Effects of parasitic infections on clinical outcomes, self-rated quality of life and physical fitness in Côte d'Ivoire

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Für meine Familie in der Schweiz und der Côte d'Ivoire

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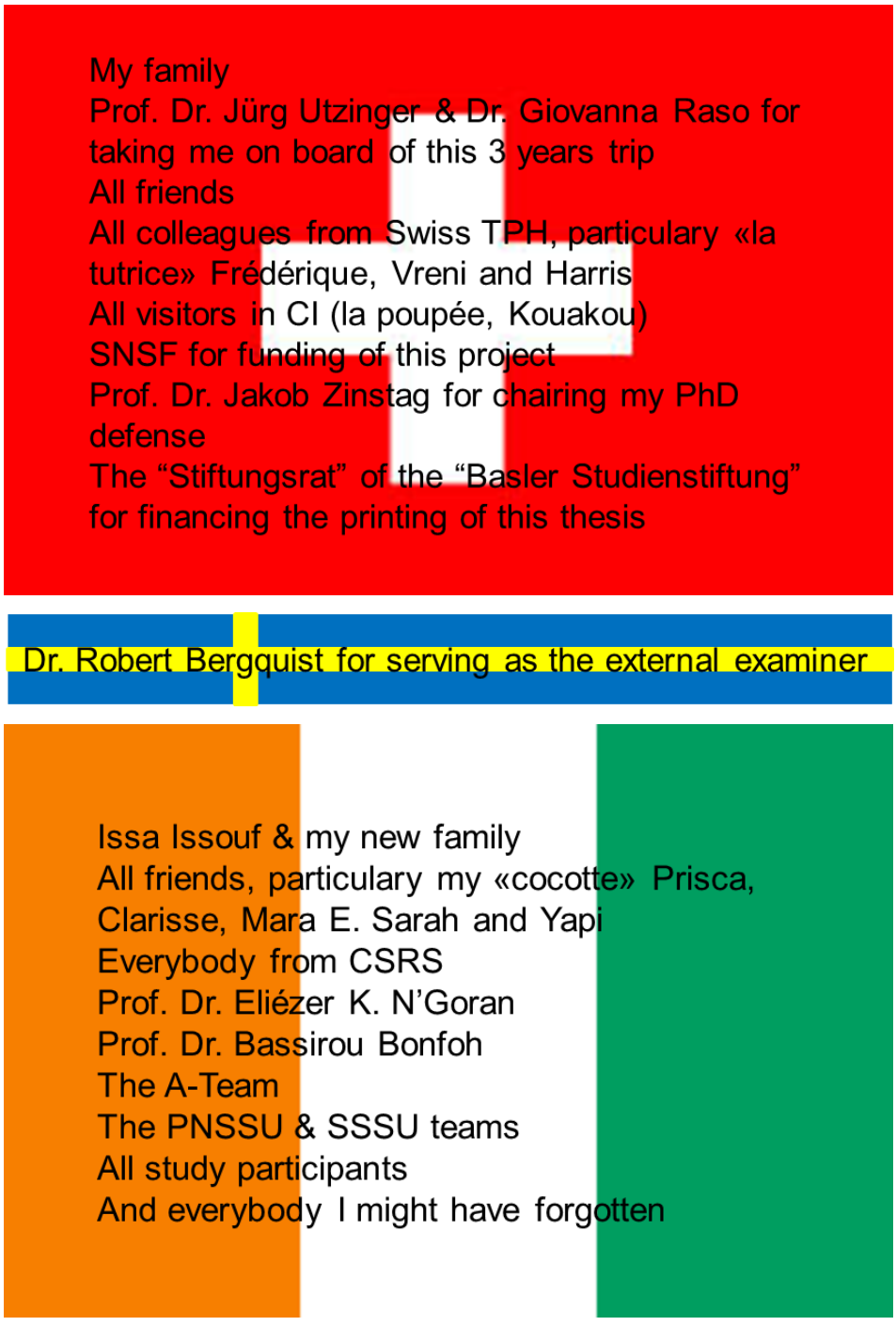

Summary

Background: Hundreds of millions of people in the developing world are at risk of parasitic diseases, such as malaria and neglected tropical diseases (NTDs). Health implications due to these diseases are manifold. Typical clinical manifestations from infection with *Plasmodium* spp. include anaemia and splenomegaly associated with erythrocyte death and splenic sequestration, respectively. Intestinal parasitic infections, such as helminth (e.g. soil-transmitted helminthiasis and schistosomiasis) and intestinal protozoa infections (e.g. amoebiasis and giardiasis) are rarely fatal, but cause long-term chronic morbidity. This may include anaemia and other nutritional deficiencies leading to impaired physical growth and cognitive development in children and reduced work capacity in adults. Concurrent infection with several of these parasites – polyparasitism – is the norm rather than the exception in countries like Côte d'Ivoire. To date, most research on parasitic disease-related morbidity, however, focused on single species infections. A deeper understanding of multiple species parasite infections and related morbidity is crucial for disease control and the reduction of the burden due to these (co-)infections.

Goal and specific objectives: The overarching goal of this Ph.D. thesis was to deepen our understanding of multiple species parasite infections and its implications for morbidity at local and regional levels in Côte d'Ivoire. The specific objectives pursued can be summarised as follows. First, to elucidate the epidemiology of single and multiple species infections with *Plasmodium* and intestinal parasites and to investigate associations and interactions between these parasitic infections among all members of entire communities and among children from public schools across Côte d'Ivoire. Second, to identify potential interrelations between parasite species and the implications of these infections on clinical and self-reported morbidity among different age groups. Third, to assess health-related quality of life among school-aged children harbouring parasitic infections and to determine age- and context-specific disability weights. Fourth, to determine clinical status, physical fitness and cognitive functioning in school-aged children and to analyse the relationships with

parasitic infections and the effect of repeated deworming on these health status measures.

Methods: The field work for this Ph.D. was split into three parts. Between July and September 2011, cross-sectional community-based surveys were conducted in nine settlements situated in the Taabo health and demographic surveillance system (HDSS) in south-central Côte d'Ivoire and in a hamlet of Azaguié town, located in the Agnéby-Tiassa region of south Côte d'Ivoire. Participants of the rural communities were asked to provide single urine, stool and finger-prick blood samples, which were examined for infection with *Plasmodium*, *Schistosoma haematobium*, *Schistosoma mansoni*, soil-transmitted helminths and intestinal protozoa. The Kato-Katz method was employed for the diagnosis of *S. mansoni* and soil-transmitted helminths, the urine filtration technique for *S. haematobium*, the formalin-ether concentration for intestinal protozoa, and microscopy of Giemsa-stained thick and blood films and rapid diagnostic testing (RDT) for *Plasmodium*. All participants additionally underwent clinical examination involving haemoglobin and anthropometric measurements to assess anaemia and nutritional status, palpation to determine liver and spleen enlargement and administration of an anamnestic questionnaire to record self-reported morbidity. Household heads were subjected to questionnaire interviews assessing local knowledge, attitude, practice and beliefs (KAPB) of parasitic diseases and information pertaining to the household's socioeconomic status.

From November 2011 to February 2012 a national cross-sectional study was implemented in 94 schools across Côte d'Ivoire. In each school, approximately 60 children were subjected to parasitological examination for *Plasmodium*, intestinal helminth (i.e. *S. mansoni* and soil-transmitted helminths) and *S. haematobium* infection. The same diagnostic approaches mentioned before were used, with the exception of *S. haematobium*, where reagent strips were applied to detect microhaematuria as a proxy for infection. Similar to the community-based surveys clinical examination for assessment of anaemia, nutritional status, splenomegaly and

hepatomegaly was conducted. Children responded to a questionnaire on household asset ownership, self-reported morbidity and health-related quality of life (HrQoL).

A 5-month prospective intervention study, involving two rounds of deworming at month 0 and 2 targetting schistosomiasis and soil-transmitted helminthiasis, was implemented in a school in Niablé in the eastern part of Côte d'Ivoire. Infections with *Plasmodium*, *Schistosoma*, soil-transmitted helminths and intestinal protozoa were determined using the same methods as in the community-based surveys. Children's clinical status, physical fitness and cognitive performance were determined at baseline (December 2012) and in a 5-month follow-up (May 2013).

Results: The rate of polyparasitism was high and peaked in school-aged children. Young children were mainly affected by *Plasmodium* infection, whilst helminth infections were more common among school-aged children and young adults. Intestinal parasitic infections were significantly associated with poor hygiene behaviour and practice of open defecation besides several sociodemographic determinants. Clinical morbidity mainly affected children and the national cross-sectional school-based survey revealed that every third child was anaemic or malnourished. *Plasmodium* infection showed strong associations with clinical manifestations, i.e. anaemia, splenomegaly and fever. Helminth infections were associated with self-reported morbidity pertaining to gastro-intestinal symptoms. Of note, *Plasmodium* and helminth infections showed significant interactions on clinical manifestations, i.e. anaemia and pallor indicating a protective effect in co-infected individuals.

The questionnaire survey on HrQoL revealed a disability weight of 0.01 for anaemia in school-aged children, whilst no difference in HrQoL measures could be identified between *Plasmodium* and helminth-infected and non-infected children. Health status rating further depended on socioeconomic status with generally lower HrQoL among the poorest.

Plasmodium-S. mansoni co-infection was related with better physical fitness, whilst haematological and nutritional deficiencies negatively impacted on children's physical performance. Repeated deworming showed only limited benefits on clinical

status, physical fitness and cognitive functioning among school-aged children in a high-transmission malaria setting.

Conclusions: Parasitic diseases remain of major public health relevance in Côte d'Ivoire and their continued transmission is governed by social-ecological systems and behavioural determinants. Our findings call for concerted efforts of established disease control programmes and implementation of integrated approaches to enhance the impact on prevalence and disease-related morbidity. The most urgent actions to be taken include the universal coverage with long-lasting insecticidal nets (LLINs) for malaria prevention, improved access to effective treatment, safe water and improved sanitation and awareness raising through setting-specific health education programmes among the affected populations. The studies conducted in the frame of this Ph.D. programme provide an extensive evidence-base determining the extent and characterising health implications due to multiple species parasite infections with an emphasis on malaria, schistosomiasis and soil-transmitted helminthiasis at local and national level. Hence, the findings reported here contribute to the planning of targeted integrative control strategies.

Résumé

Contexte: Des centaines de millions de personnes dans le monde en développement sont à risque de maladies parasitaires telles que le paludisme et les maladies tropicales négligées (MTNs). Les répercussions de ces maladies sur la santé sont multiples. Les manifestations cliniques de l'infection par les espèces de *Plasmodium* incluent typiquement l'anémie et la splénomégalie associée à la mort des érythrocytes et la séquestration splénique, respectivement. Les infections parasitaires intestinales, telles que les helminthiases (p. ex. les géohelminthiases et la schistosomiase) et les protozoaires intestinaux sont rarement fatales. Mais elles peuvent causer une morbidité chronique à long terme, comme notamment une anémie et d'autres carences nutritionnelles qui se manifestent par un retard de croissance et nuisent au développement cognitif de l'enfant ainsi qu'à la capacité de travail des adultes. L'infection concomitante par plusieurs de ces parasites – le polyparasitisme – est la norme plutôt que l'exception dans des pays comme la Côte d'Ivoire. Cependant, jusqu'à présent, la plupart de la recherche menée sur la morbidité liée aux infections parasitaires s'est concentrée sur les infections simples. Une compréhension approfondie des infections parasitaires multiples et de leur impact sur la santé est essentielle pour lutter contre ces (co-)infections et réduire leur charge de morbidité.

But et objectifs spécifiques: Le but global de cette thèse était d'approfondir notre compréhension des infections parasitaires multiples et leurs répercussions sur la morbidité au niveau local et régional en Côte d'Ivoire. Les quatre objectifs spécifiques poursuivis peuvent être résumés comme suit : Premièrement, élucider l'épidémiologie des infections simples et multiples par *Plasmodium* et les parasites intestinaux, et investiguer les associations et interactions entre les différentes espèces au niveau des communautés d'une part, et parmi les enfants au niveau des écoles publiques à travers de la Côte d'Ivoire d'autre part. Deuxièmement, identifier de potentielles interrelations entre les espèces parasitaires et les conséquences de ces infections sur la morbidité clinique et auto-évaluée parmi différentes tranches

d'âge. Troisièmement, évaluer la qualité de vie liée à la santé parmi les enfants d'âge scolaire infectés par des parasites et déterminer les poids d'incapacité spécifiques à l'âge et au contexte. Quatrièmement, déterminer le statut clinique, l'aptitude physique et le fonctionnement cognitif parmi les enfants d'âge scolaire et analyser leurs relations avec les infections parasitaires ainsi que l'effet du déparasitage répété sur ces mesures de l'état de santé.

Méthodes: Le travail de terrain pour cette thèse était divisé en trois parties.Entre juillet et septembre 2011, des enquêtes transversales communautaires ont été menées dans neuf localités situées dans le système de surveillance démographique (SSD) de Taabo au centre-sud de la Côte d'Ivoire et dans un campement d'Azaguié ville, situé dans la région d'Agnéby-Tiassa du sud de la Côte d'Ivoire. Pour chaque participant, un échantillon d'urine, de selles et de sang prélevé au bout du doigt ont été recueillis et examinés pour détecter la présence d'infections par *Plasmodium*, *Schistosoma haematobium*, *Schistosoma mansoni*, les géohelminthes et les protozoaires intestinaux. Les méthodes de diagnostic suivantes ont été utilisées: la technique de Kato-Katz pour rechercher les œufs de *S. mansoni* et des géohelminthes, la technique de filtration pour les œufs de *S. haematobium* dans les urines, la sédimentation formol-éther pour les protozoaires intestinaux et la microscopie des gouttes épaisses et frottis sanguins après coloration au Giemsa et des tests de diagnostic rapide (TDR) pour le paludisme. En outre, tous les participants ont été soumis à un examen clinique comprenant des mesures anthropométriques pour évaluer le statut nutritionnel et du taux d'hémoglobine pour dépister l'anémie, et une palpation pour déterminer la présence d'hépatomégalie ou de splénomégalie. Ils ont aussi tous été interrogés à l'aide de questionnaires anamnestiques pour enregistrer les mesures subjectives de la morbidité auto-évaluée. Les chefs de ménage ont été interrogés par questionnaires sur les connaissances, attitudes, pratiques et croyances (CAPC) liées aux maladies parasitaires et sur diverses informations relatives au statut socioéconomique du ménage.

De novembre 2011 à février 2012, une enquête transversale nationale a été réalisée dans 94 écoles dans toute la Côte d'Ivoire. Dans chaque école, un échantillon d'environ 60 enfants a été soumis aux examens parasitologiques pour vérifier la présence d'infection par *Plasmodium*, ou par les helminthes intestinaux (*S. mansoni* et géohelminthes) et par *S. haematobium*. Les approches diagnostiques précitées ont été appliquées, à l'exception de celle pour la détection de *S. haematobium*. Pour cette espèce, des bandelettes réactives ont été utilisées pour détecter la présence d'hématurie microscopique comme indicateur de l'infection. Comme pour les enquêtes communautaires, un examen clinique pour déterminer l'anémie, le statut nutritionnel, la splénomégalie et l'hépatomégalie a été effectué. Chaque enfant a répondu un questionnaire sur la propriété des actifs du ménage, la morbidité auto-évaluée et la qualité de vie liée à la santé (QVLS).

Une étude d'intervention prospective sur 5 mois qui comprenait deux tours de déparasitage contre la schistosomiase et les géohelminthiases, aux mois 0 et 2, a été réalisée dans une école à Niablé dans l'est de la Côte d'Ivoire. Les infections par *Plasmodium*, par *Schistosoma*, ou par les géohelminthes et par les protozoaires intestinaux ont été identifiées en appliquant les mêmes techniques diagnostiques que dans les enquêtes communautaires précédentes. Le statut clinique, l'aptitude physique et la performance cognitive des enfants ont été évalués deux fois, soit au début de l'étude (décembre 2012) et à l'enquête de suivi finale à 5 mois (mai 2013).

Résultats: Le taux de polyparasitisme était élevé et plafonnait parmi les enfants d'âge scolaire. Les jeunes enfants ont été principalement touchés par le paludisme, tandis que les helminthiases ont été plus fréquemment trouvées chez les enfants d'âge scolaire et les adolescents. Un lien significatif a été trouvé entre les infections parasitaires intestinales et les mauvaises pratiques d'hygiène, la défécation à l'air libre, ainsi qu'avec plusieurs autres déterminants sociodémographiques. Une morbidité clinique a été identifiée principalement chez les enfants et, en plus, l'enquête nationale transversale en milieu scolaire a révélé qu'un enfant sur trois souffrait d'anémie ou de malnutrition. Le paludisme était fortement associé aux manifestations cliniques, i.e. à l'anémie, la splénomégalie et la fièvre. Les

helminthiases étaient associées aux symptômes gastro-intestinaux rapportés. Fait à noter, une interaction significative entre l'anémie et la pâleur a été trouvée chez les cas de co-infection *Plasmodium*-helminthes indiquant un effet protecteur chez les sujets co-infectés.

L'enquête par questionnaire sur la QVLS a révélé un poids de l'incapacité de 0.01 pour l'anémie parmi les enfants d'âge scolaire, tandis qu'aucune différence n'a été observée entre les mesures QVLS des enfants avec une co-infection *Plasmodium*-helminthes et leurs homologues non-infectés. En outre, l'évaluation subjective de l'état de santé était associée avec le statut socioéconomique, les valeurs les plus basses ayant été trouvées parmi les plus pauvres.

La co-infection *Plasmodium-S. mansoni* était liée à une meilleure aptitude physique, tandis que les carences hématologiques et nutritionnelles avaient un effet négatif sur la performance physique des enfants. Le déparasitage répété a montré des bénéfices limités relatifs au statut clinique, à l'aptitude physique et au fonctionnement cognitif parmi les enfants d'âge scolaire dans une zone de forte transmission du paludisme.

Conclusion: Les maladies parasitaires continuent de constituer un problème de santé publique majeur en Côte d'Ivoire et leur transmission continuelle est régie par des systèmes socio-écologiques et des facteurs comportementaux. Nos résultats appellent à des efforts concertés des programmes de lutte contre les différentes infections, et la mise en œuvre d' approches de lutte intégrées pour renforcer l'impact sur la prévalence et la morbidité liée à ces maladies. Les mesures les plus urgentes à prendre incluent la couverture universelle des moustiquaires imprégnées d'insecticide à longue durée (MID) pour la prévention du paludisme, l'amélioration de l'accès à un traitement efficace, à l'eau potable et à des installations d'assainissement améliorées, et la sensibilisation par des programmes d'éducation à la santé ciblant les populations affectées. Les études menées dans le cadre de ce programme de doctorat fournissent une vaste base de preuves qui détermine la dimension des infections parasitaires multiples et leurs répercussions sur la santé. En mettant l'accent sur le paludisme, la schistosomiase et les géohelminthiases au

niveau local et national, ce travail contribue à la planification des stratégies de lutte ciblées et intégrées.

1. Introduction

1.1 Life cycles and biology of *Plasmodium* spp. and intestinal parasites

1.1.1 Malaria

Malaria, which means “bad air” in Italian and refers to the ancient concept that the disease is induced by emanated poisonous vapour or mist (Hempelmann & Krafts, 2013), is in fact caused by infection with parasites belonging to the genus *Plasmodium*. Five species are known to cause malaria in humans; *P. falciparum*, which is considered the most deadly, *P. vivax*, *P. malariae*, *P. ovale* and the so far known to infect monkeys species *P. knowlesi*. Malaria is a vector-borne disease and is transmitted by *Anopheles* mosquitoes. The parasite has a complex life cycle and undergoes several larval stages within the vector and the host (Figure 1.1). During blood feed of female anopheline mosquitoes hosts get infected with motile sporozoites, which enter liver cells and then multiply. The developed daughter cells, merozoites, get released in the blood stream when the liver schizont bursts, where they invade erythrocytes. The intra-erythrocyte stages of the parasite feed on the red blood cell content and reproduce, which is accompanied by rupture of erythrocytes. The newly released merozoites in turn invade more erythrocytes and repeat the cycle. When parasite densities have reached about 50/µl of blood, the parasites become detectable by usual diagnostic tests (e.g. microscopy and rapid diagnostic test (RDT)) and thus the infection becomes symptomatic. Some of the blood stages of the parasite develop into sexual forms, gametocytes, which represent the infective stages for mosquitoes. Gametocytes are taken up by a feeding *Anopheles.* The gametocytes reproduce sexually by forming an ookinete into an oocyst in the mosquito gut. Bursting of the oocyst liberates sporozoites, which migrate to the salivary glands where they await inoculation of another host at the next blood feed. An entire cycle can take approximately 1 month, while the incubation period before symptoms appear in humans is 12-14 days from an infective bite, but may vary between species (White et al., 2014).

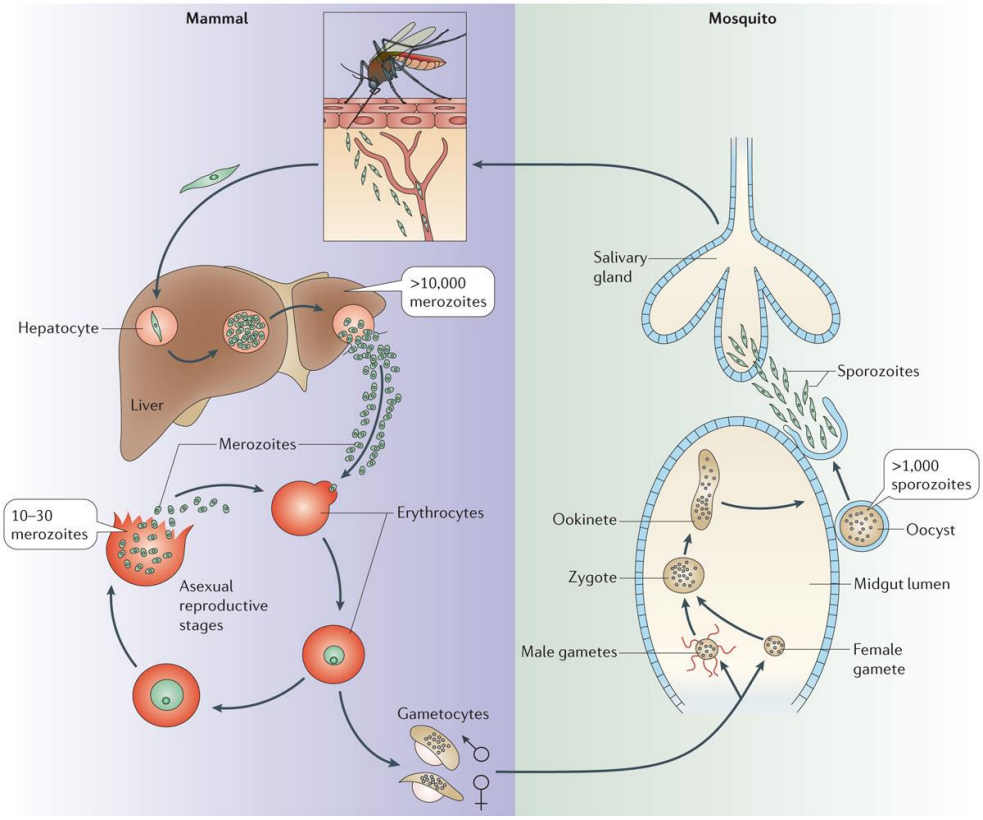

Figure 1.1 The life cycle of *Plasmodium* species that infect mammals (including humans).
Parasite stages within mammals (host) and mosquitoes (vector) (Ménard et al. 2013).

