-9 years, n = 88 (17.6%); 10-14 years, n = 67 (13.4%); 15-24 years, n = 58 (11.6%); 25-39 years, n = 95 (19.0%); and ≥40 years, n = 104 (20.8%). The number of males and females in these age groups showed no statistical significant difference (χ^2 = 9.82, degrees of freedom (df) = 5, *P* = 0.080).

Frequencies of parasites investigated

Table 1 displays the overall prevalences and sex-related differences of each of the parasites investigated. Examination of 2-3 Kato-Katz thick smears per individual revealed eggs of *S. mansoni* in 39.8% of the participants with no significant difference among males and females. The pooled data from the Kato-Katz thick smears and the SAF-conserved faecal sample revealed an overall hookworm prevalence of 45.0%. Males were significantly more often infected with this parasite than females (53.8% vs. 36.3%, *P* < 0.001). Infection prevalences of *T. trichiura* and *A. lumbricoides* were low, 6.0% and 2.0%, respectively, with no sex differences. With regard to intestinal protozoa, the highest prevalence was found for *E. coli* (64.4%).
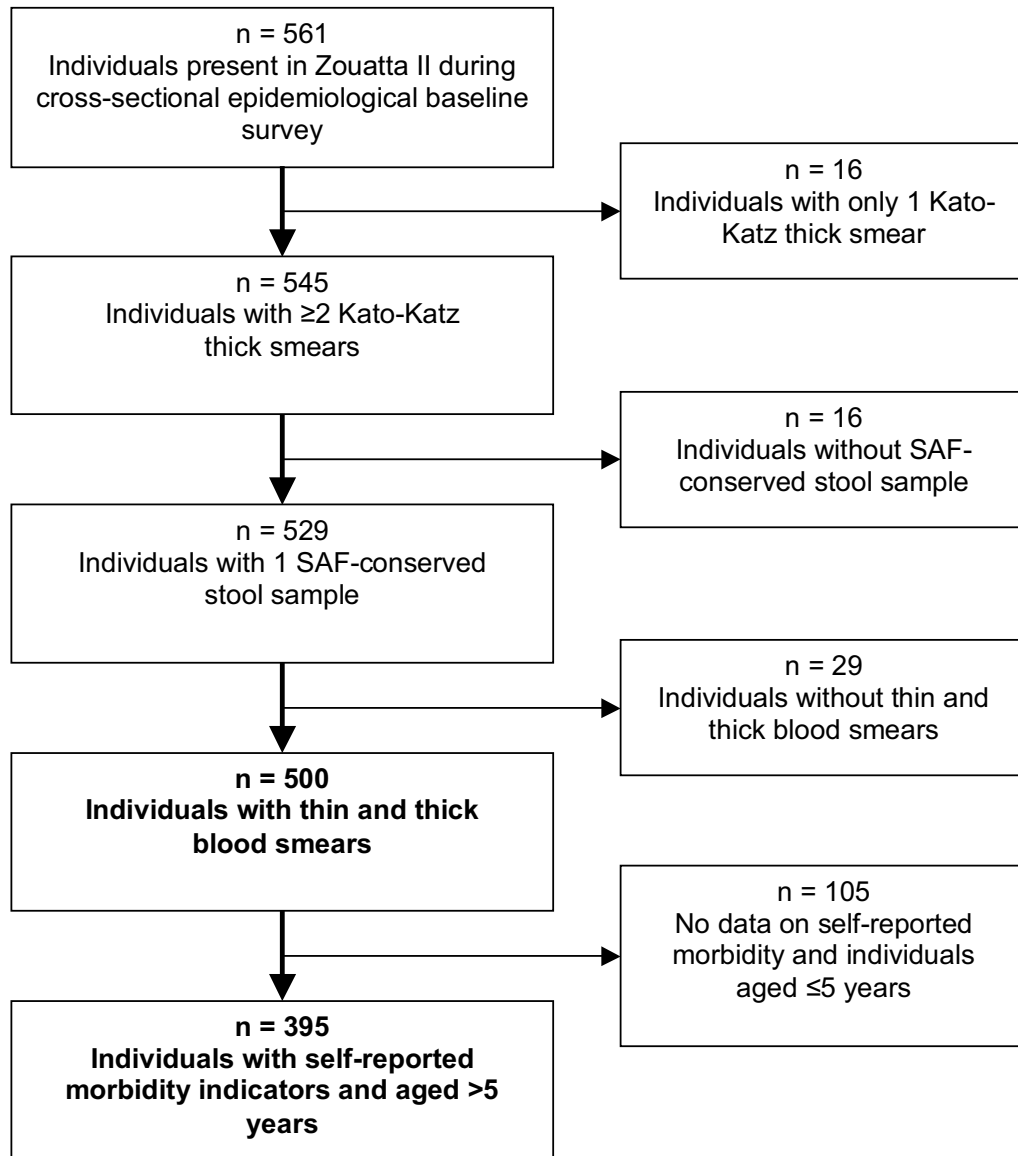
Figure 1 Compliance and study cohort for assessment of polyparasitism, parasite interactions and associations between infection and self-reported morbidity indicators in a rural community of western Côte d'Ivoire

This parasite showed a borderline significant sex difference (females: 68.5%, males: 60.2%, P = 0.053). High infection prevalences were also found for *E. histolytica/E. dispar* and *B. hominis* with 42.2% and 41.2%, respectively.

The overall prevalence of *G. duodenalis* was 10.8% with no difference among sex. A prevalence of 12.6% was found for *E. nana*. Female participants were significantly less often infected with this parasite than their male counterparts (9.6% vs. 15.7%, $P = 0.040$).

Table 1 Overall infection prevalence of each parasite investigated and sex-related differences among 500 study participants in the village of Zouatta II, western Côte d'Ivoire

Parasite	Prevalence of infection			
	Overall (95% CI)	Females (n = 251)	Males (n = 249)	*P*-value[a]
Schistosoma mansoni	39.8 (35.5-44.1)	38.3	41.4	0.507
Soil-transmitted helminths				
Hookworm	45.0 (40.6-49.4)	36.3	53.8	<0.001
Trichuris trichiura	6.0 (3.9-8.1)	5.6	6.4	0.690
Ascaris lumbricoides	2.0 (0.8-3.2)	1.6	2.4	0.515
Intestinal protozoa				
Entamoeba coli	64.4 (60.2-68.6)	68.5	60.2	0.053
Entamoeba histolytica/E. dispar	42.2 (37.9-46.5)	40.6	43.8	0.478
Blastocystis hominis	41.2 (36.9-45.5)	41.4	41.0	0.915
Entamoeba hartmanni	23.2 (19.5-26.9)	25.5	20.9	0.222
Iodamoeba buetschlii	21.2 (17.6-24.8)	22.3	20.1	0.542
Chilomastix mesnili	14.2 (11.1-17.3)	14.7	13.7	0.728
Endolimax nana	12.6 (9.7-15.5)	9.6	15.7	0.040
Giardia duodenalis	10.8 (8.1-13.5)	10.0	11.7	0.544
Plasmodia				
Plasmodium falciparum	76.4 (72.7-80.1)	75.3	77.5	0.560
Plasmodium malariae	2.2 (0.9-3.5)	2.0	2.4	0.750
Plasmodium ovale	0.2 (-0.2-0.5)	0.4	0.0	1.000[b]

[a] *P*-value based on χ^2-test.

[b] *P*-value based on Fisher's exact test.

More than three-quarters of the participants had an infection with *P. falciparum*, similarly distributed among sex. In addition, 11 (2.2%) individuals were found to harbour *P. malariae*, one of whom had a mixed infection with *P. falciparum*, and one individual had an infection with *P. ovale* singly.

Many of the parasites investigated showed significant associations with age categories, namely *S. mansoni* (χ^2 = 94.52, df = 5, P < 0.001), hookworm (χ^2 = 56.97, df = 5, P < 0.001) and five of the eight intestinal protozoa (*B. hominis, E. coli, E. histolytica/E. dispar, E. nana* and *G. duodenalis*). Figure 2 depicts the age prevalence curves for each of these seven parasites.
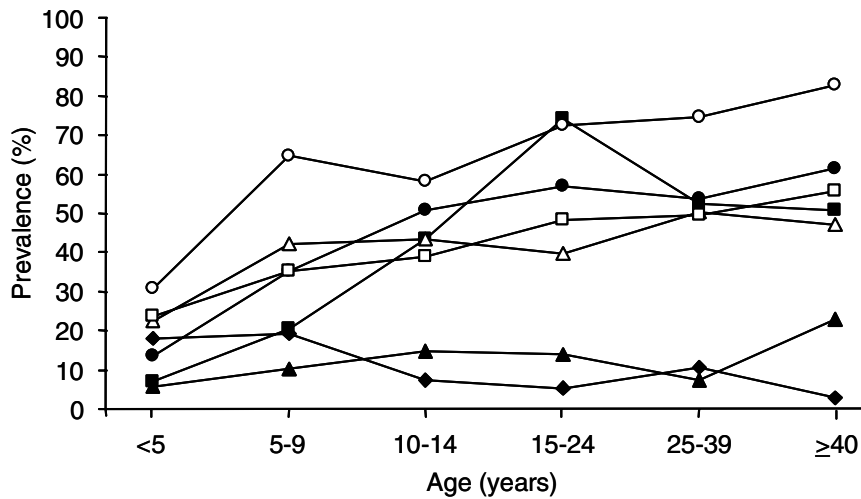
Figure 2 Age prevalence curves of Schistosoma mansoni (■), hookworm (●), *Blastocystis hominis* (Δ), *Entamoeba coli* (○), *Entamoeba histolytica/E. dispar* (□), *Endolimax nana* (▲) and *Giardia duodenalis* (◆).

Significant associations between infection prevalence and age groups were observed for *P. falciparum* (χ^2 = 16.77, df = 5, P = 0.005), and *P. malariae* (χ^2 = 32.86, df = 5, P < 0.001). Furthermore, both *P. falciparum* and *P. malariae* infection intensities were significantly associated with age, i.e. younger age groups showed consistently higher prevalences and infection intensities than their older counterparts. On the other hand, no significant associations were observed with sex (Table 2).

Table 2 Number of individuals (%) with different infection intensities of *Plasmodium falciparum*, stratified by sex and age (n = 489; individuals infected with *P. malariae* only (n = 10) or P. ovale only (n = 1) were excluded)

Variable	Infection intensity of *P. falciparum* (parasites/μl blood)					χ^2	*P*-value
	0	1-50	51-500	501-5000	>5000		
Sex							
Male	51 (10.4)	23 (4.7)	111 (22.7)	50 (10.2)	9 (1.8)		
Female	56 (11.5)	18 (3.7)	106 (21.7)	54 (11.0)	11 (2.2)	1.31	0.860
Age (years)							
<5	7 (1.4)	5 (1.0)	26 (5.3)	25 (5.1)	15 (3.1)		
5-9	10 (2.0)	6 (1.2)	33 (6.7)	35 (7.2)	4 (0.8)		
10-14	12 (2.5)	4 (0.8)	29 (5.9)	21 (4.3)	1 (0.2)		
15-24	16 (3.3)	10 (2.0)	26 (5.3)	5 (1.0)	0		
25-39	30 (6.1)	9 (1.8)	45 (9.2)	11 (2.2)	0		
≥40	32 (6.5)	7 (1.4)	58 (11.9)	7 (1.4)	0	129.00	<0.001
Total	107 (21.9)	41 (8.4)	217 (44.4)	104 (21.3)	20 (4.1)		

Parasite community

Among the 500 study participants, only 51 (10.2%) were not infected with any of the intestinal parasites (*S. mansoni*, soil-transmitted helminths and intestinal protozoa). After inclusion of *P. falciparum*, only nine individuals had no infection. There were 49 individuals (9.8%) with a mono-infection, mainly infants and young children. Three-quarters of the participants harboured three or more parasite species concurrently. There were 11 individuals with a parasite community of eight species, four individuals with nine, and one individual with 10 different species. Figure 3 shows a frequency of species of parasites from male and female participants, which was similar (χ^2 = 10.84, df = 10, *P* = 0.370).
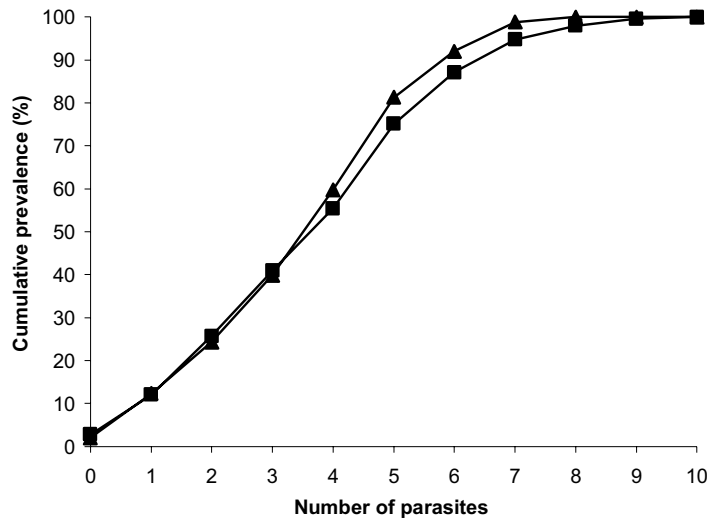
Figure 3 Cumulative frequency (%) of polyparasitism among 500 individuals from the village of Zouatta II, western Côte d'Ivoire, stratified by sex (females: ▲, males: ■). Plasmodium malariae and *P. ovale* were excluded from the analysis
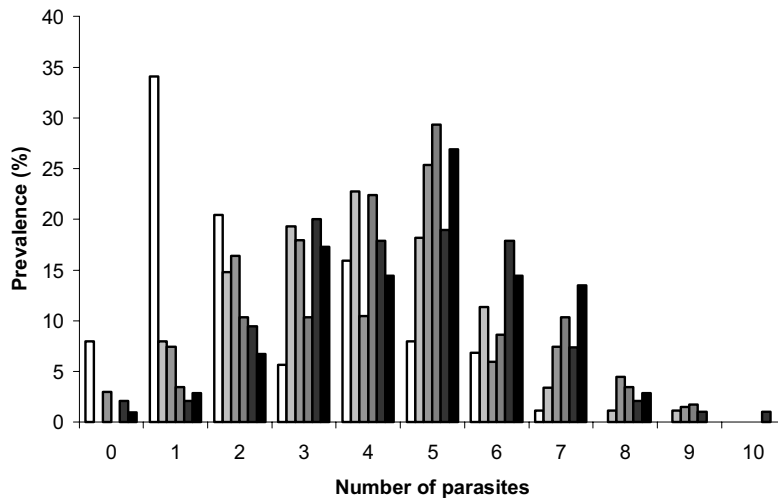
Figure 4 Frequency distribution of parasitic infections among 500 individuals from the village of Zouatta II, western Côte d'Ivoire, stratified by age (< 5 years: white bars, 5-9 years: very light grey bars, 10-14 years: light grey bars, 15-24 years: dark grey bars, 25-39 years: very dark grey bars, ≥ 40 years: black bars). *Plasmodium malariae* and *P. ovale* were excluded from the analysis.

Table 3 Association between a particular parasite investigated and sex, age group and any of the remaining parasites among 500 study participants from the village of Zouatta II, western Côte d'Ivoire

Parasite	Association	Adjusted odds ratio (95% CI)	*P*-value
Schistosoma mansoni	Hookworm	1.83 (1.22-1.75)	0.003
	Chilomastix mesnili	1.75 (1.01-3.04)	0.047
	Age group	1.46 (1.29-1.64)	<0.001
Soil-transmitted helminths			
Hookworm	*Entamoeba coli*	2.07 (1.33-3.23)	0.001
	Schistosoma mansoni	1.79 (1.18-2.68)	0.006
	Age group	1.32 (1.17-1.48)	<0.001
	Sex	0.40 (0.27-0.60)	<0.001
Ascaris lumbricoides	*Trichuris trichiura*	15.86 (3.93-63.99)	<0.001
Trichuris trichiura	*Ascaris lumbricoides*	14.60 (3.74-57.09)	<0.001
	Giardia duodenalis	3.88 (1.52-9.90)	0.005
Intestinal protozoa			
Entamoeba histolytica/E. dispar	*Entamoeba coli*	4.56 (2.86-7.27)	<0.001
	Age group	1.17 (1.04-1.31)	0.008
Entamoeba hartmanni	*Iodamoeba bütschlii*	2.56 (1.57-4.15)	<0.001
	Entamoeba coli	2.24 (1.29-3.90)	0.004
	Giardia duodenalis	2.06 (1.07-3.97)	0.030
Entamoeba coli	*Entamoeba histolytica/E. dispar*	4.36 (2.68-7.08)	<0.001
	Chilomastix mesnili	3.58 (1.60-7.99)	0.002
	Endolimax nana	2.55 (1.17-5.55)	0.018
	Sex	1.82 (1.17-2.86)	0.009
	Hookworm	1.79 (1.12-2.88)	0.018
	Age group	1.32 (1.15-1.50)	<0.001
Endolimax nana	*Entamoeba coli*	3.14 (1.49-6.60)	0.003
	Sex	0.48 (0.27-0.85)	0.012
	Chilomastix mesnili	0.31 (0.12-0.84)	0.021
Iodamoeba bütschlii	*Entamoeba hartmanni*	2.46 (1.52-3.99)	<0.001
	Entamoeba coli	1.79 (1.02-3.14)	0.043
Giardia duodenalis	*Trichuris trichiura*	3.40 (1.28-9.04)	0.014
	Entamoeba hartmanni	2.15 (1.09-4.23)	0.027
	Age group	0.67 (0.55-0.81)	<0.001
Chilomastix mesnili	*Entamoeba coli*	4.18 (1.99-8.76)	<0.001
	Schistosoma mansoni	1.84 (1.09-3.11)	0.023
	Endolimax nana	0.36 (0.14-0.96)	0.041
Blastocystis hominis	*Iodamoeba bütschlii*	1.73 (1.12-2.69)	0.014
	Age group	1.16 (1.05-1.29)	0.004
Plasmodia			
Plasmodium falciparum	Age group	0.74 (0.65-0.85)	<0.001
	Plasmodium malariae	0.01 (0.002-0.11)	<0.001
Plasmodium malariae	Age group	0.25 (0.10-0.64)	0.004
	Plasmodium falciparum	0.01 (0.001-0.13)	<0.001

Figure 4 exhibits that parasite communities among different age groups varied considerably. In general, younger age groups harboured fewer parasite species when compared to older age groups (χ^2 = 150.22, df = 50, P < 0.001).

Parasite associations

Table 3 summarizes all significant associations between a particular parasite and any other parasite, sex and age group. Infections with *S. mansoni* showed significant positive associations with hookworm (P = 0.003) and *C. mesnili* (P = 0.047). Concurrently, hookworm infections were positively associated with *S. mansoni* (P = 0.006). In addition, this parasite showed a significant positive association with *E. coli* (P = 0.001). A highly significant positive association was found between *A. lumbricoides* and *T. trichiura* (P < 0.001). The latter further showed a significant association with *G. duodenalis* (P = 0.005). Significant associations were also observed between *E. histolytica/E. dispar* and *E. coli* (P < 0.001), between *G. duodenalis* and *E. hartmanni* (P = 0.027), and between *P. falciparum* and *P. malariae* (P < 0.001).

Table 4 shows that there was a highly significant association between hookworm infections and the intensity of *S. mansoni* infections (χ^2 = 34.59, df = 3, P < 0.001). Thus, high odds ratios (OR) were observed when the number of hookworm infections was compared between *S. mansoni*-negative individuals and those with either a moderate (OR = 5.30) or a heavy *S. mansoni* infection (OR = 3.32). In addition, there was a highly significant association between *E. histolytica/E. dispar* infections and *S. mansoni* infection intensities (χ^2 = 21.53, df = 3, P < 0.001). While 36.2% of the *S. mansoni*-negative participants were infected with *E. histolytica/E. dispar*, 76.5% of those individuals with heavy *S. mansoni* infections concurrently harboured *E. histolytica/E. dispar*.

Parasite community and self-reported morbidity indicators

Self-reported morbidity indicators were available from 73 of the 75 households. Since children aged five years or below did not respond to the questions themselves, they were excluded for these analyses. On the other hand, those 17 children with an age above 5 years who had questions responded to by their mothers or guardians were retained for these analyses. The final study cohort consisted of 395 individuals (Figure 1). Table 5 summarizes the results from the multivariate analyses, with an emphasis on significant associations between an infection with a particular parasite and self-reported morbidity indicators after

adjusting for sex and age group. Infection with *S. mansoni* was strongly associated with abdominal pain (OR = 2.51, 95% CI = 1.47-4.29) and dysentery (OR = 2.12, 95% CI = 1.28-3.51).

Table 4 Relationship between the intensity of *Schistosoma mansoni* infections and the presence/absence of hookworm or the presence/absence of *Entamoeba histolytica/E. dispar* infections among 500 study participants from Zouatta II, western Côte d'Ivoire

	Schistosoma mansoni infection intensity[a]				χ^2	*P*-value
	Negative	Light	Moderate	Heavy		
Hookworm positive	107	69	38	11		
Hookworm negative	194	62	13	6		
Odds ratio	1.00	2.02	5.30	3.32	34.59	<0.001
E. histolytica/E. dispar positive	109	57	32	13		
E. histolytica/E. dispar negative	192	74	19	4		
Odds ratio	1.00	1.36	2.97	5.72	21.53	<0.001

[a] Negative: 0 epg, light: 1-100 epg, moderate: 101-400 epg, heavy: >400 epg.

On the other hand, *S. mansoni*-infected individuals were significantly less likely to report diarrhoea (OR = 0.55, 95% CI = 0.34-0.89), which is partially explained by other covariates. There was evidence of a positive association between *T. trichiura* infections and blood in stool (OR = 3.46, 95% CI = 1.20-10.02). Individuals infected with *P. falciparum* were more likely to report convulsions than their non-infected counterparts (OR = 4.84, 95% CI = 1.10-21.29). In contrast, *P. falciparum*-infected individuals were less likely to report lethargy (OR = 0.43, 95% CI = 0.21-0.92), but age played a very important role in this association.

The total number of parasites harboured in an individual was strongly associated with self-reported itching illnesses (χ^2 = 19.15, df = 10, *P* = 0.038), and malaria (χ^2 = 18.54, df = 10, *P*=0.046).

Table 5 Relationship between parasitic infections and self-reported morbidity indicators among 395 study participants from Zouatta II, western Côte d'Ivoire (analysis adjusted for sex and age groups; *Plasmodium malariae* and *P. ovale* were excluded from the models)

Parasite	Association	Adjusted odds ratio (95% CI)	*P*-value
Schistosoma mansoni	Abdominal pain	2.51 (1.47-4.29)	0.001
	Dysentery	2.12 (1.28-3.51)	0.004
	Age group	1.28 (1.10-1.49)	0.002
	Diarrhoea	0.55 (0.34-0.89)	0.015
Soil-transmitted helminths			
Hookworm	Itching illnesses	1.85 (1.04-3.30)	0.036
	Age group	1.20 (1.03-1.39)	0.016
	Sex	0.36 (0.23-0.55)	<0.001
Trichuris trichiura	Blood in stool	3.46 (1.20-10.02)	0.022
	Schistosomiasis	0.13 (0.03-0.50)	0.003
Intestinal protozoa			
Entamoeba histolytica/E. dispar	Age group	1.22 (1.06-1.40)	0.006
Entamoeba hartmanni	Respiratory problems	1.88 (1.15-3.08)	0.012
	Abdominal pain	1.80 (1.01-3.21)	0.046
	Malaria	0.50 (0.26-0.97)	0.039
Entamoeba coli	Age group	1.34 (1.14-1.57)	<0.001
Endolimax nana	Blood in stool	0.48 (0.26-0.90)	0.022
Iodamoeba bütschlii	Blood in stool	2.09 (1.28-3.41)	0.003
	Lethargy	0.50 (0.28-0.89)	0.019
Chilomastix mesnili	Headache	5.25 (1.08-25.39)	0.039
	Diarrhoea	2.30 (1.18-4.49)	0.014
	Age group	1.31 (1.05-1.63)	0.018
	Hot body	0.37 (0.17-0.78)	0.009
Giardia duodenale	Muscle aches	2.29 (1.03-5.12)	0.043
	Age group	0.62 (0.47-0.81)	0.001
Plasmodium falciparum	Convulsions	4.84 (1.10-21.29)	0.037
	Age group	0.80 (0.67-0.96)	0.015
	Lethargy	0.43 (0.21-0.92)	0.028

3.5 Discussion

The town of Man and surrounding villages and settlements in western Côte d'Ivoire are endemic for *S. mansoni* and soil-transmitted helminth infections, particularly hookworms.[31,32] Consequently, since 2000, district health authorities implement a programme to improve access to treatment with praziquantel and albendazole, primarily targeted to the school-age population, with the aim to control morbidity among this high-risk group. Unfortunately, due to socio-political unrest commencing in September 2002, treatment campaigns had to be interrupted for more than a year. Previous surveys among schoolchildren and entire communities in several villages in the region of Man also showed that polyparasitism is a

common phenomenon.[18,24,25,33] Here, we confirm these observations for the village of Zouatta II; high frequencies of *S. mansoni*, hookworms, *P. falciparum*, and several intestinal protozoa were found, and polyparasitism was very common. In fact, <2% of the 500 study participants had no infection, whereas three-quarters of them were infected with three or more species. The extent of polyparasitism was similar by sex, but was strongly associated with age. Infants and young children were less likely to harbour multiple parasite species when compared to older age groups, probably explained by lower levels and shorter durations of exposure.

Our results were obtained from a cross-sectional survey, employing standardized field procedures, and quality-controlled laboratory procedures that are widely used in population-based epidemiological surveys in the tropics.[6,34] For example, infections with *S. mansoni* were diagnosed with the Kato-Katz technique, examining multiple faecal samples. Individuals with <2 Kato-Katz thick smear readings were excluded from further analyses, because of the chances of having missed some light infections.[35-37] Diagnosis of soil-transmitted helminths was based on multiple Kato-Katz thick smears plus a single formol-ether processed faecal sample. This approach is superior to the Kato-Katz technique alone, since hookworm eggs tend to dissolve promptly once the sieved faecal samples are placed on the microscope slides and are covered with glycerine-soaked cellophane paper.[38] Indeed, while multiple Kato-Katz thick smears revealed an estimated hookworm prevalence of 35.4% for the present population sample, pooling these results with the formol-ether processed faecal examinations augmented the prevalence to 45.0%. The results of the intestinal protozoa are based on a single formol-ether processed faecal sample. Repeated faecal examinations increase the sensitivity of this diagnostic test,[39,40] hence we can assume that the prevalences reported here are underestimating the 'true' prevalences. Similarly, examination of a single thick and thin blood smear per individual is likely to have underestimated *Plasmodia* prevalence rates.

Taken together, the extent of polyparasitism, reported to be very high in this village of Côte d'Ivoire, could be even higher. However, it is conceivable that mainly light infections were missed; hence the impact on morbidity and co-morbidity of these undetected infections is likely to be small. No attempt was made to concurrently assess bacteria and viruses pathogenic to the intestine, which are also prevalent in populations living in the developing world and presumably contribute substantially to morbidity and mortality (for a recent review see Thapar & Sanderson (2004)[41]). It would be desirable to have a tool with a high sensitivity and a high specificity for accurate diagnosis of multiple species parasitic infections, which in turn would enhance our understanding of interactions between different parasites. For

example, it was recently shown in Senegal that helminth-free individuals had the same level of protection against clinical malaria attacks as that provided by the sickle-cell trait.[42] Accurate diagnosis is thus of considerable relevance for understanding morbidity and co-morbidity patterns. Recognizing the limitations of current diagnostic tools for capturing polyparasitism, we are investigating whether proton nuclear magnetic resonance (^{1}H-NMR)-based metabonomics can be developed to fill this gap. Recent advances with this approach in the field of chronic diseases (e.g. coronary heart disease)[43] might hold promise for infectious diseases.

The present study also confirms previous results obtained elsewhere in Côte d'Ivoire,[18,24] and in Brazil,[44,45] as a significant positive association was found between *S. mansoni* and hookworm infections. In addition, our recent observation that schoolchildren with higher infection intensities of *S. mansoni* are at higher risk of a concurrent hookworm infection is confirmed here for an entire community.[24] In this epidemiological setting, the interactions are likely to be ecological in nature, largely explained by the lack of clean water and sanitation.[24,32] In turn, improving access to safe drinking water and enhanced excreta disposal entails opportunities for sustainable control of schistosomiasis, soil-transmitted helminthiasis and diarrhoeal diseases among other benefits.[21,41,46] In future work, emphasis should also be placed on the nutritional level of study participants living in the developing world, as this is an important underlying risk factor by which intestinal parasites inhibit growth and development.[47,48]

Another interesting finding of the present study is the strong association between an infection with *E. histolytica/E. dispar* and the infection intensity of *S. mansoni*. This strengthens the evidence of this protozoa-helminth interaction, which had already been reported in Egypt.[49] Since light microscopy fails to separate between *E. histolytica* and *E. dispar*, it would be interesting to carry out species-specific diagnosis. Only the former parasite is pathogenic, but it is common that the latter is the predominant species.[50] In eastern Côte d'Ivoire, for example, a recent cross-sectional survey among schoolchildren revealed a ratio of *E. histolytica* to *E. dispar* of 1:46.[51] If we assume that a similar ratio occurs in western Côte d'Ivoire, hence only very few cases of E. histolytica were actually present, this might explain the lack of any significant associations between *E. histolytica/E. dispar* and self-reported morbidity indicators. In the population sample studied here, there was a positive association between *A. lumbricoides* and *T. trichiura* infections, which is in agreement with previous studies from different epidemiological settings.[15,52]

Our study also confirms previously reported associations between a particular parasitic infection and self-reported morbidity. For example, abdominal pain and dysentery were strongly associated with *S. mansoni*, which is of considerable relevance for rapid screening of high-risk populations as a means of cost-effective interventions, e.g. mass administration of praziquantel.[53] However, care is needed in the interpretation of the associations between a particular parasitic infection and any of the self-reported morbidity indicators, because polyparasitism was so common. In other words, the majority of the study participants harboured multiple parasite species concurrently; hence it is difficult to separate out which one is responsible for the self-reported morbidity over the past 2-4 weeks.

We conclude that multiple species parasitic infections are the norm rather than the exception in this community of rural Côte d'Ivoire, as is probably the case elsewhere in developing countries. Consequently, we speculate that capturing polyparasitism can serve as a basis for measuring the dynamics of morbidity and co-morbidity following specific and comprehensive interventions. In turn, this is of importance for informed decision-making with a view towards integrated control to reduce overall morbidity within a population. For example, chemotherapy-based morbidity control should only be viewed as the initial stage in a more comprehensive control approach, emphasising preventive measures. In the population studied here, only a tiny proportion currently sleeps under ITNs and house constructions are inadequate to effectively prevent the entrance of mosquitoes. Access to clean water and improved sanitation facilities is lacking. Consequently, effective information, education and communication campaigns readily adapted to this setting could raise awareness of sound preventive measures against some of the major health problems. For example, house screening, closing eaves and sleeping under ITNs will significantly reduce exposure to malaria vectors,[54] and provision of clean water and installation of improved sanitation facilities will address the root ecological problem of schistosomiasis, as well as other intestinal parasites.[21,41,46] Such a multi-stage approach could ultimately form the basis for transmission control of selected pathogens, which in turn will contribute to poverty alleviation.

3.6 Acknowledgements

3.7 References

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4. Efficacy and side effects of praziquantel against *Schistosoma mansoni* in a community of western Côte d'Ivoire

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4.1 Abstract

Praziquantel is efficacious against the adult stages of all human schistosome parasites, and has become the drug of choice for morbidity control of schistosomiasis. There is concern that resistance to praziquantel might develop or already exists, which could be further facilitated through new control initiatives relying on large-scale administration of praziquantel. Therefore, monitoring praziquantel efficacy in different epidemiological settings is required. We assessed the efficacy and side effects of praziquantel against *Schistosoma mansoni* in a rural community of western Côte d'Ivoire. Three consecutive stool specimens from 545 children and adults were examined by the Kato-Katz technique, revealing an overall prevalence of 40.9%. *S. mansoni*-infected individuals were treated with a single oral dose of praziquantel at 40 mg/kg. The most frequent side effects were abdominal pain, dizziness and diarrhoea. The overall cure rate, assessed 6 weeks post-treatment, was 60.9%. Moderate or heavy infections were only cleared in half or one-third of the individuals, respectively. The total egg count reduction was 61.4%. Infection intensity pre-treatment was significantly associated with age, cure rate, reported diarrhoea and dizziness. Our findings call for additional studies that rigorously evaluate the efficacy of praziquantel against different schistosome species in entire communities, using similarly sensitive diagnostic approaches as employed here.

Keywords: *Schistosoma mansoni*, praziquantel, efficacy, side effects, Kato-Katz thick-smear, Côte d'Ivoire

4.2 Introduction

Praziquantel is the current drug of choice for the treatment and morbidity control of schistosomiasis. Its efficacy against all five species of human schistosomes, good tolerability, ease of administration, and substantial price reduction (average cost for adult treatment in 2003 is US$ 0.30) are key features explaining the success of this drug (BERGQUIST, 2002; DOENHOFF *et al.*, 2002; FENWICK *et al.*, 2003). Using the recommended dosages, cure rates recorded in a 1984 review were: 75-85% for *Schistosoma haematobium*, 63-85% for *S. mansoni*, and 60-80% for mixed infections with *S. mansoni* and *S. haematobium* (WEGNER, 1984). More recent studies found cure rates of 60-90% for *S. mansoni* in different epidemiological settings (GRYSEELS *et al.*, 1987; DAVIS, 1993; KUMAR & GRYSEELS, 1994; UTZINGER *et al.*, 2000*a*). However, in an intensive focus in northern Senegal, the drug resulted in very low cure rates, namely 18-39% (for recent reviews see GRYSEELS *et al.*, 2001;

DANSO-APPIAH & DE VLAS, 2002). Side effects due to praziquantel usually occur in a relatively large proportion of patients (30-60%), but they are mild and transient and disappear within 24 h (JAOKO *et al.*, 1996; BERHE *et al.*, 1999; CIOLI & PICA-MATTOCCIA, 2003; N'GORAN *et al.*, 2003).

The major weaknesses of praziquantel are its lack of efficacy against immature parasites (XIAO *et al.*, 1985; SABAH *et al.*, 1986), and the risk of resistance development (DANSO-APPIAH & DE VLAS, 2002). The stage-specific susceptibility of praziquantel is thought to be the likely source of most treatment 'failures' experienced in clinical practice (CIOLI & PICA-MATTOCCIA, 2003). In areas of intense transmission people are likely to be infected simultaneously with different developmental stages of the parasite. Two praziquantel treatments, given a few weeks apart, showed promising results with elevated cure rates and egg count reductions (PICQUET *et al.*, 1998; RENGANATHAN & CIOLI, 1998; UTZINGER *et al.*, 2000*a*; N'GORAN *et al.*, 2003). Another potentially interesting approach is combination chemotherapy with praziquantel and an artemisinin derivative, because these compounds are complementary with regard to their stage-specific susceptibilities (UTZINGER *et al.*, 2003).

According to the overall endemicity of schistosomiasis in a given setting, WHO currently recommends either selected treatment of infected individuals or systematic praziquantel administration (WHO, 2002). However, the prospect of relying on a single drug for a disease affecting 200 million people is an alarming situation (CIOLI, 2000). In addition, it is anticipated that praziquantel usage will increase with the recent launch of the 'Schistosomiasis Control Initiative' (http://www.schisto.org), and the deworming and school-feeding programmes of the World Food Programme, the World Bank and WHO in already 19 of the 41 endemic African countries (SAVIOLI *et al.*, 2002; FENWICK *et al.*, 2003). Consequently, monitoring the efficacy of praziquantel in different epidemiological settings is a high priority (RENGANATHAN & CIOLI, 1998) and has to be built into operational programmes (WHO, 1999).

Recently, an attempt to interpret low *S. mansoni* cure rates from Senegal on the basis of trends from other studies was carried forward (DANSO-APPIAH & DE VLAS, 2002). The authors identified 11 studies that met the following inclusion criteria: (1) studies involved entire communities or representative sub-samples thereof; (2) participants received a single oral dose of 40 mg/kg praziquantel; (3) treatment outcomes were expressed as a cure rate; and (4) praziquantel efficacy was assessed between 1 and 12 months post-treatment. The authors concluded that even if high pre-treatment intensities of infection could partly explain low cure

rates, the suspicion about tolerance or resistance to praziquantel could not be ruled out for Senegal. The limited number of studies and the fact that they all employed slightly different designs and diagnostic procedures, call for additional studies in other settings.

This paper reports the first evaluation of the efficacy and side effects of praziquantel against *S. mansoni* in an entire community of Côte d'Ivoire. A sensitive diagnostic approach was employed by quantitative examination of 3 consecutive stool specimens per individual prior and after praziquantel administration. The study estimates cure rates and egg count reductions, and assesses side effects after treatment, placing special emphasis on associations to pre-treatment infection intensities.

4.3 Materials and methods

Study area, population and consent

The study was carried out in Zouatta II, a village situated in the district of Man in western Côte d'Ivoire. This area has been reported to be a major focus of *S. mansoni* several decades ago (Service des Grandes Endémies 1970; 1983; DOUMENGE *et al.*, 1987). This has been confirmed by a recent, large-scale, cross-sectional survey, when examination of > 5000 schoolchildren revealed an infection prevalence of *S. mansoni* of 54% (UTZINGER *et al.*, 2000*b*).

The study was launched in May 2002. First, the village chief was asked for permission to work in Zouatta II, and then a meeting was organized with the village authorities to explain the aims and procedures of the study. After consent was obtained and the village chief designated 8 persons to assist our research team, the village authorities informed the community. A detailed demographic survey was carried out in 75 households, randomly selected according to the EPI survey approach (LEMESHOW & ROBINSON, 1985). For each household, the number of inhabitants was recorded and the geographic coordinates were collected using a hand-held GPS (Magellan 320). For each individual, the name, sex and age were recorded. Identification numbers were then assigned to each household, as well as each participant. Two researchers specialized in the fields of sociology (C. A. Adjoua), socio-geography and visual communication (A. Luginbühl), together with the 8 local assistants, conducted a questionnaire survey, both at the household and the individual level. Pre-tested questionnaires were utilized for appraisal of socio-economic factors, perceived morbidity indicators and health seeking behaviour patterns.

Subsequently, a detailed parasitological survey was carried out. Due to logistic reasons and the capacity of our laboratory technicians to process a maximum of 300 stool and 300 urine specimens per day, the selected households were geographically split into 2 similarly large groups. In the evening before the first parasitological survey commenced, the study participants reassembled at the village chief's domicile and the procedures of the forthcoming survey and subsequent treatments were explained.

Field and laboratory procedures

The 8 people assisting our research team went from one household to another and distributed pre-packed plastic bags containing stool and urine containers, marked with the name and identification number of each participant in group 1. The household chiefs were asked to return the containers early in the next morning, filled with small amounts of fresh stool and urine specimens, to a central location in the village. In cases where the household head was unable to return the filled containers, another member of the same household could fulfil this task. This procedure was repeated over 3 consecutive days. It facilitated that study participants could go for work in the fields or attend school during survey days. On the third day, all study participants of group 1 were also invited for a full clinical examination and to provide finger prick blood samples for thick and thin blood films. Thereafter, treatment against *S. mansoni* and soil-transmitted helminths or other ailments, was administered. Group 2 was enrolled from day 5 onwards, adhering to the same field procedures. These activities were completed on day 7 by a full clinical examination, investigation of malaria parasites, and subsequent drug administration.

Stool, urine and blood specimens were brought to the central laboratory in Man. The stool specimens were processed as follows. First, a 1-2 g portion of each specimen was preserved in 10 ml sodium acetate – acetic acid – formalin (SAF). Second, a single Kato-Katz thick-smear, using 42 mg plastic templates, was prepared from each specimen (KATZ *et al.*, 1972). After a clearing time of 30-45 min, the Kato-Katz thick-smears were examined by light microscopy, and eggs of *S. mansoni*, hookworms, *Ascaris lumbricoides* and *Trichuris trichiura* were recorded by 4 experienced microscopists. For quality control, 10% of the slides were randomly selected at the end of each day and re-examined by the senior microscopist.

The SAF-conserved stool samples were forwarded to a reference laboratory in Switzerland for appraisal of intestinal protozoa. Urine specimens were tested with reagent strips (Nephur 6–Test®, Roche Diagnostics, Mannheim, Germany), and 3-4 ml of urine were

frozen and forwarded to a reference laboratory in the UK for NMR-based metabonomic analyses. Thick and thin blood films were stained with Giemsa and species-specific densities were estimated under a microscope, following standardized, quality-controlled procedures. These results will be presented elsewhere.

Chemotherapy, reported side effects and follow-up

After the last stool specimens had been obtained from study participants in group 1 (day 3) and group 2 (day 7), all individuals who had resulted positive for *S. mansoni* were invited for praziquantel treatment at a single oral dose of 40 mg/kg. Individuals who were *S. mansoni*-negative but had an infection with soil-transmitted helminths (hookworm, *A. lumbricoides* or *T. trichiura*) were treated with albendazole (400 mg). Adults who were infected neither with *S. mansoni* nor any soil-transmitted helminth received vitamins. In case the full clinical examination by the study physician revealed other diseases/conditions, the patients were referred for treatment to the central laboratory in the town of Man.

Individuals treated with praziquantel were instructed to report side effects within 24 h post-treatment. The list of symptoms included nausea, vomiting, abdominal pain, diarrhoea, itching, headache, urticaria and dizziness. Six weeks later a follow-up survey was carried out among these individuals. Stool specimens were again collected over 3 consecutive days and Kato-Katz thick-smears were quantitatively examined. Individuals who continued to excrete *S. mansoni* eggs in their faeces were treated with another dose of praziquantel, also administered at 40 mg/kg.

Data management and statistical analyses

Data were double entered and cross-checked using EpiInfo (version 6.04, Centers for Disease Control and Prevention, Atlanta, GA, USA). Analyses were performed with STATA (version 7.0, Stata Corporation, College Station, TX, USA). Praziquantel efficacy was calculated for those study participants who had at least 2 Kato-Katz thick-smears examined pre- and post-treatment and were administered praziquantel. To estimate the mean *S. mansoni* egg counts of each individual, the arithmetic means were calculated both at the baseline and follow-up surveys. These arithmetic means determined the infection intensity of each individual and were utilized to calculate geometric means for the population. Infection intensities were subdivided into 3 categories as follows: (1) light infection: 1-100 eggs/g of stool (epg); (2) moderate infection: 101-400 epg; and (3) heavy infection: > 400 epg (WHO, 2002). Age was

subdivided into 5 categories: < 10 years; 10-14 years; 15-24 years; 25-39 years; and ≥ 40 years. The parasitological cure rate was calculated as the proportion of individuals who had *S. mansoni* eggs in their stool prior to praziquantel administration and those who excreted no eggs after treatment. Egg count reduction was calculated as [1 - (geometric mean epg after treatment/geometric mean epg before treatment)] multiplied by a factor 100. χ^2-test, Fisher's exact test and Mann-Whitney statistics, including 95% confidence intervals (CI) of means, were used to compare groups. Side effects were analysed by examining separately each one of the 8 specific side effects.

4.4 Results

Operational results and study compliance

In May 2002, data pertaining to demographic, socio-economic, perceived morbidity indicators, infection risk- and health seeking behaviour patterns were collected from all inhabitants of 75 randomly selected households in Zouatta II. During the cross-sectional parasitological baseline survey, 561 individuals participated. The large majority, namely 545 (97.1%), provided at least 2 stool specimens, hence they were considered for detailed analyses (Fig. 1).

The numbers of male and female participants were similar (ratio = 1.04; $\chi^2 = 0.49$, $P = 0.70$). Participants' ages ranged from 5 days to 91 years, consisting of: < 10 years, n = 184 (33.8%); 10-14 years, n = 74 (13.6%); 15-24 years, n = 75 (13.8%); 25-39 years, n = 101 (18.5%); and ≥ 40 years, n = 111 (20.4%). Children aged 10-14 years consisted of twice as many boys than girls (50 vs. 24), whereas in older age categories there were slightly more women than men. This resulted in a significant effect of the association between age categories and sex ($\chi^2 = 9.88$, d.f. = 4, $P = 0.04$). The mean age between the 2 sexes was not significantly different (men = 22.4 years, women = 23.7 years; Mann-Whitney z = -0.81, $P = 0.42$).

S. mansoni infection before praziquantel treatment

Table 1 shows the prevalence, infection intensity and geometric mean egg counts of *S. mansoni* among the 545 study participants before the administration of praziquantel after sample 1, sample 2 and sample 3. Examination of the first Kato-Katz thick-smear revealed eggs of *S. mansoni* in 137 participants owing to an observed prevalence of 25.1%.
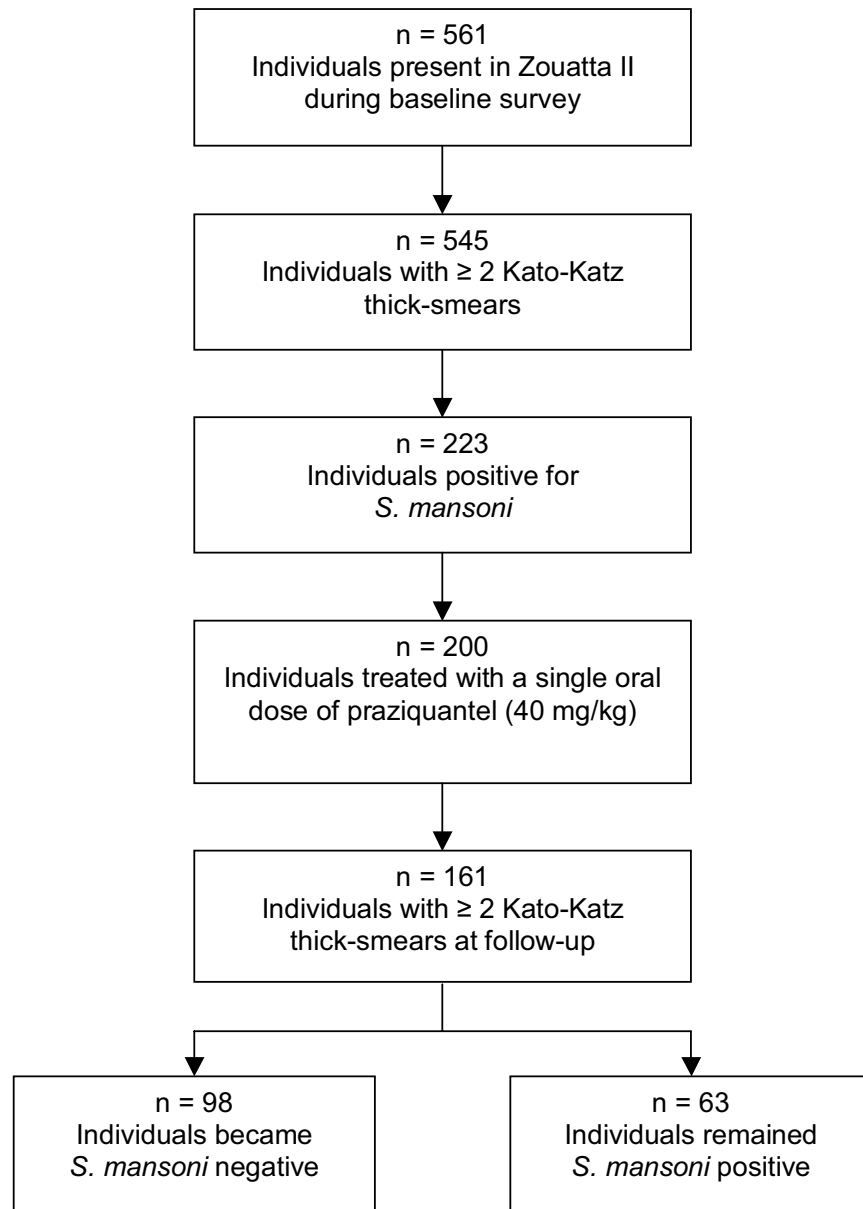
Figure 1 Study compliance for assessment of efficacy and side effects of praziquantel against *Schistosoma mansoni* in the village of Zouatta II, western Côte d'Ivoire.

Table 1 Number (%) of study participants with *Schistosoma mansoni* infections at different intensity levels and geometric mean egg count for the population before the administration of praziquantel in the village of Zouatta II, western Côte d'Ivoire (n = 545)

S. mansoni infection level (eggs/gram stool)[a]	Cumulative results: no. of individuals (%)		
	After sample 1	After sample 2	After sample 3[b]
Light infection (1-100)	74 (13.6)	123 (22.6)	145 (26.6)
Moderate infection (101-400)	36 (6.6)	49 (9.0)	57 (10.5)
Heavy infection (> 400)	27 (4.9)	25 (4.6)	21 (3.9)
All infections (> 0)	137 (25.1)	197 (36.2)	222 (40.9)
Geometric mean egg count [95% CI]	122 [98-152]	69 [57-83]	57 [47-69]

[a] For stratification into different infection intensity levels, after collection of 2 or 3 samples, arithmetic means were used to establish the mean egg count of each individual.

[b] 98 of 545 individuals provided 2 instead of 3 stool specimens.

The prevalence of infection was as high as 40.9% after the examination of 3 consecutive Kato-Katz thick-smears. A considerable number of patients displaying light infections, but to some degree also moderate infections, were missed by a single Kato-Katz thick-smear. In contrast, heavy infections were all detected after the first stool examination. After the maximum sampling effort of 3 Kato-Katz thick-smears, 26.6% of the study participants displayed light, 10.4% moderate and 3.9% heavy infections. The geometric mean egg count among all those individuals were *S. mansoni* positive was 57 epg (95% CI: 47-69).

There was no significant association between level of infection intensity and sex (χ^2 = 0.83, d.f. = 3, *P* = 0.84; Table 2). On the other hand, the association between level of infection intensity and age categories was highly significant with the age group of 15-24 years exhibiting the highest infection intensity (χ^2 = 119.29, d.f. = 12, *P* < 0.001; Fig. 2). Consequently, further analyses were performed for the 5 different age categories.