1.1.2 Intestinal parasites

Schistosomiasis

Schistosomiasis, also known as bilharziasis, is a parasitic disease caused by blood-flukes of the genus *Schistosoma.* Seven species are known to affect humans; *S. mansoni*, *S. intercalatum*, *S. guineensis*, *S. japonicum*, *S. mekongi* and *S. malayensis* cause intestinal schistosomiasis, whereas *S. haematobium* infection involves the urinary tract (Gryseels et al., 2006; Rollinson et al., 2013; WHO, 2013a). Schistosomiasis is a water-related vector-borne disease. Aquatic snails from the genus *Bulinus* act as intermediate hosts for *S. haematobium*, *S. intercalatum* and *S. guineensis*, while snails from the genus *Biomphalaria* are involved in *S. mansoni*

transmission. *Oncomelania spp.* and *Neotricula aperta* were identified as intermediate host species for *S. japonicum* and *S. mekongi*, respectively (Sturrock, 2001; Ohmae et al., 2004; Rollinson et al., 2013). The disease is characterised by a complex life cycle, which involves different free-living, within intermediate host (fresh water snails) and within definitive host (humans) stages (Figure 1.2.). Humans get infected through contact with water bodies or marshlands infested with cercariae, the free-swimming larval stages, which penetrate the skin of potential hosts. Once within the host body, they become schistosomulae and migrate in the blood via the lungs to the portal veins of the liver, where they mature to adult worms, female and male worms unite and mate. Couples of female and male adult stages further migrate to the mesenteric vessels of the bowel (intestinal schistosomiasis) or bladder (urinary form), where numerous eggs are produced. Eggs pass through adjacent tissues and get excreted in faeces or urine, while a portion of eggs remain trapped in the tissues. Excreted eggs hatch in fresh water to motile miracidia, which infect specific fresh water intermediate host snails. In the snails they develop to a next stage to produce cercariae, which get ultimately released in the water again (Gryseels et al., 2006; King, 2009; Gray et al., 2011).

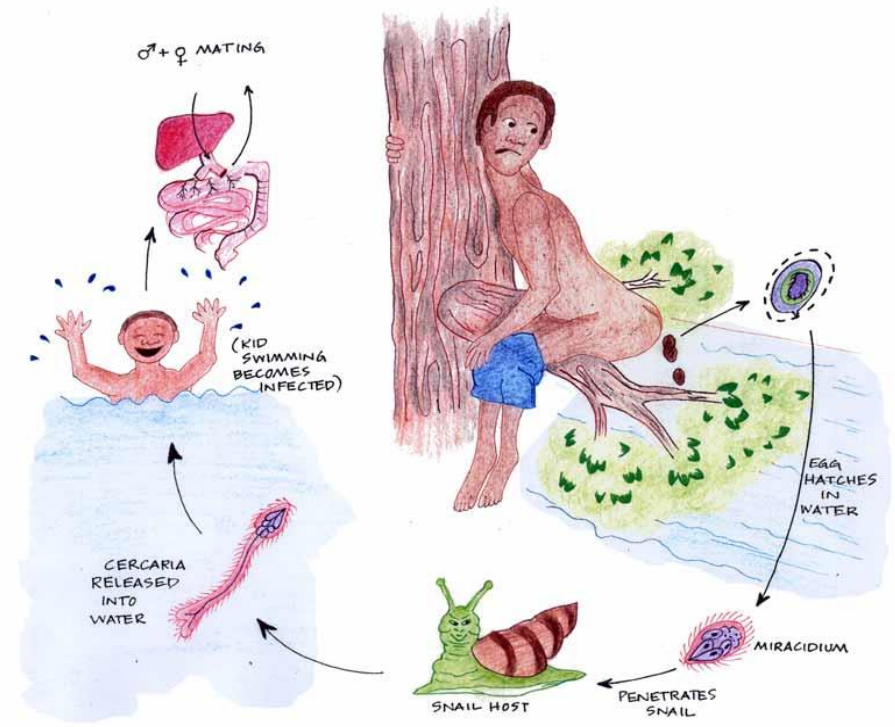

Figure 1.2 Life cycle of *Schistosoma* spp. (Source: http://parasito-nasiri.blogfa.com/post-5.aspx)

Soil-transmitted helminthiasis

Soil-transmitted helminthiasis (STH) is caused by parasitic nematodes, which inhabit the human gastrointestinal tract. *Ascaris lumbricoides*, hookworms of the genera *Ancylostoma* and *Necator*, and *Trichuris trichiura* are considered the most common species to induce soil-transmitted helminthiasis. Humans are regarded as the only major definitive host of these parasites, thus transmission does not involve any intermediate hosts. People become infected with *A. lumbricoides* and *T. trichiura* by swallowing mature eggs from contaminated food and fingers or by active penetration of the skin by larvae in the soil – in the case of hookworms (Figure 1.3). Larvae of *A. lumbricoides* and *T. trichiura* hatch from ingested eggs in the stomach and migrate to their final habitat in the intestines, where they mature to adult worms. Hookworms access blood circulation after skin penetration, migrate via the lungs to

the oesophagus, where they are swallowed, and pass through the stomach to reach the intestine. Once settled in the intestinal habitat, *A. lumbricoides* and *T. trichiura* feed on the host's intestinal food content, whereas hookworms suck blood and fluids from grasping and cutting gut tissue. Female adult worms of these parasites produce large number of eggs; up to 200,000, 25,000 and 20,000 eggs per day for *Ascaris*, hookworm and *Trichuris*, respectively. The eggs are released in faeces to the environment, where they develop to infective stages (Bethony et al., 2006; Hall et al., 2008).

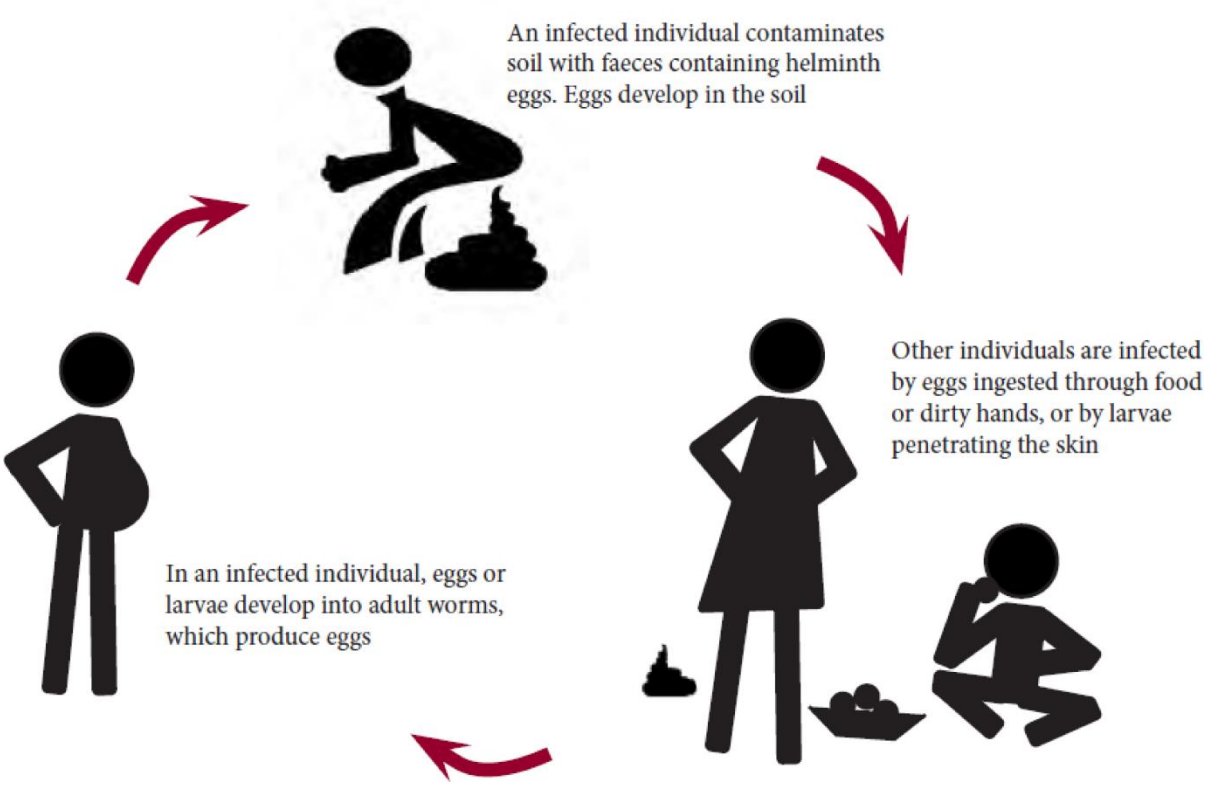

Figure 1.3 Schematic life cycle of soil-transmitted helminths (WHO, 2011)

Intestinal protozoa infections

A wide range of intestinal protozoa are known to colonize the human intestinal tract and fortunately most of them are commensals not causing any harm (Keddy et al., 2005). In this section we will focus on two diseases caused by intestinal protozoa infections, which are considered the major ones worldwide in terms of prevalence and public health importance, namely giardiasis and amoebiasis (Bouzid et al., 2008). Giardiasis is caused by infection with *Giardia intestinalis*, also known as *G. lamblia* or *G. duodenalis*, while *Entamoeba histolytica* is the causative agent of amoebiasis (Figure 1.4). Both parasite species have a simple life cycle and they either occur as cysts (long-lived infective stages) or motile trophozoites within hosts. Infection results from ingestion of faecally contaminated water or food. For *G. intestinalis*, which is highly contagious, also direct infection from person-to-person among family members or subjects in crowded conditions, as well as sexual transmission in men who have sex with men, was reported (Muhsen & Levine, 2012). Ingested cysts survive passage of the acid stomach environment and are stimulated to excystate in the intestine. The resulting trophozoite stages multiply asexually in the intestinal lumen, whereof some transform into cysts again. Cysts excreted in the stool maintain the life cycle by further faecal–oral spread (Haque et al., 2003; Muhsen & Levine, 2012).

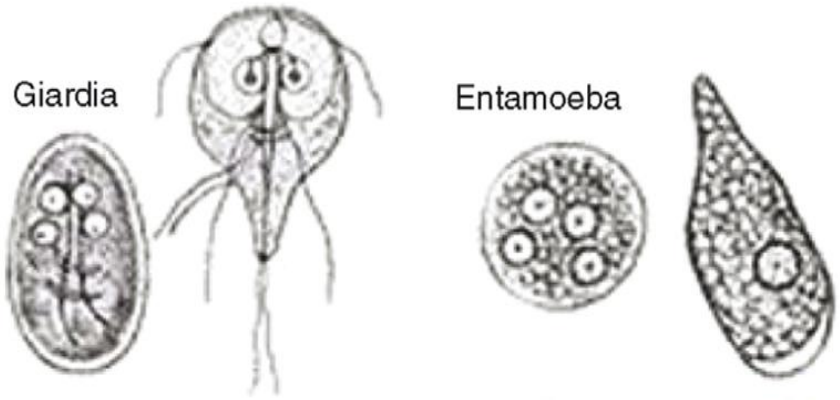

Figure 1.4 Cysts and vegetative stages (trophozoites) of *Giardia* and *Entamoeba* (Bouzid et al., 2008)

1.2 Global epidemiology of malaria, intestinal parasites and multiple species infections and situation in Côte d'Ivoire

1.2.1 Malaria

Since malaria is a vector-borne disease its abundance mainly depends on the density, longevity, biting habits and efficiency of the mosquito vector. The mosquitoes belonging to the *Anopheles gambiae* complex in Africa are considered the most effective malaria vectors among the approximately 70 anopheline species, which are capable to transmit the disease (Sinka et al., 2012; White et al., 2014). Both, the longevity of the mosquitoes and the developmental transformations of *Plasmodium* within the vector strongly depend on ambient temperature. Permissive temperatures for *Plasmodium* sporogonic development range from 16 °C to 35 °C (Lefèvre et al., 2013). Besides temperature, humidity and rainfall are major factors for mosquito abundance and thus malaria transmission. Higher humidity fosters mosquito survival rates and stagnant water bodies after seasonal rainfall provide optimal vector breeding sites (Yé et al., 2009). Consequently, tropical bioclimatic zones represent an optimal environment (Figure 1.5). Among the 97 countries that were considered to be endemic with ongoing malaria transmission, the majority (> 80%) of malaria cases identified in 2012 occurred on the African continent (WHO, 2013b).

In Côte d'Ivoire 100% of the population are considered to be at risk of *Plasmodium* infection and transmission is regarded to be stable with constant, year-round infections (Adja et al., 2011; Assi et al., 2013). *P. falciparum* is the predominant species in Côte d'Ivoire, but also *P. malariae* and *P. ovale* are endemic (Raso et al., 2012). In stable transmission settings a naturally acquired immunity is developed due to constant exposure. Infants and young children, who still lack immunity, thus are among the most affected (Doolan et al., 2009).

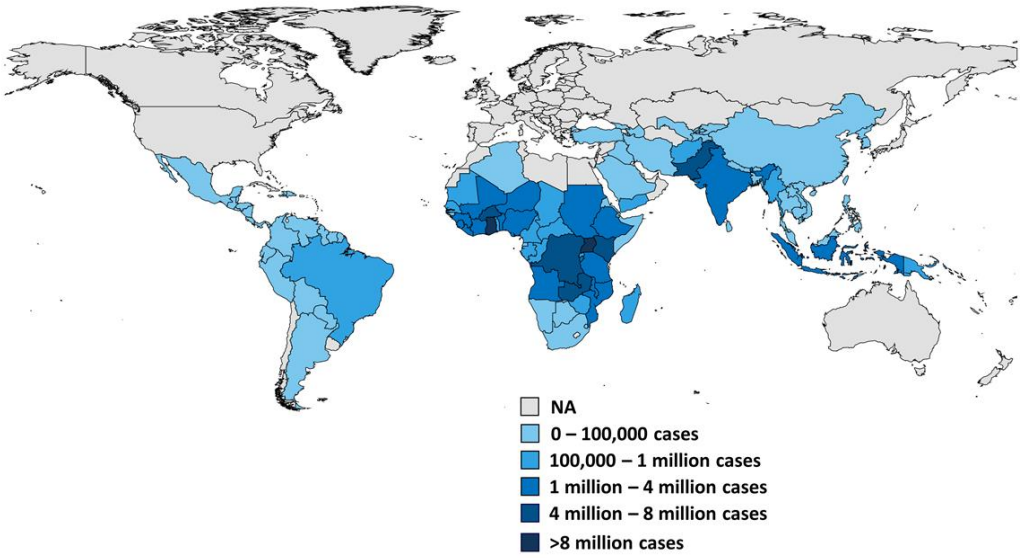

Figure 1.5 Global map of reported malaria cases in 2012 (Source: http://kff.org/globaldata/, based on (WHO 2013b))

1.2.2 Intestinal parasites

Schistosomiasis

Schistosomiasis has been reported to be endemic in 78 countries and territories situated mainly in tropical and subtropical climatic zones with almost 240 million people estimated to be infected and in need of treatment in 2011 (WHO, 2013a). Various environmental and social factors drive schistosomiasis transmission resulting in a very focal distribution of the disease. First, the transmission of the disease is geographically limited to the abundance of the respective intermediate host snails and thus only occurs in freshwater. Water flow velocity and temperature are key determinants for host snail abundance and show seasonal patterns (Gryseels et al., 2006). Water resources development such as construction of dams and irrigation schemes for agricultural land use have been shown to create favourable conditions for proliferation of intermediate host snail populations and hence, to result in transmission intensification (Steinmann et al., 2006). Further, poor sanitation and hygiene contaminate the environment and water bodies with human excrements, which if they contain parasite eggs foster the spread of the disease.

Schistosomiasis is therefore often also referred to as a poverty-related disease (Gryseels et al., 2006; King, 2010). People acquire the infection through contact with schistosome-infested water. Infection risk and intensity, thus, depends on water contact patterns including frequency, duration and body parts exposed during contact, but also on settlements' proximity to water contact sites (Rudge et al. 2008; Coulibaly et al. 2013a).

Côte d'Ivoire is listed among countries requiring preventive chemotherapy. Both, urogenital and intestinal forms of schistosomiasis are endemic to Côte d'Ivoire and are caused by *S. haematobium* and *S. mansoni*, respectively (WHO, 2013a). Peak prevalences are usually observed in school-aged children, but may be shifted to adulthood depending on setting-specific endemicity (Woolhouse, 1998).

Soil-transmitted helminthiasis

Recent estimates suggest that globally 439 million people were infected with hookworm in 2010; 819 million with *A. lumbricoides* and 464 million with *T. trichiura* (Pullan et al, 2014). Eggs and larvae especially thrive in warm and moist soils of tropical and subtropical countries (Bethony et al., 2006; WHO, 2006). Likewise to schistosomiasis, transmission of soil-transmitted helminths is fostered in resource-constrained settings with a lack of sanitation facilities and inadequate hygiene and particularly affects the most poor (de Silva et al., 2003; Raso et al., 2005; King, 2014). Age-related prevalence and intensity of infection have been found to vary between species. In *A. lumbricoides* and *T. trichiura* worm loads tend to rise in childhood and decline in adulthood, showing peaks in school-aged children. Hookworm infection, in contrast, shows a steady rise of intensity with age, peaking in adulthood (Tchuem Tchuenté, 2011).

Côte d'Ivoire, situated in the humid tropics, offers favourable climatic conditions for a year-round transmission of soil-transmitted helminthiasis. Infection prevalence with any soil-transmitted helminth species is estimated to range between 10% and 20% (Figure 1.6). Previous studies have identified hookworm as the predominant species in Côte d'Ivoire (Raso et al., 2006a; Coulibaly et al., 2012).

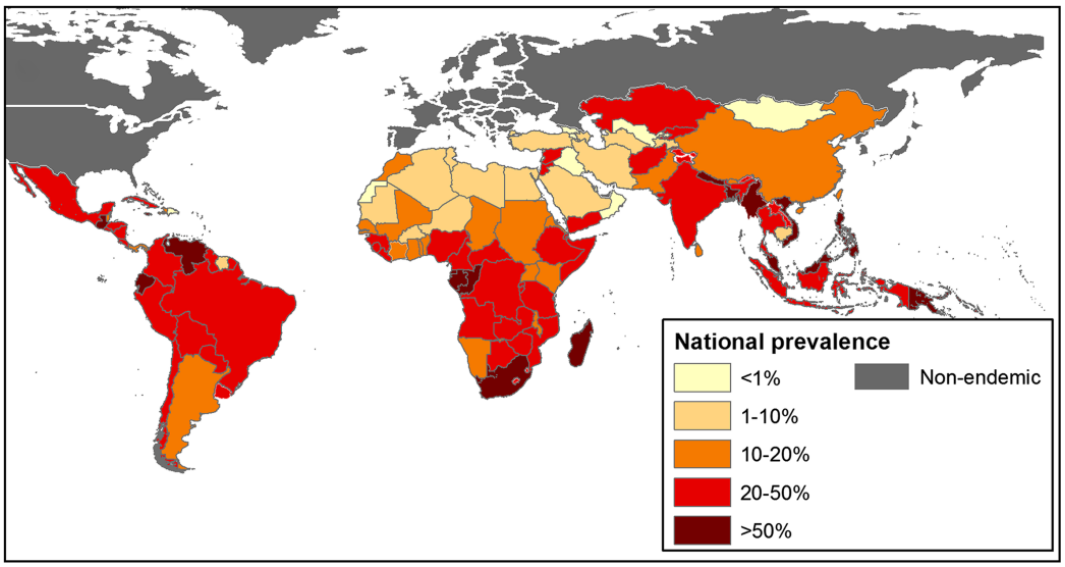

Figure 1.6 Distribution of any STH infection in 2010 (Pullan et al., 2014)

Intestinal protozoa infections (amoebiasis and giardiasis)

Infections with *Giardia intestinalis* and *Entamoeba histolytica* occur worldwide, but are much more common in developing countries where there is often a lack of adequate sanitation and access to safe water. Both species are among the most widespread protozoa causing diarrhoea. There are 200 million symptomatic *G. intestinalis* cases and over 500 million people infected with *E. histolytica* worldwide (Bouzid et al., 2008). In industrialised countries, infections often re-emerge as outbreaks due to contaminated drinking water or inappropriate and unhygienic handling or manufacturing of foods that are consumed uncooked (Dawson, 2005). Especially cysts of *Giardia* are known to persist and stay infective over months in the environment and the possibility of animal reservoirs has been indicated (Hunter & Thompson, 2005). Both, cysts of *G. intestinalis* and *E. histolytica* survive chlorination process of water, in order to avoid infection food and water therefore needs to be cooked thoroughly and boiled, respectively (Wright, 2005).

No national estimates for amoebiasis and giardiasis are available for Côte d'Ivoire. Various observational studies, nonetheless, have shown prevalences for *G. intestinalis* and *E. histolytica* in the range of 7% to 14 % and 5% to 42%,

respectively, peaking in children for giardiasis whereas *E. histolytica/E. dispar* infection prevalence increased with age (Keiser et al., 2002a; Raso et al., 2004; Ouattara et al., 2010; Coulibaly et al., 2012). Of note, the aforementioned studies did not differentiate between the two morphologically identical *E. histolytica* and *E. dispar*, though only the former is pathogenic. A study from central Côte d'Ivoire suggested the ratio *E. histolytica E. dispar* was very low (Heckendorn et al., 2002).

1.2.3 Multiple species infections

Parasitic infections share common factors that foster transmission such as climatic suitability, lack of sanitary facilities and access to clean water, as well as poor hygiene conditions. Co-existence and multiple species infections are thus rather the norm than the exception in developing and particularly in tropical countries (Molyneux et al., 2005; Hotez et al., 2007; Pullan & Brooker, 2008; Steinmann et al., 2010). Epidemiological and clinical studies formerly used to focus on specific species. In recent years, however, especially co-infections of *Plasmodium* and helminth infections got increased attention in the scientific world. Interactions on disease outcomes have been highlighted, yet findings are conflicting. While some identified synergistic interactions on morbidity others observed protective effects from co-infection (Nacher, 2011; Righetti et al., 2012; Naing et al., 2013; Lemaitre et al., 2014). Species interactions are of complex nature and may be very setting specific and further depend on infection intensities. Polyparasitism is a widespread phenomenon in Côte d'Ivoire and increases with age, being most prevalent in adolescents and young adults, and decreases in late adulthood (Keiser et al., 2002a; Raso et al., 2004; Coulibaly et al., 2012). Several studies have investigated *S. mansoni*-hookworm co-infections in the country and partly found strong associations (Keiser et al., 2002b; Raso et al., 2004, 2006b; Matthys et al., 2007).