The observed prevalences of hookworm, *T. trichiura* and *A. lumbricoides*, following examination of 3 Kato-Katz thick-smears, were 36.1%, 5.3% and 1.7%, respectively.

Table 2 Baseline characteristics of *Schistosoma mansoni* infections among 545 study participants from the village of Zouatta II in western Côte d'Ivoire with regard to sex and age

Indicator	Infection intensity				χ^2	*P*-value
	None infected	Light	Moderate	Heavy		
Sex						
Male	160	75	31	12		
Female	162	70	26	9	0.83	0.84
Age (years)						
< 10	159	22	2	1		
10-14	43	22	6	3		
15-24	17	36	19	3		
25-39	49	28	16	8		
≥ 40	54	37	14	6	119.29	<0.001

Table 3 Number (%) of study participants with *Schistosoma mansoni* infections at different intensity levels and geometric mean egg count for the population 6 weeks after praziquantel treatment (single oral dose of 40 mg/kg) among 161 people

S. mansoni infection level (eggs/gram stool)[a]	Cumulative results: no. of individuals (%)		
	After sample 1	After sample 2	After sample 3[b]
Light infection (1-100)	13 (8.1)	35 (21.7)	56 (34.)
Moderate infection (101-400)	5 (3.1)	7 (4.3)	5 (3.1)
Heavy infection (> 400)	2 (1.2)	1 (0.6)	2 (1.2)
All infections (> 0)	20 (12.4)	43 (26.7)	63 (39.1)
Geometric mean egg count [95% CI]	64 [37-112]	33 [23-48]	22 [16-30]

[a] For stratification into different infection intensity levels, after collection of 2 or 3 samples, arithmetic means were used to establish the mean egg count of each individual.

[b] 23 of 161 individuals provided 2 instead of 3 stool specimens
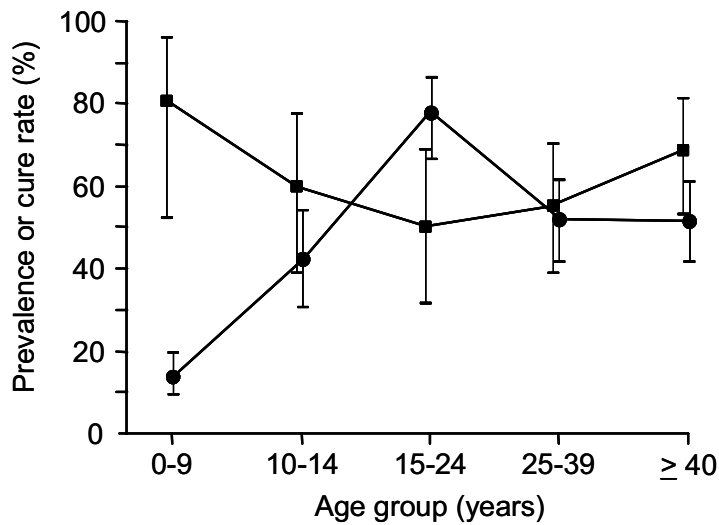
Figure 2 Age-prevalence curve of *Schistosoma mansoni* infections (●) and age-cure rate curve (■) following a single oral dose of 40 mg/kg praziquantel (vertical bars indicate 95% confidence intervals).

Effect of praziquantel and parasitological cure rate

From those 223 participants found with *S. mansoni* eggs during the baseline survey, 200 (89.7%) were treated with a single oral dose of 40 mg/kg praziquantel. The remaining 23 individuals missed treatment, because they were travelling, preferred to work or refused to take the drugs. Six weeks later, 161 participants provided at least 2 stool specimens, hence this cohort was utilized for estimation of the parasitological cure rate. Table 3 summarizes the prevalence, infection intensity and geometric mean egg counts among this cohort after examination of sample 1, sample 2 and sample 3. The observed prevalence after examination of the first Kato-Katz thick-smear was 12.4%. It increased 3-fold, reaching an overall prevalence of 39.1% after 3 Kato-Katz thick-smears had been examined. This translates to an overall cure rate of 60.9%. Had only the first Kato-Katz thick-smear been examined, the cure rate would result in 87.6%, and hence would have been significantly overestimated. The geometric mean egg count among egg positive individuals was 22 epg (95% CI: 16-30), resulting in a total egg count reduction of 61.4%. There were still 7 individuals displaying either moderate or heavy infections. The overall cure rates among individuals who had light, moderate or heavy infections pre-treatment were 70.3%, 50.0% and 33.3%, respectively (Table 4). Consequently, there was a significant association between cure rates and level of

infection intensities prior to treatment ($\chi^2 = 11.58$, d.f. = 2, $P = 0.003$), but cure rates were independent of sex and age categories (Table 5).

Table 4 Effect of single oral dose of 40 mg/kg praziquantel administered to 161 *S. mansoni*-infected patients in Zouatta II, western Côte d'Ivoire in relation to infection intensity pre-treatment

S. mansoni infection level pre-treatment (eggs/gram stool)	No. of patients treated	No. of patients cured (%)	No. of patients non-cured			
			Light infection	Moderate infection	Heavy infection	All infections
Light infection (1-100)	101	71 (70.3)	27	2	1	30
Moderate infection (101-400)	42	21 (50.0)	18	3	0	21
Heavy infection (> 400)	18	6 (33.3)	11	0	1	12
All infections (> 0)	161	98 (60.9)	56	5	2	63

Table 5 Parasitological cure rate after praziquantel treatment in relation to sex and age

Indicator	No. of patients infected	No. of patients cured (%)	χ^2	*P*-value
Sex				
Male	90	56 (62.2)		
Female	71	42 (59.2)	0.16	0.69
Age				
< 10	15	12 (80.0)		
10-14	27	16 (59.3)		
15-24	30	15 (50.0)		
25-39	42	23 (54.8)		
≥ 40	47	32 (68.1)	5.51	0.24

As shown in Table 4, most of the moderate and heavy *S. mansoni* infections were either cleared or reversed to light infections after praziquantel treatment with 4 exceptions. First, 1

heavy infection (mean egg count prior to treatment 3920 epg) remained heavy, although with a sharp reduction in mean egg counts (928 epg post-treatment). Second, 3 moderate infections remained moderate. Third, there were 2 individuals with light infections pre-treatment who were found with moderate infections post-treatment (mean egg counts changed from 32 and 24 epg pre-treatment to 208 and 304 epg post-treatment, respectively). Fourth, 1 light infection pre-treatment was diagnosed as a heavy infection post-treatment (mean egg counts were 36 and 1008 epg pre- and post-treatment, respectively).

Table 6 Number of reported side effects among those 25 individuals who complained of 1 or more side effects within 24 h after praziquantel administration in relation to *Schistosoma mansoni* infection intensity pre-treatment

Reported side effects	Infection intensity pre-treatment			Total records	χ^2	*P*-value
	Light	Moderate	Heavy			
Abdominal pain	6	3	3	12		0.13*
Dizziness	4	5	3	12		0.04*
Diarrhoea	4	0	4	8	16.85	<0.001
Itching	2	2	1	5		0.35*
Vomiting	1	0	1	2	4.09	0.13
Headache	1	1	0	2		0.59*
Nausea	1	1	0	2		0.59*
Urticaria	1	0	0	1	0.57	0.75
Total	20	12	12	44		

*P-value was based on Fisher's exact test

Side effects

Among the 200 treated individuals, 25 (12.5%) reported 1 or more side effects within 24 h post-treatment (Table 6). Individuals with high infection intensities reported significantly more often diarrhoea ($\chi^2 = 16.85$, d.f. = 2, $P < 0.001$) and dizziness (Fisher's exact test, $P = 0.036$) than individuals with light or moderate infections. None of the other side effects showed an association with infection intensity, and side effects showed no association with

age categories. Abdominal pain was the only reported side effect that was significantly associated with sex (women 9 cases vs. men 3 cases, $\chi^2 = 4.02$, $P = 0.045$).

4.5 Discussion

The parasitological cure rate of 60.9% following the administration of a single oral dose of 40 mg/kg praziquantel to *S. mansoni*-infected individuals from western Côte d'Ivoire was at the bottom end of the usually reported cure rates of 60-90% in different epidemiological settings, using the same antischistosomal drug against this schistosome species (DAVIS, 1993; KUMAR & GRYSEELS, 1994; UTZINGER *et al.*, 2000*a*). However, markedly lower cure rates of only 18-39% were observed in a recent and very intense focus of *S. mansoni* in northern Senegal. After in-depth analysis of these data several explanations were carried forward to interpret the unexpectedly low cure rates (GRYSEELS *et al.*, 2001; DANSO-APPIAH & DE VLAS, 2002). In the study presented here the overall egg count reduction was also low (61.4%), which is considerably lower than the normally reported egg count reductions of over 80% or 90% (GRYSEELS *et al.*, 1987; UTZINGER *et al.*, 2000*a*). It was even lower than in Senegal where 2 different studies revealed egg count reductions of 71% (PICQUET *et al.*, 1998) and 86% (STELMA *et al.*, 1995). Recent work employing a 2-treatment protocol against *S. mansoni* in Senegal resulted in a cure rate of 76% and egg count reduction of 88% (PICQUET *et al.*, 1998). These findings indicated that intense disease transmission is an important factor explaining, at least partially, the apparent lack of praziquantel efficacy in the Senegal focus (KUSEL & HAGAN, 1999). Other studies drew the same conclusions although they remained short on quantification (for a review see DANSO-APPIAH & DE VLAS, 2002). Pre-patent infections may also account for some of the 'treatment failures', since juvenile stages of *S. mansoni* are largely insensitive to praziquantel (XIAO *et al.*, 1985; SABAH *et al.*, 1986). The design of the present study did not allow quantification of the extent of pre-patent infections.

Concurrent infections with other parasites and host-related factors have also been found to influence cure rates and egg count reductions. Van Lieshout and colleagues, working in Senegal, investigated different host-related factors between those persons who were cured and those who remained *S. mansoni* egg positive despite treatment. Interestingly, age and pre-treatment infection intensities were the only 2 factors that were significantly associated with low cure rates, while humoral immune responses showed no apparent association (VAN LIESHOUT *et al.*, 1999). Other studies also demonstrated that cure rates depend on infection

intensities prior to praziquantel chemotherapy. These findings are consistent both for *S. mansoni* and *S. haematobium* (ANDREWS, 1981; STELMA *et al.*, 1995; PICQUET *et al.*, 1998; BERHE *et al.*, 1999; UTZINGER *et al.*, 2000a; N'GORAN *et al.*, 2003). Our study adds to this body of evidence, as there was also a highly significant association between *S. mansoni* infection intensity prior to treatment and cure rates. While heavy infections (> 400 epg) showed a low cure rate of only 33.3%, light infections were cleared in 70.3% of the cases. Comparably low cure rates of 42.9% among heavy infections (401-1000 epg) were found by Piquet *et al.* (1998) in Senegal after the initial praziquantel treatment. The overall cure rate in this group increased to 86% after the second praziquantel treatment. Infections with more than 1000 epg resulted in cure rates of 27% and 78% after the first and second round of treatment, respectively. Consequently, administration of 2 doses of praziquantel within a few weeks was proposed for the treatment of heavy infections in this setting. This approach also showed success against *S. haematobium* in a highly endemic area of Côte d'Ivoire with an overall cure rate of 93% after two praziquantel treatments (N'GORAN *et al.*, 2003).

The peak in *S. mansoni* prevalence was observed in the age group of 15-24 years, which is consistent with previous studies where the overall prevalence and intensity of infection were relatively low (FULFORD *et al.*, 1992). The evidence suggests that in populations where the level of infection is high, immunity will be acquired more rapidly, and consequently the peak prevalence will occur at younger age. In contrast, immunity will be acquired later when levels of infections are low and consequently the peak will be expected in older ages (ANDERSON & MAY, 1985; WOOLHOUSE, 1998).

In most community-based studies, cure rates have been estimated on the basis of only 1 or 2 Kato-Katz readings, usually derived from single stool specimens (GRYSEELS *et al.*, 2001; DANSO-APPIAH & DE VLAS, 2002). Our study is based on examination of 3 Kato-Katz thick-smears derived from consecutive stool specimens, both prior and after praziquantel administration, to overcome the inherent problem of day-to-day variation in *S. mansoni* egg outputs (BARRETO *et al.*, 1990; DE VLAS & GRYSEELS, 1992; DE VLAS *et al.*, 1992; ENGELS *et al.*, 1996; UTZINGER *et al.*, 2001). In our previous work carried out in a neighbouring village we showed that examination of at least 3 consecutive Kato-Katz thick-smears results in a high diagnostic sensitivity (UTZINGER *et al.*, 1998). Examination of multiple stool specimens is of particular relevance after implementation of chemotherapeutic interventions, when the overall geometric mean egg count is likely to be low. The present study reiterates that adherence to such sensitive diagnoses is mandatory for estimation of 'true' cure rates (DE VLAS &

GRYSEELS, 1992; DOENHOFF, 1998; UTZINGER *et al.*, 2000*a*). The apparent cure rate after examination of single stool specimens before and after praziquantel administration was 87.6%, whereas the 'true' cure rate was only 60.9%. Several authors emphasised the lack of uniform methodologies when assessing praziquantel efficacies and imply the need to standardize treatment and evaluation protocols, so that findings can be readily compared between different settings, facilitating the interpretation of cure rates and in turn elucidating the potential development of resistance to praziquantel (GEERTS & GRYSEELS, 2000; UTZINGER *et al.*, 2001; DANSO-APPIAH & DE VLAS, 2002).

Side effects due to praziquantel were mild and transient, i.e. they occurred in the first hours after treatment and gradually resolved within 24 h. They were consistent with those side effects reported in other schistosome-endemic areas. Abdominal pain was the most prominent side effect, followed by dizziness and diarrhoea. Among these both diarrhoea and dizziness showed significant associations with pre-treatment infection intensities. Previous studies also found higher frequencies of side effects among the heaviest infection intensities, which had been explained by dying schistosomes and the release of their products. Therefore, severe side effects are mainly encountered in the highest intensity areas (POLDERMAN *et al.*, 1984; STELMA *et al.*, 1995; CIOLI & PICA-MATTOCCIA, 2003).

There is another important aspect of the present study worth discussing. It was carried out among all inhabitants of 75 randomly selected households, thus containing the entire age range. Most previous studies pertaining to the epidemiology of schistosomiasis focused on a particular age group, usually school-age children (HOWARD *et al.*, 2001). This is explained by the fact that this age group is at highest risk of acquiring the disease, which is primarily driven by behavioural and immunological factors. Consequently, school-age children display high prevalences and intensities of schistosome-infections, and often show lower cure rates than adults (VAN LIESHOUT *et al.*, 1999). Therefore, care is needed in the interpretation of cure rates following praziquantel administration because of this potential age bias. To our knowledge this is the first detailed study evaluating praziquantel efficacy against *S. mansoni* in an entire community of Côte d'Ivoire. It complements those 11 studies that were previously carried out in different settings of Africa, and that were included in a recent meta-analysis for interpretation of praziquantel cure rates (DANSO-APPIAH & DE VLAS, 2002). To this end, our work is an important contribution to the ongoing monitoring of praziquantel efficacy against different schistosome species in various endemic areas.

Finally, our findings may raise suspicion about the possible development of praziquantel tolerance or resistance, and this is the subject of current analyses. Although laboratory experiments showed that the degree of praziquantel resistance exhibited by drug-insusceptible isolates is relatively low, and may not be clinically relevant, failure to monitor these developments may have serious consequences in the longer term (DOENHOFF *et al.*, 2002). With the launch of new control initiatives that focus on large-scale administration of praziquantel in countries of sub-Saharan Africa (FENWICK *et al.*, 2003), monitoring drug efficacy over time must become an integral part of control programme evaluation in general, and the surveillance of praziquantel resistance development in particular.

4.6 Acknowledgements

4.7 References

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5. Disparities in parasitic infections, perceived ill health and access to health care among poorer and less poor schoolchildren of rural Côte d'Ivoire

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5.1 Summary

Differences in the state of health between rural and urban populations living in Africa have been described, yet only few studies analysed inequities within poor rural communities. We investigated disparities in parasitic infections, perceived ill health and access to formal health services among more than 4000 schoolchildren from 57 primary schools in a rural area of western Côte d'Ivoire, as measured by their socioeconomic status. In a first step, we carried out a cross-sectional parasitological survey. Stool specimens and finger prick blood samples were collected and processed with standardized, quality-controlled methods, for diagnosis of *Schistosoma mansoni*, soil-transmitted helminths, intestinal protozoa and *Plasmodium*. Then, a questionnaire survey was done for the appraisal of self-reported morbidity indicators, as well as housing characteristics and household assets ownership. Mean travel distance from each village to the nearest health care delivery structure was provided by the regional health authorities. Poorer schoolchildren had significantly higher infection prevalence of hookworm when compared to better-off children. On the other hand, higher infection prevalences of intestinal protozoa (i.e. *Blastocystis hominis, Endolimax nana* and *Iodamoeba bütschlii*) were found with increasing socioeconomic status. Significant negative associations were observed between socioeconomic status and light infection intensities with hookworm and *S. mansoni*, as well as with several self-reported morbidity indicators. The poorest school-attending children lived significantly further away from formal health services when compared to their richer counterparts. Concluding, our study provides evidence for inequities among schoolchildren's parasitic infection status, perceived ill health and access to health care in a large rural part of Côte d'Ivoire. These findings call for more equity-balanced parasitic disease control interventions, which in turn might be an important strategy for poverty alleviation.

5.2 Introduction

It is estimated that the current global burden of malaria is 46.5 million disability adjusted life years (DALYs) (WHO 2004). Strikingly, more than half of this burden is concentrated among the poorest 20% of people, whereas only 0.2% of the total DALYs due to malaria are lost by

the richest 20% (Gwatkin & Jones 2000). Soil-transmitted helminthiasis and schistosomiasis might be responsible for up to 39.0 and 4.5 million DALYs lost, respectively (WHO 2002; Utzinger & Keiser 2004). For sub-Saharan Africa, it is estimated that infections with *Plasmodium,* schistosomes, soil-transmitted helminths and other parasites are responsible for 42.5% of the total DALYs lost in this region (Murray & Lopez 1996). The poorest of the poor are at particular high risks for infections, and hence associated morbidity and mortality, through a multiplicity of factors, including polluted water, lack of improved sanitation, crowding, poor housing conditions, high exposure to pathogens and disease vectors, and poor coverage to other essential services, e.g. education (Victora *et al.* 2003; World Bank 2004). Consequently, in areas where such conditions occur at high frequencies, they delay the social and economic development (WHO 2001; Jha *et al.* 2002; World Bank 2004).

In a study carried out in Madagascar, Kightlinger and colleagues (1998) could show that children from poorer families had higher *Ascaris lumbricoides* worm burdens. Other studies investigated the relationship between infections with *Schistosoma mansoni*, soil-transmitted helminths or *Plasmodium* and socioeconomic variables and found significant associations (Tshikuka *et al.* 1996; Kightlinger *et al.* 1998; Biritwum *et al.* 2000; Carneiro *et al.* 2002). However, these studies employed slightly different methodologies rendering comparisons between one epidemiological setting to another difficult. In recent studies estimations of wealth have been made without expenditure or consumption data. Instead, data on household assets ownership (e.g. possession of a radio or a television) and housing characteristics (e.g. type of walls and roofing material) are used to construct an asset index, which is used as proxy for wealth (Gwatkin *et al.* 2000; Filmer & Pritchett 2001; Armstrong Schellenberg *et al.* 2003; Brooker *et al.* 2004). Using this methodology, Filmer (2002) investigated the relationship of fever incidence, as proxy for malaria, with household poverty and found a weak but significant positive association across different African countries.

Unfortunately, in many parts of the world, the poorest people are the least likely to benefit from health interventions and adequate service delivery (World Bank 2004). For example, studies have demonstrated that children from the poorest households are less likely than their richer counterparts to be reached by preventive interventions such as the supplementation of vitamin A or insecticide-treated nets (ITNs) (Hanson & Jones 2000; Victora *et al.* 2003). A recent study conducted in a rural area of the United Republic of Tanzania found that care-seeking behaviour is worse among poorer families and those children from poorer families made significantly longer journeys to attend the nearest health

facility. Interestingly, though, reported morbidity indicators were not significantly associated to the socioeconomic status of study participants (Armstrong Schellenberg *et al.* 2003). For this rural setting it was thus concluded that the main difference in the poorest and the better-off is the unequal access to adequate treatment.

During the school year 2001/2002 more than 4000 schoolchildren in a rural area of Côte d'Ivoire were screened for *Plasmodium*, *S. mansoni*, soil-transmitted helminths and intestinal protozoa. Children were also interviewed about perceived morbidity indicators, housing characteristics and a set of assets in their homes. The purpose of the present report was to investigate disparities in parasitic infections, schoolchildren's self-reported ill health (i.e. symptoms and diseases) and access to health care in relation to children's socioeconomic status. We also examined associations between parasitic infections and individual household assets, habits of hand washing and location of residency. Our findings may facilitate a more equity-balanced planning of parasitic disease control interventions in western Côte d'Ivoire, and elsewhere in sub-Saharan Africa, with the objective to reach those at highest need.

5.3   Materials and methods

Study area and population

The study was carried out in the region of Man, western Côte d'Ivoire, between October 2001 and July 2002. This mountainous region has distinct climate conditions within Côte d'Ivoire. The majority of the population is engaged in subsistence agriculture. Coffee and cacao are the predominant cash crops providing an important income source for the people (Utzinger *et al.* 2000). Our own preceding epidemiological studies in this region have shown that multiple species parasitic infections are very common (Keiser *et al.* 2002a, b; Raso *et al.* 2004). The study presented here consists of two different surveys. Firstly, a comprehensive parasitological survey was carried out in all primary schools of two education inspections in the region of Man that fulfilled our inclusion criteria. Secondly, a questionnaire survey was conducted to collect data on self-reported morbidity, housing characteristics and household assets ownership to estimate schoolchildren's socioeconomic status.

Parasitology: field and laboratory procedures

After the two education officers were contacted and the aim and procedures of the study were explained, they provided our research team with maps and lists of all primary schools. Those

schools situated in the town of Man and schools in rural areas with less than 100 pupils registered were excluded from this survey. Hence, the remaining 57 schools were enrolled. In a next step, school directors were informed by the education officers about our study and invited to prepare class lists with name, age and sex of each child. Subsequently, only schoolchildren attending grades 3-5 were considered.

During several weeks the research team visited one school after the other in the morning and distributed plastic containers to all study participants. The children were asked to return the containers with a small portion of their own stool, and unique identification numbers were attached to the filled containers. Following, a finger prick blood sample was taken from each child. Thin and thick blood smears were prepared on microscope slides, immediately marked with the child's identification number. Finally, geographical coordinates of each school were determined using a hand-held Magellan 320 global positioning system (GPS; Thales Navigation, Santa Clara, CA, USA).

Stool specimens and blood smears were transferred to the central laboratory in the town of Man. Small portions of stool (1-2 g) were placed into small plastic tubes containing a 10 ml solution of sodium-acetic acid-formalin (SAF), and were shaken rigorously for 20-30 seconds. Then, a single 42 mg Kato-Katz thick smear was prepared from each stool specimen on microscope slides (Katz *et al.* 1972). After a clearing time of 30-45 minutes they were examined by one of four experienced laboratory technologists under a light microscope at low magnification. The numbers of ova of *S. mansoni*, *A. lumbricoides*, hookworm, and *Trichuris trichiura* were counted and recorded separately. The blood smears were stained with Giemsa (Hira & Behbehani 1984).

SAF-conserved stool specimen and Giemsa-stained blood smears were forwarded to a reference laboratory in Abidjan. They were processed with standardized, quality-controlled methods and analysed by four experienced laboratory technologists under a light microscope at high magnification. Presence or absence of the following intestinal protozoa was recorded separately: *Blastocystis hominis*, *Chilomastix mesnili*, *Entamoeba coli*, *Entamoeba hartmanni*, *Entamoeba histolytica/E. dispar*, *Endolimax nana*, *Iodamoeba bütschlii* and *Giardia duodenalis*. Blood smears were examined for species-specific density of *Plasmodium*, assuming for a standard white blood cell count of 8,000/µl blood, according to standard procedures (see for example N'Goran *et al.* 2003).

Questionnaire: self-reported morbidity and socioeconomic status

The questionnaire employed in this study was a further developed version of an existing one, which had been used previously in the region of Man for the rapid assessment of individuals and communities at highest risk of *S. mansoni* infections (Utzinger *et al.* 2000). It included questions on symptoms and diseases that are common in this epidemiological setting. The recall period was one month. We added, for the first time, a section on socioeconomic indicators. After pre-testing in a nearby school that was not enrolled in the present study, the questionnaire was readily adapted and then distributed to all 57 schools. The final questionnaire consisted of three main sections, namely (i) a list of 10 symptoms (headache, hot body, abdominal pain, dysentery, blood in urine, blood in stool, breathing problems, vomiting, lethargy and diarrhoea), (ii) a list of 7 diseases (skin disease, eye disease, schistosomiasis, worms, malaria, malnutrition and cold) and (iii) a list of 12 socioeconomic indicators (wearing shoes, sleeping under a bednet, living in a cement house, living in a house with electricity, and the household assets soap, radio, television, refrigerator, fan, bicycle, motorbike and car). Two additional questions were asked, namely (i) "do you live inside or outside the main village?" and (ii) "do you wash your hands after defecation?"

A separate sheet of detailed instructions and copies of printed class lists accompanied the questionnaire. Teachers were instructed to interview children individually in an empty class room, and to record their answers as "yes", "no" and "don't know", whereas "don't know" was treated as a "no" answer.

Treatment

At the end of the parasitological survey all schoolchildren were treated against an overall fee of FCFA 200 (approximately US$ 0.35) according to the existing treatment schedule recently developed by the regional health authorities. In brief, this fee covers the transport costs of the designated health worker to the schools and the costs of the drugs, namely praziquantel for treatment of *S. mansoni*, and albendazole for treatment of soil-transmitted helminth infections. Standard doses are administered according to the World Health Organization (WHO 2002). Children who complained of malaria-related symptoms and had an axillary temperature $\geq 37.5°$ C were administered Nivaquine® and paracetamol.

Analyses

Data were double entered and validated with EpiInfo v. 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA). All statistical analyses were performed with STATA v. 8.0 (Stata Corporation, College Station, TX, USA). Only participants who had complete parasitological data records (one Kato-Katz thick smear, one SAF-conserved faecal sample, and one blood smear), or complete results from the questionnaire survey, or both, were included in the final analyses. The information on mean travel distances from the study villages to the nearest formal health service was employed for the spatial analysis of access to health care.

Schoolchildren were subdivided into two age groups, namely (i) 6-10 years and (ii) 11-16 years. Infections with hookworm and *S. mansoni* were further stratified into light, moderate or heavy infection intensities, according to thresholds set forth by WHO (2002). Infections with *P. falciparum* were stratified into three intensity categories, namely (i) 1-500, (ii) 501-5000 and (iii) > 5000 parasites/µl of blood.

For the calculation of schoolchildren's socioeconomic status, an household asset-based approach was adopted, which proved valid for rapidly estimating household wealth and income during health surveys carried out in rural Côte d'Ivoire (Morris *et al.* 2000). Principal component analysis was used to define household asset weights, whereby missing values were replaced by the mean of the respective asset. Household assets used in this survey had only dichotomous character. The procedure of this analysis was done according to technical notes put forward by the HNP/Poverty Thematic Group of the World Bank (Gwatkin *et al.* 2000). The first principal component explained 24.1% of the variability and gave greatest weight to living in households possessing a television (0.45), followed by the presence of a fan (0.42) and a refrigerator (0.39). After standardisation of these weighed asset variables, living in households possessing a car had the highest scores (1.62), followed by the presence of a refrigerator (1.57), fan (1.38), motorbike (1.01) and television (0.88). Lowest scores were attached to households without a radio (-0.31) and without electricity (-0.31). The asset scores were summed to a total score for each schoolchild and the children ranked according to their total score. Thereafter, individuals' total scores were divided into wealth quintiles, as follows: (i) most poor, (ii) very poor, (iii) poor, (iv) less poor and (v) least poor.

χ^2-statistics were used to test for associations between a particular parasitic infection, polyparasitism and single and multiple self-reported morbidity indicators with sex and age group. The concentration index (CI), as proposed by Wagstaff *et al.* (1991), was used to

measure inequities in parasitic infection prevalence and intensities, and self-reported morbidity indicators (http://www.worldbak.org/poverty/health/wbact/health_eq.htm) related to schoolchildren's socioeconomic status. One of the strengths of the CI is that it facilitates examination of the direction of the association. The association between socioeconomic status with the presence or absence of a formal health service in the village, and the nearest distance to such services was also measured by the CI. Statistical significance was shown by the standard error (SE) of the measured inequality. Kruskal-Wallis tests were used to compare the number of multiple species parasitic infections or multiple self-reported morbidity indicators between the five socioeconomic strata.

To investigate the relationship of any single parasite with household assets, location of residency and habits of hand washing after defecation, we fitted logistic regressions for each parasite species. The covariates used were the set of household assets, location of residency and habits of hand washing after defecation. The models were adjusted for sex and age group whenever necessary. Covariates were included at a significance level of 0.2. The covariates, which were not significantly related to the parasite under investigation, were removed in a stepwise backward elimination procedure. Odds ratios (OR), adjusted for sex and age group, were computed for associations with P-values < 0.05, including 95% confidence intervals.

5.4 Results

Study compliance and operational results

From 5448 schoolchildren who were registered on the class lists in grades 3-5 in the 57 schools, 5019 were present during the cross-sectional parasitological survey. Figure 1 shows that among these children, 264 lacked a Kato-Katz thick smear and another 419 had no SAF-conserved faecal sample, primarily due to insufficient amounts of stool being collected. Another 294 children missed finger prick blood collection. Consequently, 4042 schoolchildren had complete parasitological data records, owing to an overall compliance of 74.2%. There were 2444 boys and 1598 girls with 2192 children aged 6-10 years and 1850 with an age of 11-16 years.

One school failed to return the filled-in questionnaires. Overall, 1072 schoolchildren were either absent or were not interviewed by the teachers, resulting in 4376 (80.3%) schoolchildren interviewed (for sex and age see Figure 1).
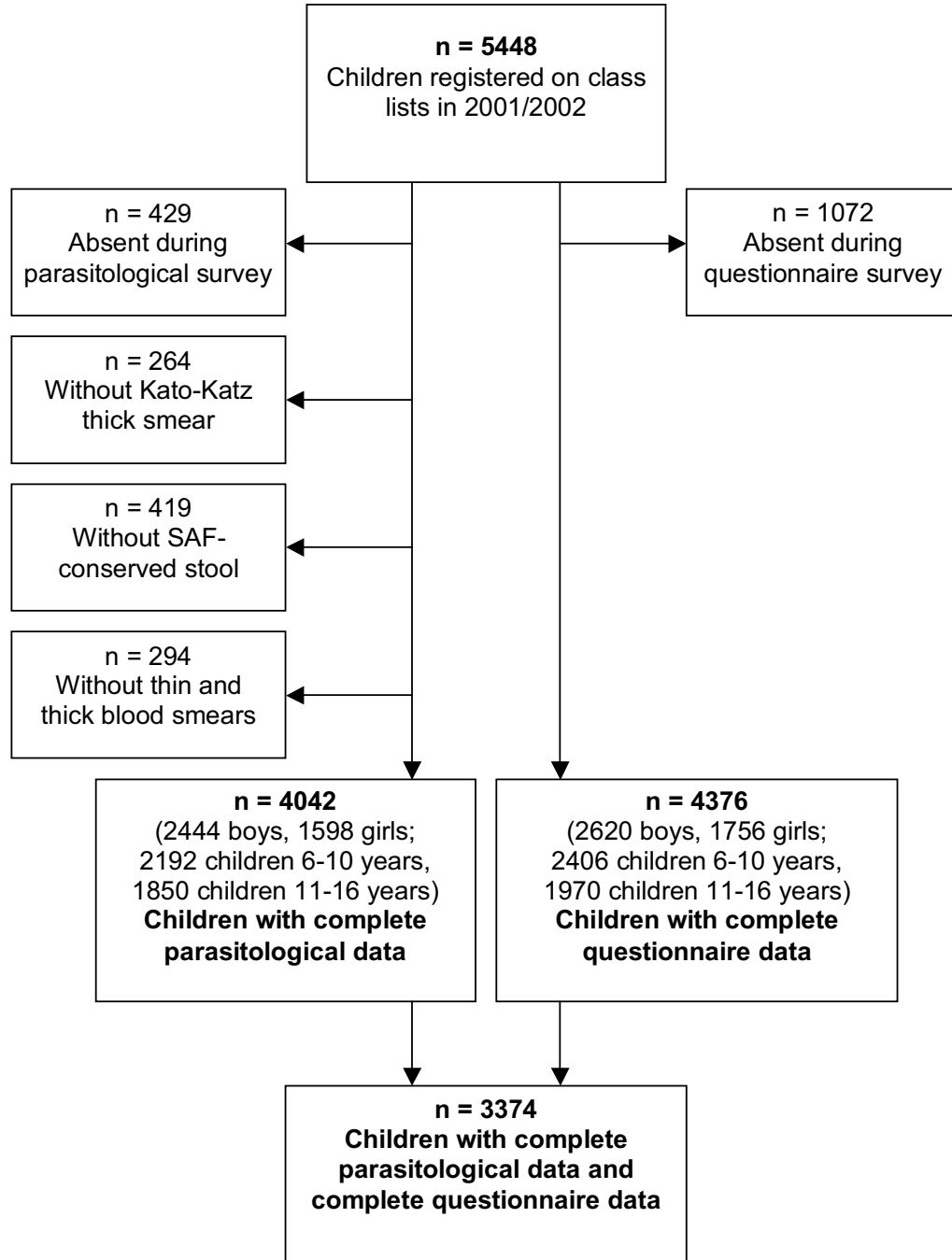
Figure 1 Study cohort with an emphasis on those schoolchildren who had either complete parasitological data, or complete questionnaire data, or both.

Parasitic infections

Table 1 summarizes the results from the cross-sectional parasitological survey. Examination of a single Kato-Katz thick smear revealed infection prevalences with *S. mansoni*, hookworm, *A. lumbricoides* and *T. trichiura* of 38.7%, 30.5%, 2.2% and 1.3%, respectively. The most frequent intestinal protozoa, as examined by a formol-ether concentration method, were *E. nana* (82.6%) and *E. coli* (74.9%). Pathogenic intestinal protozoa, namely *G. duodenalis* and *E. histolytica/E. dispar*, were found in 17.4% and 11.0% of the children, respectively. Infection with *P. falciparum* was common; microscopic analysis of a single blood smear revealed a point prevalence of 64.0%. Infections with *P. malariae* and *P. ovale* were rare; observed prevalences were 3.0% and 0.2%, respectively.

Boys were significantly more likely to be infected with *S. mansoni* than girls (40.5% *vs.* 36.0%; $\chi^2 = 8.19$, degree of freedom (df) = 1, $P = 0.004$). Hookworm infections were much more prominent among boys than girls (35.9% *vs.* 22.2; $\chi^2 = 86.49$, df = 1, $P < 0.001$). None of the other parasites showed a significant association with sex.

Table 1 Parasitic infections, stratified by sex and two age groups, among 4042 schoolchildren in the region of Man, western Côte d'Ivoire.

Parasite	Total (%)	Sex				Age group			
		Females	Males	χ^2	*P*-value	6-10 years	11-16 years	χ^2	*P*-value
S. mansoni	38.7	36.0	40.5	8.19	0.004	36.4	41.4	10.57	0.001
Soil-transmitted helminths									
Hookworm	30.5	22.2	35.9	86.49	<0.001	28.9	32.3	5.48	0.019
A. lumbricoides	2.2	2.6	1.8	2.84	0.092	2.4	1.8	1.60	0.206
T. trichiura	1.3	1.1	1.4	1.03	0.310	1.3	1.3	0.01	0.955
Intestinal protozoa									
E. nana	82.6	81.7	82.9	0.91	0.339	82.6	82.6	0.01	0.998
E. coli	74.9	76.2	74.1	2.09	0.148	75.0	74.9	0.01	0.921
G. duodenalis	17.4	16.7	17.9	0.92	0.337	18.5	16.1	4.06	0.044
I. bütschlii	17.2	17.5	17.0	0.20	0.656	17.2	17.2	0.01	0.940
C. mesnili	15.1	15.2	15.1	0.03	0.854	14.8	15.6	0.48	0.487
B. hominis	10.5	11.0	10.2	0.77	0.379	10.7	10.3	0.18	0.676
E. histolytica/E. dispar	11.0	10.6	11.2	0.28	0.597	9.9	12.3	6.00	0.014
E. hartmanni	7.1	7.0	7.2	0.13	0.721	6.9	7.4	0.26	0.608
Plasmodium									
P. falciparum	64.0	63.1	64.5	0.76	0.385	67.4	59.8	25.07	<0.001
P. malariae	3.0	2.6	3.2	1.06	0.302	3.5	2.4	4.13	0.042

With regard to age, older children had significantly higher infection prevalences with *S. mansoni* (χ^2 = 10.57, df = 1, *P* = 0.001), hookworm (χ^2 = 5.48, df = 1, *P* = 0.019) and *E. histolytica/E. dispar* (χ^2 = 6.00, df = 1, *P* = 0.014). On the other hand, younger children were significantly more likely to be infected with *P. falciparum* (χ^2 = 25.07, df = 1, *P* < 0.001), *P. malariae* (χ^2 = 4.13, df = 1, *P* = 0.042) and *G. duodenalis* (χ^2 = 4.06, df = 1, *P* = 0.044).

Polyparasitism was very common, as four children out of five harboured ≥ 3 parasites concurrently. Overall, 308 (7.6%) children had six, 88 (2.2%) children had seven, seven children had eight and two children had nine parasites. Only eight children were free of parasitic infections. Polyparasitism was significantly associated with sex (χ^2 = 24.19, df = 9, *P* = 0.004), but not with age (χ^2 = 6.93, df = 9, *P* = 0.644).

Table 2 Self-reported morbidity indicators among 4376 schoolchildren, stratified by sex and two age groups, in the region of Man, western Côte d'Ivoire.

Morbidity indicator	Total (%)	Sex				Age group			
		Females	Males	χ^2	*P*-value	6-10 years	11-16 years	χ^2	*P*-value
Symptoms									
Headache	66.5	71.3	63.4	29.77	<0.001	63.8	69.9	17.56	<0.001
Hot body	57.9	59.7	56.7	3.79	0.052	57.6	58.3	0.20	0.656
Abdominal pain	56.2	58.9	54.5	8.34	0.004	54.7	58.2	5.45	0.020
Lethargy	46.5	45.8	46.9	0.48	0.488	45.4	47.8	2.57	0.109
Vomiting	40.6	41.6	39.2	1.18	0.276	41.5	39.5	1.77	0.183
Diarrhoea	33.8	36.3	32.2	7.75	0.005	34.3	33.3	0.47	0.491
Blood in stool	30.9	32.0	30.2	1.45	0.228	30.9	30.9	0.01	0.995
Dysentery	29.6	29.0	30.0	0.50	0.480	29.9	29.3	0.21	0.647
Breathing problems	14.1	14.6	13.7	0.82	0.365	13.6	14.7	1.13	0.289
Blood in urine	8.2	7.5	8.7	1.84	0.175	8.9	7.4	2.99	0.084
Diseases									
Worms	45.2	48.6	42.8	14.32	<0.001	45.6	44.7	0.34	0.559
Malaria	45.1	45.6	44.8	0.24	0.626	44.3	46.2	1.54	0.214
Cold	32.6	32.9	32.4	0.14	0.704	33.7	31.3	2.83	0.092
Malnutrition	25.7	24.8	26.3	1.35	0.246	24.7	27.0	2.91	0.088
Skin disease	20.2	18.7	21.3	4.34	0.037	20.7	19.7	0.62	0.431
Eye disease	15.9	17.3	15.0	4.34	0.037	16.1	15.7	0.13	0.719
Schistosomiasis	14.6	13.7	15.2	1.81	0.178	15.5	13.6	3.16	0.075

Self-reported morbidity

Table 2 shows the frequencies of self-reported morbidity indicators among schoolchildren, stratified by sex and age groups. The most frequently reported symptom was headache (66.5%), followed by hot body (57.9%) and abdominal pain (56.2%). Girls reported significantly more often to have had a headache, abdominal pain or diarrhoea in the one month preceding the interview. Older children reported significantly more often to have suffered from headache and abdominal pain. The most frequently reported diseases were worms (45.2%) and malaria (45.1%). While sex was significantly associated with self-reported worms, skin disease and eye disease, no significant associations were found with age.

Only 325 (7.4%) schoolchildren reported no suffering from any of the morbidity indicators investigated. In contrast, more than two-thirds of the schoolchildren reported at least four different symptoms or diseases concurrently. There were 28 children reporting 16, and five children reporting 17 symptoms and diseases. Age was positively associated with the total number of reported morbidity indicators ($\chi^2 = 33.59$, df = 17, $P = 0.009$).

Housing characteristics, household assets ownership and wealth quintiles

Table 3 displays the wealth quintiles for each household asset. Most of the schoolchildren had shoes (92.4%) and soap (87.5%). About 4 out of 10 of the school-attending children lived in households with electricity, but only 0.2% of these children belonged to the most poor households, while 91.8% of the least poor had electricity at home. While more than half of the schoolchildren reported to have a radio at home, only one out of five children had a television. None of the children of the most poor households slept under bednets, while 22.4% of the least poor gave a positive answer. Finally, none of the children of the most and very poor quintiles lived in households that owned a television, fan, refrigerator, motorbike or a car.

With regard to age, strong positive associations were found for wearing shoes ($\chi^2 = 13.37$, df = 1, $P < 0.001$) and having soap at home ($\chi^2 = 12.62$, df = 1, $P < 0.001$). On the other hand, negative associations were found for living in a house with electricity ($\chi^2 = 5.04$, df = 1, $P = 0.025$), built with cement ($\chi^2 = 7.01$, df = 1, $P = 0.008$), and the possessions of a refrigerator ($\chi^2 = 6.26$, df = 1, $P = 0.012$) and a television ($\chi^2 = 6.79$, df = 1, $P = 0.009$). Girls were significantly more often associated with the assets bicycle, shoes, soap and fan, as well as having electricity at home and living in a cement house.

Table 3 Wealth quintiles of 12 different household assets among 4376 schoolchildren in the region of Man, western Côte d'Ivoire.

Household asset variable	Total (%)	Wealth quintiles (%)				
		Most poor (n = 864)	Very poor (n = 887)	Poor (n = 867)	Less poor (n = 878)	Least poor (n = 880)
Wears shoes	92.4	86.7	90.8	93.0	94.8	96.7
Has soap	87.5	82.2	80.6	87.3	94.0	93.3
Has radio	57.5	0.7	74.1	49.3	73.9	88.3
Has electricity	42.2	0.2	7.1	33.8	77.6	91.8
Lives in a cement house	35.5	1.3	6.8	44.7	45.7	78.8
Has television	20.8	0.0	0.0	0.7	21.9	80.7
Has bicycle	16.4	0.2	5.4	17.7	19.5	38.9
Sleeps under a bednet	10.4	0.0	4.4	9.2	16.1	22.4
Has fan	8.4	0.0	0.0	0.0	0.5	41.4
Has refrigerator	6.0	0.0	0.0	0.0	0.0	29.7
Has motorbike	6.0	0.0	0.0	0.5	6.7	22.7
Has car	1.1	0.0	0.0	0.0	0.2	5.5

Associations between parasitic infections and household assets, location of residency and hygiene behaviour

Table 4 summarizes all significant associations between a particular parasitic infection and single household assets, location of residency and hygiene behaviour. Those children who reported washing hands after defecation were less likely to have an infection with *S. mansoni* (OR = 0.82, P = 0.005), *C. mesnili* (OR = 0.66, P < 0.001) and *P. malariae* (OR = 0.46, P = 0.001), but more likely to have an infection with *G. duodenalis* (OR = 1.21, P = 0.014). Sleeping under a bednet was a protective factor against infections with *P. falciparum* (OR = 0.78, P = 0.041), and showed a borderline significance for *P. malariae* (OR = 0.36, P = 0.050). Schoolchildren living in houses constructed with cement were less likely to have infections with soil-transmitted helminths.

Washing hands after defecation was significantly associated to children's socioeconomic status (χ^2 = 21.78, df = 4, P < 0.001), but was not associated to the number of parasites harboured. In general, schoolchildren living in the main village belonged significantly more often to the least poor group than those living in settlements outside (χ^2 = 13.01, df = 4, P = 0.011). The number of parasites harboured by an individual was not associated to the location of residency.

Table 4 Significant associations between parasitic infections and household assets, habits of hand washing, and location of residency, as assessed by a stepwise logistic regression analysis. Models were adjusted for sex and two age groups, whenever necessary, among 3374 schoolchildren in the region of Man, western Côte d'Ivoire (values in brackets indicate 95% confidence interval).

Parasite	Significant association	Adjusted odds ratio	P-value
S. mansoni	Has bicycle	1.42 (1.17-1.72)	<0.001
	Has soap	1.38 (1.10-1.73)	0.005
	Age group	1.22 (1.06-1.41)	0.006
	Sex	0.84 (0.72-0.97)	0.017
	Washes hands after defecation	0.82 (0.72-0.94)	0.005
	Lives in village	0.75 (0.60-0.94)	0.012
	Has fan	0.70 (0.53-0.93)	0.013
Soil-transmitted helminths			
Hookworm	Age group	1.17 (1.00-1.36)	0.044
	Lives in a cement house	0.76 (0.64-0.90)	0.001
	Has soap	0.73 (0.59-0.91)	0.005
	Has fan	0.65 (0.47-0.89)	0.007
	Has motorbike	0.64 (0.44-0.91)	0.014
	Sex	0.52 (0.44-0.61)	<0.001
A. lumbricoides	Has soap	4.06 (1.27-13.05)	0.018
	Lives in a cement house	0.43 (0.24-0.77)	0.005
Intestinal protozoa			
E. histolytica/E. dispar	Has soap	1.58 (1.09-2.30)	0.016
	Age group	1.28 (1.03-1.59)	0.024
E. nana	Has motorbike	1.75 (1.13-2.72)	0.013
I. bütschlii	Has fan	0.69 (0.48-0.99)	0.044
	Wears shoes	0.56 (0.41-0.76)	<0.001
G. duodenalis	Has soap	1.49 (1.11-2.02)	0.009
	Washes hands after defecation	1.21 (1.04-1.41)	0.014
	Age group	0.81 (0.67-0.97)	0.022
	Has motorbike	0.66 (0.43-0.99)	0.047
C. mesnili	Has soap	1.47 (1.08-2.00)	0.015
	Washes hands after defecation	0.66 (0.54-0.80)	<0.001
B. hominis	Has bicycle	1.49 (1.13-1.95)	0.005
Plasmodium			
P. falciparum	Sleeps under a bednet	0.78 (0.62-0.99)	0.041
	Has bicycle	0.75 (0.62-0.92)	0.006
	Age group	0.71 (0.61-0.81)	<0.001
	Has motorbike	0.69 (0.52-0.92)	0.013
P. malariae	Has television	1.68 (1.06-2.69)	0.029
	Sex	0.65 (0.41-0.99)	0.050
	Washes hands after defecation	0.46 (0.29-0.73)	0.001
	Sleeps under a bednet	0.36 (0.13-1.00)	0.050

Table 5 Frequencies of parasitic infections among 3374 schoolchildren according to their socioeconomic status in the region of Man, western Côte d'Ivoire (CI: concentration index; SE: standard error)

Parasite	Total (%)	Wealth quintiles (%)					CI	SE (CI)	*t*-test (CI)
		Most poor (n = 676)	Very poor (n = 663)	Poor (n = 660)	Less poor (n = 679)	Least poor (n = 696)			
S. mansoni	38.7	39.2	41.2	40.8	38.7	34.1	-0.0267	0.0170	-1.58
Soil-transmitted helminths									
Hookworm	30.5	33.9	34.2	32.4	29.2	23.2	-0.0701	0.0268	-2.61*
A. lumbricoides	2.2	2.1	2.9	2.0	3.1	0.9	-0.0873	0.1101	-0.79
T. trichiura	1.4	0.7	1.4	1.5	1.9	1.2	0.0882	0.0884	1.00
Intestinal protozoa									
E. nana	82.3	82.1	80.5	82.1	82.8	83.8	0.0056	0.0024	2.32*
E. coli	75.7	75.6	76.6	76.2	76.3	73.9	-0.0040	0.0038	-1.05
G. duodenalis	17.7	16.7	17.8	19.9	16.6	17.4	0.0007	0.0146	0.05
I. bütschlii	17.3	15.1	18.1	16.1	18.7	18.4	0.0336	0.0162	2.08*
C. mesnili	15.3	15.4	14.2	17.6	15.2	14.4	-0.0058	0.0156	-0.37
B. hominis	10.4	9.3	7.8	9.2	12.1	13.4	0.0970	0.0216	4.49*
E. histolytica/E. dispar	11.5	10.8	15.2	10.9	10.2	10.6	-0.0371	0.0321	-1.16
E. hartmanni	7.5	7.3	6.2	9.1	6.3	8.6	0.0291	0.0286	1.02
Plasmodium									
P. falciparum	64.3	64.6	64.6	69.1	64.1	59.3	-0.0142	0.0124	-1.15
P. malariae	3.0	2.2	3.2	3.5	3.1	3.2	0.0500	0.0429	1.16

* *t*-test is significant

Table 6 Different infection intensity thresholds for *S. mansoni* and hookworm, stratified by socioeconomic status, among 3374 schoolchildren in the region of Man, western Côte d'Ivoire (epg: eggs per gram of stool; CI: concentration index; SE: standard error)

Infection intensity	Total (%)	Wealth quintiles (%)					CI	SE (CI)	*t*-test (CI)
		Most poor (n = 676)	Very poor (n = 663)	Poor (n = 660)	Less poor (n = 679)	Least poor (n = 696)			
S. mansoni									
No infection	61.3	61.0	58.8	59.2	61.3	66.0	0.0166	0.0104	1.60
1-100 epg	15.6	17.5	15.8	17.0	14.0	13.9	-0.0465	0.0117	-3.96*
101-400 epg	12.6	11.1	15.5	12.7	12.7	11.2	-0.0167	0.0361	-0.46
> 400 epg	10.5	10.5	9.8	11.1	12.1	8.9	-0.0080	0.0315	-0.25
Hookworm									
No infection	69.5	66.1	65.8	67.6	70.8	76.7	0.0305	0.0092	3.34*
1-2000 epg	29.1	32.4	32.6	30.0	27.8	22.7	-0.0674	0.0226	-2.98*
2001-4000 epg	1.0	0.9	1.4	1.7	0.8	0.4	-0.1269	0.1242	-1.02
> 4000 epg	0.5	0.6	0.3	0.8	0.6	0.1	-0.1227	0.1534	-0.80

* *t*-test is significant

Table 7 Frequency of self-reported morbidity indicators, stratified by socioeconomic status, among 4376 schoolchildren in the region of Man, western Côte d'Ivoire.