1.3 Morbidity and burden due to malaria and intestinal parasitic infections

1.3.1 Malaria

Malaria is ranked on first position with regard to mortality among parasitic diseases. In 2012 there were an estimated 627,000 malaria deaths worldwide. The majority (90%) of these occurred in Africa, where *P. falciparum*, the most dangerous *Plasmodium* species, predominates (WHO, 2013b). The global burden in 2010 was estimated to be more than 80 million disability-adjusted life years (DALYs) (Murray et al., 2012). Infants and children under five years of age are at particular risk of severe, complicated malaria involving dysfunction of vital organs, cerebral malaria and coma. Pregnant women are at higher risk to develop severe malaria and for still birth. In high-transmission areas children develop a protective immunity against clinical malaria. Infection therefore usually is of uncomplicated nature and can even be asymptomatic. Typical features of uncomplicated clinical malaria are non-specific symptoms accompanied by irregular fever. Several days after mild anaemia due to erythrocyte death, a palpable spleen and enlargement of the liver can occur (Doolan et al., 2009; White et al., 2014).

1.3.2 Intestinal parasites

Schistosomiasis

Global burden due to schistosomiasis has been estimated to be 3.3 million DALYs in 2010 (Murray et al., 2012). The disease is rarely lethal and is associated with more subtle morbidity. The pathology of the infection is mainly related to migrating eggs, which get trapped in tissues and provoke lesions. Infection with *S. haematobium* usually leads to haematuria and egg deposition in the genital tract can result in female genital schistosomiasis (Hotez & Whitham, 2014). Typical symptoms of uncomplicated chronic intestinal schistosomiasis are diarrhoea (sometimes containing blood) and abdominal pain. Intestinal species like *S. mansoni*, *S. japonicum* and *S. mekongi* can cause hepatic schistosomiasis when eggs are

trapped in the liver. More fatal disease outcome such as bladder cancer and kidney failure in urogenital schistosomiasis or bleeding from gastrointestinal varices as a consequence of liver fibrosis in intestinal schistosomiasis have been reported. Schistosomiasis can be associated with anaemia due to internal blood loss from lesions (Gryseels et al., 2006).

Soil-transmitted helminthiasis

Infection with hookworm, *Ascaris* and *Trichuris* were estimated to have accounted for about 3.2, 1.3 and 0.6 million DALYs in 2010 (Murray et al., 2012). Soil-transmitted helminthiasis is usually not lethal, but chronic infection is thought to impair child growth and cognitive development as well as work capacity in adults. Thus, soil-transmitted helminthiasis is of major health and economic significance. Malabsorption and competition for nutrients, feeding on host tissues, diarrhoea, intestinal bleeding, loss of appetite and reduction of food intake consequently leads to nutritional deficiencies and anaemia (Hall et al., 2008; WHO, 2011). Morbidity of worm infections is generally considered to be positively related to infection intensities (Hotez et al., 2006).

Intestinal protozoa (amoebiasis and giardiasis)

G. intestinalis and *E. histolytica* are major water-borne parasitic diseases and responsible for considerable morbidity and mortality associated with diarrhoeal disease in both the developed and developing world. Infection with *E. histolytica* can cause amoebic colitis, while the morphologically indistinguishable *E. dispar* is a harmless commensal. In rare cases extraintestinal spread of *E. histolytica* throphozoites to the liver can involve development of liver abscesses. Persisting diarrhoea in *G. intestinalis* infections can result in intestinal malabsorption and thus can be associated with retardation of growth and development in children. Most infections with *E. histolytica* and *G. intestinalis* stay asymptomatic, but infected individuals are cyst carriers and excreters and thus add to the transmission of the disease (Haque et al., 2003; Farthing, 2006).

1.4 New disability assessment approaches

Current disability estimates of parasitic diseases, which are based on DALY metrics, were often criticised to underestimate the actual burden of these health conditions and there is ongoing discussion and debate (Hotez et al., 2014; King, 2014). Particularly for helminth infections, which involve rather subtle morbidities, a re-assessment using novel approaches was requested. Main critical points were the separation of infection-associated morbidity (e.g. anaemia) from infection-attributable disability, non-consideration of cultural and socioeconomic context, additive burden due to concurrent infections and ignorance of community and patient-based appraisal (Reidpath et al., 2003; Pullan & Brooker, 2008; Payne et al., 2009; King, 2010). Some of these issues meanwhile have been addressed by the global burden of disease consortium and new DALY estimates for the year 2010 have been presented (Murray et al., 2012; Salomon et al., 2012). Major alternative disability measures and their use for burden assessment in parasitic infections are summarised in the following section.

1.4.1 Quality-adjusted life years (QALYs)

QALY is an alternative health assessment metric based on health related quality of life (HrQoL). HrQoL is usually determined by administration of questionnaires that inventory respondent's status in different performance domains. These domains typically include aspects on social, mental, physical and environmental wellbeing. Many HrQoL instruments further include a visual analogue scale (VAS) asking respondents to rate their general health status on a scale from 0 (worst imaginable health) and 100 (perfect health), which are used to derive disability weights (DW). The QALY approach is meant to measure multiple potential impacts from a particular disease and concurrently incorporate personal perception of the overall health state. HrQoL scores are then normally scaled on a 0 to 100 scale as well and compared between healthy and disease-affected respondents to calculate disease-related disability (Skevington et al., 2004; King & Hinds, 2011; King, 2014). This approach has recently been used in disability assessment of helminth infections such as

S. japonicum, *S. haematobium*, *S. mansoni* and soil-transmitted helminths providing conflicting results (Ziegelbauer et al., 2010; Fürst et al., 2011, 2012; Jia et al., 2011; Samuels et al., 2012; Terer et al., 2013).

1.4.2 Physical fitness

To further assess the physical impact, besides traditional clinical measurements like anaemia and growth retardation, more and more recent epidemiological studies started to use standardised physical fitness test batteries. A range of different test batteries exist (AAHPERD, 1980; EUROFIT, 1993; FITNESSGRAM, 1994).The most prominent test used with regard to parasitic infections was a multistage 20 m shuttle run test, which determines aerobe capacity and cardio-respiratory endurance, expressed as VO_2 max (Léger et al., 1988). The test was already successfully applied in Kenyan, Ivorian and Chinese school-aged children with multiple species parasitic diseases (Bustinduy et al., 2011; Müller et al., 2011; Samuels et al., 2012; Yap et al., 2012). Exercise testing further may be complemented by tests assessing strength and explosive power, as done in cohorts of China and Mozambique (Nhantumbo et al., 2013; Yap et al., 2014)

1.4.3 Cognition and school performance

Parasitic infections and disease-related consequences are considered to negatively affect children's mental and cognitive development, be related with school absenteeism and decrease school performance (Fernando et al., 2010; King, 2010; WHO, 2011). Evidence for clear relationships and to what extent treatment of infection improves performance is, however, still sparse (Taylor-Robinson et al., 2012). A multitude of cognitive test batteries assessing attention, working memory, reasoning, verbal fluency, writing abilities and IQ were developed in industrialised countries (Wright, 2010). Not all of them may be valid in culturally different settings and adaptions might be needed (Baddeley et al., 1995). Few found application and are now increasingly used in observational and intervention studies in the developing world (Ezeamama et al., 2005, 2012; Brooker et al., 2010; Nankabirwa et al., 2013).

1.5 Diagnosis and treatment

1.5.1 Malaria

Microscopic examination of Giemsa-stained thick and thin blood films remains the gold standard among diagnostic techniques for malaria diagnosis in endemic countries (Hira & Behbehani, 1984). Thick blood films are very sensitive for parasitaemia detection and are therefore used for quantification, whereas methanol-fixed thin blood films allow for precise species identification. This diagnostic technique further enables morphological assessment and differentiation between developmental stages. Specific parasite stages can serve as indicators for the severity of the disease and thus may indicate the need for drug administration modification (from oral to intravenous route) (Moody, 2002). A considerable number of pan-malaria or species-specific RDTs with high sensitivity and specificity are available and their use has rapidly increased over the past years (White et al., 2014). While microscopic diagnosis implies well-trained laboratory technicians and availability of adequate chemical substances, antigen-detecting RDTs provide access to malaria diagnosis in areas where these means are not available. Consequently, their use forms an important part of the WHO recommended malaria case management strategy that all cases have to be based on parasite-based diagnosis (WHO, 2012).

Drugs of choice against malaria largely depend on endemicity, species distribution and the severity of the disease. *P. falciparum* has developed resistance to all established single compound treatments. Consequently, Artemisinin combination therapy (ACT) is the recommended first-line therapy for uncomplicated falciparum malaria in all endemic areas. For non-falciparum malaria Chloroquine is still an effective agent and considered as standard treatment. The spread of Chloroquine resistance in *P. vivax*, however, has been indicated implying a need for adaptions of treatment policies in the respective settings (WHO, 2013b; White et al., 2014).

1.5.2 Intestinal parasites

Schistosomiasis

Direct detection of eggs in urine (*S. haematobium*) and stool (all intestinal schistosome species) specimens is the most widely used approach for parasitological diagnosis. Preparation of faecal thick smears with the Kato-Katz technique for subsequent examination under a microscope is the recommended standard measure for intestinal schistosomiasis by the WHO (Katz et al., 1972). This technique allows for quantification of helminth eggs per gram of stool and stratification into infection intensities according to given species cut-offs (Table 1.1) (WHO, 2011). This method, however, has been found to be not very sensitive, particularly in light-intensity infections. Kato-Katz thick smears only use a small portion of stool (41.7 mg) and egg output shows day-to-day and intra-specimen variation. It is therefore recommended to prepare duplicate thick smears, ideally from several day consecutive stool sampling (Utzinger et al., 2001). Urine specimens are routinely subjected to a filtration technique. 10 ml of urine is pressed through small-meshed filters, lugol solution is added to the filter paper for staining and filter slides then examined under a microscope (Savioli et al., 1990). Detection of microhaematuria using reagent strips serves as a proxy for *S. haematobium* infection. This diagnostic approach is justified by *S. haematobium* infection causing lesions in the bladder tissue leading to presence of blood in urine of patients. Diagnostic performance of reagent strips has been evaluated in various settings and showed high sensitivity and specificity compared to egg detection (Bogoch et al., 2012; King & Bertsch, 2013). Several immunodiagnostic assays for detection of anti-schistosome antibodies exist. While circulating cathodic antigen (CCA) is detected in urine, recently developed RDTs assess specific antibodies in blood samples (Coulibaly et al., 2013b).

Praziquantel is the only recommended treatment for all forms of schistosomiasis by WHO. After patent expiry considerable cost reductions have followed. Nonetheless, praziquantel ranges amongst the most expensive available anthelminthic treatments. The amount of praziquantel tablets produced are below

the actual need in endemic countries, thus limited access to praziquantel is a major issue for control (WHO, 2013a). Praziquantel is orally effective with a single-day treatment and mainly affects the earliest stages (first few days after infection) and adult worms, but does not have any effect on eggs and juvenile worms. Treated patients therefore might continue to excrete eggs for several weeks after drug administration. Side-effects are mild and it is judged safe for treatment of young children and pregnant women. So far, no strong evidence for resistance has been observed, however, several therapeutic failures attributed to *S. mansoni* strains or possible drug resistance have been indicated (Gryseels et al., 2006; Thétiot-Laurent et al., 2013). Meanwhile Artemisinin derivatives have been identified as possible new candidates for schistosomiasis treatment. Especially juvenile stages have shown high susceptibility to ACTs, in contrast to praziquantel. Combined therapy with praziquantel thus promises high impact. ACTs, however, are first-line treatment for falciparum malaria and discussion on their use for schistosomiasis control in the light of induced *Plasmodium* resistance is ongoing (Utzinger et al., 2010).

Table 1.1 Classes of intensity for soil-transmitted helminths and schistosomes (WHO, 2011)

Parasite	Light-intensity infections	Moderate-intensity infections	Heavy-intensity infections
A. lumbricoides	1-4,999 epg	5,000-49,999 epg	≥ 50,000 epg
T. trichiura	1-999 epg	1,000-9,999 epg	≥ 10,000 epg
Hookworms	1-1,999 epg	2,000-3,999 epg	≥ 4,000 epg
S. mansoni	1-99 epg	100-399 epg	≥ 400 epg
S. haematobium	1-50 eggs/10 ml of urine		≥ 50 eggs/10ml of urine

Soil-transmitted helminthiasis

Likewise to *S. mansoni*, microscopic examination of Kato-Katz thick smears for eggs is considered the gold standard for diagnosis of soil-transmitted helminth infections. Egg counts can be expressed as infection intensity according to thresholds similarly (Table 1.1). Female soil-transmitted helminth worms usually produce large amounts of eggs per day, thus intensity cut-offs are set higher than for schistosomiasis (Bethony et al., 2006). Although the diagnostic technique remains the same, the applied procedure has to be adapted if prevalence of soil-transmitted helminths is to be investigated in addition to intestinal schistosomiasis. Hookworm eggs tend to dissolve promptly once faecal samples are placed on the microscope slides and covered with glycerine-soaked cellophane paper. The interval between preparation of Kato-Katz smears and examination under the microscope should therefore ideally not exceed 30-45 min to avoid underestimation of hookworm prevalence (Martin & Beaver, 1968). FLOTAC, a recently developed flotation method, showed promising results and was found to be more sensitive for hookworm infections than the Kato-Katz technique (Knopp et al., 2014).

Routine deworming drugs are albendazole, mebendazole or levamisole (WHO, 2011). In *Trichuris* infection, however, treatment efficacy of these standard medications has been shown to be unsatisfactory. Yet, combined treatment with standard drugs plus ivermectin, which is usually recommended to treat filariasis, improved significantly cure and egg reduction rates (Knopp et al., 2010). Very recently the potential of an originally in veterinary applied drug was discovered for chemotherapy of *T. trichiura* (Speich et al., 2014).

Intestinal protozoa

There is no routine diagnostic procedure for intestinal protozoa, but microscopic examination is probably the most widely used technique (Becker et al., 2013). To increase sensitivity different concentration methods evolved, thereunder sedimentation or formalin-ether flotation methods are the most commonly applied. Concentration methods need certain minimal laboratory standards and equipment

and are usually not feasible in improvised field laboratories. A small portion (1-2 g) of the fresh stool sample may therefore be fixed in a sodium acetate-acetic acid-formalin (SAF) solution, which allows for storage and later transport to a better equipped laboratory (Marti & Escher, 1990). The formalin-ether concentration method is considered to have a decreased sensitivity. Microscopic morphological species classification, additionally, is difficult and needs experienced laboratory technicians. Faecal antigen detecting rapid diagnostic tests exist for *G. intestinalis* and *E. histolytica*, and are further able to distinguish between the latter and the morphologically similar species *E. dispar*. Highly sensitive PCR techniques have been developed. These techniques, however, are related with high costs and a need of highly equipped laboratory facilities, which restricts their use to reference laboratories in industrialised countries (Becker et al., 2013).

Preferred treatment of amoebiasis and giardiasis depends on the clinical course of the disease. Non-invasive stages of *E. histolytica* may be treated with paromomycin. Nitroimidazoles, particularly metronidazole, are the drugs of choice for giardiasis and invasive *E. histolytica*, which involve amoebic colitis or liver abscess (Haque et al., 2003; Farthing, 2006).

1.6 Control strategies and national programmes

1.6.1 Chemotherapy

Access to efficacious treatment against malaria is a key feature in malaria control. Treatment policies, however, depend on the setting-specific endemic situation. Chemotherapy for malaria control in endemic areas mainly relies on case management rather than mass drug administration (MDA). Recently, however, WHO updated policies towards the use of intermittent preventive treatment (IPT) in infants and during pregnancy in moderate to high malaria transmission areas (WHO, 2013b; WHO MPACS, 2013). IPT in children, additionally, has been proposed as a valuable tool to significantly decrease clinical malaria episodes and mortality in settings with markedly seasonal transmission (Wilson, 2011). Huge investment and efforts further have been put in the development of vaccines. Yet there are no licensed malaria vaccines. Nonetheless, several candidates are being evaluated in clinical trials, thereof one currently in Phase 3 clinical trials (RTS,S/AS01) and about 20 more in Phase 1 or Phase 2 clinical trials (WHO, 2013b).

In human helminthiasis including soil-transmitted helminths and schistosomiasis, preventive chemotherapy is the cornerstone of control strategies. Frequency, implementation and targeted population for preventive chemotherapy depend on thresholds of community risk based on infection prevalence in school-aged children (Table 1.2). Most deworming campaigns are planned and implemented on school level or targeting school-aged populations. This approach and eventually higher impact through community-based treatment plans are highly debated (Anderson et al., 2013). Indeed, it may be more appropriate to address helminth infections on community-level, where peak shifts are observed and prevalences are higher among adults, as for example in hookworm and *S. mansoni* infections (Woolhouse, 1998; Raso et al., 2004; Tchuem Tchuenté, 2011). According to WHO the main focus of helminth control through preventive chemotherapy is morbidity reduction by eliminating high-intensity infections.

Table 1.2 Recommended control strategies for schistosomiasis and soil-transmitted helminthiasis (adapted from WHO, 2006, 2011)

Category	Prevalence in school-aged children (SC)*	Control strategy	
		Preventive chemotherapy	**Additional actions**
Schistosomiasis			
High-risk area	≥ 50%	Treat all SC (enrolled and not enrolled) once a year	Also treat adults considered at risk
Moderate-risk area	≥ 10% and < 50%	Treat all SC (enrolled & not enrolled) once every 2 years	Also treat adults considered at risk
Low-risk area	≥ 1% and < 10%	Treat all SC (enrolled & not enrolled) twice during primary schooling age	Praziquantel should be available in dispensaries and clinics for treatment of suspected cases
Soil-transmitted helminths			
High-risk area	≥ 50%	Treat all SC (enrolled and non-enrolled) twice a year	Also treat: - Pre-schoolers - Women of childbearing age (2^{nd}/3^{rd} trimester pregnant and lactating included) - Adults in high-risk occupations
Low-risk area	≥ 20% and < 50%	Treat all SC (enrolled and non-enrolled) once a year	Also treat: - Pre-schoolers - Women of childbearing age (2^{nd}/3^{rd} trimester pregnant and lactating included) - Adults in high-risk occupations

*Based on parasitological methods

Chemotherapy in diarrhoeal diseases from intestinal protozoa infection has been discussed in the previous section on treatment. Control strategies to tackle amoebiasis and giardiasis rather focus on prevention and water safety actions, which will be elaborated in the following sections.

1.6.2 Vector control

Vector control is an important component in malaria control and incorporates several measures. Insecticide treated mosquito nets (ITNs) protect the user from infective bites but also have been shown to indirectly protect the community by killing mosquitoes (White et al., 2014). The impact of ITN on malaria prevalence and morbidity is strongly linked with its coverage. ITN use is therefore highly promoted and coverage particularly progressed in African countries where ITN distribution was promoted free of charge compared to those that rely on cost recovery or subsidised public-sector promotion (Noor et al., 2009). In recent years, long-lasting insecticide treated nets (LLINs) have replaced ITNs, hence have overcome the problem of re-impregnation of ITNs needed at a 6-month interval in order to keep effectiveness. Transmission control through indoor residual spraying (IRS) also remains a powerful control tool. A total of 88 countries, including 40 in the African region, recommended IRS for malaria control in 2012. Resistance of anopheline mosquitoes to insecticides represents a major issue for vector control. Its monitoring and surveillance and policy adaption, if required, are thus highlighted (WHO, 2013b). Another measure of vector control is mosquito larval source management (LSM), which focuses on management of potential larval habitats. This involves larviciding, biological control (e.g. by introduction of natural enemies of mosquitoes into the aquatic habitat), habitat manipulation and modification (e.g. drainage of surface water, coverage of water storage recipients, drain cleaning, etc.) (Tusting et al., 2013).

Complementary interventions which aim at interruption of schistosomiasis transmission like environmental snail control and focal molluscicide have been recommended in areas approaching elimination. To date, only few countries have implemented these measures in conjunction with preventive chemotherapy (e.g. Burkina Faso, Cambodia, China, Egypt, Mauritius and Morocco) (WHO, 2013a). Chemical snail control using niclosamide, however, has certain drawbacks. Molluscicide is considered costly, needs to be applied in discrete water bodies and besides snails also kills non-target water organisms like fish, thus acceptability of implementation in target communities may be low (Takougang et al., 2007).

1.6.3 Health education and preventive measures

Parasitic diseases are preventable diseases, but affected populations often lack adequate knowledge on disease and its transmission. Furthermore, own local concepts for diseases may exist, which foster risk-related behaviour. A study on knowledge, attitude, practice and behaviour (KAPB) revealed that lack of knowledge of intestinal schistosomiasis negatively affected help seeking and highlighted the public concern of not being able to seize measures to avoid infection due to unawareness (Acka et al., 2010). Health education together with standard measures of control (e.g. preventive chemotherapy) can enhance impact of control strategies by increasing acceptance for treatment and prevention tools and create awareness for a disease and its symptoms and thus change help seeking behaviour. Knowledge about mosquitoes as causative agent in malaria transmission for example was positively associated with ITN use in Côte d'Ivoire (Ouattara et al., 2011). Especially in diseases with fast and high reinfection rates due to inadequate hygiene behaviour, such as soil-transmitted helminthiasis, health education proved to be very valuable (Bieri et al., 2013). As a complementary measure in disease control it is therefore highly recommended by WHO (WHO, 2011, 2013b).

1.6.4 Improved sanitation and access to clean water

By the end of 2011 2.5 billion people lacked access to an improved sanitation facility, 768 million did not use an improved source for drinking-water, including 185 million who relied on surface water to meet their needs, and 1 billion (15%) still practiced open defecation. Worst progress in sanitation and drinking-water was observed in Sub-Saharan Africa, particularly in rural areas (WHO & UNICEF, 2013). Improving sanitation and provide access to safe water would concurrently tackle a wide range of parasitic infections, which are usually referred to as poverty-related diseases. The risk of soil-transmitted helminthiasis has been shown to be significantly decreased if improved sanitary facilities are present and used (Ziegelbauer et al., 2012). In Côte d'Ivoire small-scale heterogeneity of schistosomiasis prevalence in pre-school children was related to access to safe

water supply and proximity to open water sources (Coulibaly et al. 2013a). In many countries only very limited resources for water supply and sanitation development are available, thus environmental conditions which favour the spread of water-borne and hygiene-related diseases persist. One way forward in such areas would be the implementation of community-led approaches to total sanitation (CATS) (UNICEF, 2009). A range of methods under the CATS umbrella exist, “community-led total sanitation” (CLTS) (Kar & Chambers, 2008) is probably the most widely and successfully implemented among them. The main goal of CLTS is the community-wide elimination of open defecation through awareness raising and affordable sanitation options. A further core element of CLTS is the strengthening of solidarity and cooperation between households resulting in collective actions taken and benefits perceived and thus high acceptance within the community.

1.6.5 Current control programmes in Côte d’Ivoire

Côte d’Ivoire maintains a national malaria control programme, which is supported by the Global Fund. Due to a post-electoral crisis in late 2010, however, control strategies suffered a setback (Bonfoh et al., 2011). The programme has set an ambitious agenda to be implemented based on an integrated approach which comprises recommended region-specific aspects on treatment policies, vector control and prevention (RBM, 2008). Current and future activities financed by a new Global Fund grant will focus on acquisition of 12 Million LLINS for scaling-up distribution and replacing used LLINs, thus allowing a universal coverage across the population.