Morbidity indicator	Total (%)	Wealth quintiles (%)					CI	SE (CI)	*t*-test (CI)
		Most poor	Very poor	Poor	Less poor	Least poor			
		(n = 864)	(n = 887)	(n = 867)	(n = 878)	(n = 880)			
Symptoms									
Headache	66.5	63.9	65.8	65.7	71.4	65.8	0.0112	0.0082	1.37
Hot body	57.9	52.4	55.1	54.8	62.8	64.3	0.0435	0.0067	6.48*
Abdominal pain	56.2	55.4	51.8	55.0	58.1	60.9	0.0248	0.0094	2.62*
Lethargy	46.5	38.4	47.2	43.1	47.3	56.1	0.0610	0.0251	2.43*
Vomiting	40.6	35.7	37.7	40.3	44.4	44.9	0.0494	0.0070	7.06*
Diarrhoea	33.8	33.9	33.7	33.5	34.7	33.4	0.0000	0.0034	0.00
Blood in stool	30.9	29.5	30.3	28.5	34.1	32.2	0.0238	0.0067	3.55*
Dysentery	29.6	34.1	29.1	27.8	31.0	26.3	-0.0368	0.0217	-1.69
Breathing problems	14.1	10.8	12.5	12.9	15.5	18.5	0.1048	0.0224	4.69*
Blood in urine	8.2	6.6	7.7	8.2	8.9	9.7	0.0719	0.0178	4.04*
Diseases									
Worms	45.2	45.1	43.3	44.6	47.3	45.5	0.0086	0.0050	1.72
Malaria	45.1	47.9	52.7	43.8	40.6	40.6	-0.0001	0.0092	-5.19*
Cold	32.6	28.0	37.3	33.6	31.0	33.0	0.0086	0.0286	0.30
Malnutrition	25.7	31.9	30.6	22.5	23.8	19.8	-0.0965	0.0175	-5.52*
Skin disease	20.2	19.4	24.5	17.0	21.9	18.3	-0.0194	0.0261	-0.74
Eye disease	15.9	13.7	12.5	15.8	18.1	19.4	0.0857	0.0114	7.53*
Schistosomiasis	14.6	15.1	14.7	14.2	16.0	13.2	-0.0137	0.0176	-0.78

* *t*-test is significant

Table 8 Travel distance to the nearest health care delivery structure according to wealth quintiles among 4376 schoolchildren in the region of Man, western Côte d'Ivoire (this table does not differentiate between schoolchildren living within a village and those in settlements outside the main village).

Distance to nearest health care delivery structure	Total (%)	Wealth quintiles (%)					CI	SE (CI)	*t*-test (CI)
		Most poor (n = 864)	Very poor (n = 887)	Poor (n = 867)	Less poor (n = 878)	Least poor (n = 880)			
< 1 km	28.0	21.7	15.9	26.9	36.9	38.8	0.1580	0.0251	6.31*
1-5 km	31.9	36.8	33.9	31.7	28.6	28.3	-0.0559	0.0091	-6.12*
> 5 km	40.1	41.5	50.2	41.4	34.5	31.9	-0.0704	0.0264	-2.66*

* *t*-test is significant

Association between socioeconomic status and parasitic infections

Overall, 3374 schoolchildren had complete parasitological and socioeconomic data, as shown in Figure 1. Table 5 gives an account of the relationships and directions between individual parasites and schoolchildren's socioeconomic status. The prevalence of hookworm infection was significantly higher among poorer schoolchildren when compared to their richer counterparts (CI = -0.0701, SE = 0.0170). On the other hand, prevalences of three intestinal protozoa, namely *B. hominis* (CI = 0.0970, SE = 0.0216), *E. nana* (CI = 0.0056, SE = 0.0024) and *I. bütschlii* (CI = 0.0336, SE = 0.0162), were significantly higher in better-off groups.

Table 6 shows that not only the prevalence of hookworm infection, but also its intensity was significantly associated with schoolchildren's socioeconomic status; poorer children had higher frequencies of light infection intensities than their better-off counterparts (CI = -0.0674, SE = 0.0226). In addition, poorer children also had higher frequencies of light infection intensities of *S. mansoni* (CI = -0.0465, SE = 0.0117). No statistically significant associations were found for neither of these two parasites with regard to moderate or heavy infection intensities.

Figure 2 displays the relationship between polyparasitism and socioeconomic status (Kruskal-Wallis H = 15.26, df = 4, *P* = 0.004). The number of parasites harboured by the least poor schoolchildren was significantly lower when compared to their poorer peers.

Association between socioeconomic status and self-reported morbidity

Table 7 shows that 10 out of 17 self-reported morbidity indicators were significantly associated with schoolchildren's socioeconomic status. Most of the significant indicators, with the exception of malaria and malnutrition, were reported more often from better-off groups. Self-reported headache, diarrhoea, dysentery, worms, cold, skin diseases and schistosomiasis showed no association to socioeconomic status.

As shown in Figure 3, significant differences were found among the five socioeconomic groups and the mean number of reported symptoms (Kruskal-Wallis H = 22.39, df = 4, *P* < 0.001) and the number of reported diseases (Kruskal-Wallis H = 19.28, df = 4, *P* < 0.001).
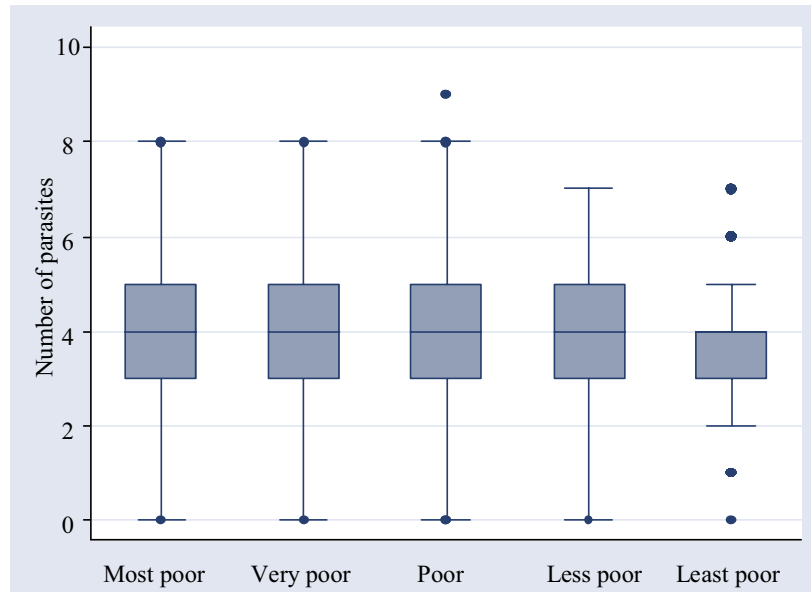
Figure 2 Boxplot displaying frequency of parasites among 3374 schoolchildren, stratified by wealth quintiles, in the region of Man, western Côte d'Ivoire.
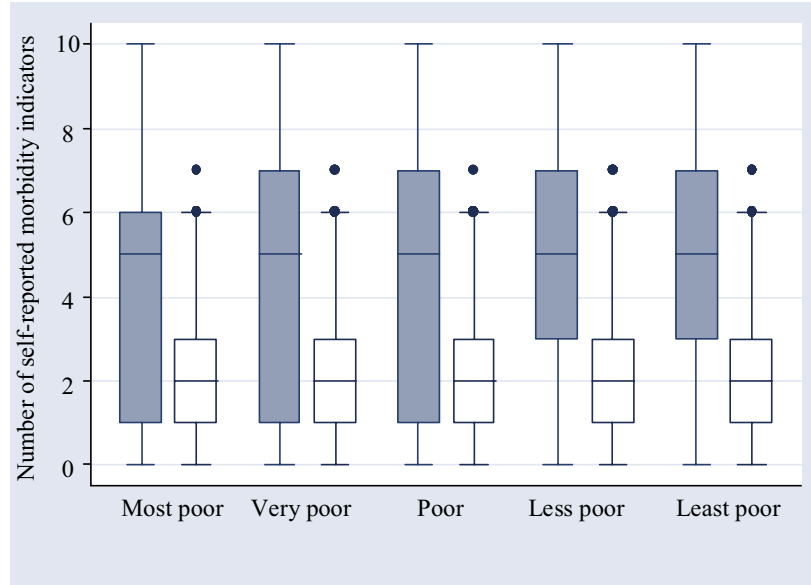

Figure 3 Boxplot displaying self-reported symptom (dark coloured box) and disease (light coloured box) indicators among 4376 schoolchildren, stratified by socioeconomic status, in the region of Man, western Côte d'Ivoire.

Figure 4 Map displaying the proportion of school-children belonging to different wealth quintiles in each study village of the region of Man, western Côte d'Ivoire. The town of Man is situated in the centre of the study area and is marked as red triangle.
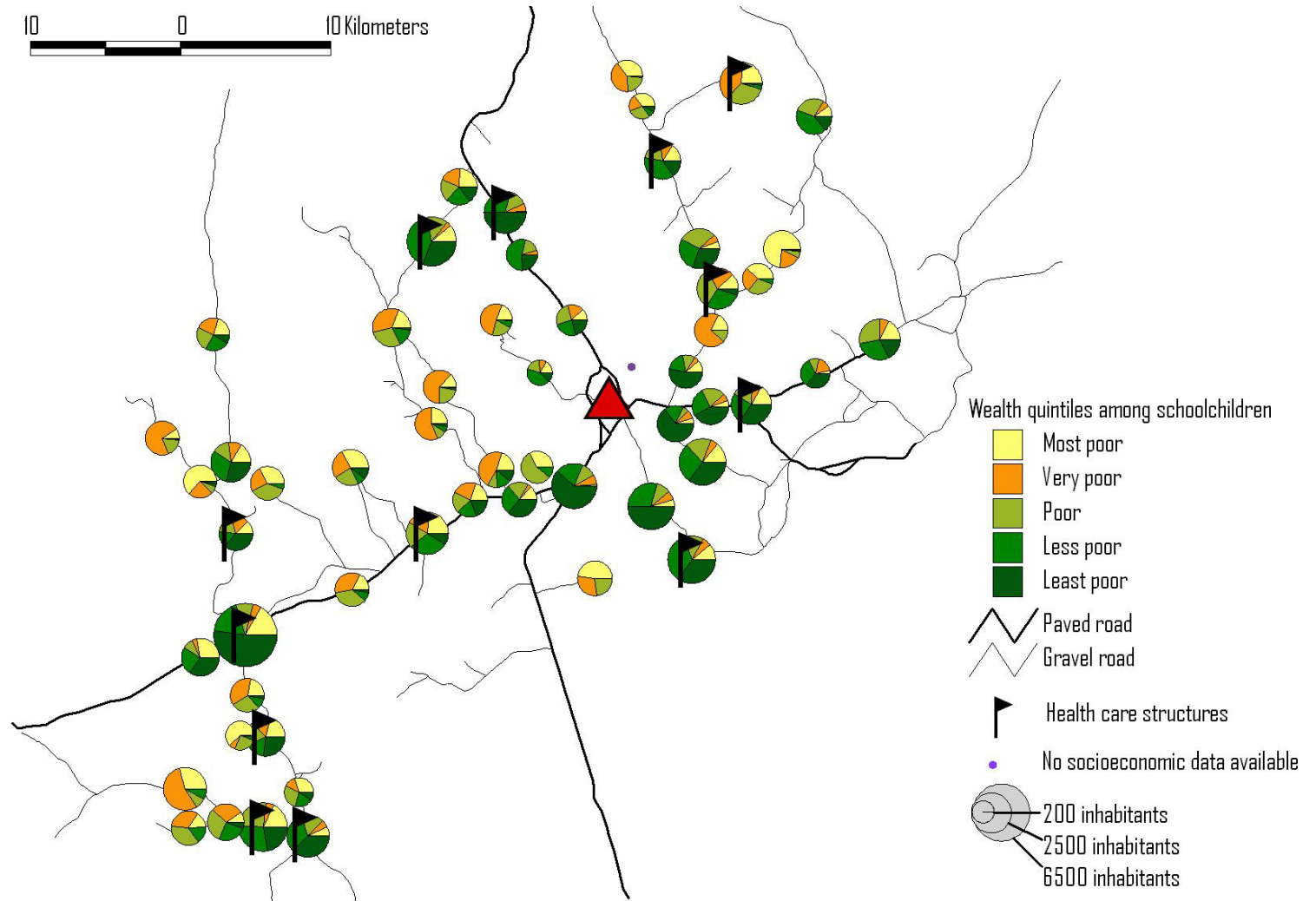
Access to formal health services

Figure 4 shows the proportion of schoolchildren belonging to different wealth quintiles in each study village and the spatial location of the formal health services currently in place in the rural parts of the region of Man. A significant association was found between the presence of a health care delivery facility in a village and schoolchildren's socioeconomic status. Table 8 shows that the distance to the closest facility is negatively associated to schoolchildren's socioeconomic status; poorer schoolchildren live significantly further away than their richer counterparts.

The presence of a health care delivery structure in a village was a protective factor against infections with hookworm (OR = 0.83, P = 0.034), *E. histolytica/E. dispar* (OR = 0.75, P = 0.025), *P. falciparum* (OR = 0.84, P = 0.032) and *P. malariae* (OR = 0.44, P = 0.004). There was also a significant negative association between the presence of a health care facility and infection intensities of *P. falciparum* (χ^2 = 8.81, df = 3, P = 0.032), as well as hookworms (χ^2 = 8.34, df = 3, P = 0.040).

5.5 Discussion

Our study shows that there are significant disparities in parasitic infections and perceived ill health among schoolchildren living in a vast geographical area of rural Côte d'Ivoire. For example, school-attending children from poorer households had significantly higher prevalences and intensities of infections with hookworms, and harboured more parasite species concurrently than their better-off counterparts. Moreover, schoolchildren living in richer households had better access to formal health services, as measured by travel distance We employed the CI for measuring inequalities in health, which has been identified as one of only two viable techniques to carry forward such analyses (Wagstaff *et al.* 1991). Hence, our results are likely to display an accurate picture of current inequalities in health in western Côte d'Ivoire.

Our findings that schoolchildren from the richest quintile harboured significantly fewer parasites than their poorer peers is consistent with results from a study carried out in the city of Lubumbashi, Democratic Republic of the Congo, where polyparasitism with *P. falciparum* and different helminths was investigated (Tshikuka *et al.* 1996). There, a significant difference was also apparent between communities of different socioeconomic status, as the poorest people harboured more parasite species compared to richer ones. In addition,

epidemiological surveys conducted in Brazil, Honduras, Madagascar and Panama also revealed significant associations between people's socioeconomic status and infection intensities of hookworm, as well as with *A. lumbricoides* (Holland *et al.* 1988; Kightlinger *et al.* 1998; Smith *et al.* 2001; Carneiro *et al.* 2002). Two possible explanations of these findings are offered for discussion. First, the construction material of houses and latrines, particularly the use of cement on floors and slabs, acts as a protective factor for the transmission of soil-transmitted helminths. Previous studies from Panama and Democratic Republic of the Congo already reported significant associations between housing characteristics and infections with soil-transmitted helminths (Holland *et al.* 1988; Tshikuka *et al.* 1995). In many parts of the developing world, cement houses are an indicator of wealth, so it is conceivable that these households have pit latrines and, if resources allow, are probably constructed with cement slabs. This in turn prevents environmental contamination with the larval stages of soil-transmitted helminths, hence reduces the risk of infection (Winblad & Kilama 1985; Asaolu & Ofoezie 2003). Consequently, improving housing condition and sanitation facilities by cementing soils is an important and relatively inexpensive control measure to reduce or prevent transmission of soil-transmitted helminths. Second, it is likely that richer population segments have easier access to inexpensive and highly efficacious single dose oral anthelmintic drugs, e.g. albendazole (Utzinger & Keiser 2004).

Previous work revealed that *P. falciparum* infections were significantly associated to socioeconomic status, as shown in studies carried out in The Gambia and Democratic Republic of the Congo (Tshikuka *et al.* 1996; Clarke *et al.* 2001). In the current epidemiological setting of western Côte d'Ivoire, however, the CI revealed no inequity in the prevalence of *P. falciparum*. This finding might be explained by the high overall infection prevalence of this parasite and the small proportion of schoolchildren who reported to sleep under a bednet (10.4%). Nonetheless, it is noteworthy that there was a clear gradient from 0% bednet coverage among the poorest quintile to 22.4% among the richest quintile. This observation underscores that preventive expenditure is related to socioeconomic status, as repeatedly shown for bednet use (de Savigny *et al.* 2002; Mushi *et al.* 2003; Wardlaw 2003). Recent findings from southern United Republic of Tanzania, where coverage rates of ITNs are relatively high, showed that only 8% of the people in the poorest quintile owned bednets, compared to 51% in the best-off quintile (de Savigny *et al.* 2002). Another study compared bednet use in relation to wealth among children under five and found that in eight out of ten countries of sub-Saharan Africa the richest quintile had a significantly higher proportion of

children sleeping under a bednet (Wardlaw 2003). Interviews with a random sample of household chiefs in 25 villages in our study area revealed that the high price of ITNs is a key factor that inhibits utilization at a larger scale among these rural populations. Our findings imply that strategies such as social marketing, as it has been successfully implemented in the United Republic of Tanzania, or providing ITNs free to the most vulnerable groups (i.e. pregnant women and young children), could have a highly beneficial effect in increasing access to ITNs in this region, and hence reduce malaria-related morbidity and mortality (Curtis *et al.* 2003; Guyatt & Ochola 2003; Hanson *et al.* 2003; Mushi *et al.* 2003).

In the present study, three intestinal protozoa, namely *B. hominis*, *E. nana* and *I. bütschlii*, were found to be positively associated to socioeconomic status. To our knowledge, these associations are described for the first time for sub-Saharan Africa. These findings might be a consequence to rich-poor differentials in hygiene behaviour or nutritional habits. However, these intestinal protozoa are not pathogenic (*E. nana* and *I. bütschlii*) or rarely pathogenic (*B. hominis*) to humans, hence from a public health point of view, these results are of no significance.

In the current setting, the infection prevalence of *S. mansoni* was not associated to socioeconomic status. However, after stratification by infection intensities, the CI showed a clear significance contra-poor for light infections. This finding confirms studies from Brazil, where it has been shown that monthly income and several socioeconomic factors were significantly related to *S. mansoni* infections (Kloos *et al.* 1998; Bethony *et al.* 2001). However, other underlying factors that are of behavioural, environmental, genetic and immunological nature may also play important roles.

The large majority of children interviewed reported suffering from several diseases and/or symptoms in the preceding month, which means that they do not perceive themselves as being healthy. On average, children reported 5-6 morbidity indicators out of the 17 included in the questionnaire. Similar results have been reported recently by Moestue and colleagues (2003) after administration of morbidity questionnaires to several thousand schoolchildren from Ghana, Mozambique and United Republic of Tanzania. An important aspect of our study is that within schoolchildren from a given geographical area, there are significant variations of self-reported morbidity indicators in accordance to children's socioeconomic status. Initially, we were surprised that schoolchildren from better-off households perceive themselves less healthy than poorer children, as the former reported significantly more symptoms concurrently. However, consulting the literature, we noticed that

this observation had been made before in many different epidemiological settings; richer people are more likely to complain (for two recent examples see van Doorslaer & Gerdtham 2003; Zere & McIntyre 2003). This might also suggest that schoolchildren from better-off households have higher expectations for their own health that make them more sensitive to distress resulting from the same level of pathology, which they identify as symptoms. Illness experience of children with lower expectations are ignored, and hence not reported as symptoms. Consequently, our findings may suggest that perceived needs are an inadequate guide for preventive and curative interventions. Relying only on symptom, self-report may ignore health-care needs of poorer segments of the population, despite greater needs based on objective assessment of infection rates. Consequently poorer children could be at an elevated risk of severe disease, because they recognise disease signals less clearly, hence might fail to seek care in time. In turn, advocacy on behalf of this segment of the population is required for a health system to function equitably.

Barriers to access to health care delivery structures can be physical, cost-related in terms of travel and treatment expenditures, and capacity-related in terms of health facilities being able to meet current and projected demands (Ensor & Cooper 2004; Rosero-Bixby 2004). In the present study area there is only one hospital, situated in the town of Man, and 13 rural health care facilities, mainly located in the highest populated villages. Employing demographic data from the 1998 census to estimate the proportion of the population residing in villages with or without formal health services, we found that approximately 40% of the rural population lived more than 5 km away from such services. Interestingly, health care delivery structures were primarily located in villages where the mean socioeconomic status, as measured among school-attending children, was high. It follows that poorer families had longer journeys to the nearest health facilities when seeking care and, consequently, may incur higher travelling costs. The long journeys might further increase the risk of severe morbidity and mortality, i.e. due to malaria or acute respiratory infections, as villagers living far away from health care delivery structures are likely to seek treatment significantly less often than those living nearby (Müller *et al.* 1998; Becher *et al.* 2004; World Bank 2004). The poorer may therefore experience a greater vulnerability to the consequences of severe parasitic infections as a result of different health seeking behaviour. Our findings therefore imply that there is a great need to improve access to early diagnosis and effective treatment for this rural population. The establishment and use of a geographic information system could

provide a tool to readily guide interventions, so that access to health care can be improved (Noor *et al.* 2003, 2004; Rosero-Bixby 2004).

One shortcoming of our study is that the sample of children attending school may not be representative for the whole society in this area. Clearly, there is a sampling bias related to non-enrolled school-age children. In India, for example, it has been shown that on average a rich child was 31% more likely to be enrolled in school than a poor child (Filmer & Pritchett 2001). For the whole of Côte d'Ivoire a similar pattern has been found, and hence similar results can be expected for our study area, although the difference might be smaller in only rural parts of a single region. To address this issue, household surveys are needed. We speculate that the gap currently observed between poor and rich school-attending children in this study area might be even higher following the proposed approach.

In conclusion, some of the parasitic infections investigated here showed clear associations to schoolchildren's socioeconomic status, as assessed by a simple asset-based approach (Morris *et al.* 2000). However, disease outcomes in this epidemiological setting are also driven by a myriad of other factors, notably behavioural and ecological, which may play equal or even more important roles for transmission. Importantly, better-off children were more likely to live in villages with health care delivery structures in place, and seemed to have better access to preventive measures such as ITNs. Our results call for concerted efforts to reach the most disadvantaged segments of populations in this part of rural Côte d'Ivoire, as well as elsewhere in the developing world. The task ahead is immense, but improving access to preventive and curative medicine, clean water and improved sanitation, coupled with sound hygiene behaviour education, will have significant effects in decreasing the intolerable burden of parasitic diseases, and thereby contribute to poverty alleviation in an equitable and sustainable manner.

5.6 Acknowledgements

5.7 References

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6. Spatial risk prediction and mapping of *Schistosoma mansoni* infections among schoolchildren living in western Côte d'Ivoire

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6.1 Summary

The objectives of this study were (1) to examine risk factors for *Schistosoma mansoni* infection among schoolchildren living in western Côte d'Ivoire, and (2) to carry forward spatial risk prediction and mapping at non-sampled locations. First, demographic and socio-economic data were obtained from 3818 children, aged 6-16 years, from 55 schools. Second, a single stool sample was examined from each child by the Kato-Katz technique to assess infection status of *S. mansoni* and its intensity. Third, remotely sensed environmental data were derived from satellite imagery and digitized ground maps. With these databases a comprehensive geographical information system was establish. Bayesian variogram models were applied for spatial risk modelling and prediction. The infection prevalence of *S. mansoni* was 38.9%, ranging from 0% to 89.3% among schools. Results showed that age, sex, the richest wealth quintile, elevation and rainfall explained the geographical variation of the school prevalences of *S. mansoni* infection. The goodness of fit of different spatial models revealed that age, sex and socio-economic status had stronger influence on infection prevalence than environmental covariates. The generated risk map can be used by decision-makers for the design and implementation of schistosomiasis control in this setting. If successfully validated elsewhere, this approach can guide control programmes quite generally.

Keywords

6.2 Introduction

Schistosomiasis is a parasitic disease caused by trematode worms belonging to the genus of *Schistosoma*. In sub-Saharan Africa, schistosomiasis remains of major public health and economic significance. Recent estimates for this region of the world suggest that 436 million people live at risk of infection with *Schistosoma haematobium*, and 393 million people are at risk of *S. mansoni*. The estimated annual mortality rate might exceed 200 000 (Chitsulo *et al.* 2000; WHO, 2002; van der Werf *et al.* 2003). Transmission occurs in freshwater bodies, where specific aquatic snails act as intermediate host. Infected snails release cercariae that can penetrate the skin of humans during occupational and/or recreational activities. Cercariae rapidly develop into schistosomula which, after several weeks, become adult schistosome worms that constantly produce eggs. A part of the eggs are released into the environment by

human excreta. The other part is trapped in the tissues of the host organs causing inflammations, which can lead to severe morbidity. Suitable climatic and environmental conditions for both the parasite and intermediate host snail, coupled with inadequate water supply and sanitation and low hygiene conditions, are the root causes for the persistence of schistosomiasis (Utzinger *et al.* 2003).

Progress has been made over the past 10-15 years with the application of geographical information system (GIS) and remote sensing (RS) for risk mapping of parasitic diseases, including schistosomiasis. Hence, these techniques have become important tools for the design and implementation of control programmes (Brooker, Hay & Bundy, 2002*a*). In addition, developments in Bayesian methods and Markov chain Monte Carlo (MCMC) inference (Gelfand & Smith, 1990) have advanced spatial modelling. GIS allows identification and visualization of demographic, environmental and socio-economic covariates for infection risk. Linked to Bayesian spatial statistics, GIS can save scarce resources of otherwise expensive parasitological surveys for detection and monitoring of high-risk areas (Brooker & Michael, 2000; Robinson, 2000). Several climate and environmental factors have been linked with schistosome infections at broad scale (Brooker *et al.* 2001; Malone *et al.* 2001; Zhou *et al.* 2001; Yang *et al.* 2005). For example, a model had been developed for the distribution of *Biomphalaria pfeifferi*, the intermediate host snail of *S. mansoni*, in Ethiopia. The model, based on the normalized difference vegetation index (NDVI) and maximum land surface temperature (LST), predicted *B. pfeifferi* distribution, and hence infection prevalence of *S. mansoni* (Kristensen, Malone & McCarroll, 2001). Using RS to derive rainfall and maximum LST data, a model had been produced for prediction of *S. haematobium* infections in Cameroon (Brooker *et al.* 2002*b*). This model, however, only showed good prediction within the boundaries of a given ecozone (Brooker *et al.* 2002*a*).

Previous research has shown that analyses on broad scale render it difficult to capture the small-scale focality, which is a typical epidemiological feature of schistosomiasis (Bavia *et al.* 2001; Brooker, 2002; Lengeler, Utzinger & Tanner, 2002). Several factors, primarily acting at a local scale, may be at the origin of such heterogeneity (Husting, 1983; Kloos *et al.* 1997; Watts *et al.* 1998). Consequently, appropriate models are required which can capture potential risk areas at different scales (Bavia *et al.* 2001; Brooker, 2002). The need for broad scale analyses derives from decision-making often taking place at the district level, while locally distinct needs can drive small-scale analyses.

The objectives of this study were (1) to identify risk factors explaining the geographical distribution of *S. mansoni* infections in a mountainous region of western Côte d'Ivoire, and (2) to make predictions at non-sampled locations. A cross-sectional survey was done among several thousand schoolchildren. They were screened for *S. mansoni*, and interviewed for demographic and socio-economic indicators. Environmental factors were obtained and a GIS was established. Finally, Bayesian geostatistics were employed for prediction of *S. mansoni* infection. This work contributes to an ongoing parasitic disease research and integrated control programme with emphasis on schistosomiasis, soil-transmitted helminthiasis and malaria in the western part of Côte d'Ivoire.

6.3 Material and methods

Study area and population

The study region is a 40 x 60 km area situated in the mountainous region of Man, western Côte d'Ivoire. This setting has been known to be a *S. mansoni* focus for over 3 decades (Doumenge *et al.* 1987). There are 2 wet seasons; the main one between April and June and a shorter one in September. Mountains, inselbergs (remnants of erosion processes forming isolated, typically rounded mountains which can range in elevation from a few to several hundreds of meters) and small valleys dominate the northern part of the study area, while the southern part is a plain that acts as a drain for the numerous small rivers. Recent studies confirmed that this setting is indeed endemic for *S. mansoni* with all age-groups concerned (Utzinger *et al.* 2000; Keiser *et al.* 2002; Raso *et al.* 2005).

The study protocol was approved by the internal review boards of the Swiss Tropical Institute (Basel, Switzerland) and the Centre Suisse de Recherches Scientifiques (Abidjan, Côte d'Ivoire). It received ethical clearance from the Ministry of Health in Côte d'Ivoire. Data presented here are derived from a cross-sectional epidemiological survey carried out between October, 2001 and February, 2002. All schools located in the town of Man and those schools in rural areas with less than 100 pupils on the education registries were excluded from the survey. In the remaining 57 rural schools, all schoolchildren attending grades 3-5 were enrolled for parasitological screening and an interview by the teachers.

Parasitological data

Details of the parasitological surveys have been described elsewhere (Raso *et al.* 2005). In brief, after explaining the objectives and procedures of the study to the education officers,

class lists for the school year 2001/2002 were obtained, containing the name, age and sex of each pupil. Unique identification numbers were assigned to all schoolchildren attending grades 3-5. Schoolchildren were examined for *S. mansoni*, soil-transmitted helminths, intestinal protozoa and *Plasmodium* infections. Emphasis here is placed on the field and laboratory procedures pertaining to *S. mansoni*.

The research team visited one school after another. Small containers were distributed to all study participants. They were invited to return the containers with a small portion of their own morning stool. The stool specimens were collected and transferred to the central laboratory in the nearby town of Man. A single 42 mg Kato-Katz thick smear was prepared from each stool specimen on microscope slides (Katz, Chaves & Pellegrino, 1972) and allowed to clear for 30-45 min. The slides were examined by experienced laboratory technologists under a light microscope at low magnification. All *S. mansoni* eggs were counted and recorded.

Treatment

At the end of the parasitological survey, schoolchildren who were egg-positive for *S. mansoni* were treated according to the existing treatment schedule recently developed by the regional health authorities, as described before (Raso *et al.*, 2005). Praziquantel was administered at a single oral dose of 40 mg/kg (WHO, 2002).

Socio-economic data

Socio-economic data were obtained from a pre-tested and validated questionnaire, which was administered by teachers to all schoolchildren who were previously examined for parasite infections. Neither teachers nor schoolchildren had prior knowledge on *S. mansoni* infection status. The questionnaire included a list of 17 morbidity indicators, 12 household assets and the question "do you live within the main village or in settlements outside?" A simple asset-based approach was adopted to stratify schoolchildren into socio-economic groups (Filmer & Pritchett, 2001). Household asset variables were weighed using principal component analysis. Schoolchildren were ranked according to their total sum of asset scores. Finally, schoolchildren were attached to wealth quintiles, namely most poor, very poor, poor, less poor, and least poor.

Environmental data

The geographical locations of schools (longitude, latitude and elevation) were recorded in the field using a hand-held global positioning system (GPS; Thales Navigation, Santa Clara, CA, USA).

In the absence of recent digital maps, available maps from the 1960s (scales: 1:200 000 and 1:50 000) were georeferenced. Streets, village boundaries, rivers and elevation lines were digitized. Satellite imagery data from Digital Enhanced Landsat Thematic Mapper (ETM+) (image dates: January 27, 2002; November 11, 2002) and GPS-referenced control points taken in the field served as validation of the digitized maps. Maps of soil types (scale: 1:500 000) were georeferenced and major soil types of the study area digitized. These were either ferrallitic soils slightly regenerated, modal, complex and fairly desaturated) or hydromorphic mineral soils. Land cover types were obtained from satellite image (Advanced Very High Resolution Radiometer (AVHRR) satellite, U.S. Geological Survey (USGS) Africa Land Cover Characteristics Database v.2: Africa Seasonal Cover Regions, USGS Earth Resources Observation System (EROS) Data Centre) at a 1x1 km spatial resolution (http://edcdaac.usgs.gov/glcc/glcc.asp). Land cover types were field-validated by taking GPS control points of relevant vegetation classes. An interpolated digital elevation model (DEM) was obtained from the USGS EROS Data Center (http://lpdaac.usgs.gov/gtopo30/gtopo_links.asp). LST and NDVI data were downloaded from Moderate Resolution Imaging Spectroradiometer (MODIS) from USGS EROS Data Center (http://edcdaac.usgs.gov/dataproducts.asp). Rainfall estimate (RFE) data with a 8x8 km spatial resolution from Meteosat 7 satellite were obtained from the Africa Data Dissemination Service (ADDS) (http://edcw2ks21.cr.usgs.gov/adds/). LST, NDVI and RFE were downloaded for the period of September, 2001 to August, 2002 and processed as suggested by Hay (2000). LST day and night data were available as 8-day-maximum value composites at 1x1 km spatial resolution. Monthly minimum, mean and maximum composites were calculated and values extracted for each pixel corresponding to the school locations. Monthly maximum value composites were also calculated from NDVI 16-day-maximum value composites at 1x1 km spatial resolution and values were extracted for each school location. The same procedure was applied for the RFE data. Annual LST and NDVI were obtained as average of the monthly mean values. For LST, annual estimates from monthly minimum and maximum values were also obtained. RFE was calculated as the total amount of rainfall over

the one-year period. Distance from schools to the nearest permanent water body was computed.

Georeferencing of maps, processing of the environmental data and distance calculation were done in IDRISI 32 (Clark Labs, Clarks University, Worcester, MA, USA). Data were displayed in ArcView GIS v.3.2 (Environmental Systems Research Institute, Inc., Redlands, CA, USA).

Statistical analysis

Parasitological and questionnaire data were double-entered and validated with EpiInfo v.6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Schoolchildren were subdivided into two age groups; namely 6-10 years, and 11-16 years.

Elevation at each school location was defined as the mean of the GPS value and the elevation derived from the digitized maps. Environmental covariates with continuous values, which showed a non-linear relationship to *S. mansoni* infection prevalence, were categorized. Logistic regression models were fitted to *S. mansoni* infection prevalence to identify significant demographic (age and sex), socio-economic and environmental covariates in STATA v.8.0 (Stata Corporation, College Station, TX, USA). Those covariates with a significance level below 0.15 were fitted into logistic geostatistical models using WinBUGS v.1.4 (Imperial College & Medical Research Council, London, UK). To take into account the spatial heterogeneity, location-specific random effects were integrated in the logistic models, assuming that they are distributed according to a multivariate normal distribution with variance-covariance matrix related to the variogram of the spatial process (Gelfand, Ravishanker & Ecker, 1999). MCMC simulation was employed to estimate the model parameters (Gelfand & Smith, 1990). Significant covariates from the final model were selected to generate a smooth map of *S. mansoni* infection risk using Bayesian kriging (Diggle, Tawn & Moyeed, 1998). For appraisal of the best fitting model, the deviance information criterion (DIC) was applied (Spiegelhalter *et al.*, 2002). A smaller DIC indicates a better model. Further details on the spatial models are given in the Appendix.

6.4 Results

Compliance and school elevation

Fig. 1 shows that in the school year of 2001/2002, 5448 schoolchildren were listed on the education registries for grades 3-5 in the 57 rural schools in the region of Man included in this

study. During the parasitological surveys 5019 children were actually at school, but 264 failed to provide sufficient quantities of stool for diagnosis of *S. mansoni*. Hence, 4755 schoolchildren had a single Kato-Katz thick smear examined by light microscopy.

Questionnaire data were obtained from 55 schools. One school returned uncompleted forms, and 1 school failed to send back the questionnaires. Overall, the teachers interviewed 4376 schoolchildren. For spatial risk prediction and mapping, the final cohort consisted of 3818 schoolchildren, who had both parasitological and questionnaire data. There were 1528 girls (40.0%) and 2290 boys, with 2093 children aged 6-10 years (54.8%) and 1725 children aged 11-16 years.

School elevations ranged between 291 m and 842 m. Forty schools (73%) were located at elevations below 400 m, while the remaining 15 schools were located at higher elevations. The highest annual rainfall value (1128 mm) was observed at the school with the highest elevation. Rainfall and elevation were found to be positively correlated ($P < 0.001$), however there was no collinearity.
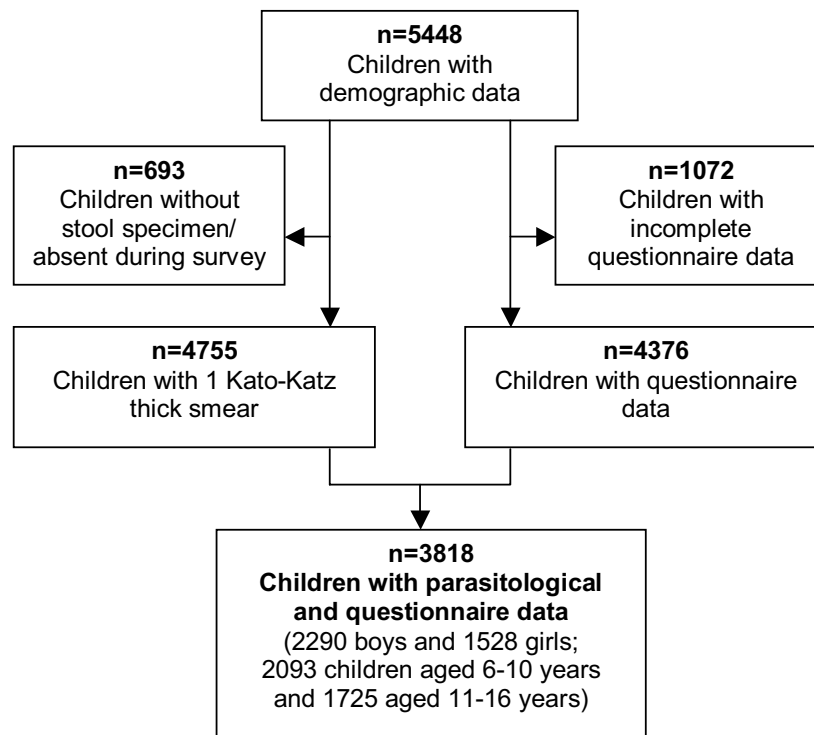
Figure 1 Study profile and compliance

S. mansoni infection

The overall *S. mansoni* infection prevalence was 38.9%. Infection prevalences of girls and boys were 36.3% and 40.6%, respectively. Infection prevalences among the 6-10 year and the 11-16 year old children were 36.4% and 41.9%, respectively.

The cumulative infection prevalences in the schools ranged from 0% to 89.3%. Fig. 2 displays mean school *S. mansoni* infection prevalences at the 55 sampled locations. The highest prevalences were found in the south-western part of the study area. Some distinct foci of high prevalence were also found in the central part. In contrast, mean school prevalences of *S. mansoni* infections were below 20% in the north-eastern part of the study area.

Figure 2 Distribution of mean *S. mansoni* infection prevalences in 55 rural schools in the mountainous region of Man, western Côte d'Ivoire.

Associations between *S. mansoni* and demographic, socio-economic and environmental covariates

Table 1 shows the results of the non-spatial bivariate logistic regression analyses. Schoolchildrens' age and sex were significantly associate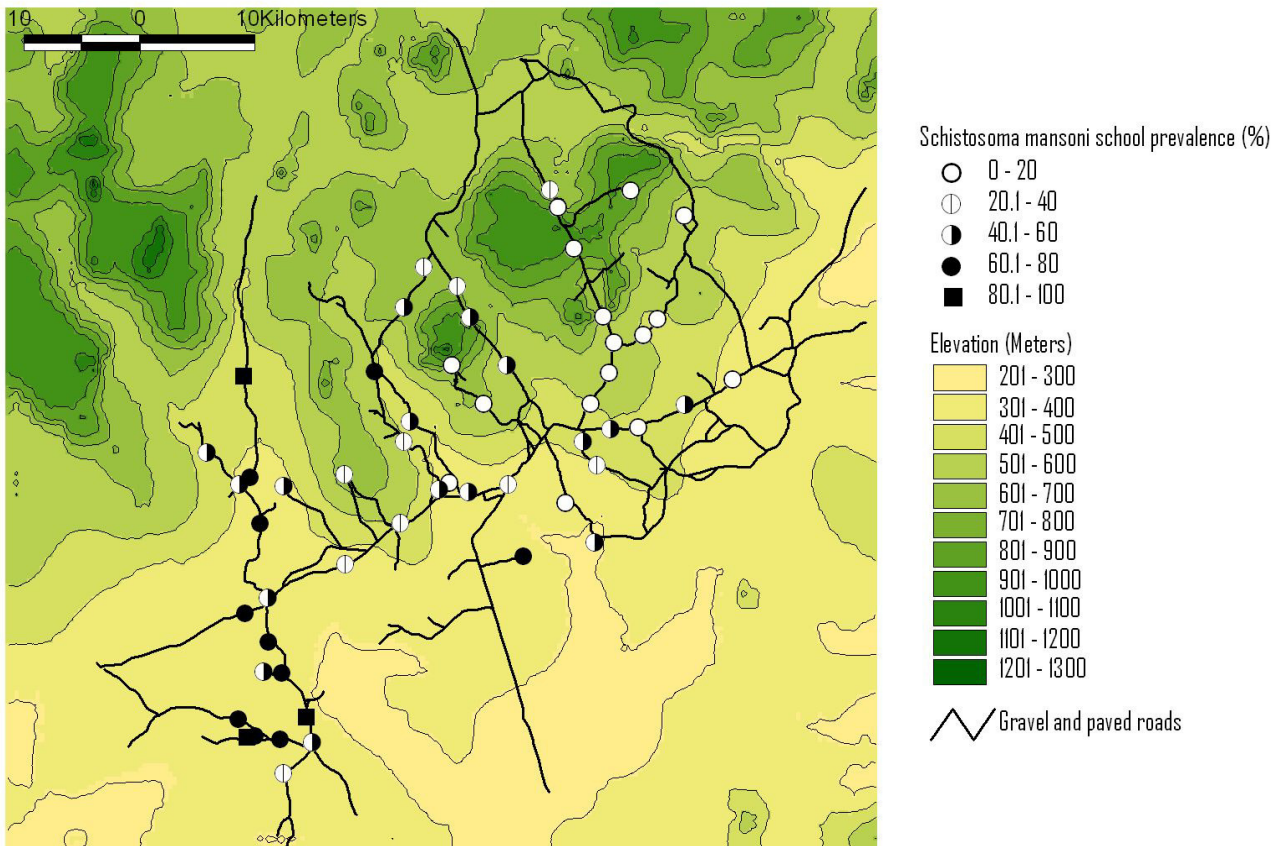d with an *S. mansoni* infection. Children from the older age group were significantly more likely to be infected with *S. mansoni* than their younger counterparts (odds ratio (OR) = 1.22, *P* = 0.001). Boys had significantly higher infection prevalence than girls (OR = 1.19, *P* = 0.008). There was no significant association between *S. mansoni* infection and socio-economic status.

Children living within the boundaries of the main villages showed significantly lower *S. mansoni* infection prevalences than those living in settlements outside (OR = 0.72, *P* = 0.001). Children living 1-4.9 km away from the nearest health facility were more likely to be infected with *S. mansoni* than those living in close proximity (OR = 1.27, *P* = 0.002).

Table 1 Bivariate associations between *S. mansoni* infection prevalence and demographic, socio-economic and physical environment-related indicators arising from non-spatial and spatial logistic models. Odds ratios (OR) are displayed with their respective 95% confidence intervals (CI).

Indicator	Non-spatial models[*]		Spatial models[†]	
	OR	95% CI	OR	95% CI
Demography				
Age group				
6-10 years	1.00		1.00	
11-16 years	1.22	1.09, 1.37	1.48	1.26, 1.73
Sex				
Female	1.00		1.00	
Male	1.19	1.06, 1.34	1.24	1.06, 1.45
Socio-economic status				
Most poor	1.00		1.00	
Very poor	1.08	0.88, 1.33	1.12	0.86, 1.44
Poor	1.10	0.89, 1.35	1.12	0.86, 1.43
Less poor	0.99	0.80, 1.22	0.93	0.70, 1.19
Least poor	0.89	0.72, 1.10	0.73	0.55, 0.95
Physical environment				
Household within main village	0.72	0.59, 0.88	0.84	0.65, 1.08
Travel distance to nearest health facility				
<1km	1.00		1.00	
1-4.9 km	1.27	1.09, 1.47	1.84	0.88, 3.44
≥5 km	0.88	0.76, 1.01	1.85	0.76, 3.78

[*] Non-spatial models were fitted in STATA

[†] Spatial Bayesian models were fitted in WinBUGS

The results from the non-spatial bivariate logistic regression between *S. mansoni* and the environmental covariates are summarized in Table 2. The annual mean NDVI showed the strongest association with *S. mansoni* infection among the set of covariates investigated. The association was highly negative; hence high *S. mansoni* infection prevalences were characterized by low NDVI values (OR = 0.001, *P* < 0.001). Elevation was also negatively associated with the prevalence of *S. mansoni* infections at the school level (OR = 0.21, *P* < 0.001). Children living above 400 m elevation were less likely to be infected with *S. mansoni*.

Spatial analysis of *S. mansoni* infection

The results of the bivariate spatial models between *S. mansoni* and demographic, socio-economic and physical environment covariates are shown in Table 1, and the results between *S. mansoni* and environmental covariates are summarized in Table 2. Age group, sex, annual rainfall and elevation remained significant covariates when spatial correlation was taken into account. The fifth wealth quintile was significant in this bivariate spatial model. NDVI, which was highly significant in the non-spatial model, was not related to *S. mansoni* infection prevalence after accounting for spatial correlation.

Results of a multivariate non-spatial model (Model 1) and three different multivariate spatial models (Models 2-4) are summarized in Table 3. The lower DICs of the spatial models underscore the importance of taking into account spatial correlation when analyzing data in space. To assess whether environmental factors or socio-economic status had a more pronounced effect on *S. mansoni* infection, the goodness of fit of Models 2-4 were compared with each other. Model 2 included schoolchildren's demographic covariates and their socio-economic status. Model 3 was built on demographic covariates and elevation alone, whereas Model 4 additionally had schoolchildren's socio-economic status included. Model 4 showed the best fit. Schoolchildren's socio-economic status was more important than elevation in explaining the spatial distribution of *S. mansoni* infection. Adding the rainfall covariate to Model 4 did not improve the fit of this model.

In the current epidemiological setting, the minimum distance at which spatial correlation between two locations was below 5% was 7.5 km.

Table 2 Bivariate associations between *S. mansoni* infection prevalence and environmental indicators arising from non-spatial and spatial logistic models. Odds ratios (OR) are displayed with their respective 95% confidence intervals (CI).

Environmental indicator	Non-spatial models[*]		Spatial models[†]	
	OR	95% CI	OR	95% CI
Minimum LST				
<25.0°C	1.00		1.00	
25.0-26.4°C	0.81	0.71, 0.91	0.99	0.43, 1.91
≥26.5°C	0.34	0.27, 0.42	0.43	0.10, 1.21
Mean LST				
<25.0°C	1.00		1.00	
25.0-26.4°C	0.57	0.50, 0.66	1.06	0.48, 2.02
≥26.5°C	0.27	0.22, 0.33	1.88	0.33, 5.89
Maximum LST				
<25.0°C	1.00		1.00	
25.0-26.4°C	1.48	1.03, 2.11	3.05	0.41, 10.88
≥26.5°C	1.02	0.71, 1.45	4.83	0.64, 17.31
Day-night temperature difference				
<6.0°C	1.00		1.00	
6.0-7.9°C	0.50	0.44, 0.58	1.85	0.63, 4.21
≥8.0°C	0.33	0.26, 0.42	1.90	0.27, 6.41
Rainfall	0.99	0.99, 0,99	0.99	0.99, 0.99
NDVI	0.001	0.0003, 0.006	6.35	0.00, 183.6
Land cover				
Forest	1.00		1.00	
Savannah	0.99	0.87, 1.12	1.12	0.59, 1.96
Cropland	0.75	0.61, 0.90	0.74	0.30, 1.55
Soil types				
Ferrallitic soils	1.00		1.00	
Hydromorphic mineral soils	1.96	1.63, 2.36	1.08	0.38, 2.50
Elevation				
<400 m	1.00		1.00	
≥400 m	0.21	0.17, 0.25	0.22	0.08, 0.54
Slope of the landscape				
<20°	1.00		1.00	
20-39°	0.03	0.01, 0.08	0.23	0.01, 1.21
40-59°	0.32	0.24, 0.43	1.22	0.19, 4.10
≥60°	0.18	0.07, 0.43	0.34	0.02, 1.70
Distance to permanent water bodies				
<500 m	1.00		1.00	
500-999 m	1.33	1.14, 1.55	1.02	0.45, 2.03
≥1000 m	1.21	1.05, 1.40	0.58	0.24, 1.19

[*] Non-spatial models were fitted in STATA

[†] Spatial Bayesian models were fitted in WinBUGS

Table 3 Comparison of four Bayesian models fitted in WinBUGS. Values represent mean odds ratios (OR) with 95% confidence intervals (CI).