The national schistosomiasis, soil-transmitted helminthiasis and lymphatic filariasis control programme in collaboration with the “Schistosomiasis Control Initiative” (SCI) started implementation of school-based deworming in 2012. First activities focused on known high-risk areas in the western and southern part of Côte d’Ivoire aiming at implementation on national scale. Also in the western part of the country, which was known as a “hot spot” for intestinal schistosomiasis (Raso et al., 2005; Matthys et al., 2007), a schistosomiasis consortium for operational research and evaluation (SCORE, http://score.uga.edu/)-funded project on sustaining

schistosomiasis control is taking place since 2010, including annual preventive chemotherapy targeting schoolchildren with praziquantel and albendazole in collaboration with the national schistosomiasis, soil-transmitted helminthiasis and lymphatic filariasis control programme. It maintains 50 sentinel sites and conducts regular community and school-based treatment rounds against schistosomiasis with the purpose to investigate the impact of different treatment strategies.

1.7 References

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2. Goals

The overarching goals of this Ph.D. study were (i) to elucidate the epidemiology of multiple species parasitic infections at community level and on regional scale; (ii) to investigate implications of single and multiple species infections for clinical and self-reported morbidity; (iii) to assess health-related quality of life among school-aged children with parasitic infections and to determine age- and context-specific disability weights; (iv) to determine physical fitness and cognitive functioning in school-aged children and to analyse for relationships with parasitic infections; and (v) to study the effect of repeated deworming on different health status measures in school-aged children

2.1 Specific objectives

The current Ph.D. thesis pursued the following specific objectives:

- To assess the prevalence and intensity of infection of *Plasmodium*, *Schistosoma*, soil-transmitted helminths and intestinal protozoa and to identify sociodemographic and behavioural determinants within rural communities.
- To investigate for associations and interactions between different parasite species.
- To study the epidemiology of *Plasmodium* and helminth infections among school-aged children across Côte d'Ivoire.
- To assess implications of single and multiple species infections for clinical and self-reported morbidity among different age groups and to identify potential interactions between parasite species.
- To evaluate differences in self-rated quality of life between non-infected and *Plasmodium*- or helminth-infected school-aged children and to derive disease-related disability weights
- To investigate the relationship between (co-)infections of *Plasmodium*, helminth and intestinal protozoa and physical and cognitive performance in school-aged children.

- To quantify the effect of repeated treatment with praziquantel and albendazole against schistosomiasis and soil-transmitted helminths on clinical, physical fitness and cognition outcomes among school-aged children after a 5-month period.

3. Study sites and designs

The surveys presented in this work were carried out in ten rural communities in south and south-central Côte d'Ivoire and in ninety-two schools across the country.

In more detail, between July 2011 and September 2011 cross-sectional community based parasitological, clinical and questionnaire-based surveys were conducted in nine settlements situated in the Taabo HDSS and in a hamlet of Azaguié town, Agnéby-Tiassa region. The communities Sahoua (Taabo HDSS) and Ancien Carrefour (Azaguié area) are indicated on the map below (Figure 3.1.)

A national cross-sectional school-based survey was carried out in 92 schools across Côte d'Ivoire between November 2011 and February 2012. In each school approximately 60 children were subjected to parasitological, clinical and questionnaire investigation.

In one of the 92 schools, Niablé, located at the border to Ghana in the eastern part of Côte d'Ivoire, a 5-month follow-up intervention study was carried out. Approximately 300 children underwent parasitological and clinical examination and were subjected to physical fitness and cognition testing at baseline (December 2012) and at a 5-month follow-up (May 2013). All participating children received two rounds of treatment against schistosomiasis and soil-transmitted helminths at month 0 and 2.

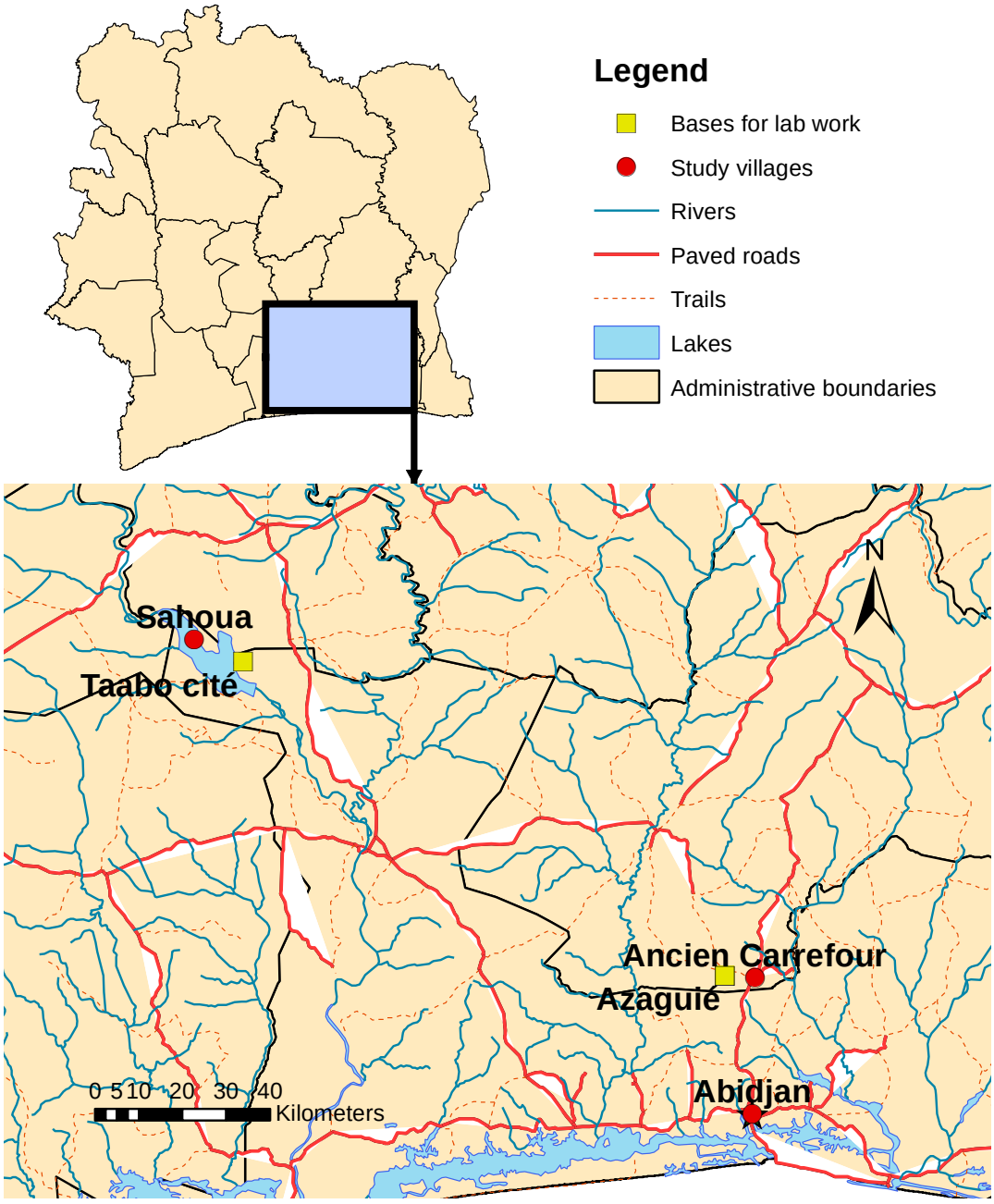

Figure 3.1. Study locations for community-based surveys; Sahoua (located in the Taabo HDSS), Ancien Carrefour (hamlet of Azaguié town)

4. The epidemiology of polyparasitism and implications for morbidity in two rural communities of Côte d'Ivoire

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4.1 Abstract

Polyparasitism is still widespread in rural communities of the developing world. However, the epidemiology of polyparasitism and implications for morbidity are poorly understood. We studied patterns of multiple species parasite infection in two rural communities of Côte d'Ivoire, including associations and interactions between infection, clinical indicators and self-reported morbidity. Between August and September 2011, two purposely selected rural communities in southern Côte d'Ivoire were screened for helminth, intestinal protozoa and *Plasmodium* infection, using a suite of quality-controlled diagnostic methods. Additionally, participants were examined clinically and we measured haemoglobin level, height, weight and mid-upper arm circumference to determine nutritional status. An anamnestic questionnaire was administered to assess people's recent history of diseases and symptoms, while a household questionnaire was administered to heads of household to collect socioeconomic data. Multivariate logistic regression models were applied for assessment of possible associations between parasitic (co-) infections and morbidity outcomes. 912/1,095 (83.3%) study participants had complete parasitological data and 852 individuals were considered for in-depth analysis. The rate of polyparasitism was high, with *Plasmodium falciparum* diagnosed as the predominant species, followed by *Schistosoma haematobium*, *Schistosoma mansoni* and hookworm. There were considerable differences in polyparasitic infection profiles among the two settings. Clinical morbidity such as anaemia, splenomegaly and malnutrition was mainly found in young age groups, while in adults, self-reported morbidity dominated. High parasitaemia of *P. falciparum* was significantly associated with several clinical manifestations such as anaemia, splenomegaly and fever, while light-intensity helminth infections seemed to have beneficial effects, particularly for co-infected individuals. Clinical morbidity is disturbingly high in young age groups in rural communities of Côte d'Ivoire and mainly related to very high *P. falciparum* endemicity. Interactions between helminth infections and *P. falciparum* burden (parasitaemia and clinical morbidity) are evident and must be taken into account to design future interventions.

Keywords: Anaemia, Côte d'Ivoire, Helminth, Malnutrition, Morbidity, *Plasmodium*, Polyparasitism, Splenomegaly

4.2 Introduction

Hundreds of millions of people in the developing world are at risk of parasitic diseases, such as malaria and neglected tropical diseases (NTDs) (Snow et al., 2005; Pullan & Brooker, 2012; Walker et al., 2013). Among the NTDs, parasitic worm (helminth) infections are particularly important in terms of number of people infected and estimated global burden, as expressed in disability-adjusted life years (DALYs) (Hotez et al., 2008; Murray et al., 2012; Utzinger, 2012; Pullan et al., 2014). In Côte d'Ivoire, an estimated 33,600 deaths and 2.5 million DALYs were attributable to malaria and NTDs in 2010. These estimates represent 16.6% of the total DALYs and 14.8% of all deaths in Côte d'Ivoire in 2010 (IHME, 2013). Typical clinical manifestations from infection with *Plasmodium* spp. include anaemia and splenomegaly associated with erythrocyte death and splenic sequestration, respectively (Föller et al., 2009; del Portillo et al., 2012). Helminth infections (e.g. soil-transmitted helminths, *Schistosoma mansoni* and *Schistosoma haematobium*) are rarely fatal, but cause long-term chronic morbidity (King et al., 2005; Knopp et al., 2012). This may include anaemia due to blood loss from intestinal or urinary tract bleeding, iron-deficiency linked to nutritional impairment such as malabsorption and other digestive disorders like diarrhoea (WHO, 2011). Nutritional impairment and competition for nutrients with intestinal parasites further affect the nutritional status leading to malnutrition and impaired child growth (Stephenson et al., 2000). *Schistosoma* spp. infections may cause tissue damage, and hence have been associated with organ pathology mainly driven by migrating parasite eggs in the human body.

To date, most research on parasitic disease-related morbidity focused on single species infections, whilst the health impact due to polyparasitism remains poorly understood (Steinmann et al., 2010). For countries like Côte d'Ivoire where

polyparasitism is still widespread (Keiser et al., 2002a; Raso et al., 2004; Coulibaly et al., 2012), a deeper mechanistic understanding of multiple species parasite infections is crucial for disease control and the reduction of the burden due to these (co-)infections. Findings from recent studies in different parts of the world are conflicting. For instance, while some studies reported a higher frequency of anaemia in individuals co-infected with *Plasmodium* and helminths, other studies found high anaemia rates in individuals with single species *P. falciparum* infections (Brooker et al., 2007; Righetti et al., 2012; Naing et al., 2013). Intensity of infection plays an important role in shaping morbidity patterns. Ezeamama and colleagues (Ezeamama et al., 2008) showed strong additive or even multiplicative effects on anaemia in children with high-intensity hookworm and *Schistosoma japonicum* co-infections in the Philippines. In another study carried out in Senegal, light-intensity infections of *S. haematobium* were associated with lower malaria parasitaemia in children, but the opposite was found in Kenyan children where high-intensity infections of both parasites were positively associated (Briand et al., 2005; Florey et al., 2012). Thus, associations and possible inhibitory or favouring mechanisms between species and depending on intensity are of considerable interest, and new research is needed to shed additional light on these issues.

Health effects from multiple species infections are complex due to associations between parasites and possible synergism/antagonism on disease outcome. Additionally, associations are further complicated due to a diversity of proximal and distal risk factors (e.g. socioeconomic status and poor nutrition), as well as demographic, exposure and immunological factors. The aim of the study presented here was to deepen the understanding of the epidemiology of polyparasitism and its implications for morbidity. Residents from two purposely selected communities in Côte d'Ivoire were examined with a suite of diagnostic methods, interviewed with a pre-tested questionnaire and subjected to detailed clinical examinations.

4.3 Materials and methods

Ethics statement

The study protocol was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland) and received clearance from the ethics committees of Basel (EKBB, reference no. 30/11) and Côte d'Ivoire (reference no. 09-2011/MSHP/CNER-P). District health authorities and village chiefs were informed about the objectives, procedures and potential risks and benefits of the study. Written informed consent was obtained from each individual (and parents/guardians of children aged below 18 years), emphasising that participation is entirely voluntary and that participants can withdraw from the study at any time without further obligation.

At the end of the survey, albendazole (400 mg for participants >2 years and 200 mg for children aged 1-2 years) against soil-transmitted helminthiasis irrespective of infection status was administered. Individuals with a *Schistosoma* infection received praziquantel (40 mg/kg). Participants with clinical malaria (i.e. fever and a positive malaria rapid diagnostic test (RDT)) were given artemisinin-based combination therapy (artesunate-amodiaquine for adults and artemether-lumefantrine for children) and paracetamol against fever. An anti-anaemic treatment in severely anaemic individuals with no malaria symptoms was provided. All treatment regimens were offered free of charge. Data were coded and confidentially treated.

Study area and population

We purposely selected two rural communities in south and south-central Côte d'Ivoire based on different helminthiases endemicity profiles; Sahoua (Fürst et al., 2012) and Ancien Carrefour (Coulibaly et al., 2013). Sahoua borders the Bandama River approximately 160 km north-west of Abidjan and is located in the Taabo health and demographic surveillance system (HDSS) (geographical coordinates: 6°19'20" N latitude, 5°10'30" W longitude) (Fürst et al., 2012). Ancien Carrefour is a hamlet of Azaguié town in the region of Agnéby-Tiassa, intersected by numerous small rivers and stagnant water bodies and is situated approximately 40 km north of Abidjan

(5°37'40" N, 4°01'15" W) (Coulibaly et al., 2013). Previous studies in nearby villages revealed high helminth infection prevalences; along the Bandama River mainly *S. haematobium* and hookworm (N'Goran et al., 1997, 2001; Glinz et al., 2010) in the Azaguié area mainly *S. mansoni* and soil-transmitted helminth infections (Coulibaly et al., 2012, 2013). Using a cross-sectional epidemiological design, in both villages, all inhabitants (872 in Sahoua and 498 in Ancien Carrefour) were invited to participate, involving parasitological and clinical examinations and questionnaire interviews. The field work was conducted in August and September 2011.

Field and laboratory procedures

In Sahoua, detailed demographic data (number of household members, names, age, sex and specific identification numbers for each household) were readily available from the Taabo HDSS database. In Ancien Carrefour, a demographic survey was carried out with the assistance of four designated local people to identify all households and to collect demographic information. Additionally, socioeconomic data and behavioural aspects regarding parasitic diseases were gathered at the unit of the household, by administering a pre-tested questionnaire (Acka et al., 2010). Finally, a pre-screening among 60 school-aged children was undertaken to clarify the extent of helminth infections. Urine examinations revealed no microhaematuria, which is a useful proxy for *S. haematobium* (Kahama et al., 1999). Hence, in the subsequent parasitological survey, participants from Ancien Carrefour were asked to provide stool samples exclusively, while participants from Sahoua provided stool and urine samples.

Pre-packed plastic bags containing one stool container (and an additional urine container in Sahoua), labelled with the name and unique identifiers for each participant, were distributed among all households. Household members were asked to return filled containers the next morning. Participants were invited for a finger-prick blood sample that was subjected to an RDT for malaria (ICT ML01 Malaria Pf kit; ICT Diagnostics, Cape Town, South Africa) and thick and thin blood films prepared on microscope slides.

Stool (and urine) specimens and thick and thin blood films were transferred to nearby laboratories. The stool samples were processed as follows. First, a small portion of stool (1-2 g) was fixed in sodium acetate-acetic acid-formalin (SAF) (Marti & Escher, 1990). Second, duplicate Kato-Katz thick smears using 41.7 mg templates (Katz et al., 1972) were prepared from each stool sample. For the detection of *S. haematobium*, urine samples were subjected to a filtration method (Savioli et al., 1990). Kato-Katz thick smears and urine filters were examined under a microscope by experienced laboratory technicians. The number of *S. haematobium* (urine filters) and *S. mansoni*, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm eggs (Kato-Katz thick smears) were counted and recorded separately. Thick and thin blood films were stained with Giemsa.

For quality control, 10% of the slides were randomly selected and re-examined by a senior microscopist. In case of discordant results, the respective slides were re-examined and the results discussed among the concerned technicians until agreement was found. If results from a specific technician had an error rate above 10%, all slides on that day were re-read.

The SAF-fixed stool specimens and the Giemsa-stained blood films were forwarded to a laboratory in Abidjan. SAF-fixed stool samples were subjected to an ether-concentration technique and the spectrum of intestinal protozoa investigated included *Entamoeba histolytica/E. dispar*, *Entamoeba hartmanni*, *Entamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, *Giardia intestinalis*, *Chilomastix mesnili* and *Blastocystis hominis*. Helminth eggs were also recorded for each species separately (Utzinger et al., 2010). Giemsa-stained blood films were assessed for parasitaemia (parasites/µl of blood) and *Plasmodium* species identification under a microscope, following standardised, quality-controlled procedures (N'Goran et al., 2003).

Disease-related morbidity was classified into two types; clinical manifestations and self-reported morbidity. Clinical manifestations were assessed during a medical examination by two experienced clinicians. These included liver and spleen enlargement determined by palpation and in case of splenomegaly graded according to the Hackett's scale (Ruzagira et al., 2010), anaemia through haemoglobin (Hb) measurement using a HemoCue analyser (HemoCue Hb 301 system; Angelholm,

Sweden) and pallor through examination of the inferior conjunctiva. Additionally, body temperature was measured in all participants using an ear thermometer (Braun ThermoScan IRT 4520; Kronberg, Germany) to identify fever cases (≥ 38.0 °C). Anthropometric measurements, including mid-upper arm circumference (MUAC) (in cm, precision mm), height (in cm) and body weight (in kg, precision 0.5 kg) were taken for subsequent calculation of the nutritional status. Self-reported morbidity was assessed through an anamnestic questionnaire recalling major disease-related symptoms (transient morbidity) such as diarrhoea, abdominal pain, blood in the stool and blood in urine, using a recall period of 2 weeks, and self-reported chronic morbidity (persistent morbidity: discomfort, pain or any other disabling health condition persisting over longer periods).

Statistical analysis

Data were double-entered and cross-checked in EpiInfo version 3.5.3 (Centers for Disease Control and Prevention; Atlanta, USA). Statistical analysis was performed in Stata version 10.1 (Stata Corp.; College Station, USA). A two-sample analytical approach was employed. The first sample consisted of individuals who had complete parasitological datasets, which was used for basic parasitological and polyparasitism frequency analysis. The second sample included those individuals who, additionally, had complete socioeconomic data and clinical measurements, thus allowed investigating the relationship between co-infection, socioeconomic status and morbidity using multivariate regression models. Two different age cut-offs were used; (i) < 5, 5-9, 10-14, 15-24, 25-39 and ≥ 40 years in sample 1 and (ii) < 5, 5-9, 10-18, 19-39 and ≥ 40 years in sample 2. The rationale to use slightly different age groups was to account for common parasite peak prevalences in sample 1 (Keiser et al., 2002a; Raso et al., 2004) and have suitable cut-offs for the calculation of morbidity indicators (e.g. anaemia and malnutrition) in sample 2.

Classes of intensity for *Schistosoma* spp. and soil-transmitted helminth infections were grouped according to guidelines of the World Health Organization (WHO) (WHO, 2002). Intestinal protozoa were recorded semi-quantitatively, distinguishing between light (one to five cysts or trophozoites per slide); moderate

(one cyst or trophozoite per observation field at a magnification of ×400 or 500); and heavy (more than one cyst or trophozoite per observation field at a magnification of ×400 or 500) (Utzinger et al., 2010). The severity of anaemia was categorised according to WHO guidelines and taking into account for 10 g/l lower cut-offs in African populations (Johnson-Spear & Yip, 1994; WHO, 2001). The nutritional status for children <5 years and children aged 5-18 years was determined according to available macros for Stata with the new child growth standards and references published by WHO (Duggan, 2010). Indicators for malnutrition in children aged <5 years included wasting (weight-for-height), stunting (height-for-age), underweight (weight-for-age), thinness (body mass index (BMI)-for-age) and malnutrition assessed by arm circumference (MUAC-for-age), while for children aged 5-18 years only stunting, thinness and underweight, the latter only up to the age of 10 years, could be applied as reference measures for nutritional status. BMI and MUAC classes were determined according to Eddleston *et al*. (Eddleston et al., 2008) and were used as measures for malnutrition in adults. All nutritional indicators were classified as (i) mild (Z-score < -1 > -2); (ii) moderate (Z-score < -2 > -3); and (iii) severe (Z-score < -3). Splenomegaly was defined as having a palpable spleen of grade 1 or higher according to Hackett's scale.

A household-based asset approach was used to determine participants' socioeconomic status, which allowed stratifying individuals into economic groups (wealth quintiles). This approach has been successfully applied and a detailed description is given in a previous study conducted in Côte d'Ivoire (Schmidlin et al., 2013). As a measure for the magnitude of inequality between health (here: parasitic infections) and the socioeconomic status, the concentration index (C) was used (O'Donnell et al., 2008).

For frequency statistics to compare for infection and morbidity rates between different strata, χ^2 and Fisher's exact test were applied, as appropriate, while for the mean number of concurrent infections two non-parametric tests (Kruskal-Wallis and the Mann-Whitney test) were used to account for skewed distributions. Comparison for Hb levels by *Plasmodium*-helminth co-infection categories was executed with one-way ANOVA or Kruskal-Wallis test in case of unequal variances.

To estimate associations between parasitic infections and morbidity indicators, multivariate logistic regression models were used. In a first step, bivariate associations were assessed and parasite species and morbidity indicators not showing any significant relationships were removed from further analyses. In each model (poly-) parasitic infection status or infection intensity served as covariates, depending on better fit of the model. A forward stepwise elimination approach was applied for each model, including covariates at a significance level of 0.15. All models were adjusted by age group, sex and socioeconomic status. Significant relationships between an outcome (infection with a parasite or a specific morbidity indicator) and explanatory variables (infection status or intensity of infection) were expressed as adjusted odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Regression analysis for clinical outcomes was performed for children/adolescents aged < 18 years and adults aged ≥ 18 years separately, accounting for age-specific morbidity and parasitic infection patterns.

Co-infection and co-morbidity pairs of the multivariate regression models for children/adolescents comprised (i) *P. falciparum*-malnutrition comorbidity; (ii) *P. falciparum*-hookworm co-infection; and (iii) *P. falciparum*-*S. haematobium* co-infection. Considering the high prevalence of *P. falciparum* in this age range and that morbidity depends on infection intensity, *P. falciparum* infections with a parasitaemia of >500 parasites/µl of blood were used instead, while malnutrition only included moderate and severe cases. For the adult models, co-infection with *P. falciparum*-*S. mansoni* was of main interest as well as hookworm-*S. mansoni* co-infection. All significant associations were presented for the four co-infection categories: (i) none of the two conditions; (ii) mono-infection species 1; (iii) mono-infection species 2; and (iv) co-infection and were expressed as OR with 95% CI.

4.4 Results

Study participation and operational results

Overall, 1,095 out of a total of 1,370 inhabitants in the two communities participated in the survey, resulting in an overall compliance of 79.9%. Considering inter-village differences, the study participation was higher in Ancien Carrefour (88.0%) compared to Sahoua (75.3%). Figure 4.1 gives a flow chart, showing the compliance to different stages of the study. For 912 individuals, complete parasitological data and full records from the clinical examination were available. These data were summarised as sample 1 and used for the analysis of parasite prevalence profiles and polyparasitism. Sixty individuals were excluded either for missing information on socioeconomic status or for implausible anthropometric measurements, resulting in 852 records assigned to sample 2, which was utilised for further analyses on parasite associations and relationships with morbidity indicators. Female and male participants were equally distributed in both samples used: 463 females and 449 males in sample 1, while sample 2 consisted of 431 female and 421 male participants. The distribution among males and females within the age groups showed no significant difference in both samples (sample 1: $\chi^2 = 6.79$, degrees of freedom (d.f.) = 5, $p = 0.236$; sample 2: $\chi^2 = 4.50$, d.f. = 4, $p = 0.343$).

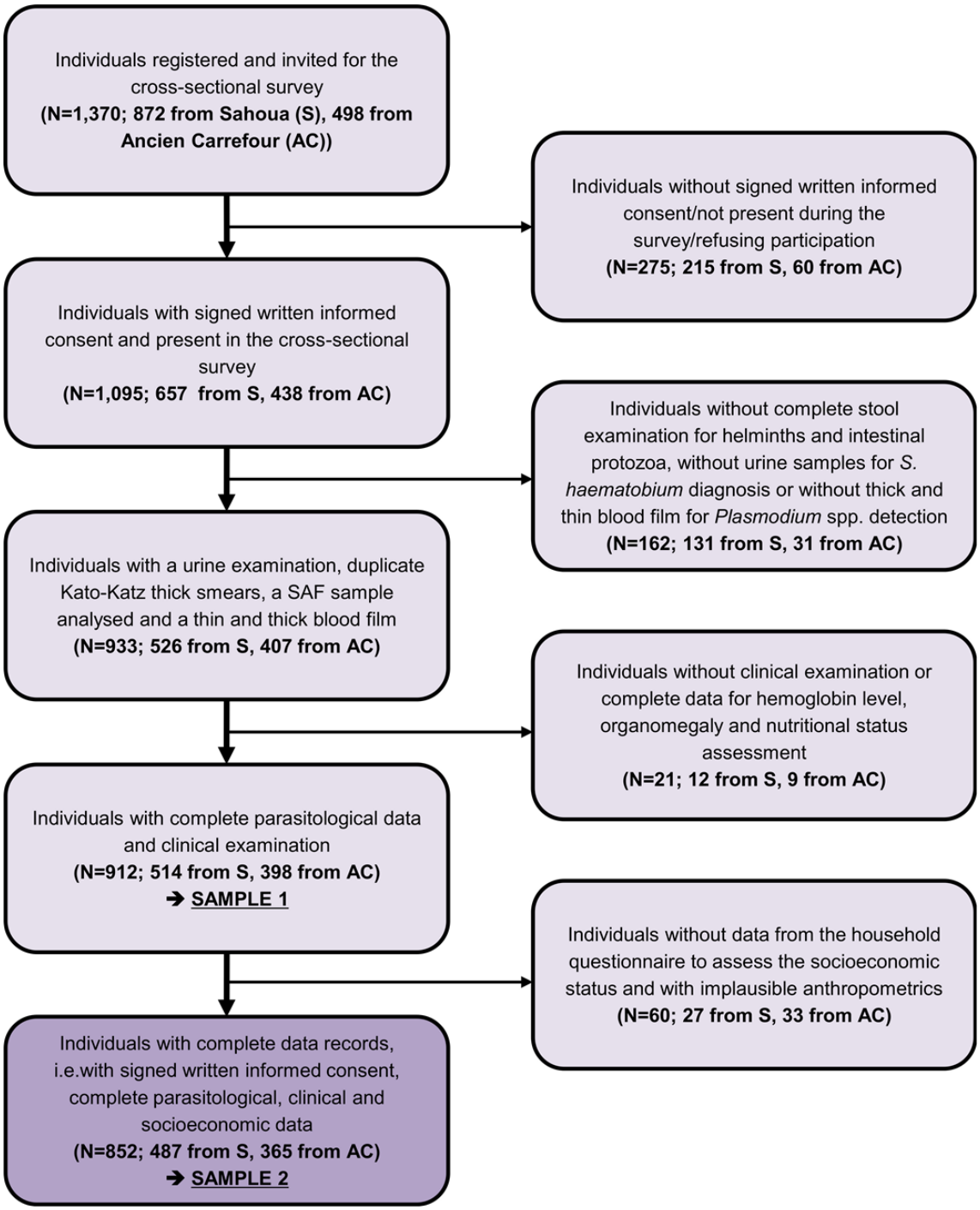

Figure 4.1 Flow chart illustrating study participation and compliance

The cross-sectional surveys were conducted in two rural settings in south and south-central Côte d'Ivoire in August and September 2011. Urine examination was done in Sahoua exclusively. AC = Ancien Carrefour, S = Sahoua.

Frequencies of parasitic infections and comparison for sociodemographic variables

The *S. haematobium* prevalence in Sahoua was 25.9%. *S. mansoni* was much more prevalent in Ancien Carrefour than in Sahoua (28.4% *vs.* 1.9%, $p < 0.001$). Concerning infection rates with soil-transmitted helminths, similar patterns were found in both communities; *A. lumbricoides* and *T. trichiura* infections were rarely found, hookworm being the predominant species with prevalences of 32.4% and 26.8% in Ancien Carrefour and Sahoua, respectively. Most of the soil-transmitted helminth infections were of light intensity. The endemicity profiles of the two settings differed not only for *Schistosoma*, but also for intestinal protozoa infections. In Sahoua a higher affection by intestinal protozoa was found in terms of prevalence and intensity of infection. Overall, the three most common intestinal protozoa species were *E. coli*, *B. hominis* and *E. nana*, with prevalences of 33.2%, 28.7% and 22.3%, respectively. The known pathogenic protozoan species *G. intestinalis* and *E. histolytica/E. dispar* were detected in 105 (11.5%) and 86 (9.4%) participants, respectively. In both localities three different *Plasmodium* species were identified; *P. falciparum* was the predominant species (overall prevalence in Ancien Carrefour and Sahoua was 70.1% and 59.7%, respectively), whilst *P. malariae* and *P. ovale* were detected in 33 (3.6%) and 3 (0.3%) of the participants, respectively.

Males were significantly more often infected with hookworm (males: 34.7%, females: 24.0%, $p < 0.001$) and *S. mansoni* (males: 15.8%, females: 11.2%, $p = 0.043$) than females.

Several parasites showed significant associations with age, such as both *Schistosoma* species (*S. haematobium*: $\chi^2 = 93.15$, d.f. = 5, $p < 0.001$; *S. mansoni*: $\chi^2 = 51.49$, d.f. = 5, $p < 0.001$), hookworm ($\chi^2 = 61.07$, d.f. = 5, $p < 0.001$) and three intestinal protozoan species (*E. coli*, *E. nana* and *G. intestinalis*). *S. haematobium*, *S. mansoni* and hookworm infections showed peak prevalences in the age groups of 10-14 years (prevalence: 35.0%), 15-24 years (prevalence: 24.0%) and 25-39 years (prevalence: 39.7%), respectively. The pathogenic intestinal protozoon species *G. intestinalis* was more often found in young children (peak in the age group of 5-9 years with 22% infected), while the prevalence of non-pathogenic intestinal protozoa

increased with age (peak in individuals aged 25-39 years). Malaria parasites were significantly more often found in younger individuals, and the three individuals identified to harbour *P. ovale* all belonged to the youngest age group of under-fives. Hookworm and *S. mansoni* were significantly associated with a lower socioeconomic status and more prevalent in participants from poorer households (concentration indices and standard errors: C = -0.0624, SE = 0.0306 and C = -0.2126, SE = 0.0427, respectively).

Polyparasitism

Polyparasitism was common; on average, a study participant harboured 2.5 concurrent parasitic infections. The maximum number of parasite species found in the same host was nine. The median number of infections significantly differed between age groups (Kruskal-Wallis, $p < 0.001$) illustrated in Figure 4.2, showing a peak in the age groups of 5-9 years (median: 3 parasites/individual, range: 1-8 parasites) and 10-14 years (median: 3 parasites/individual, range: 0-7 parasites), hence school-aged children showed the highest extent of polyparasitism. Females were slightly less affected by multiple species parasite infections compared to their male counterparts (Wilcoxon rank-sum with $p = 0.020$). Considering the high endemicity of *P. falciparum* (overall prevalence: 64.3%), the major co-infections identified were concurrent infections with *P. falciparum* and helminths, particularly hookworm (18.1%), *S. haematobium* (10.0%) and *S. mansoni* (8.9%). Co-infection patterns differed with age group. In younger age groups, where infections with helminths were less common, concurrent infections with *P. falciparum* and pathogenic intestinal protozoa like *G. intestinalis* and *E. histolytica/E. dispar* added up to the polyparasitic burden with a peak prevalence of 19.2% and 9.0%, respectively, in children aged 5-9 years.

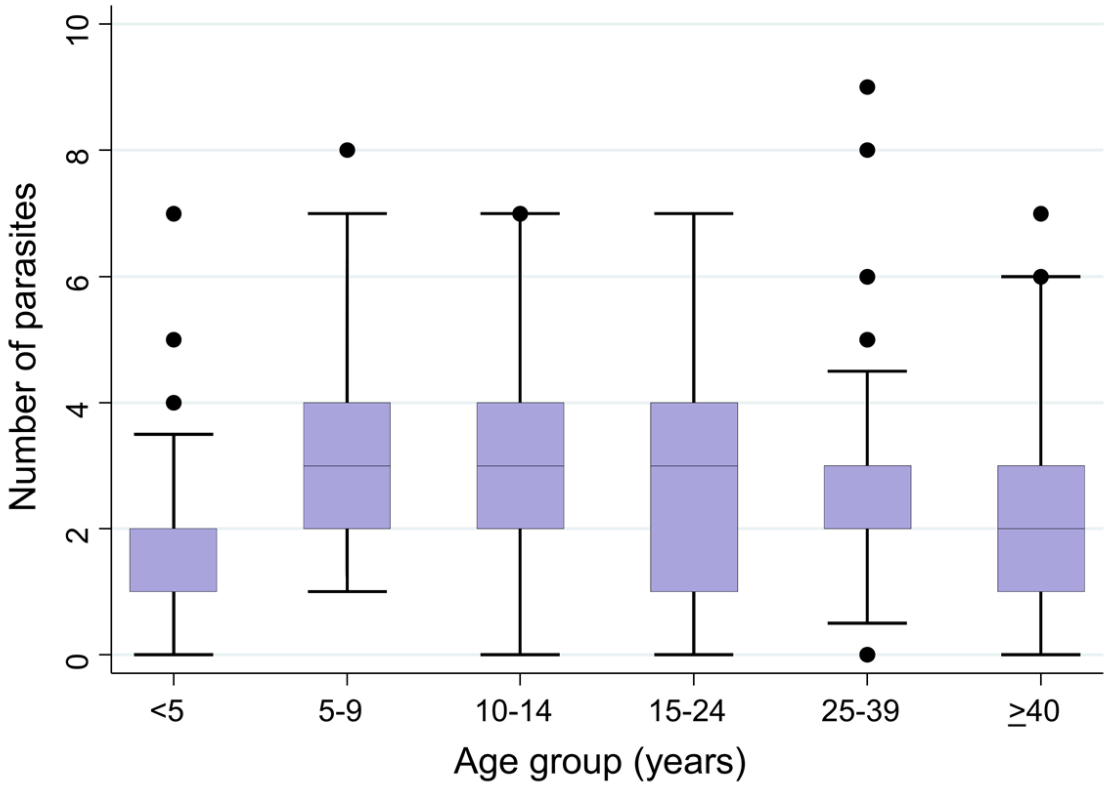

Figure 4.2 Number of concurrent parasitic infections, stratified by age group.

Rate of polyparasitism among 912 study participants with complete parasitological data. Box plot: boxes illustrate the 25th and 75th percentiles (ptile), while the whiskers indicate the adjacent lower and upper values (most extreme values which are within 25th ptile - 1.5*(75th-25th ptile) and 75th ptile + 1.5*(75th-25th ptile), respectively). The median is shown by the line within the boxes, outliers are indicated with dots.

Paired associations between parasite species

The findings from the multivariate regression analysis revealed a significant positive association in both ways for *P. falciparum* and *S. mansoni* with adjusted ORs of 2.03 and 2.14, respectively. All 78 (66.7%) *S. mansoni*-positive individuals who were co-infected with *P. falciparum*, exclusively had low *P. falciparum* parasitaemia with <500 parasites/µl blood (data not shown). *S. mansoni* infection shared a strong positive association with hookworm infection (OR = 2.78, p < 0.001) and *vice versa* (OR = 2.78, p < 0.001). Most intestinal protozoa species showed significant positive associations between each other. *E. coli*, for example, was associated with *E. histolytica/E. dispar* (OR = 3.19), *E. nana* (OR = 4.68) and *I. bütschlii* (OR = 5.93) (all p < 0.001). Significant associations from multivariate regression models between a particular parasite species and any other parasite, sex, age group and socioeconomic status are presented in Appendix 10.1.1.

Clinical and self-reported morbidity

The assessment for clinical morbidity revealed the prevalence for hepatomegaly, splenomegaly and anaemia of 0.2%, 15.7% and 19.5%, respectively among 852 individuals from both communities. Figure 4.3 depicts the extent of clinical manifestations assessed and self-reported symptoms and recent histories of disease reported during clinical examination, stratified by age group. Clinical morbidity mainly affected young age groups, while self-reported morbidity was common among all age groups. Besides age, morbidity patterns also differed by sex. In the most affected group of children under 10 years of age, splenomegaly and malnutrition were significantly more often found in boys than in girls (38.8% *vs.* 28.0% and 42.4% *vs.* 28.7%, respectively). Anaemia did not differ between boys and girls, but was associated with female sex in adulthood (age >18 years). 19% of all study participants showed any sign of moderate or severe nature for malnutrition (for details see Appendix 10.1.2). Stunting with a prevalence of 40% was by far the most common sign for malnutrition in the youngest and most affected age group. Additionally, stunting was significantly more often found in males than in females (49.4% *vs.* 29.0%) (Figure 4.4).

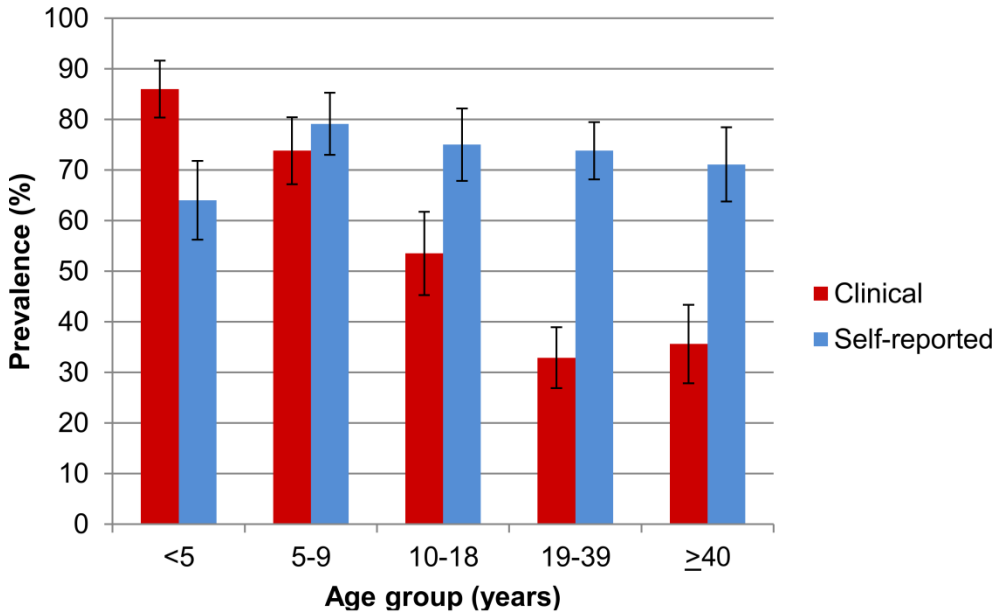

Figure 4.3 Prevalence of at least one clinical and/or self-reported morbidity indicator, stratified by age group.

Clinical outcomes included: anaemia, splenomegaly, pallor, fever and malnutrition (z-scores < -2), while self-reported morbidity comprised reported symptoms of diarrhoea, abdominal pain, blood in the stool, blood in urine and chronic morbidity. All morbidity data were assessed during medical examination in 852 study participants.

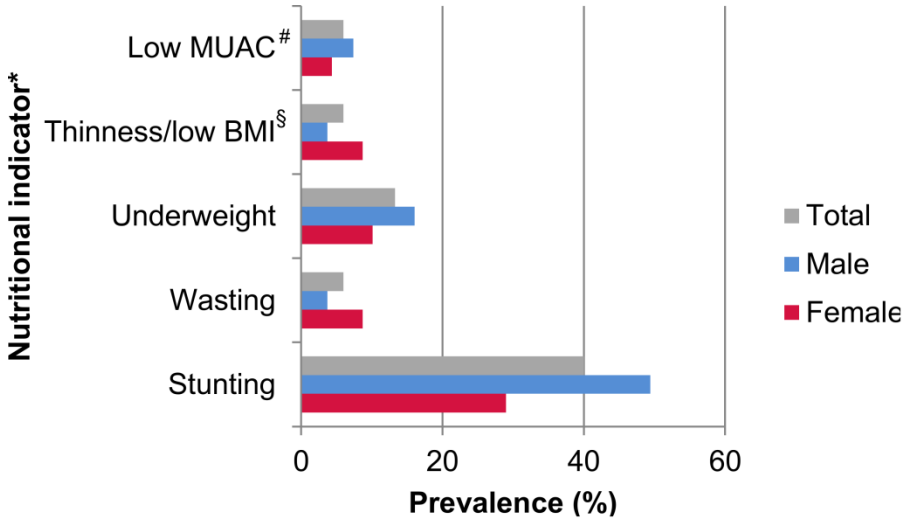

Figure 4.4 Prevalence of different nutritional indicators used to assess malnutrition in children aged <5 years, stratified by sex.

* Considered are only individuals showing moderate or severe signs for malnutrition;

MUAC = mid-upper-arm-circumference;

§ BMI = body mass index (weight / height^2)

Associations between parasitic infections and morbidity

Table 4.1 highlights all significant associations of clinically assessed and self-reported morbidity with any parasite infection status or intensity derived from the multivariate regression analysis. Anaemia, splenomegaly and fever all showed a strong positive association with *P. falciparum* parasitaemia. Splenomegaly was significantly associated with *P. malariae*, which was predominantly found in young age groups.

Table 4.1 Statistically significant associations between parasitic infections and clinically assessed or self-reported morbidity from multivariate regression analysis

Morbidity[§]	Association	Adjusted OR (95% CI)	p-value
Clinically assessed morbidity			
Anaemia[2+,7i,8i,9i,10i,12i,14]	*P. falciparum* intensity (> 5,000 parasites/µl of blood)	3.54 (1.63, 7.66)	0.001
	E. histolytica/E. dispar intensity (++)	4.43 (1.79, 10.97)	0.001
	S. mansoni intensity (+)	0.29 (0.10, 0.86)	0.025
	Hookworm intensity (+)	0.60 (0.37, 0.99)	0.045
Splenomegaly[3+,6+,7i,9i,10i,14]	*P. falciparum* intensity (> 5,000 parasites/µl of blood)	6.26 (2.60, 15.03)	<0.001
	P. falciparum intensity (501-5,000 parasites/µl of blood)	3.71 (1.80, 7.64)	<0.001
	P. malariae	2.28 (1.01, 5.18)	0.048
Malnutrition (z-score < -2)[9+,13,14,15]	*S. mansoni* intensity (+)	0.32 (0.11, 0.93)	0.036
	S. haematobium	0.55 (0.31, 0.98)	0.041
Pallor[7i,11i,14]	*S. mansoni*	0.40 (0.23, 0.70)	0.001
	E. histolytica/E. dispar	0.52 (0.29, 0.93)	0.026
Fever (≥ 38.0 °C)[3+,6+,14]	*P. falciparum* intensity (> 5,000 parasites/µl of blood)	4.19 (1.35, 13.03)	0.013
Self-reported morbidity			
Diarrhoea[6i,9+,11i,12i,14,16]	*S. mansoni* intensity (++)	3.33 (1.35, 8.23)	0.009
Abdominal pain[9i,14,15]	*S. mansoni*	2.54 (1.66, 3.89)	<0.001
	G. intestinalis intensity (++)	2.14 (1.17, 3.93)	0.013
Blood in the stool[7+,9+,10i,12i,13,14]	*S. haematobium*	2.20 (1.34, 3.62)	0.002
	S. mansoni	2.13 (1.27, 3.57)	0.004
	P. falciparum	0.58 (0.38, 0.89)	0.013
Blood in urine[7i,8i,9i,11i,12i,13,14]	*S. haematobium* intensity (+)	2.77 (1.61, 4.77)	<0.001
	S. haematobium intensity (+++)	11.08 (4.75, 25.83)	<0.001
	S. mansoni	0.29 (0.12, 0.72)	0.008
Chronic morbidity[6i,7i,8i,9i,11i,12i,14,15]	No. of concurrent pathogenic infections (≥ 3 parasites)	0.43 (0.21, 0.88)	0.022
	S. haematobium	2.25 (1.35, 3.75)	0.002
	S. mansoni	0.38 (0.19, 0.74)	0.004

Reference categories: parasite infection status or intensity: no infection. Helminth infection intensities: (+) = light, (++) = moderate, (+++) = heavy.

[§] Covariates further included in each model for adjustment (+ = infection with, i = intensity of infection): 1 = *P. falciparum*, 2 = *P. malariae*, 3 = *S. haematobium*, 4 = *S. mansoni*, 5 = Hookworm, 6 = *E. histolytica/E. dispar*, 7 = *E. coli*, 8 = *E. nana*, 9 = *I. bütschlii*, 10 = *G. intestinalis*, 11 = *C. mesnili*, 12 = *B. hominis*, 13 = sex, 14 = age group, 15 = socioeconomic status, 16 = number of concurrent pathogenic infections (0, 1, 2, and 3 or more).