Covariate	Model 1 (non-spatial)		Model 2 (spatial)		Model 3 (spatial)		Model 4 (spatial)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Demography								
Age								
6-10 years	1.00		1.00		1.00		1.00	
11-16 years	1.27	1.11, 1.46	1.45	1.24, 1.71	1.47	1.25, 1.72	1.46	1.24, 1.71
Sex								
Female	1.00		1.00		1.00		1.00	
Male	1.18	1.03, 1.36	1.21	1.03, 1.42	1.22	1.04, 1.43	1.21	1.03, 1.41
Socio-economic status								
Most poor	1.00		1.00				1.00	
Very poor	1.06	0.84, 1.31	1.12	0.85, 1.44			1.12	0.85, 1.44
Poor	1.07	0.86, 1.33	1.09	0.83, 1.40			1.08	0.82, 1.39
Less poor	0.97	0.78, 1.20	0.92	0.70, 1.84			0.91	0.69, 1.18
Least poor	0.72	0.58, 0.89	0.74	0.56, 0.96			0.73	0.55, 0.95
Environment								
Elevation								
<400 m	1.00				1.00		1.00	
≥400 m	0.20	0.16, 0.24			0.22	0.07, 0.53	0.20	0.07, 0.50
Sigma[*]			2.80	1.37, 6.44	1.73	0.92, 3.71	1.72	0.93, 3.53
u[†]			0.0001	0.00004, 0.0003	0.0003	0.00007, 0.002	0.0004	0.00008, 0.002
DIC[‡]	**4717.09**		**4025.43**		**4029.26**		**4025.03**	

[*] Sigma is the estimate of the geographical variability.

[†] u is the smoothing parameter (correlation decay), measuring the range of the geographical dependency. $3/u$ indicates the minimum distance at which spatial correlation between two locations becomes less than 5%.

[‡] DIC is the measure for the model fit. A smaller DIC indicates a better fit of the model data.

Prediction of *S. mansoni* infection

The predicted prevalence of *S. mansoni* infection among school-age children for the non-sampled locations is shown in Figure 3. Low prevalences were predicted for the north-eastern part, whereas high prevalences were predicted for the south-western part of the study area.

The standard deviation of the predicted *S. mansoni* infection prevalences is given in Figure 4. This figure shows that with increasing distance from surveyed locations the error of the prediction increased.

Figure 5 shows the random effects of the predictive model at non-sampled locations. It indicates how much is explained by other factors, which are not included in Model 4. In areas of low predicted *S. mansoni* infection prevalence, the random effect had the highest negative values, whereas in areas of high predicted prevalence, the random effect had the highest positive values.
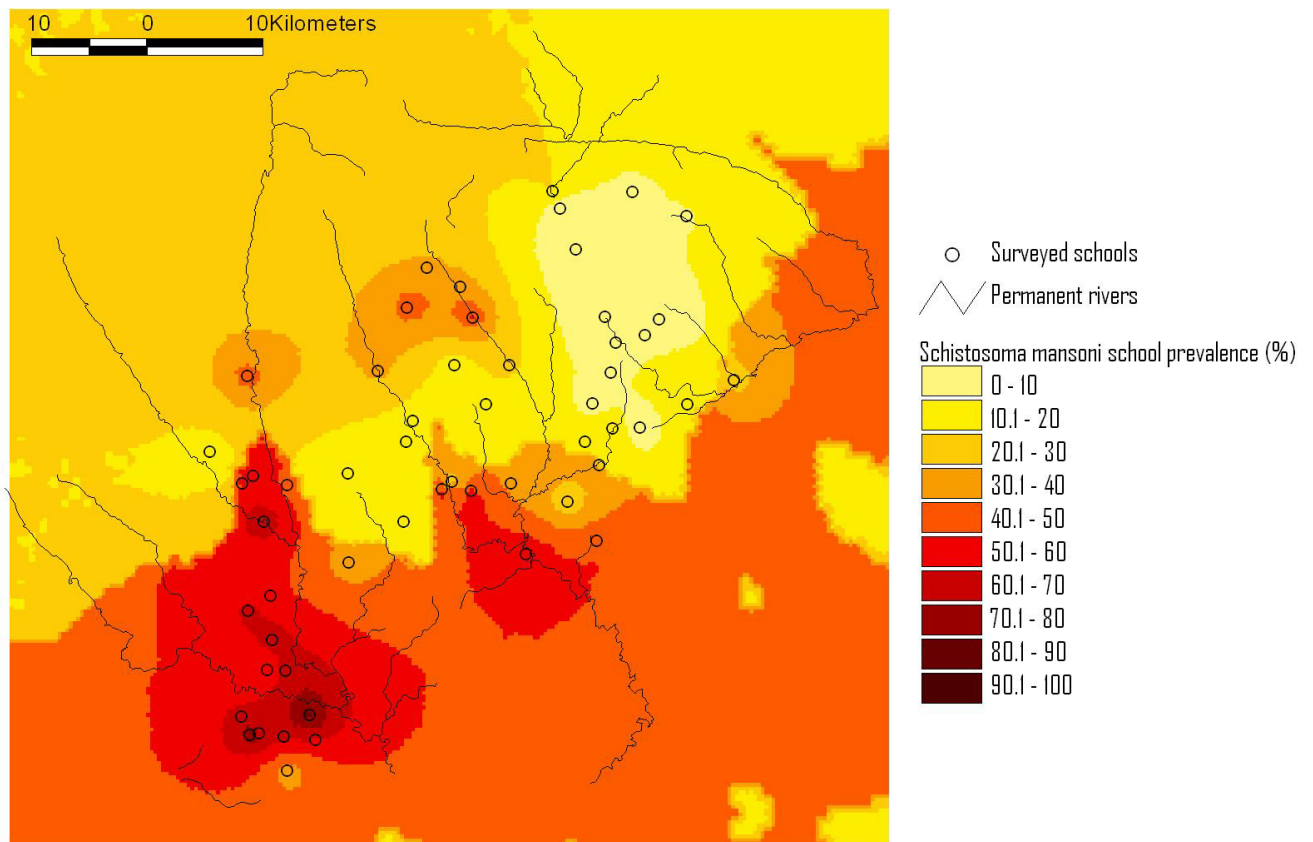

Figure 3 Smoothed map of the predicted *S. mansoni* infection prevalence based on Model 4 and produced with Bayesian kriging for the region of Man, western Côte d'Ivoire.

Figure 4 Smoothed map with standard errors of the predicted *S. mansoni* infection prevalence based on Model 4 and produced with Bayesian kriging for the region of Man, western Côte d'Ivoire.
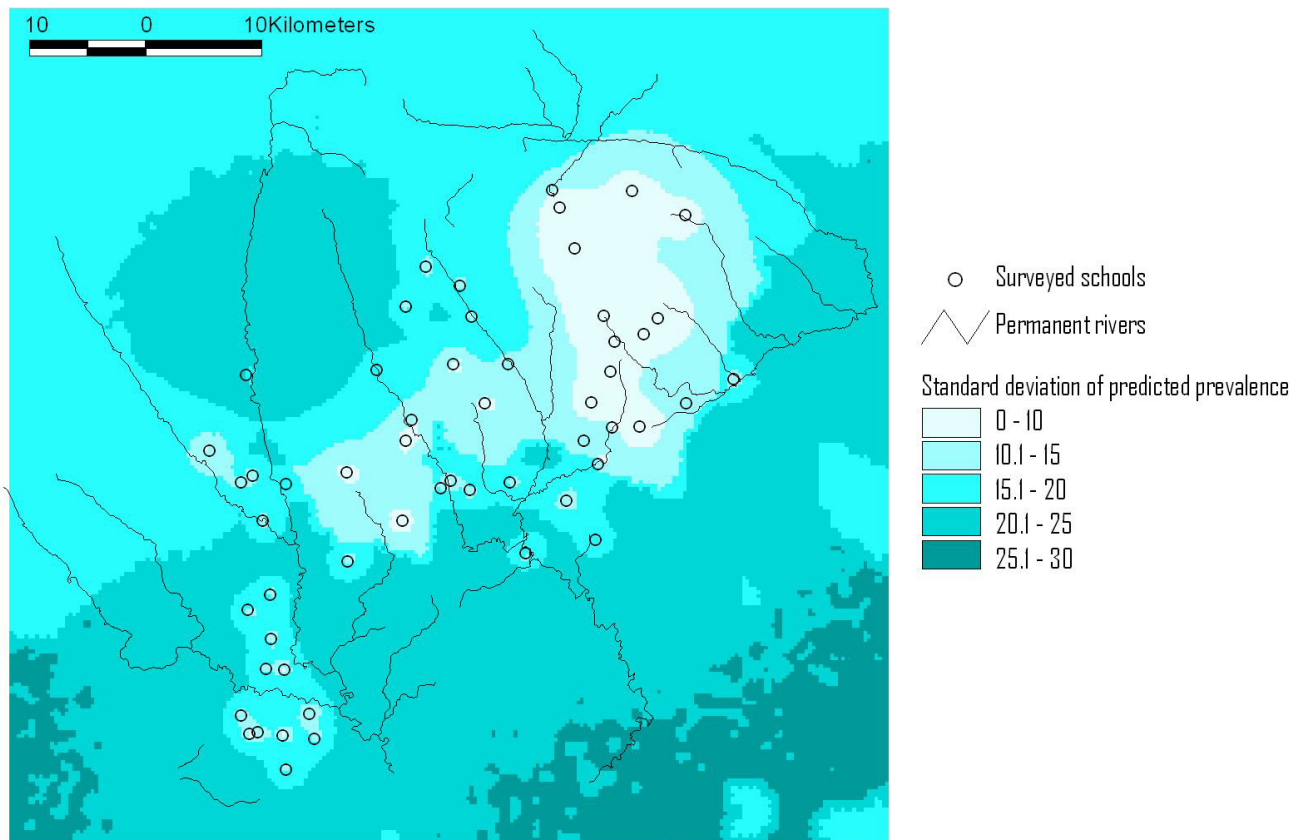
6.5 Discussion

Microscopic examination of a single Kato-Katz thick smear from a population sample of 3818 schoolchildren in rural western Côte d'Ivoire revealed an overall *S. mansoni* infection prevalence of 38.9%. These results confirm the high endemicity of this parasitic infection in this epidemiological setting (Utzinger *et al.* 2000; Raso *et al.* 2005). Analysis at the school level showed that the infection prevalence of *S. mansoni* varied considerably from one location to another, often within short distances, confirming small-scale focality of this parasite (Greer *et al.* 1990; Ratard *et al.* 1990; Gryseels, 1991; Lengeler *et al.* 2002).
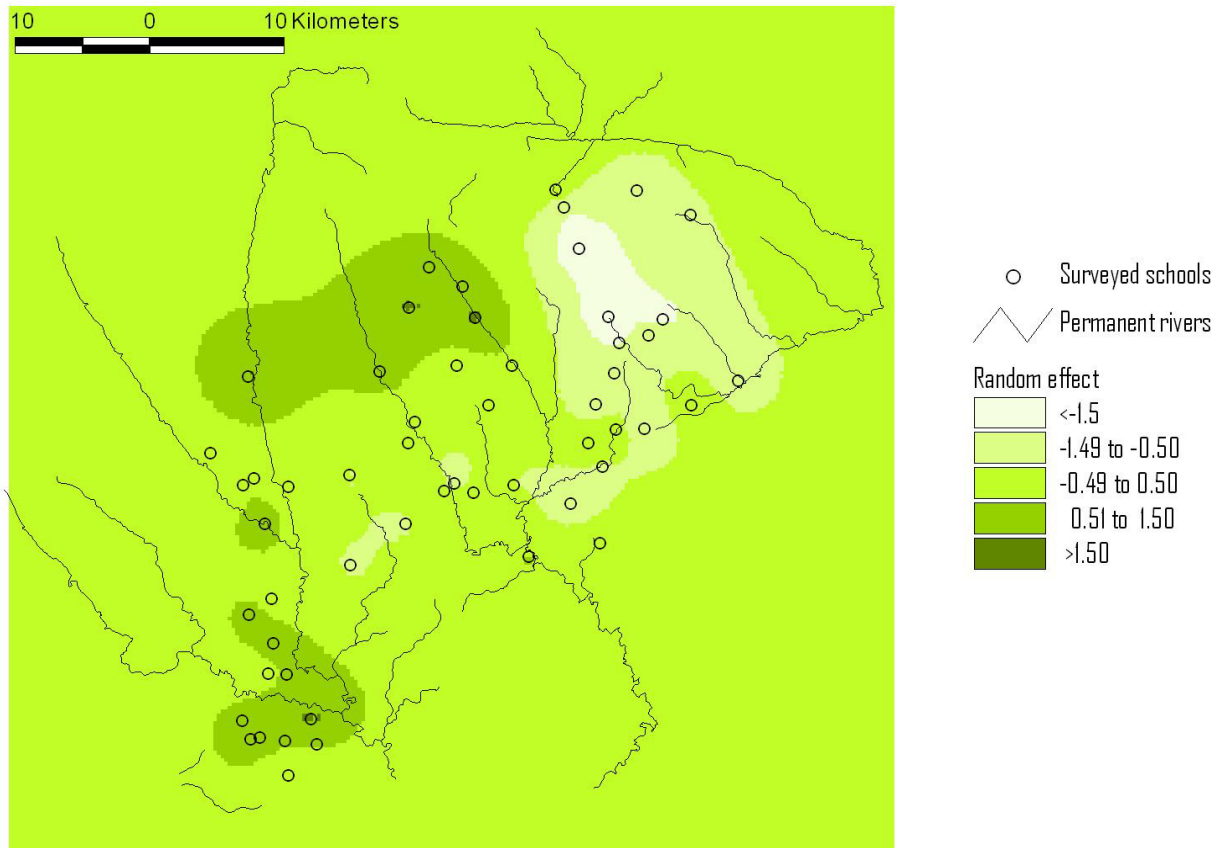
Figure 5 Smoothed map with location-specific random effects based on Model 4 and produced with Bayesian kriging for the region of Man, western Côte d'Ivoire.

Bayesian spatial statistical analysis revealed that the covariates age, sex, 5th wealth quintile, elevation and rainfall explainined the geographical variation of *S. mansoni* infection prevalence. Interestingly, age, sex and socio-economic status showed stronger influence on the geographical variation of this parasite at small-scale when compared to the environmental covariates investigated.

Three methodological shortcomings are important to note. First, diagnostic sensitivity of a single Kato-Katz thick smear is low due to the significant day-to-day and intra-specimen variation of *S. mansoni* egg counts in stool samples (Engels, Sinzinkayo & Gryseels, 1996; Utzinger *et al.* 2001; Booth *et al.* 2003; Berhe *et al.* 2004; Raso *et al.* 2004). However, mainly light infections are being missed, while moderate and heavy infections, which are particularly important from a public health point of view, are more likely to be detected. Second, we have

employed environmental factors with different spatial resolutions. Using data at different spatial resolutions may affect the model results by introducing bias (Levin, 1992). Third, the alignment between the environmental covariates and the locations where the *S. mansoni* prevalence data have been collected (i.e. school) introduces a bias, since the environmental features correspond to a wider geographical area than single point measures (Curran *et al.* 2000).

Previous work has been done to investigate how exposure affects *S. mansoni* infection. Distance to transmission sites, human water contact patterns, and household characteristics were associated with individuals or groups at highest risk of *S. mansoni* infections (Husting, 1983; Bethony *et al.* 2001, 2004; Gazzinelli *et al.* 2001). For example, micro-geographical studies carried out in Brazil and Kenya showed that distance from households to streams was negatively associated with *S. mansoni* infection (Kloos *et al.* 1997; Kloos, Gazzinelli & van Zuyle, 1998). With a view to approximate exposure related to water contact, we have used the distance to permanent rivers. In the non-spatial logistic regression analysis, this feature was significantly associated to an *S. mansoni* infection. However, in the spatially-explicit models, distance to permanent water bodies showed no significant association. It follows that water contact activities approximated by distance to rivers may be more complex than previously proposed. Consequently, it seems difficult to capture water contact activities adequately with this single variable at a meso-geographical scale, because the relationship may be biased by uneven distribution of intermediate host snails.

The results of the best fitting spatial model (Model 4) showed that the 5th wealth quintile explained a significant part of the geographical variation of *S. mansoni*, as shown by the improved goodness of fit of the model. One possible explanation arises from distance to health care delivery centres. However, as previously shown and confirmed in this study, there is no significant association with the school prevalence of *S. mansoni* infection and physical access, as measured by travel distance, to the nearest health facility (Raso *et al.* 2005). Household decision-making processes may play an equal or even more important role in this case. Underlying reasons include health seeking behaviour, transport costs to a dispensary, treatment costs, and knowledge and perception of the disease by household members, as recently shown in studies from Tanzania (Armstrong Schellenberg *et al.* 2003). On the other hand, socio-economic status may also be an indicator for the presence of a latrine in the richer wealth quintiles, which in turn is a protective factor for *S. mansoni* transmission. Taken together, socio-economic status plays a role in the small-scale distribution of *S. mansoni*, but

it acts as an aggregate measure for multiple risk factors that are connected to well-being and equity.

Elevation and rainfall were the 2 most important environmental covariates to capture the spatial distribution of *S. mansoni* infection prevalence. These 2 covariates were highly correlated. It is plausible that the mountains in the northern part of the study area are responsible for the higher rainfall observed there, generating distinct regional climate conditions. It is speculated that both covariates have an effect on the flow velocity of rivers, which in turn is likely to influence the presence of *B. pfeifferi*, the intermediate host snail of *S. mansoni*. Malacological studies have shown that *B. pfeifferi* tolerate a maximum flow speed of up to 0.3 m/s (Appleton, 1978; Kloos *et al.* 2001). Recently, the slope of the landscape has been proposed as a proxy for water flow (Brooker & Michael, 2000). Although this covariate showed no significant association to *S. mansoni* infection prevalence in the spatial models presented here, the elevation, which is related to the slope, was significant. Elevation thus is a useful covariate to indicate higher flow speed in the mountainous part of the present study area. In addition to elevation, rainfall is another covariate that is likely to increase the flow speed of rivers (Brooker & Michael, 2000). It follows that *B. pfeifferi* are less likely to populate rivers in mountainous parts. Snails are more likely to proliferate in the plain, which would explain why children living at locations below 400 m were at a 5-fold higher risk of an *S. mansoni* infection when compared to those living at altitudes above this threshold.

In contrast to previous GIS/RS applications with an emphasis on schistosomiasis, the study presented here is, to our knowledge, one of the first attempts to carry forward Bayesian geostatistics for assessment of environmental risk factors. The results underscore the importance of using appropriate geostatistical approaches for the analysis of spatially-explicit data. Different environmental covariates were tested for their association with the school prevalence of *S. mansoni* infection. The majority of the environmental factors were significantly associated to *S. mansoni* infection in the non-spatial logistic regressions. However, when adding a spatial component to the logistic models, NDVI, LST, and day-night temperature difference were not significant anymore. This finding is expected since omission of spatial correlation underestimates the standard errors of the covariate coefficient (Cressie, 1993).

The results show that demographic covariates and socio-economic status play a more important role on the small-scale variation of *S. mansoni* infection than any of the environmental covariates investigated, as assessed by the DIC. However, in areas of either

low or high prevalences, model predictions were less certain, suggesting that other risk factors (e.g. of behavioural or genetic nature, which were not included in the analysis) might also play important roles in these areas. Furthermore, ecological details at small-scale that are important for *B. pfeifferi* habitat formation (Genner & Michel, 2003), and which are suppressed in RS studies at broad or regional scale, may also be at the origin of the uncertainty expressed in our model. Although the random effects and standard errors of the predictions teach us about the model uncertainty, our model needs to be validated in order to assess its reliability, e.g. by random sampling at other school locations that have not been currently included.

Concluding, smoothed maps of *S. mansoni* infection risk, together with the standard error of the predictions and random effects displaying uncertainties, were generated for the region of Man. These maps can be used by decision-makers for the design and implementation of *S. mansoni* control interventions reaching those at highest risk. An important next step is to validate the models in similar ecozones. If they perform well, predictive modelling can guide schistosomiasis control programmes in similar eco-epidemiological settings of Côte d'Ivoire and elsewhere in sub-Saharan Africa.

6.6 Acknowledgments

Appendix

Let Y_{ij} and p_{ij} be the status and probability of *S. mansoni* infection, respectively, of schoolchild j in village i. We assume that Y_{ij} arises from a Bernoulli distribution, $Y_{ij} \sim Be(p_{ij})$. We model covariates $\underline{X}_{ij}$ and village-specific random effect ϕ_i on the $\log it(p_{ij})$, that is $\log it(p_{ij}) = \underline{X}_{ij}^T \underline{\beta} + \phi_i$, where $\underline{\beta}$ is the vector of regression coefficients. We introduce the spatial correlation on the ϕ_i's by assuming that $\underline{\phi} = (\phi_1, \phi_1, ..., \phi_N)^T$ has a multivariate normal distribution, $\underline{\phi} \sim MVN(\underline{0}, \Sigma)$, with variance-covariance matrix Σ. We also assume an isotropic stationary spatial process, where $\Sigma_{kl} = \sigma^2 \exp(-u d_{kl})$, d_{kl} is the Euclidean distance between villages *k* and *l*, σ^2 is the geographical variability known as the sill, *u* is a smoothing parameter that controls the rate of correlation decay with increasing distance and measures the range of geographical dependency. The range is defined as the minimum distance at which spatial correlation between locations is below 5%. This distance can be calculated as $\frac{3}{u}$ meters.

Following a Bayesian model specification, we adopt prior distributions for the model parameters. We choose vague Normal distributions for the $\underline{\beta}$ parameters with large variances (i.e. 10 000), an inverse gamma prior for σ^2 and a uniform prior for *u*. MCMC simulation was applied to fit the models. We run a single chain sampler with a burn-in of 5000 iterations. Convergence was assessed by inspection of ergodic averages of selected model parameters. The chain thereafter sampled every single iteration until a sample size of 5000 had been attained.

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7.   Discussion and conclusions

The overarching goal of this thesis was to enhance our understanding of demographic, ecologic, environmental and socio-economic factors that influence disease distribution in space in the region of Man, western Côte d'Ivoire. This knowledge base, in turn, facilitated the creation of risk maps and predictions of parasitic infections. The results from our cross-sectional surveys confirmed that several parasitic diseases were common among schoolchildren, as well as in younger and older study participants (Utzinger *et al.*, 2000b; c; Keiser *et al.*, 2002b). For example, in our community-based study carried out in 561 villagers of all ages living in a single village, the prevalences of *P. falciparum*, hookworm, *E. histolytica/E. dispar* and *S. mansoni* were 76.4%, 45.0%, 42.2% and 39.8%, respectively. Polyparasitism was very common. Treatment of *S. mansoni* infected people with a single oral dose of praziquantel at 40 mg/kg was efficacious. The overall cure and egg reduction rates were 60.9% and 61.4%, respectively. Several parasitic infections showed strong associations with age, sex and other parasite species. Of considerable importance from public health and control points of view were the significant positive association between *S. mansoni* and hookworm infections. This result confirmed previous observations made in neighbouring villages (Keiser *et al.*, 2002a; b). In the above-mentioned population sample, and after adjusting for age and sex, several parasitic infections were associated with different morbidity indicators. A relationship between socio-economic status and parasitic infections and self-reported morbidity, as well as physical access to health care, was identified. In the case of *S. mansoni* infections, results showed that age, sex, socio-economic status, rainfall and elevation explained the spatial distribution of this parasite in our epidemiological setting. Interestingly, demographic factors and socio-economic status had stronger influence on the model fit than environmental factors.

Our work is attached with several methodological shortcomings that have been discussed in the previous chapters. The key limitations are briefly summarized here. First, because of the large number of parasites examined, it was not feasible to carry out repeated sampling of stool and blood specimen during our regional cross-sectional parasitological survey that enrolled more than 4,000 schoolchildren. Similarly, in the community-based survey carried out in a single village, *Plasmodium* and intestinal protozoa were examined by a single blood or stool sample, respectively. It follows that parasite prevalences and the frequency of polyparasitism were underestimated due to the sensitivities of the diagnostic tools, which are below 100% (Raso *et al.*, 2004a; b). Second, inequities in parasitic infections,

perceived ill health and access to health care were measured among schoolchildren only. By focussing on this segment of the population, the results may not be representative of the whole population in this region (Raso *et al.*, 2005b). Implications of this selection bias and approaches to remedy this shortcoming have been discussed, which are in line with previous research carried out elsewhere (Filmer & Pritchett, 2001). Third, for the spatial analysis of *S. mansoni* infection risk, we have used data of different sources and at different spatial resolutions. Hence, this may have introduced bias into the model results (Raso *et al.*, 2005a). A fourth shortcoming is that the recall period of the school questionnaires pertaining to morbidity indicators was one month. This may have biased the results, as schoolchildren may not report mild disease events, which are likely to be forgotten, compared to more severe events or chronic morbidity (Moestue *et al.*, 2003; Zere & McIntyre, 2003). However, it has been shown recently that for reported "blood in urine", recall periods of two or four weeks produced similar results (van der Werf *et al.*, 2003).