Individuals presenting light-intensity infection with *S. mansoni* and hookworm had significantly lower ORs for anaemia. Effects on Hb levels due to infection with these helminths and co-infection with *P. falciparum* are illustrated in Figure 4.5. Children aged 5-11 years overall had significantly higher Hb levels if co-infected with hookworm (mean: 122.3 g/l, 95% CI: 119.0, 125.5 g/l) compared to individuals with *P. falciparum* mono-infection (mean: 115.4 g/l, 95% CI: 113.2, 117.5 g/l). In adolescents and adults aged 16-39 years, individuals with *S. mansoni* mono-infection were found to have significantly higher Hb values (mean: 143.7 g/l, 95% CI: 136.3, 151.1 g/l) compared to individuals with neither infection (mean: 131.2 g/l, 95% CI: 127.6, 134.8 g/l) or with *P. falciparum* mono-infection (mean: 132.1 g/l, 95% CI: 127.9, 136.2 g/l). This effect from helminth infections on Hb was found in male individuals only. Malnutrition was negatively associated with both *Schistosoma* species. The association between pallor and *S. mansoni* confirmed the negative direction also observed in the association between anaemia and light-intensity infections of this helminth species.

Although *S. mansoni* (particularly light-intensity infections) seemed to have beneficial effects on clinical outcomes like anaemia and malnutrition, a significantly positive association was found with several symptoms involving the digestive tract such as diarrhoea, abdominal pain and blood in the stool (all $p < 0.05$). Self-reported blood in urine and blood in the stool were both positively associated with *S. haematobium* infection. Eggs of *S. haematobium* are expected to be excreted in urine, but five out of the 126 (4%) individuals identified with *S. haematobium* were found to excrete eggs also in the stool.

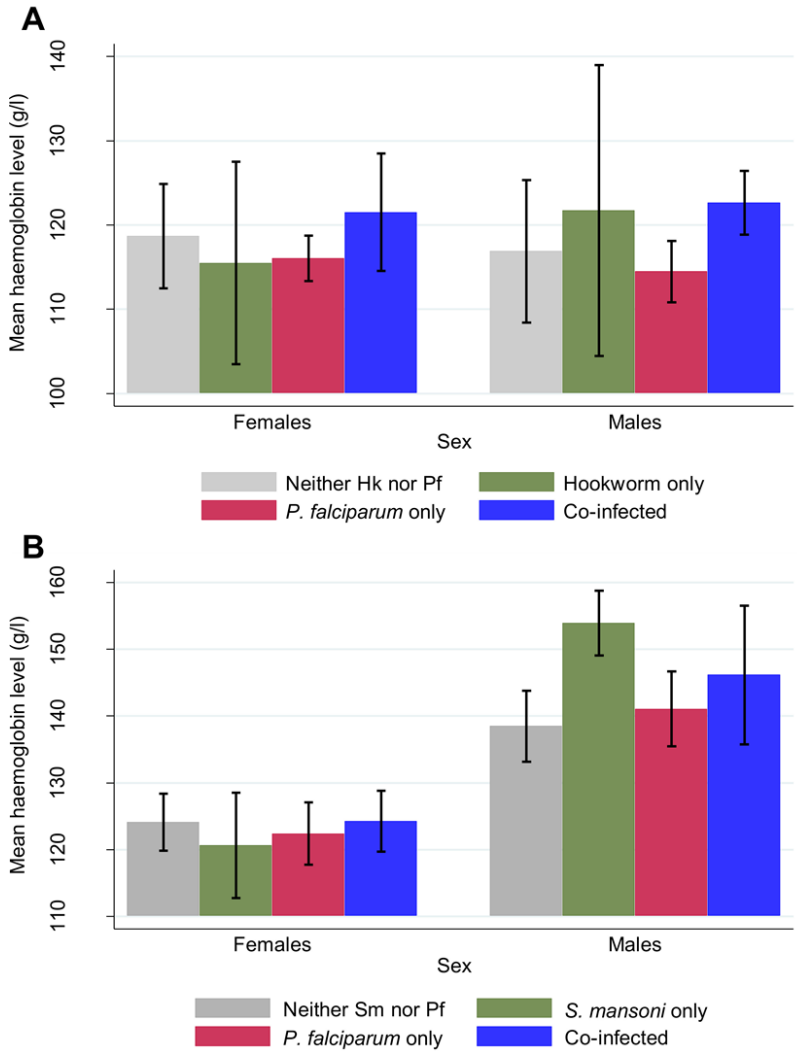

Figure 4.5 Comparison of mean haemoglobin levels in different age groups, stratified by sex in relation to helminth-*P. falciparum* co-infection from one-way ANOVA and Kruskal-Wallis test.

A: Comparison of mean haemoglobin (Hb) level in children aged 5-11 years (n = 224) shown for both sexes with different infection categories for hookworm (Hk) and *P. falciparum* (Pf). One-way ANOVA analysis showed significant differences in Hb for the total sample (not shown, p = 0.008) and in males (p = 0.028) **B:** Comparison of mean Hb level in participants aged 16-39 years (n = 243) shown for both sexes with different infection categories for *S. mansoni* (Sm) and *P. falciparum* (Pf). Kruskal-Wallis test showed significant differences in Hb for the total sample (not shown, p = 0.021) and in males (p = 0.015). Pregnant women (n = 31) were excluded from the analysis since pregnancy significantly lowers the Hb levels. In this sample of women aged 16-39 years, pregnant women had a mean Hb of 113.5 g/l *vs.* 123.3 g/l in non-pregnant women (p = 0.001).

Self-reported chronic morbidity was negatively associated with the highest category of concurrent infections with pathogenic parasites (non-pathogenic intestinal protozoa excluded), harbouring three or more pathogenic species ($p = 0.022$), but the association was of positive direction for *S. haematobium*-infected individuals ($p = 0.002$).

Table 4.2 shows the key findings from the multivariate regression analysis for the relationship between co-infection and selected morbidities in children/adolescents and adults. An infection with *P. falciparum* (> 500 parasites/µl of blood) in combination with malnutrition added up to the risk of having clinical manifestations such as anaemia and splenomegaly in children <18 years of age. The relationship of *P. falciparum* (> 500 parasites/µl of blood)-*S. haematobium* co-infection was not of an additive nature but all three infection categories showed significantly higher ORs for splenomegaly among children.

The pattern of negative associations between helminth infections and several morbidity outcomes found in the single-species models were confirmed in the co-infection models. Children infected with hookworm only had significantly lower ORs for anaemia. Additionally, hookworm had an antagonistic effect in relation to *P. falciparum* infection since co-infection with hookworm and a *P. falciparum* parasitaemia >500 parasites/µl of blood was not associated with anaemia. In adults, an antagonistic relationship was found for *P. falciparum*-*S. mansoni* co-infections; individuals co-infected had significantly lower ORs for several morbidities such as anaemia, pallor and recent history of chronic disease.

S. mansoni infection among adults, on the other hand, was found to be significantly positively associated with self-reported abdominal pain in combination with other parasites, such as *P. falciparum* and hookworm.

Table 4.2 Associations between specific morbidities and co-infection/co-morbidities in children/adolescents aged <18 years and adults ≥18 years from multivariate regression analysis

Morbidity[§]	N	Co-infection/co-morbidity category	Adjusted OR (95% CI)	p-value
Children/adolescents aged <18 years (n = 466)				
Anaemia[3i,4i,6i,14,16]	188	No hookworm/no *P. falciparum*$_{500+}$[Δ]	1.00 (reference)	
	170	*P. falciparum*$_{500+}$ only	1.90 (1.15, 3.14)	0.012*
	66	Hookworm only	0.29 (0.09, 0.88)	0.030*
	42	Co-infected	0.84 (0.34, 2.05)	0.699
Anaemia[3i,4i,5+,6i,14]	181	Not malnourished/no *P. falciparum*$_{500+}$	1.00 (reference)	
	141	*P. falciparum*$_{500+}$ only	1.94 (1.05, 3.57)	0.034*
	73	Malnourished only	2.21 (1.11, 4.41)	0.024*
	71	Malnourished/*P. falciparum*$_{500+}$	4.62 (2.32, 9.21)	<0.001*
Splenomegaly[2+,6i,13,14]	191	No *S. haematobium*/no *P. falciparum*$_{500+}$	1.00 (reference)	
	174	*P. falciparum*$_{500+}$ only	3.10 (1.80, 5.31)	<0.001*
	63	*S. haematobium* only	2.47 (1.11, 5.49)	0.026*
	38	Co-infected	3.48 (1.49, 8.15)	0.004*
Splenomegaly[2+,3+,6i,13,14]	181	Not malnourished/no *P. falciparum*$_{500+}$	1.00 (reference)	
	141	*P. falciparum*$_{500+}$ only	1.91 (1.08, 3.40)	0.027*
	73	Malnourished only	0.70 (0.32, 1.53)	0.374
	71	Malnourished/*P. falciparum*$_{500+}$	3.26 (1.66, 6.40)	0.001*
Adults aged ≥18 years (n = 384)				
Anaemia[6i,8+,13]	186	No *S. mansoni*/no *P. falciparum*	1.00 (reference)	
	121	*P. falciparum* only	1.03 (0.52, 2.03)	0.940
	34	*S. mansoni* only	0.47 (0.10, 2.15)	0.332
	43	Co-infected	0.13 (0.02, 0.99)	0.049*
Pallor[8i,12i,13,15]	186	No *S. mansoni*/no *P. falciparum*	1.00 (reference)	
	121	*P. falciparum* only	0.81 (0.47, 1.41)	0.458
	34	*S. mansoni* only	0.74 (0.28, 2.01)	0.560
	43	Co-infected	0.23 (0.07, 0.71)	0.011*
Chronic morbidity[5+,9i,11i,13,14,15]	186	No *S. mansoni*/no *P. falciparum*	1.00 (reference)	
	121	*P. falciparum* only	0.61 (0.37, 1.01)	0.052
	34	*S. mansoni* only	0.53 (0.22, 1.31)	0.168
	43	Co-infected	0.16 (0.06, 0.46)	0.001*
Abdominal pain[14,15]	186	No *S. mansoni*/no *P. falciparum*	1.00 (reference)	
	121	*P. falciparum* only	0.63 (0.36, 1.10)	0.105
	34	*S. mansoni* only	1.74 (0.80, 3.78)	0.163
	43	Co-infected	2.79 (1.38, 5.64)	0.004*
Abdominal pain[14,15]	206	No *S. mansoni*/no hookworm	1.00 (reference)	
	34	*S. mansoni* only	2.15 (0.99, 4.64)	0.051
	101	Hookworm only	1.02 (0.59, 1.78)	0.946
	43	Co-infected	3.27 (1.61, 6.63)	0.001*

*Statistically significant with a p-value < 0.05.
[Δ]*P. falciparum* parasitaemia of > 500 parasites/µl of blood.
[§]Not significant covariates further included in each model for adjustment (+ = infection with, i = intensity of infection): 1 = *P. falciparum*, 2 = *P. malariae*, 3 = *S. haematobium*, 4 = *S. mansoni*, 5 = Hookworm, 6 = *E. histolytica/E. dispar*, 7 = *E. coli*, 8 = *E. nana*, 9 = *I. bütschlii*, 10 = *G. intestinalis*, 11 = *C. mesnili*, 12 = *B. hominis*, 13 = sex, 14 = age group, 15 = socioeconomic status, 16 = malnutrition (moderate or severe).

4.5 Discussion

Since 2010, the population of Sahoua, located at the northern edge of the Taabo HDSS in south-central Côte d'Ivoire, has benefitted from multiple rounds of deworming, targeting schistosomiasis, soil-transmitted helminthiasis and lymphatic filariasis, which are all endemic in this zone (N'Goran et al., 2001; Adjami et al., 2004). However, the prevalence of hookworm infection was still considerable (26.8%), but most of the infections were of light intensity. One quarter of all *S. haematobium* infections were of high intensity (≥ 50 eggs/10 ml of urine). The second study location, Ancien Carrefour, has not been subjected to regular deworming before. We found hookworm and *S. mansoni* prevalences of 32.4% and 28.4%, respectively, indicating a moderate-risk community (WHO, 2006). *Plasmodium* infection was common in both settings. Consequently, polyparasitism was prevalent, even though varying in parasite species composition, which is in line with earlier studies conducted in rural areas of Côte d'Ivoire (Keiser et al., 2002a; Raso et al., 2004; Coulibaly et al., 2012). In fact, less than 10% of the study participants who had complete parasitological data were free of any of the parasites investigated. It should be noted that the true dimension of multiple-species infections is likely to be higher, considering that only single stool, urine and finger-prick blood samples were taken and analysed microscopically. It is widely acknowledged that egg-output of *S. mansoni* shows important intra-stool and day-to-day variation, and hence multiple Kato-Katz thick smears are necessary to increase diagnostic sensitivity (de Vlas & Gryseels, 1992; Booth et al., 2003; Utzinger et al., 2011; Coulibaly et al., 2012). Despite the likely underestimation of the true prevalence of single and multiple species parasitic infections, we consider the data as meaningful to reveal implications on disease-related morbidity taking into account that those

infections missed were most likely of light intensity, whereas disease burden is a consequence of infection intensity (Staubli Asobayire et al., 2001; Sacko et al., 2011). The extent of polyparasitism and species combinations showed significant variation by age group. School-aged children were found to have the highest number of concurrent infections, while preschool-aged children less often showed co-infection with three parasites or more (11.2%). The youngest individuals were mainly affected by *Plasmodium* and intestinal protozoa. Helminth infections were most common in the school-aged and adult populations. Peak prevalences varied from species to species; for instance children aged 10-14 years for *S. haematobium*, adolescents and young adults for *S. mansoni* and adults for hookworm. These findings are consistent with previous observations (Woolhouse, 1998; Keiser et al., 2002a; Raso et al., 2004).

Several significant associations between parasite species are worth highlighting; some of which have been discussed before, particularly the co-infection of hookworm and *S. mansoni* (Keiser et al., 2002b; Brooker et al., 2006; Matthys et al., 2007). It is conceivable that this is strongly related to shared risk factors, such as poor sanitation and hygiene behaviour (Raso et al., 2007; Coulibaly et al., 2013; Schmidlin et al., 2013). The same conclusions might be drawn for the various associations found between different intestinal protozoa species. Transmission occurs mainly through the faecal-oral pathway, thus by ingestion of contaminated food and water (Newell et al., 2010). This issue might explain why we could detect several protozoa species already in early childhood compared to helminth infections that were more prevalent in older age groups. Infants are restricted in their movement and are less exposed to schistosome-infested water bodies or open defecation grounds. Tackling poor hygiene and improve access to clean water and sanitation offers the opportunity to fight these infectious diseases. New approaches, such as community-led total sanitation (CLTS) exist and a pilot project conducted in the Taabo HDSS showed promising results (Schmidlin et al., 2013).

As parasite species varied for different age groups, so did clinical manifestations (i.e. anaemia and splenomegaly), which mainly occurred in children. In adults, morbidity patterns were mainly driven by chronic, yet subtle but still disabling morbidities, as

assessed by self-reported symptoms and recent history of chronic disease. The transition from acute and clinical morbidity to more chronic conditions in older age is explained by the constant exposure to these parasites in highly endemic areas, and the slowly acquired protective immunity (Marsh & Kinyanjui, 2006; Pinot de Moira et al., 2013). Our findings confirm that the burden of disease increases with parasite load. Interestingly, *S. mansoni* infection seemed to have a beneficial effect on anaemia in case of light-intensity infection, but was at the same time associated with a number of gastro-intestinal symptoms with higher intensity. Furthermore, anaemia was significantly associated with high-intensity infections of *P. falciparum*, *E. histolytica/E. dispar* in all individuals and *S. haematobium* in children aged <9 years (data not shown). These results are in line with findings from earlier studies on disease outcome in relation to parasite intensity on *P. falciparum* and *S. haematobium* (Staubli Asobayire et al., 2001; Sacko et al., 2011) and justify one of the defined primary goals of control programmes for high endemicity areas, namely to reduce morbidity by periodic deworming, and thus eliminate moderate and high infection intensities but not necessarily cure all infections. Malnutrition could not directly be associated with any parasitic infection and indicates the need to integrate information on local dietary habits for future surveys on assessing this health consequence. In our study, malnutrition nevertheless served as an additional condition that was shown to be associated with other disease outcomes, as it is the case for anaemia and splenomegaly in children. Malaria remains the most important parasitic disease in terms of prevalence and clinical outcomes, high parasitaemia being associated with splenomegaly and fever besides anaemia. Long-lasting insecticidal nets (LLINs) as preventive measures are promoted in health centres and efforts have been made to increase coverage (Ouattara et al., 2011; WHO, 2012). Two out of three of the surveyed households possessed a LLIN, but coverage differed between study sites with 90% in Ancien Carrefour and 43% in Sahoua. Despite a high coverage in Ancien Carrefour, the malaria parasite rate, and consequently the disease-related burden, was striking. In recent years, bed net coverage has increased considerably in Côte d'Ivoire (Noor et al., 2009; WHO, 2012), but still needs further scaling up actions (Flaxman et al., 2010; WHO, 2012).

Additionally, local knowledge, attitudes and practices towards malaria and other parasitic diseases must be addressed, as they influence the use of preventive measures, help-seeking and treatment behaviour, and risk-related behaviour (Acka et al., 2010; Ouattara et al., 2011).

Another interesting finding of this study is the indication of protective effects on anaemia in individuals co-infected with helminths (i.e. hookworm and *S. mansoni*). This confirms earlier studies carried out in comparable rural settings elsewhere in West Africa (Briand et al., 2005; Righetti et al., 2012). The underlying mechanisms of such a protective effect need further scientific inquiry, but might be explained by the immunomodulatory effects that helminths are known for (van Riet et al., 2007). In Senegalese school-aged children, chronic schistosomiasis influenced the humoral immune response against malaria antigens by specifically increasing IgG1 and IgG3 Abs levels, which are thought to play a role in protection during human malaria (Diallo et al., 2010). Moreover, one has to keep in mind that most helminth infections detected were of light intensity, a different relationship may be observed if worm loads are higher. In general, our findings suggest that a co-infection does not automatically mean more disease-related pathology. Individuals who were diagnosed with three or more concurrent pathogenic infections were found to be negatively associated with a recent history of chronic disease. Co-infecting parasites are competing for the limited resources within a single host, thus some combinations of parasite species may reduce disease burden due to inhibition of growth of the other one, especially if the inhibited species causes more pronounced morbidity. In our study we present mainly the inhibitory effects of interactions between parasite species, while in other studies co-infection was related with a higher risk of disease-related morbidity (i.e. anaemia) (Naing et al., 2013). In a next step, we will further investigate the directions of interactions between species and its implications for morbidity and additionally investigate their magnitude. Interaction measures like the synergistic index, as defined by Rothman (Rothman, 1974) and initially used in clinical case-control studies, offer new ways to assess the magnitude and direction of additive interaction due to co-infection. Ezeamama and colleagues (Ezeamama et al., 2008), for example, showed that moderate- to heavy-intensity infections of

S. japonicum and *T. trichiura* were associated with higher odds of anaemia with a synergy index (SI) of 2.9. As a consequence of existing interactions between parasites, treatment campaigns against specific diseases should be conducted with caution and should consider local patterns of co-infection with other species since treating one parasite may exacerbate the consequences to another one. Furthermore, co-infection may influence the effect and efficacy of drugs or vaccines (Su et al., 2006). Specific drugs and agents have been shown to have an impact on different kinds of parasites, like artemisinin-based combination therapy, which is used to treat malaria but also has an effect on *Schistosoma* infection (Keiser et al., 2010; Abay et al., 2013) or ivermectin that is used in the global programme for eliminating lymphatic filariasis, and also impacts on soil-transmitted helminthiasis (Knopp et al., 2010). Thus considering the co-occurrence of different parasitic species should also influence the choice of treatment to be applied.

We conclude that multiple-species parasite infections are common in rural parts of Côte d'Ivoire, explained by social-ecological contexts that foster the presence and transmission of these diseases, but that there is small-scale heterogeneity. Taken together, our findings and other recent studies on polyparasitism imply the need for adaptation of future interventions towards integrated control. Treatment campaigns will serve as the backbone of interventions, but must be combined with other control interventions to reduce parasite intensity and thus morbidity (Tchuem Tchuenté et al., 2013). Furthermore, the interactions between parasites are evident and may have implications on morbidity, such as anaemia. Treatment plans should therefore be adapted to local co-infection risk profiles in terms of combined treatment of several infections to avoid exacerbation of one disease by treating the other and in terms of most appropriate drugs to profit of substances active against a range of different species. The fact that infectious diseases like malaria, schistosomiasis, soil-transmitted helminthiasis and intestinal protozoa infections are mainly driven by social-ecological systems (Raso et al., 2006, 2012; Ouattara et al., 2011; Utzinger et al., 2011; Schmidlin et al., 2013) provides an opportunity to fight them as a whole by addressing these factors. It goes without saying that issues of underdeveloped infrastructure and water and sanitation provision in rural areas of Côte d'Ivoire

cannot be improved from one day to another, but new promising approaches do exist. Interventions like CLTS incorporate whole communities and place emphasis on hygiene education taking into account local knowledge, attitudes and practices and schedule concrete action plans determined by the community themselves with the overall goal to achieve open defecation-free status in their village (Schmidlin et al., 2013). Programmes like this target on higher acceptance and involvement of the local population and present new ways of more integrated control of parasitic infections that may serve as an example to be adapted.

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5. Effects of hygiene and defecation behaviour on helminths and intestinal protozoa infections in Taabo, Côte d'Ivoire

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5.1 Abstract

More than 1 billion people are currently infected with soil-transmitted helminths and schistosomes. The global strategy to control helminthiases is the regular administration of anthelmintic drugs to at-risk populations. However, rapid re-infection occurs in areas where hygiene, access to clean water and sanitation are inadequate.

In July 2011, inhabitants from two villages and seven hamlets of the Taabo health demographic surveillance system in south-central Côte d'Ivoire provided stool and urine samples. Kato-Katz and ether-concentration methods were used for the diagnosis of *Schistosoma mansoni*, soil-transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) and intestinal protozoa. Urine samples were subjected to a filtration method for the diagnosis of *Schistosoma haematobium*. A questionnaire was administered to households to obtain information on knowledge, attitude, practice, and beliefs in relation to hygiene, sanitation and defecation behaviour. Logistic regression models were employed to assess for associations between questionnaire data and parasitic infections.

A total of 1894 participants had complete data records. Parasitological examinations revealed prevalences of hookworm, *S. haematobium*, *T. trichiura*, *S. mansoni*, and *A. lumbricoides* of 33.5%, 7.0%, 1.6%, 1.3% and 0.8%, respectively. *Giardia intestinalis* and *Entamoeba histolytica*/*E. dispar* were detected in 15.0% and 14.4% of the participants, respectively. Only one out of five households reported the presence of a latrine, and hence, open defecation was common. Logistic regression analysis revealed that age, sex, socioeconomic status, hygiene and defecation behaviour are determinants for helminths and intestinal protozoa infections.

We found that inadequate sanitation and hygiene behaviour are associated with soil-transmitted helminths and intestinal protozoa infections in the Taabo area of south-central Côte d'Ivoire. Our data will serve as a benchmark to monitor the effect of community-led total sanitation and hygiene education to reduce the transmission of helminthiases and intestinal protozoa infections.

Running title: Hygiene, Defecation Behaviour and Intestinal Parasites

5.2 Introduction

Hundreds of millions of people are still affected by neglected tropical diseases (NTDs), particularly in the developing world due to parasitic worm infections (helminthiases) (Hotez et al., 2006; Utzinger et al., 2012). Taken together, soil-transmitted helminthiasis and schistosomiasis are responsible for 8.5 million disability-adjusted life years (DALYs) with more than 1 billion people infected (Steinmann et al., 2006; Hotez et al., 2008; Murray et al., 2012). Diseases caused by intestinal protozoa infections, such as giardiasis and amebiasis also cause considerable morbidity and mortality (Walsh, 1986; Minenoa & Avery, 2003; Savioli et al., 2006; Becker et al., 2013).

Current helminthiases control programs focus on preventive chemotherapy, that is the regular administration of anthelmintic drugs to at-risk populations, particularly school-aged children (WHO, 2002, 2006). However, preventive chemotherapy does not prevent re-infection, which might occur rapidly (Quinnell et al., 1993; Jia et al., 2012). Additionally, there is considerable concern about the development of drug resistance in the era of preventive chemotherapy, as experience has shown in livestock (Geerts & Gryseels, 2000). Although, the importance of integrated control approaches for the interruption of transmission of helminthiases is well established since almost a century (Cort et al., 1929; Stiles, 1939), current control efforts emphasize drug interventions, and do not give sufficient attention to hygiene behaviour, clean water and adequate sanitation (Utzinger et al., 2003; Singer & de Castro, 2007; Ziegelbauer et al., 2012). Indeed, data from 2010 suggest that 2.6 billion people lacked access to some kind of improved sanitation (WHO & UNICEF, 2010). To contribute to the achievement of several of the millennium development goals (MDGs), ongoing efforts to control NTDs have to be maintained and further intensified, including complementary approaches for prevention and control (Utzinger et al., 2011).

In July 2011, a project emphasizing an integrated control approach targeting intestinal parasites was launched in the Taabo health demographic surveillance system (HDSS) in south-central Côte d'Ivoire. The main objective is to assess the impact of community-led total sanitation (CLTS) and health education on the incidence of helminths and intestinal protozoa infections, implemented alongside preventive chemotherapy. CLTS not only focuses on the construction of latrines, but also on local knowledge, attitude, practice and beliefs (KAPB) related to hygiene and defecation behaviour, which play a key role for sustainability (Acka et al., 2010). Through a participatory grassroots approach, CLTS aims to achieve and sustain an open defecation-free status of communities (Kar & Chambers, 2008). To our knowledge, the effect of CLTS on re-infection patterns with helminths and intestinal protozoa infections has yet to be determined. Here, we present helminth and intestinal protozoa infection profiles in a selection of villages and hamlets of the Taabo HDSS, including associations between infection and people's KAPB related to hygiene and defecation behaviour during the baseline cross-sectional survey. Our data will serve as a benchmark for monitoring the longer term impact of CLTS on people's health and wellbeing.

5.3 Materials and methods

Ethics statement

This study received clearance from the ethics committees of Basel (Ethikkommission beider Basel; reference no. 177/11) and Côte d'Ivoire (Comité National de l'Ethique et de la Recherche; reference no. 13324 MSLS/CNER-P). Study participants were informed about the aims, procedures and potential risks and benefits. Participants and parents/guardians of minors provided written informed consent (signature of a witness for illiterate participants). Participation was voluntary and people could withdraw from the study at any time without further obligation. To guarantee anonymity, each study participant was given a unique identification number.

At the end of the parasitological survey, anthelmintic treatment was administered to all people in the study villages and hamlets regardless of infection

status and participation (single 400 mg oral dose of albendazole for individuals aged ≥ 2 years) (WHO, 2002, 2006). Additionally, participants aged ≥ 4 years who were diagnosed for *Schistosoma* spp. were given a single oral dose of praziquantel (40 mg/kg, using a "dose pole") (WHO, 2002, 2006). Individuals who required other specific medical interventions were referred to the next health care facility. No treatments were given to participants identified with intestinal protozoa infections, as the results from the sodium acetate-acetic acid-formalin (SAF)-fixed stool samples subjected to an ether-concentration method were only available several weeks after completion of the field work and intestinal protozoa infection are often self-limiting.

Study area and population

The study was conducted in the Taabo HDSS, located in a primarily rural part of south-central Côte d'Ivoire (Fürst et al., 2012; Righetti et al., 2012, 2013). General living standards are low. For instance, 71% of households lacked a toilet facility and two-third of the households used unprotected surface water (e.g. rivers and lakes) as drinking water according to the latest available data from the Taabo HDSS. Soil-transmitted helminthiasis, schistosomiasis, onchocerciasis and lymphatic filariasis control activities have been implemented within the Taabo HDSS (preventive chemotherapy, using albendazole, praziquantel and ivermectin) since 2008 and are on-going with yearly drug interventions. At the time of the execution of the current study, preventive chemotherapy was the main strategy implemented in the study area.

The study presented here was implemented in two villages (i.e. Katchénou and Sahoua) and seven hamlets of different villages, Yobouékro (belonging to Sahoua), Ouattafouékro and Kouadio Kouamékro (Ahondo), Boussoukro (Tokohiri), Amani Kouadiokro (Sokrogbo), and Beh N'Guessankro and Allah Thérèsekro (Léléblé) (Figure 1). These villages and hamlets were purposely selected because of their small population sizes and the relatively homogeneous population structure. All inhabitants of the villages and hamlets were targeted as study population, using the readily available Taabo HDSS database.

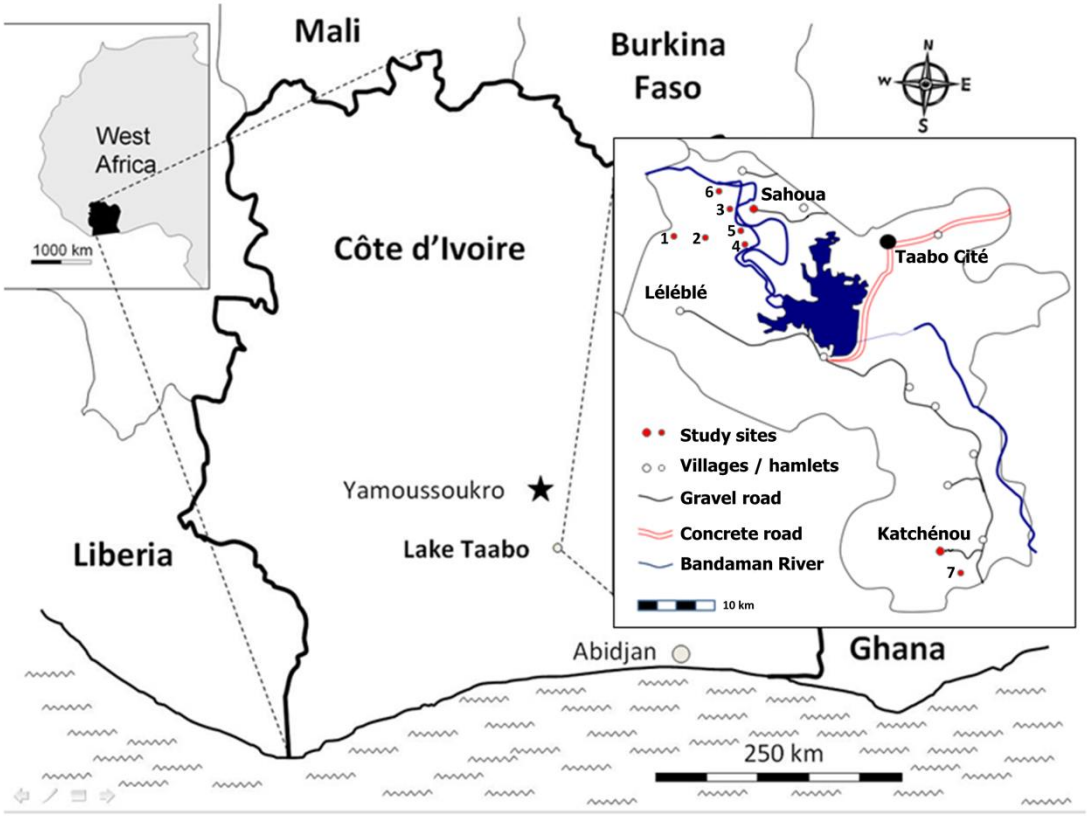

Figure 5.1 Map of the study area in Taabo, situated in south-central Côte d'Ivoire

The study was carried out in two villages (Sahoua, Katchénou) and seven hamlets (1 = Beh N'Guessankro, 2 = Allah Thérèsekro, 3 = Yobouékro, 4 = Ouattafouékro, 5 = Kouadio Kouamékro, 6 = Boussoukro, 7 = Amani Kouadiokro) that are part of the Taabo health demographic surveillance system. Results presented here pertain to the baseline cross-sectional parasitological and questionnaire surveys conducted in July 2011.

Study design and procedures

In July 2011, just shortly before the annual round of preventive chemotherapy, a cross-sectional survey was carried out to assess the baseline parasitological and KAPB situation. This cross-sectional survey was part of a larger, still ongoing project aiming to assess the effect of CLTS on helminths and intestinal protozoa re-infection patterns. This larger project consists of a baseline cross-sectional survey (design, field and laboratory procedures, questionnaire survey and results are presented in

this paper), implementation of CLTS combined with health education, and a 1-year follow-up survey.

Before commencement of the study, villages/hamlets were visited by the research team to get approval from the local authorities and to announce the exact date of the sampling day. The day before the sampling, participants were given empty plastic containers for stool and urine collection. Participants were invited to bring early morning stool and urine samples to a central place in the village/hamlet. For parasitological examinations, faecal and urine samples were transferred to our mobile field laboratories in Léléblé and Sokrogbo or the laboratory of the hospital in Taabo-Cité.

Duplicate Kato-Katz thick smears were prepared on microscope slides from each stool sample, using standard templates holding 41.7 mg of faeces. Slides were examined under a microscope and the eggs of *Schistosoma mansoni*, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm were counted by experienced laboratory technicians the same day, and recorded for each species separately (Katz et al., 1972). Ten millilitres of vigorously shaken urine were filtered, the filter placed on a microscope slide and a drop of Lugol's iodine added. The slides were examined under a microscope and the number of *S. haematobium* eggs counted (Savioli et al., 1990). For quality control, all slides were read independently by different laboratory technicians. When inconsistencies were detected, the discordant slides were re-examined and the results discussed until agreement was reached.

Additionally, from each stool sample, 1-2 g was transferred into a small plastic tube filled with 10 ml of SAF (Marti & Escher, 1990). The SAF-fixed stool samples were forwarded to a laboratory at the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS; Abidjan, Côte d'Ivoire) for further examination to detect intestinal protozoa infections. In brief, samples were processed using an ether-concentration method and the slides were analysed by experienced laboratory technicians under a microscope (Allen & Ridley, 1970). Standard protocols were followed and intestinal protozoa (*Blastocystis hominis*, *Chilomastix mesnili*, *Entamoeba coli*, *Entamoeba hartmanni*, *Entamoeba histolytica/E. dispar*, *Endolimax*

nana, *Giardia intestinalis* and *Iodamoeba bütschlii*, recorded semi-quantitatively (Utzinger et al., 2010).

A questionnaire was administered to all households at the day of stool and urine sampling. The households were visited by a researcher accompanied by a trained field enumerator who speaks the local languages. Whenever the head of a household was present, he/she was interviewed; otherwise a present adult household member was interviewed. The questionnaire was designed in a structured manner with closed questions to obtain quantitative data for the analyses. The questionnaire consisted of basic questions on demographic factors (e.g. age, sex, ethnicity and education), socioeconomic indicators (e.g. possession of a number of household assets) and KAPB. Topics covered by the KAPB were: (i) sanitation and defecation behaviour (e.g. place of defecation, use of latrine); (ii) open defecation (e.g. reasons for open defecation, problems of open defecation); (iii) hygiene behaviour (e.g. hand washing after defecation); (iv) opinions, taboos, and beliefs (e.g. preoccupations, gender-specific latrine use); and (v) intestinal parasitic infections (e.g. prevention, transmission, signs, and symptoms). The questionnaire was piloted in 10 households in a village not otherwise involved.

Statistical analysis

Data were double-entered and cross-checked in EpiInfo version 3.5.1 (Centers for Disease Control and Prevention; Atlanta, United States of America) and analysed in STATA version 10.0 (Stata Corp.; College Station, United States of America). Only participants with complete datasets (i.e. those with duplicate Kato-Katz thick smears, one SAF-fixed faecal sample, and one urine filtration) were included in the final analyses. For each participant, the arithmetic mean egg count was calculated and used to stratify the infection intensities (mean number of eggs per gram of stool (EPG)) into light, moderate and heavy infections using cut-offs commonly employed by the World Health Organization (WHO) (Montresor et al., 1998). Participants were stratified into five age groups (i.e. < 5; 5-14; 15-24; 25-40; and > 40 years). For the variables and summary statistics of the KAPB questionnaire, frequency tables with indicators of central value and dispersion were calculated. Furthermore, the several categories of the KAPB questionnaire were coded for their importance with a value of 0 if the category was not mentioned at all, a value of 1 after a probed answer and a value of 2 after a spontaneous answer (Kouadio et al., 2013). The KAPB questionnaire data gathered at the unit of the household served as individual values for every participant living in a specific household, which might slightly distort our results for logistic regression. Participants with a particular helminth or intestinal protozoan infection were compared to participants not infected with that species. Test statistics included chi-square (χ^2), Fisher's exact test, Wilcoxon rank-sum, Kruskal-Wallis, two sample *t*-tests, and logistic regression models adjusted for participants' socioeconomic status, age group, and sex. Hence, these characteristics were included wherever these parameters showed significant association with infection. Furthermore, all logistic regressions were corrected for potential clustering at the unit of the village/hamlet.

The socioeconomic status was calculated using a household asset-based approach (Gwatkin, 2000). Household asset weights were determined using principal component analysis (PCA). Missing values were replaced by the mean of the particular asset. Only binary variables were used for household assets. Household assets were excluded to make the first principal component (PC) stand

for more than 30% of the variability. Greatest weight were given to the possession of a television (0.34), followed by the presence of a shower with cement floor (0.33) and the possession of a video recorder (0.33). The calculated scores were added up for each household and subsequently ranked according to the total score. The households were then separated into wealth quintiles: (i) poorest, (ii) very poor, (iii) poor, (iv) less poor and (v) least poor. To estimate inequities in parasitic infection prevalence related to the participants' socioeconomic status, the concentration index (CI) was used (Wagstaff et al., 1991), that arises from the concentration curve. It quantifies the degree of socioeconomic-related inequality in a health variable and is twice the area between the concentration curve and the 45-degree line that is called the line of equality. The CI is 0 if there is no socioeconomic-related health variable. When the CI becomes negative then the curve lies above the line of equality indicating that there is a disproportionate concentration of the health variable among the poor and, *vice versa*, it takes a positive value if the concentration of the health variable is among the wealthier. Significance of the CI was assessed using standard deviations (Kakwani et al., 1997).

5.4 Results

Study participation

From 3,420 registered people in 485 households in the selected villages and hamlets in the Taabo HDSS, 2,514 individuals were present during the cross-sectional parasitological survey. As shown in Figure 5.2, 213 participants lacked duplicate Kato-Katz thick smears (no stool sample was provided), 183 had no urine filtration done (no urine sample was provided) and 116 had missing ether-concentration data (insufficient stool provided to perform the test). Complete parasitological data were available from 1,992 individuals (58.2% based on the registered population).

In 54 households, adult members were either absent or refused to participate in the questionnaire survey. Interviews were conducted in the remaining 431 households (88.9%). For regression analysis, 98 participants dropped, due to missing questionnaire data leading to a final study sample of 1894 people (55.4% of the registered population).

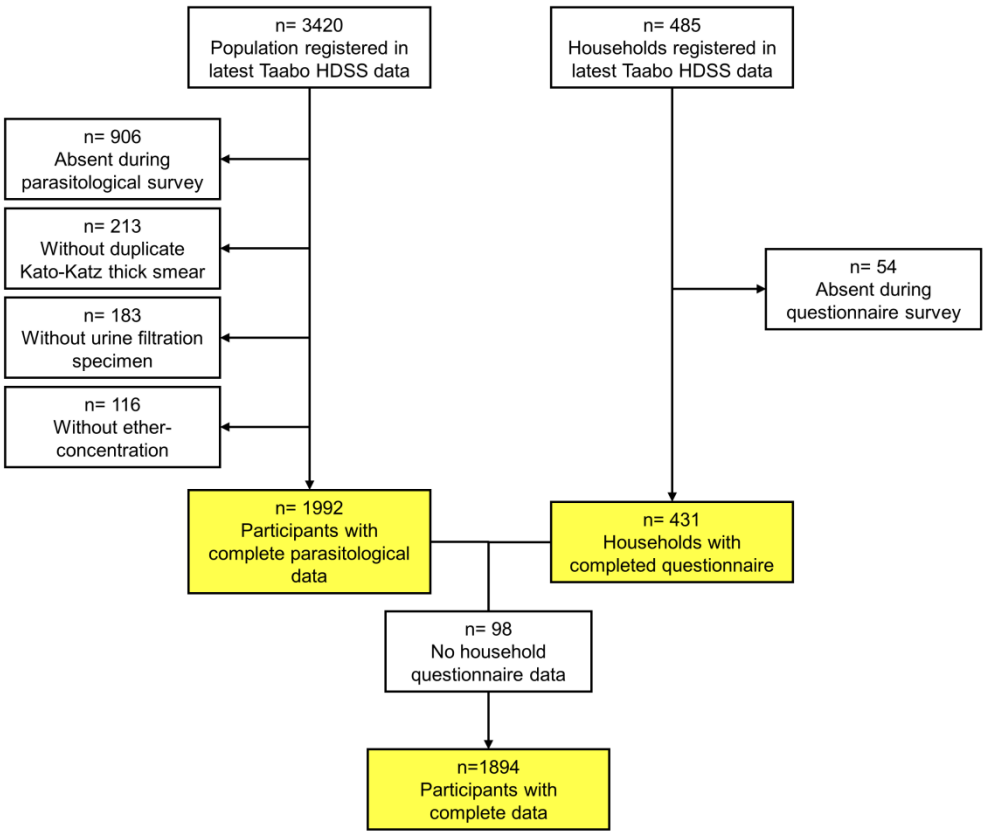

Figure 5.2 Flow chart showing the study cohort and compliance with emphasis on the three different samples considered in the analysis

The study was carried out in the Taabo health demographic surveillance system in south-central Côte d'Ivoire in July 2011. The three sub-samples pertain to participants with complete parasitological data, households with complete questionnaire data and participants with complete parasitological data from a household with complete questionnaire data.

Parasitological results

Among those 1,992 participants with complete parasitological data, we found prevalences for hookworm, *S. haematobium*, *T. trichiura*, *S. mansoni* and *A. lumbricoides* of 33.5%, 7.0%, 1.6%, 1.3%, and 0.8%, respectively (Table 5.1). Only very few individuals were identified with moderate or heavy helminth infection intensities, with the exception of *S. haematobium* (25.9% of the infections were classified as heavy, i.e. ≥ 50 eggs/10 ml of urine). The prevalences of the pathogenic intestinal protozoa *G. intestinalis* and *E. histolytica*/*E. dispar* were 15.0% and 14.4%, respectively. The most common intestinal protozoa were *E. coli* and *B. hominis* with respective prevalences of 45.0% and 35.4%.

Males were significantly more likely to be infected with hookworm than females (38.8% *vs.* 28.2%; $\chi^2 = 25.49$, $p < 0.001$). The same patterns were found for *E. coli* (50.6% *vs.* 39.4%; $\chi^2 = 25.08$, $p < 0.001$) and *E. nana* (31.8% *vs.* 25.2%; $\chi^2 = 10.69$, $p = 0.001$). In contrast, females were more likely to be infected with *T. trichiura* compared to males (2.2% *vs.* 1.0%; $\chi^2 = 4.62$, $p = 0.032$).

Table 5.1 Helminth infection prevalence and intensity among 1,992 participants in Taabo, south-central Côte d'Ivoire, in July 2011

Parasite species	Infected (%)	Minimum egg count	Maximum egg count	Infection intensity[a]		
				Light	Moderate	Heavy
Hookworm	667 (33.5)	12	13,584	616 (96.7)	14 (2.2)	7 (1.1)
T. trichiura	32 (1.6)	12	2,316	25 (83.3)	5 (16.7)	0 (0.0)
S. mansoni	26 (1.3)	12	2,520	20 (87.0)	2 (8.7)	1 (4.3)
A. lumbricoides	15 (0.8)	24	5,832	8 (88.9)	1 (11.1)	0 (0.0)
S. haematobium	139 (7.0)	1	685	103 (74.1)	n.d.	36 (25.9)

Infection intensities (mean egg count) were split into light, moderate, and heavy infections using WHO guidelines (WHO, 2002, 2006).

[a]Number of infected participants stratified by infection intensities (values in brackets as percentage, %)

n.d., not defined

Several intestinal parasites were significantly associated with age group, including hookworm ($\chi^2 = 123.35$, degree of freedom (d.f.) = 4, $p < 0.001$), *S. mansoni* ($\chi^2 = 14.11$, d.f. = 4, $p = 0.007$), *S. haematobium* ($\chi^2 = 74.68$, d.f. = 4, $p < 0.001$) and six of the eight encountered intestinal protozoa (*E. histolytica*/*E. dispar*,

E. coli, *E. nana*, *I. bütschlii*, *G. intestinalis* and *B. hominis*). Age-prevalence curves are shown in Figure 5.3. Participants of poorer households were significantly more often infected with hookworm (CI = -0.0266, standard error (SE) = 0.0085), *T. trichiura* (CI = -0.2774, SE = 0.1230), *E. histolytica/E. dispar* (CI = -0.1072, SE = 0.0242), *I. bütschlii* (CI = -0.0414, SE = 0.0189) and *G. intestinalis* (CI = -0.0548, SE = 0.0162). However, the prevalence of *S. haematobium* was significantly higher in the richer participants compared to their poorer counterparts (CI = 0.2249, SE = 0.0382).