One of the strengths of this work was that in the community-based parasitological survey, three consecutive stool specimens were collected and analysed for the presence of *S. mansoni* and soil-transmitted helminth infections. Such a rigorous diagnostic approach increased the sensitivity, and hence the accuracy at which associations between parasite species and the cure and egg reduction rates of praziquantel against *S. mansoni* were assessed (Raso *et al.*, 2004a; b). Furthermore, the questionnaire developed for the rapid assessment of socio-economic status among schoolchildren, allowed to approximate – through a simple household asset-based approach – socio-economic status without the need to collect data on household expenditure (Raso *et al.*, 2005b). This asset-based approach has been validated by Filmer & Prichett (2001) in a study conducted with Indian schoolchildren. Similar questionnaires with a set of household assets have been used in different African settings, which showed a good correlation to income (Morris *et al.*, 2000). Another strength of the study was that we used Bayesian geostatistical methods to predict infection risk at unsampled locations (Raso *et al.*, 2005a). This statistical analysis enabled at the same time to evaluate the model precision (e.g. standard deviation of prediction) without the need to refer to external (e.g. split sample) or internal (e.g. jack-knifing) data-based validation procedures (Graham *et al.*, 2004).

The results of the work presented here are of significance for the control of parasitic diseases and can find direct application in the region of Man and, perhaps, elsewhere. In

addition, our research has identified important gaps where more pointed emphasis is needed. These aspects are offered for discussion in the next sections.

7.1 Polyparasitism

A consistent finding of our work is that polyparasitism currently is very common in the western part of Côte d'Ivoire. In the community under investigation, from a single village, more than three-quarters of the participants, including all age groups, harboured three or more parasitic infections concurrently (Raso *et al.*, 2004a). A similar observation was made among schoolchildren from 57 rural schools; four out of five children harboured three or more parasite species concurrently (Raso *et al.*, 2005b). Our results are in full agreement with previous studies carried out in western Côte d'Ivoire (Utzinger *et al.*, 1999; Keiser *et al.*, 2002a; b). That polyparasitism is the norm rather than the exception has also been shown in other recent studies done elsewhere in sub-Saharan Africa, and other tropical and subtropical regions of the world (Guignard *et al.*, 2000; Waikagul *et al.*, 2002; Tchuem Tchuenté *et al.*, 2003). In view of these findings it is surprising that polyparasitism is a relatively neglected topic of research (McKenzie, 2005). However, there now seems to be growing interest in public health circles for integrated and combined control, because such an approach can substantially decrease morbidity and co-morbidity, in a timely and cost-effective manner (Molyneux & Nantulya, 2004). The results pertaining to polyparasitism in our study area have important implications for the control of parasitic diseases quite generally, hence it is discussed in more detail below.

In the community-based study a positive significant association between *S. mansoni* and hookworm infections was found. Our findings suggest that a combined and integrated control strategy, as recommended by WHO, would have great beneficial effects by decreasing the burden of disease due to these two parasite infections concurrently, in the region of Man.

Furthermore, we have observed a positive association between the intestinal protozoa *G. duodenalis* and *T. trichiura.* To our knowledge, this pairwise parasite association had not been described in the literature before. A similar association between *T. trichiura* and the intestinal protozoa *E. histolytica* has been described before (Dönges, 1988; Gilles, 2003). A light infection with *T. trichiura* usually does not result in clinical manifestations, but if associated with *E. histolytica,* it can lead to severe colitis. This suggests that *T. trichiura* facilitates the invasion of *E. histolytica* into the tissues. Our diagnostic approaches, i.e. light microscopy, did not allow to differentiate between the pathogenic *E. histolytica* and the

morphologically identical non-pathogenic, and probably more prevalent *E. dispar,* which might explain why we did not find this association (Heckendorn *et al.*, 2002; Raso *et al.*, 2004a). Further research is warranted to elucidate whether *G. duodenalis* and *T. trichiura* interact in a similar way as described above. If that is the case, control strategies targeted against soil-transmitted helminths (i.e. through administration of albendazole or mebendazole), could reduce helminth-induced morbidity, but also co-morbidity due to concurrent infections with pathogenic intestinal protozoa.

Another significant parasite association that emerged from our epidemiological setting, which had already been described in the literature, is the one between *P. falciparum* and *P. malariae*. For example, it has been shown that individuals experiencing a *P. falciparum* infection following an infection with *P. malariae*, had lower parasitaemia and clinical disease (Black *et al.*, 1994; Collins & Jeffery, 1999). Hence it has been suggested that, factors that remain to be elucidated, limit mixed *Plasmodium* species infections, and that mixed-species infections protect against severe *P. falciparum* malaria (Zimmerman *et al.*, 2004). However, the opposite had been reported almost 40 years ago, namely an increased parasitaemia in mixed *Plasmodium* infections compared to single species infections (Jeffery, 1966). The current understanding of mixed species infections is rudimentary. New research is required, as enhancement of our understanding of mixed infections has implications for the development and testing of vaccines and new antimalarial drugs. Hence, concerted efforts are needed to understand the biological mechanisms that regulate mixed *Plasmodium* infections (Zimmerman *et al.*, 2004).

At this point it should be re-emphasised, that one of the limitations in elucidating patterns of parasite-parasite associations is that the diagnostic results strongly depend on the technique employed. For example, the sensitivity of the widely used microscopical blood film examination can vary considerably between laboratories, yet results may be affected in different ways across epidemiological settings, rendering comparison between studies difficult. To remedy the lack of sensitivity of commonly employed diagnostic techniques repeated sampling is necessary. This approach, however, is time consuming, expensive and reduces study compliance. Another limitation of investigating polyparasitism is that different diagnostic techniques have to be employed for different biofluids to detect multiple parasites. For example, we have used three different techniques to investigate 15 different parasite species. Yet, additional parasitic infections, such as *Cryptosporidium,* bacterial infection (e.g. *Escherichia coli*) or viral infections, which were not investigated in our study, may be

responsible for an important part of the disease burden. Recent work in neighbouring Ghana has shown that *Cryptosporidium* and *E. coli* causes considerable distress in children (Addy *et al.*, 2004). There is a pressing need to develop novel and non-invasive diagnostic tools with high sensitivity and high specificity, which allow to investigate several parasites at the same time (Raso *et al.*, 2004a). We are currently in the process of assessing whether nuclear magnetic resonance (NMR) spectroscopy-based and mass spectrometry (MS)-based metabonomic approaches can play a seminal role for biomarker identification (Lindon *et al.*, 2004). This could reveal the overall metabolic consequences of evolving human internal ecosystems that encompass microfloral-parasite-host interactions. Recently, the metabolic signature of an *S. mansoni*-infected mouse by analysing urine samples with high-field ^{1}H NMR and multivariate pattern recognition techniques was reported (Wang *et al.*, 2004).

To conclude, our results, and those from others, indicate that polyparasitism is very common in the developing world, but only few studies examined pair-wise parasite associations, its effect on morbidity and co-morbidity and the ecologic and immunological nature regulating relationships among multiple parasites and their hosts. The development of novel diagnostic tools with high sensitivity and high specificity is of great relevance and will be of leverage to approach some of these goals. Finally, addressing the root ecologic and behavioural causes, i.e. improvement of access to clean water and sanitation, coupled with sound hygiene behaviour, will decrease the frequency of polyparasitism (Utzinger *et al.*, 2003a).

7.2 Monitoring drug efficacy

Recently launched large-scale chemotherapy-based control programmes for schistosomiasis and soil-transmitted helminthiasis have resulted in the increased use of praziquantel and albendazole/mebendazole, respectively, in many parts of sub-Saharan Africa. Large-scale administration of anthelmintic drugs is well known to have been an important factor in the development of drug resistance in animals. Hence there is considerable concern that anthelmintic drug resistance may develop and spread also in human populations (Geerts & Gryseels, 2000, 2001; Albonico *et al.*, 2004).

Yet, reports on praziquantel resistance of clinical significance are limited and the potential negative effect of the large-scale chemotherapy campaigns on the development and spread of praziquantel resistance is unknown (Hagan *et al.*, 2004). There are several reports of treatment failures in Egypt (Cioli, 2000; Doenhoff *et al.*, 2002) and Senegal (Gryseels *et al.*,

1994; Gryseels *et al.*, 2001). Isolates of schistosomes from Egypt and Senegal that had survived praziquantel treatment, were shown to have a lower susceptibility to praziquantel than either freshly isolated schistosomes or laboratory parasites (Ismail *et al.*, 1994; 1996; 1999; Fallon *et al.*, 1997; Liang *et al.*, 2001; Doenhoff *et al.*, 2002). The low cure rates observed in Senegal, however, can also be explained by the stage-specific efficacy of praziquantel (Xiao *et al.*, 1985; Sabah *et al.*, 1986; Danso-Appiah & de Vlas, 2002). The excretion of eggs from an adult worm that was in a drug unsusceptible juvenile stage at the time of praziquantel treatment can give the impression of treatment failure. Hence, repeated treatment significantly increases the efficacy of the drug compared to a single dose (Picquet *et al.*, 1998; Utzinger *et al.*, 2000a; N'Goran *et al.*, 2003). Our results showed that the cure rate of a single oral dose of praziquantel at 40 mg/kg in our community under investigation was 60.9%, which is somewhat lower than expected. In addition, the overall egg count reduction was also lower (61.4%) than what is usually reported in the literature (WHO, 2002; Utzinger & Keiser, 2004). Although our findings may raise suspicion of resistance, we could show that low cure rates were mainly observed among patients with heavy and moderate infections, whereas light infections had a cure rate of 70.3%, which is in the expected range. Since we analysed three consecutive stool samples per individual, we have assessed a 'true' cure rate, compared to other studies that relied on one or two examinations, normally derived from a single stool specimen (de Vlas & Gryseels, 1992; Utzinger *et al.*, 2000a). Strikingly, the cure rate based on only one stool examination would have been 87.6% for our setting. Clearly, the different methodological approaches to assess cure rates make it difficult to compare cure rates across settings, and hence decrease the likelihood of identifying the onset of resistance development (Geerts & Gryseels, 2000; Danso-Appiah & de Vlas, 2002).

Concluding, monitoring drug efficacy by using 'gold standard' laboratory techniques adopting repeated stool sample examination or other sensitive diagnostic tools should become an integral part of schistosome and helminth control programme evaluations.

7.3 Perception of disease and access to health care

The use of simple questionnaires for the rapid assessment of parasitic infections proved useful for the identification of significant associations between several parasitic infections and self-reported morbidity indicators in the community-based survey, after adjusting for age and sex. Yet, one of the limitations that must be discussed is that, in view of polyparasitism being so common (almost 90% of the study population harboured a polyparasitic infection), it is

difficult to determine which infection is responsible for the experienced morbidity, particularly for the non-specific indicators, such as abdominal pain and/or diarrhoea.

In addition, in the regional school-based study we found a relationship between socio-economic status and self-reported morbidity. This observation suggests that the results of such morbidity questionnaires addressed to schoolchildren can be biased by socio-economic status. This important observation is in agreement with previous work. Different studies assumed that poorer people living in disease-endemic areas may consider the suffering to be part of their life, and hence report illness episodes frequently in severe cases, compared to economically better-off groups who even may over-report symptoms (Murray *et al.*, 1999; Zere & McIntyre, 2003). Importantly, in our research the morbidity indicator "blood in urine", which is widely used for the rapid appraisal of *S. haematobium*, and "blood in stool", which is an indicator for *S. mansoni*, showed significant positive linear relationships with children's socio-economic status. However, particularly for "blood in urine" it is difficult to make any conclusion from our findings, as the overall self-report of this indicator was very low due to hypo-endemicity of *S. haematobium* in the western part of Côte d'Ivoire. Hence, there is a need to investigate in an endemic area, over a large-scale, whether socio-economic status shows a similar significant relationship to *S. haematobium*. If this relationship is confirmed in other settings, calculations of the burden due to *S. haematobium* that make use of the indirect morbidity indicator "blood in urine", may underestimate disease burden because of a lower sensitivity of this indicator in poorer groups. Similar conclusions can be drawn for other morbidity indicators that are related to specific diseases. Adding socio-economic indicators to morbidity school questionnaires might provide a good means to adjust for socio-economic status when estimating burden of disease.

An implication of differential perception of ill health by socio-economic status is that it may represent an important barrier to seek care in poorer groups. Yet, one of the prerequisite for integrated schistosomiasis control through the existing health care delivery system (i.e. self-report of "blood in urine" and "blood in stool") is adequate health seeking behaviour. Strikingly, results from a study in Ghana that investigated different steps from perceived symptoms to receiving proper treatment has shown that less than 5% of the cases with "blood in urine" or "blood in stool" finally received praziquantel from a health care service. Reasons for not seeking care include lack of financial means (transport costs) or the symptoms not being perceived as serious enough (Danso-Appiah *et al.*, 2004; de Vlas *et al.*, 2004). Studies from northern Senegal and Mali that evaluated case management of schistosomiasis at

primary health care, showed that once a patient with schistosomiasis-related symptoms sought care at a health facility, the subject could expect to receive adequate diagnoses and treatment (van der Werf *et al.*, 2002; Landouré *et al.*, 2003). In our study area we have seen that *S. mansoni* shows a distinct spatial distribution, which was related to schoolchildren's socio-economic status. Hence, we have speculated that people living in villages without health facilities are less likely to receive praziquantel because they are less likely to seek care, as they do not perceive the symptoms to be severe enough. This could explain why schoolchildren from the fifth wealth quintile, who were more likely to live in villages with a health care facility, were at a lower risk of *S. mansoni*. It seems fundamental to introduce health education at community level and to reinforce it at school, so that knowledge about the disease is enhanced, facilitating that people can make the link between serious disease or symptoms and risk factors, which in turn may increase people's perception by changing their risk behaviour and health seeking behaviour. In the village where we have carried out the community-based survey, another two PhD studies are currently underway with an emphasis on social sciences and visual communication to readily adopt sound health messages. The aim of this complementing research is to understand the perception of disease in the community that will facilitate the development of communication tools. These communication tools will consist of messages addressed to the local population, which are based on knowledge, attitude and beliefs towards distinct diseases and risk factors, elicited through results from interviews at individual and household level, as well as focus group discussions.

However, access to health care from the health seeker side is also limited by costs, such as travelling, diagnosis at health care facilities, treatment and preventive measures. Although, we have measured access to health care simply by means of travel distance, we could show that poorer schoolchildren had longer journeys to the nearest health care delivery facility, which in turn results in higher travel costs. Hence, providing free treatment at health care structures to high risk groups, i.e. young children and pregnant women, might improve access to health services.

Our results pertaining to schoolchildren sleeping under a bednet showed a strikingly low coverage, as only 10.4% of the more than 4,000 interviewed schoolchildren gave a positive response. When our surveys were done, initiatives were under way to promote insecticide-treated nets (ITN) through local health facilities. Similarly low bednet use has been reported from Nigeria, where only 10-12% of household owned at least one untreated net (Onwujekwe *et al.*, 2004). Hence, our results suggest that social marketing, which has been successfully

implemented in Tanzania, should be considered as a strategy to increase the use of ITNs in our study region, which in turn will reduce the burden due to malaria (Abdulla *et al.*, 2001; Armstrong Schellenberg *et al.*, 2001). There has been some concern that social marketing employing discount voucher systems may not reach the ones at highest need (Mushi *et al.*, 2003). The ownership of ITNs showed a clear relationship to household economic status in our study setting, as none of the children from the poorest group reported sleeping under a bednet, whereas 22.4% of the wealthiest group gave a positive answer. Interviews with different key informants in selected villages confirmed that the current high costs of ITNs are a major barrier impeding its wider use. In a recent study from Tanzania, social marketing has also been shown to increase equity in ITN use; three years after a social marketing campaign commenced, the ratio of net ownership between the poorest and the richest quintile increased from 0.3 to 0.6 (Nathan *et al.*, 2004).

Concluding, it is known that public spending on health care favours the better-off disproportionally, hence targeting equity cannot be solved only by adjusting subsidy allocations. Our results, and those of others, suggest that constraints that prevent the poor from taking advantage of these services must be addressed. Further work should investigate the sequential steps from perceiving a symptom/disease to seek care at the formal health care sector. Results from such studies will allow to design more equitable and integrated disease control that is readily adapted to local needs.

7.4 Predicting the risk of parasitic infections

Schistosomiasis remains a public health problem in the developing world, especially in Africa south of the Sahara (WHO, 2002). There have been successful national control programmes. However, after external support for these programmes has ceased and large-scale administration of praziquantel came to an end, the pre-intervention levels of prevalence and infection intensity were often reached quite shortly (Engels *et al.*, 2002; Utzinger *et al.*, 2003a). Predicting the infection risk using GIS and RS has become an important tool to identify environmental factors at broad scale related to the risk of infection, and consequently allowing decision makers to allocate scarce resources in a cost-effective manner (Brooker *et al.*, 2002; Yang *et al.*, 2005). However, small scale focality is not captured by such broad scale approaches (Bavia *et al.*, 2001; Brooker, 2002). Needs are often locally distinct because of the small scale focality of schistosomiasis that has been observed also in our study region (Utzinger *et al.*, 2000c; Raso *et al.*, 2005a). Hence, we have modelled *S. mansoni* infection

risk in the region of Man by using age, sex, socio-economic status and elevation as covariates. The generated predictive models are valid for the region of Man and can guide schistosomiasis interventions in this setting. The produced maps indicate that predictions of the model are excellent in close proximity to sampled locations, but their validity is reduced as distance from the sampled locations increases. There is now a need to validate these models in other settings of Côte d'Ivoire and neighbouring countries with similar ecozones to assess their predictive accuracy. In case the models perform well, they can be used in *S. mansoni* endemic settings with similar ecologic features. However, we speculate that in settings with distinctly different ecologic features in other parts of Côte d'Ivoire (e.g. dryer savannah zone), they will probably fail to predict *S. mansoni*. Such a pattern has already been demonstrated with other models aiming to predict *S. haematobium* in Cameroon, which did not fit in ecologically different settings (Brooker *et al.*, 2002).

Interestingly, our model shows that demographic and socio-economic factors have much more influence on the model fit than environmental factors for prediction of *S. mansoni* risk. We have suggested in the previous chapter that household decision-making processes that influences health seeking behaviour, transport costs to the nearest formal health care system, treatment costs, knowledge and perception of diseases, as well as the use of latrines may be underlying factors explaining the spatial distribution (Raso *et al.*, 2005a). People's socio-economic status is of particular interest, as it may represent a macroscopic aggregate measure that inform the higher levels (regional scale) about lower level behaviour (fine scale). This suppression of details represents a strength rather than a weakness, as it allows to explain the observed ensemble without reference to details (Levin, 1992).

At first sight, we were surprised to learn from our results that environmental factors did not much improve the model fit as we expected, because previous research suggested that environmental and climatic factors are particularly useful covariates for prediction of schistosomiasis (Bavia *et al.*, 2001; Kristensen *et al.*, 2001; Malone *et al.*, 2001; Zhou *et al.*, 2001; Brooker *et al.*, 2002). Starting from the assumption that system description varies with the choice of scale (Levin, 1992), we believe that environmental and climatic factors explain rather mechanisms responsible for intermediate host snail distribution driven at broad scale than at regional scale, and hence predictability of models including environmental factors is better at broad scale. Thus, at regional scale, ecologic details may be suppressed, which are important for snail habitat formation at finer scale. In contrast, socio-economic status may increase the predictability, as it probably represents a part of an individual's behaviour at fine

scale. We now speculate that socio-economic status may loose its predictability at broad scale. A deeper understanding of the mechanisms acting at different scales is warranted to inform risk assessment and prediction of schistosomiasis and to guide its control. There is a clear need to explore the potential of socio-economic status on predicting the distribution of schistosomiasis in other settings at a district/regional scale, as well as at broader scale, by utilizing the existing corpus of school-based questionnaires. In addition, analysis of the spatial distribution of schistosomiasis within single communities, i.e. at the individual and household level, will further elucidate the underlying mechanism that are acting at a fine scale (Utzinger *et al.*, 2003b). It will be interesting to assess and quantify the association between socio-economic status and specific behavioural factors to explain the findings of such work. If socio-economic status performs well also in other settings, we propose a two step approach to predict schistosomiasis. First, at broad scale by the use of remotely sensed data, second, at regional scale using questionnaire data.

In a subsequent step, it will be interesting to adopt such models as described in our work (Raso *et al.*, 2005a) and by others (Brooker *et al.*, 2001; Kristensen *et al.*, 2001; Malone *et al.*, 2001) for other diseases that are common in our epidemiological setting, e.g. hookworm disease and malaria and disease combinations. An important aspect of this work will be the differential contribution of climatic and environmental factors, and socio-economic status that influence spatial and temporal distribution of disease. We anticipate that for hookworm infection, a similar pattern as for *S. mansoni* infection will be recognized, because soil-transmitted helminth infections and schistosomiasis have common epidemiological risk factors, most notably lack of improved sanitation, which is intimately connected to socio-economic status. For malaria, a similar socio-economic approach has been adopted by Brooker and colleagues (2004) to model malaria risk by cluster analysis, but socio-economic status did not explain the geographical distribution. The complex epidemiology of several parasitic infections varies from setting to setting and, probably, cultural aspects are relevant too for their geographical distribution. We are currently interested in including bednet coverage in spatial models to see how this covariate influences the spatial distribution of malaria in our epidemiological setting. Studies have been carried to assess the impact of ITN coverage in populations (Binka *et al.*, 1998; Hawley *et al.*, 2003). Recently, a study has been carried out by Abdulla and colleagues (2005) in a village of western Tanzania, to elucidate spatial effects of the social-marketing of ITNs on malaria morbidity. They could show that (i) children living in areas of moderately to high ITN coverage were at a lower risk of

moderate/severe anaemia and splenomegaly, irrespective of their net use, and (ii) the use of untreated nets had neither coverage nor short distance effects on anaemia or parasitaemia.

Finally, the commonness of polyparasitism implies the need to develop spatial multinomial regression models that can capture the risk of multiple parasite infections.

7.5 Conclusions

The starting point of this work was the assessment of parasitic infections and perception of morbidity within a community living in a single village. After rigorous parasitological screening of a random population sample, praziquantel treatment was offered to *S. mansoni*-infected study participants and its efficacy and side-effects were assessed. Adding a list of household assets, so that schoolchildren's socio-economic status could be captured, expanded an existing morbidity school questionnaire that was developed in the region of Man for the rapid assessment of *S. mansoni* infections several years ago (Utzinger *et al*., 1998; 2000c). This questionnaire, along with large-scale screening, was administered to all rural schools targeting over 4,000 schoolchildren. Finally the risk of *S. mansoni* infections in the region of Man was mapped and predicted by applying Bayesian geostatistical methods. Based on the results of the work presented in this thesis, the following conclusions can be drawn:

- Three-quarters of the screened population were infected with at least three parasites concurrently and several associations between parasites and between parasites and self-reported morbidity indicators were found. The results suggest that it is difficult to attribute non-specific morbidity indicators (e.g. abdominal pain or diarrhoea) to specific parasitic infections, as polyparasitism was very common.

- In view of polyparasitism being so common, interventions should address the root behavioural and ecologic causes of these parasitic infections to reduce the burden caused by these parasites.

- Praziquantel was efficacious against *S. mansoni* infections, curing 60.9% of *S. mansoni* positive study participants after one single oral dose at 40 mg/kg. Although there is no clear evidence that resistance to praziquantel exists, monitoring drug efficacy should become an integral part of disease control interventions that heavily rely on chemotherapy.

- Disparities in parasitic infections, perceived ill health and access to health care were identified between schoolchildren of different socio-economic groups. Our results suggest that interventions such as social-marketing for promoting ITNs, provision of effective treatment to high risk groups and health education are key for equitable and integrated disease control.

- *S. mansoni* spatial distribution analysis revealed that age, sex, socio-economic status, elevation and rainfall were explaining the spatial variation in the region of Man. The produced risk maps can be used by decision makers to guide schistosomiasis control interventions in this region.

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Curriculum vitae

1973	Born on 12 October in Grenchen (SO), Switzerland
1980-1989	Primary and secondary school in Grenchen (SO), Switzerland
1989-1992	**Commercial apprenticeship**
	Sphinxwerke Müller AG, Solothurn, Switzerland
1992-1995	**High School**
	Feusi Schulzentrum, Bern, Switzerland
• D-levels	Modern languages
1995-2001	**MSc in Biology**
	University of Basel, Swiss Tropical Institute (STI) & Cantonal Laboratory Baselland, Switzerland
• MSc thesis	"Microbiological changes of karst spring water: influence of rainfall and manure application"
	Supervision by Prof. Dr. Marcel Tanner (STI) and Dr. Adrian Auckenthaler (Cantonal Laboratory Baselland)
2001-2004	**PhD in Epidemiology**
	Swiss Tropical Institute (STI), Basel, Switzerland, Department of Public Health and Epidemiology
• PhD thesis	"Assessment, mapping and prediction of the spatial distribution of parasitic infections in western Côte d'Ivoire and implications for integrated control"
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