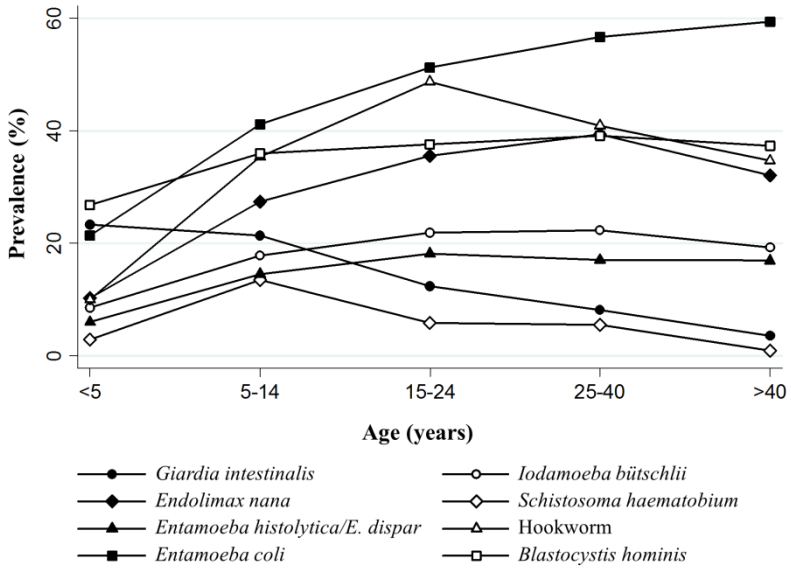

Figure 5.3 Age-prevalence curves of investigated parasites.

The results of the intestinal protozoa and helminth infections arise from the baseline cross-sectional survey carried out in July 2011 among community members of two villages and seven hamlets in the Taabo health demographic and surveillance system, south-central Côte d'Ivoire. *Trichuris trichiura*, *Schistosoma mansoni* and *Ascaris lumbricoides* are not displayed due to very low prevalence.

Results of the KAPB survey

Table 5.2 shows the demographic and socioeconomic characteristics among the households, stratified by wealth quintiles. Muslims were more frequently part of the least poor quintile, compared to Christians and animists. Household size steadily increased from poorest to least poor. Household assets such as electricity, latrine, television, and a motorcycle were more often reported by the least poor quintile. The poorest more often obtained their drinking water from the nearby Bandama river or other unprotected open surface water bodies than their wealthier counterparts who were more likely to use a village pump as source of drinking water.

Most interviewees (98.6%) said that they would wash their hands regularly. The most frequently mentioned occasions to wash hands were before a meal (99.8%), after a meal (92.5%), after defecation (85.3%) and when hands looked dirty (75.6%). Among these four categories, before eating was most often spontaneously mentioned (proportion 86%). Hand washing after defecation was only spontaneously mentioned by 27% of the interviewees (Table 5.3).

Place and defecation frequency index assessed on a semi-quantitative scale, stratified by possession of a latrine, are summarised in Table 5.4. Study participants frequently reported to defecate into the nearby bush or open plantations. People living in households with latrines mostly used them, but they also practiced open defecation. Members of households without a toilet most of the time defecated in the open. A significant difference of defecation frequency could only be found between households possessing a latrine and households without a latrine for the nearby bush (0.73 *vs.* 3.28, $p < 0.001$) and latrine (3.38 *vs.* 0.05, $p < 0.001$), while no significant difference was found for the plantation (1.64 *vs.* 1.68, $p = 0.969$). Most household members said that they need a latrine (98.5%) and nine out of 10 interviewees perceived open defecation as a problem. The most frequently stated reasons why a household does not have a latrine were the high cost (51.1%), traditional habit of open defecation (24.0%), not all of the required material locally available (12.0%) and soil not stable enough or the groundwater table too high for a durable construction (9.9%).

Table 5.2 Characteristics of the 431 households, participating in the knowledge, attitude, practice and beliefs survey, stratified by wealth quintiles

Characteristics	Total (n = 431)	Wealth quintiles (%)					Ratio (poorest / least poor)
		Poorest (n = 85)	Very poor (n = 85)	Poor (n = 81)	Less poor (n = 91)	Least poor (n = 89)	
Sex (%)							
Male	59.2	50.6	55.3	61.7	65.9	61.8	0.82
Female	40.8	49.4	44.7	38.3	34.1	38.2	1.29
Age (years)							
Mean (SD)	40.3 (14.2)	37.6 (12.3)	39.4 (13.4)	37.5 (11.8)	43.3 (16.1)	43.0 (15.8)	
Median (Q1-Q3)	39 (30-48)	36 (29-45)	38 (30-47)	37 (27-45)	41 (31-53)	40 (32-55)	
Status of the respondent (%)							
Household chief	57.3	55.3	56.5	59.3	60.4	55.1	1.00
Wife	28.5	37.7	31.8	24.7	26.4	22.5	1.68
Son or daughter	7.7	3.5	5.9	9.9	8.8	10.1	0.35
Other	4.4	3.5	4.7	2.5	4.4	7.9	0.44
Brother or sister	2.1	0.0	1.2	3.7	0.0	4.5	0.00
Religion (%)							
Christian	44.1	47.6	45.8	46.9	51.1	29.6	1.61
Muslim	23.0	17.4	12.2	15.3	17.4	37.8	0.46
Animist	29.1	29.8	34.9	32.1	26.7	22.7	1.31
Other religions	3.8	5.2	7.1	5.7	4.8	9.9	0.53
Educational attainment (%)							
No education	66.6	64.7	64.7	72.8	68.1	62.9	1.03
Primary school	18.3	25.9	22.4	17.3	13.2	13.5	1.92
Secondary school	9.7	7.1	9.4	8.6	11.0	12.4	0.57
Koranic school	4.4	2.4	3.5	1.2	5.5	9.0	0.27
University	0.9	0.0	0.0	0.0	2.2	2.2	0.00
Reading-writing ability (%)							
Reading	27.8	31.8	32.9	24.7	24.2	25.8	1.23
Writing	27.6	31.8	32.9	23.5	24.2	25.8	1.23
Household size							
Mean (SD)	7.3 (4.3)	5.5 (2.8)	6.5 (3.5)	6.7 (3.8)	8.5 (5.0)	9.2 (4.9)	0.60
Median (Q1-Q3)	6 (5-9)	5 (4-7)	6 (5-8)	6 (4-9)	8 (5-11)	8 (6-11)	0.75

Household assets (%)							
Shower	88.4	88.2	92.9	70.0	91.2	97.7	0.90
Bicycle	79.3	64.7	80.0	77.5	82.4	91.0	0.71
Radio	72.8	65.9	77.7	70.0	72.5	77.5	0.85
Motorcycle	22.3	4.7	17.7	20.0	24.2	43.8	0.11
Latrine	20.7	10.6	9.4	12.5	33.0	38.2	0.28
Television	18.4	1.2	1.2	3.8	17.6	65.2	0.02
Electricity	14.2	1.2	1.2	4.9	11.0	51.1	0.02
Drinking water source (%), multiple answers possible							
Pond/river	58.8	81.5	61.9	50.6	55.0	46.0	1.77
Pump	37.7	18.5	35.7	43.0	37.4	52.9	0.35
River	37.4	60.5	40.5	27.9	27.5	32.2	1.88
Pond	21.6	21.0	21.4	22.8	28.6	13.8	1.52
Cistern	19.4	16.1	17.9	17.7	30.8	13.8	1.17
Well	1.9	1.2	3.6	0.0	2.2	2.2	0.55

The study was carried out in the Taabo health demographic surveillance system in south-central Côte d'Ivoire in July 2011. Questionnaires were conducted with the household chief if present; otherwise the next higher household member was interviewed.
Q1-Q3 stands for first quartile to third quartile, defining the interquartile range.

Table 5.3 Knowledge, attitude, practice and beliefs related to hygiene behaviour, latrine possession and open defecation mentioned by the respondents

	Total reported %	Proportion spontaneous[a]	Mean prominence[b]
Reasons to possess a latrine (n = 89)			
Safety	75.3	0.42	1.07
Clean environment	68.5	0.51	1.03
Comfort	67.4	0.53	1.03
Avoid diseases	67.4	0.27	0.85
Privacy	65.2	0.29	0.84
Visitors	70.8	0.10	0.78
Elderly people	51.7	0.26	0.65
Modern lifestyle	18.1	0.12	0.31
Time point of hand washing (n = 427)			
Before a meal	99.8	0.86	1.85
After a meal	92.5	0.53	1.42
Always when dirty	75.6	0.64	1.24
After defecation	85.3	0.27	1.08
Before preparing a meal	50.8	0.31	0.67
Before nourishing a child	42.2	0.13	0.48
After cleaning a child	38.9	0.10	0.43
Problems associated with open defecation (n = 384)			
Safety	85.2	0.65	1.40
Clean environment	71.1	0.28	0.91
Hygiene	63.5	0.36	0.86
No comfort	62.2	0.37	0.85
Privacy	58.3	0.25	0.73
Elderly people	56.8	0.11	0.63
Visitors	58.6	0.05	0.62
Drinking water	52.1	0.04	0.54
Reason not to possess a latrine (n = 334)			
No technical comprehension	51.1	0.91	0.98
Traditional habit	24	0.70	0.41
Soil not stable	9.9	1.00	0.20
No material	12	0.60	0.19
Low priority	6.9	0.52	0.10
Reason to practice open defecation (n = 320)			
No latrine in the household	88.8	0.95	1.73
Traditional habit	43.4	0.66	0.56

The study was carried out in the Taabo health demographic surveillance system in south-central Côte d'Ivoire in July 2011.

[a] Proportion of categories reported spontaneously.

[b] Mean prominence based on values assigned to each category (0 = not mentioned, 1 = probed, 2 = spontaneous).

Table 5.4 Defecation behaviour assessed with the parameters place and frequency, stratified by the abundance of household-owned latrines

Place and defecation frequency index	Total households[a]	With latrine[a]	Without latrine[a]	P-value[b]
Near bush	2.75 (0.09)	0.73 (0.14)	3.28 (0.08)	<0.001
Plantation	1.67 (0.08)	1.64 (0.15)	1.68 (0.10)	0.969
Latrine	0.74 (0.07)	3.38 (0.12)	0.05 (0.02)	<0.001
Shared latrine	0.28 (0.04)	0.17 (0.08)	0.30 (0.05)	0.069
River/pond	0.06 (0.02)	0.03 (0.03)	0.07 (0.02)	0.207
Behind the house	0.05 (0.02)	0	0.07 (0.03)	0.173

The study was carried out among 431 households in the Taabo health demographic surveillance system in south-central Côte d'Ivoire in July 2011.

[a]Frequency of defecation (defecation frequency index) assessed on a semi-quantitative scale (0 = never, 1 = irregular, 2 = regular, 3 = often, 4 = always) for each place of defecation. Frequency is indicated as means (standard error in brackets).

[b]P-value assessed with Wilcoxon rank-sum test.

There was poor knowledge of schistosomiasis and parasitic worms in general (Table 5.5). Only 64.0% and 49.3% stated that prevention of schistosomiasis and parasitic worms, respectively, is possible. Open defecation was most frequently practiced because households simply did not have a latrine (88.8%) or because of a deeply rooted tradition of open defecation (43.4%). Perceived problems with open defecation were safety issues with regard to different dangers lurking in the bush such as snakes (85.2%), pollution of the environment (71.1%), lack of hygiene (63.5%), lack of comfort (62.2%) and lack of privacy (58.3%). The only category with more than the half spontaneous answers of all reports was safety (65.0%). The top reasons to own a latrine were safety (75.3%), provide a decent place to defecate for visitors (70.8%), keep the environment clean (68.5%), preventing the spread of diseases (67.4%), enhanced comfort (67.4%) and higher level of privacy (65.2%).

Table 5.5 Knowledge of prevention of urogenital schistosomiasis and intestinal helminthiases

Schistosomiasis		
Can you prevent yourself from getting schistosomiasis?		
(n = 253)	Yes	64.0%
	Don't know	32.0%
	No	4.0%
If yes, how? (multiple answers allowed)		
(n = 162)	Do not bath	58.0%
	Do not drink dirty water	53.7%
	Do not defecate in the water	22.2%
	Do not eat overripe fruits	4.9%
	Do not eat washed fruits	1.2%
Soil-transmitted helminthiasis		
Can you prevent yourself from getting parasitic worms?		
(n = 424)	Yes	49.3%
	Don't know	45.3%
	No	5.4%
If yes, how? (multiple answers allowed)		
(n = 209)	Do not eat overripe fruits	38.8%
	Wash hands before eating	38.3%
	Eat candies	34.0%
	Wash hands after defecation	33.5%
	Taking medication	27.8%
	Wash fruits	27.3%
	Do not eat meat	13.9%
	Wearing shoes	4.3%

The study was carried out among 431 households in the Taabo health demographic surveillance system in south-central Côte d'Ivoire in July 2011. Questionnaires were applied on a household level and the question only asked if the participant stated to know the disease.

Association of parasitic infection with hygiene and defecation behaviour

All significant associations between a specific parasite infection and hygiene and defecation behaviour and demographic factors are summarised in Table 5.6. For several different parasitic infections (*A. lumbricoides*, *E. coli*, *E. nana*, *I. mesnili*, and *C. bütschlii*) Muslims had lower odds of an infection than their counterparts with other religious beliefs. Besides demographic characteristics, place of defecation and hand washing behaviour showed statistically significant associations with intestinal parasitic infections, including hookworm, *T. trichiura*, *E. hartmanni*, *E. nana*, and *B. hominis*.

Table 5.6 Significant associations between parasitic infections and household assets, hygiene and defecation behaviour

Parasite	Association	Adjusted odds ratio (95% CI)	P-value[a]
S. haematobium	Christian	1.00	
	Muslim	7.18 (2.60-19.80)	<0.001
	Animist	2.10 (1.47-3.00)	<0.001
	Hand washing for personal hygiene	3.50 (1.68-7.28)	0.001
	Use of pond water for hand washing	3.76 (1.75-8.08)	0.001
	Hand washing time points spontaneously correct answered	2.61 (1.52-4.49)	<0.001
Soil-transmitted helminths			
Hookworm	Latrine	0.63 (0.40-1.00)	0.050
	Hand washing to prevent diseases	0.75 (0.58-0.98)	0.037
	Defecation in the bush	1.70 (1.07-2.69)	0.025
	Children defecating in latrine	0.53 (0.34-0.83)	0.006
	Children defecating in the bush	1.64 (1.06-2.54)	0.027
A. lumbricoides	Christian	1.00	
	Muslim	0.27 (0.09-0.87)	0.028
	Knowledge of parasitic worms	0.39 (0.20-0.78)	0.008
T. trichiura	Hand washing to prevent diseases	0.37 (0.16-0.86)	0.020
	Children defecating in latrine	0.50 (0.25-1.00)	0.048
Intestinal protozoa			
E. hartmanni	Farmer	0.54 (0.33-0.90)	0.019
	Drinking water from pump	0.52 (0.29-0.94)	0.031
	Defecation in latrine	0.27 (0.11-0.67)	0.005
E. coli	Christian	1.00	
	Muslim	0.75 (0.64-0.88)	<0.001
	Fisher	1.57 (1.30-1.91)	<0.001
E. nana	Christian	1.00	
	Muslim	0.81 (0.67-0.99)	0.039
	Latrine	0.80 (0.66-0.97)	0.027
I. mesnili	Christian	1.00	
	Muslim	0.58 (0.40-0.85)	0.004
	Animist	0.80 (0.65-1.00)	0.047
	Other religion	0.57 (0.44-0.75)	<0.001
C. bütschlii	Christian	1.00	
	Muslim	0.51 (0.29-0.91)	0.023
	Animist	1.53 (1.09-2.15)	0.014
B. hominis	Fisher	0.61 (0.37-0.98)	0.041
	Hand washing to prevent diseases	0.71 (0.51-0.97)	0.030

The study was carried out among 431 households in the Taabo health demographic surveillance system in south-central Côte d'Ivoire in July 2011. Logistic regression analysis was used with village

level exchangeable random effects. Variables included as potential confounders were age groups (< 5, 5-14, 15-24, 25-40, and > 40 years), wealth quintiles and sex whenever age, sex, and socioeconomic status were significantly associated with a given parasitic infection.
No significant associations for *E. histolytica/E. dispar* and *G. intestinalis* with household assets, hygiene, and defecation behaviour have been found after correction for potential confounders (sex, age group, or wealth quintile).
[a]P-value based on Wald test

5.5 Discussion

The global strategy for the control of helminthiases emphasizes preventive chemotherapy (WHO, 2002, 2006; Hotez et al., 2008; Savioli et al., 2009). The impact of this strategy on morbidity control is undeniable (Touré et al., 2008). However, there is rapid re-infection after deworming, and hence the importance of improved sanitation is widely acknowledged in the literature dating back almost 100 years (Cort et al., 1929; Stiles, 1939; Jia et al., 2012; Ziegelbauer et al., 2012). Yet, compared to preventive chemotherapy, relatively little attention is paid on improving sanitation and clean water in contemporary helminthiases control programs (Utzinger et al., 2003, 2009; Singer & de Castro, 2007). In the present study we assessed the prevalence (and intensity) of helminths and intestinal protozoa infections and associated these findings with the local KAPB in nine purposely selected villages/hamlets of the Taabo HDSS in south-central Côte d'Ivoire, where annual preventive chemotherapy against helminth infections is administered to the entire population. The most prevalent helminth infection was hookworm (33.5%), followed by *S. haematobium* (7.0%). Other helminths were encountered only rarely.

The investigated parasitic infection prevalences and intensities were much lower than some 10 years ago; initial hookworm infections in the Taabo area in the late 1990s/early 2000s were high (34.4-54.0%), while initial prevalences for *A. lumbricoides* and *T. trichiura* infections were low; 0-1.3% and 3.3-7.5%, respectively (N'Goran et al., 2003). The reduction of the highly prevalent infections can partly be explained by the interventions carried out within the Taabo HDSS as well as preceding research and control activities against schistosomiasis (N'Goran et al., 2001; Becker et al., 2011; Fürst et al., 2012, 2013; Righetti et al., 2012, 2013). Indeed, our continuous research-cum-action activities pertaining to helminthiases in

selected localities in the study area might have had a positive influence by reducing the incidence through improved knowledge about these otherwise neglected disease in the population. In previous work on schistosomiasis in western Côte d'Ivoire we found that our research activities considerably improved knowledge in the community (Acka et al., 2010). Furthermore, while *S. haematobium* and *S. mansoni* infections are a problem for only certain localities due to the focal distribution of the disease, it can be tackled comparably easy once these foci are identified. In contrast, hookworm infections are more homogeneously distributed throughout the Taabo HDSS and considerable in- and out-migration and the challenge to reach high coverage with preventive chemotherapy are important underlying issues. It should be noted that, despite continuous control efforts through annual deworming, re-infection with hookworm occurs rapidly. Hence, there is a need to continue preventive chemotherapy, coupled with additional control measures to prevent rapid re-infection (Scherrer et al., 2009; Knopp et al., 2010; Jia et al., 2012).

Two limitations of our study are offered for discussion. First, although duplicate Kato-Katz thick smears were performed on single stool samples in order to increase sensitivity of the technique (Booth et al., 2003) it is conceivable that the reported helminth infection prevalences are an underestimation of the “true” situation in the study area. The issue of missing low infection intensities based on microscopic examination of single specimens has been discussed before (Knopp et al., 2008), partially explained by considerable day-to-day variation of helminth egg output (Utzinger et al., 2001; Coulibaly et al., 2012). Other new diagnostic tools such as the FLOTAC technique (Cringoli et al., 2010), molecular approaches (i.e. polymerase chain reaction (PCR) (Verweij et al., 2007)), or the collection of samples over several days should be considered in future studies to increase sensitivity (Glinz et al., 2010). Second, the low prevalence of infections with *T. trichiura* and *A. lumbricoides* made it difficult to draw conclusive evidence about the direction and strength of association between these helminth species and risk factors.

Several intestinal parasite infections showed significant association with socioeconomic status, confirming observations from western Côte d’Ivoire of significant disparities of parasitic infection status among study participants (Raso et

al., 2005). Hookworm, *T. trichiura*, *E. histolytica/E. dispar*, *G. intestinalis*, and *I. bütschlii* were more prevalent among the poorer wealth quintiles. Surprisingly, *S. haematobium* was positively associated with higher socioeconomic status and Muslim showed a higher risk than people with other religious beliefs. However, these observations might be explained by the focal distribution of urogenital schistosomiasis; 97% of all *S. haematobium* cases were found in Sahoua, situated in close proximity to the Bandama River. In this village, the majority of inhabitants are Muslims. Moreover, the average socioeconomic status of Sahoua is considerably higher than other study village and hamlets.

A generally good hygiene behaviour (e.g. not drinking dirty water, hand washing after defection) was recorded, which undoubtedly impacts on parasitic worms. Knowledge of schistosomiasis transmission (e.g. swimming and bathing in Lake Taabo) is widely known (58%), while wearing shoes to prevent hookworm infections was rarely mentioned (4.3%). This lack of knowledge about hookworm transmission might explain the relatively high prevalence of this helminth species despite several rounds of deworming.

Open defecation was commonly reported by the study participants. Indeed, the habit of open defecation is so deeply rooted that it was also reported (at least partially) among households having a latrine. As expected, we found a significant negative association between hookworm infection and the use of a latrine, confirming results from a systematic review and meta-analysis (Ziegelbauer et al., 2012). Literally all variables related with latrine availability or use were associated with a significantly lower odds of certain helminth infections (most importantly hookworm), but also some intestinal protozoa infections (e.g. *E. hartmanni* and *E. nana*) (Esrey et al., 1991; Fewtrell et al., 2005).

Sanitation and hygiene behaviour have proven to be substantial contributors to a sustainable control of soil-transmitted helminthiasis, schistosomiasis, diarrhoea and other faecal-orally transmitted diseases (Bartram & Cairncross, 2010). However, the promotion of sanitary solutions and the improvement of hygiene behaviour are of a higher complexity than the regular administration of anthelmintic drugs to at-risk populations, as the former entail many locally rooted socio-cultural idiosyncrasies.

For example, the possession of a latrine does not necessarily mean that it is being used (Mara et al., 2010; Ziegelbauer et al., 2012). In the current study, however, the participants living in a household with a latrine reported its use, but we did not further verify these self-reports through direct observations. Open defecation is still commonly practiced among households possessing a latrine, particularly when people pursue agricultural activities, often several kilometres away from home. Importantly though, open defecation while pursuing agricultural activities was not associated with a higher odds of helminths and intestinal protozoa infections, which is in contrast to open defecation within a human settlement (village or hamlet) and in close proximity to open water sources. Population density in human settlements is higher than on plantations, and hence contaminated faeces in the village/hamlet are a source of infection for villagers. Nevertheless, open defecation is not desirable in any case and should be stopped for the reason that plantations and agricultural fields in close proximity to open water bodies could contaminate the environment.

The main reasons advanced by interviewees regarding the possession of a latrine were issues of safety, privacy, enhanced comfort, clean environment and hygiene. Indeed, households that attributed importance to these issues were more likely to have a latrine. Health-related reasons such as hygiene or prevention of disease were frequently reported, but only a small number of interviewees mentioned such reasons spontaneously, indicating that health-related issues were perceived as less important. Although, everyone stated the need for a latrine, not everyone was motivated to build one, mainly because the construction of latrines was perceived as an expensive undertaking. Furthermore, open defecation was seen as a traditional behaviour that the whole village/hamlet is practicing. Overall, we found that health-related reasons played a minor role in the decision-making process. Therefore, health education interventions are necessary to increase the motivation of change or sanitation promotion focusing on these socio-cultural and socioeconomic reasoning and taking the whole spectrum of the villagers' concerns into account (Jenkins & Scott, 2007; Kar & Chambers, 2008; Kouadio et al., 2013).

Most people reported that they regularly wash their hands, but myriad reasons for hand washing were given. However, villagers seemed not to associate disease

prevention with general cleanliness as the two elements were mentioned separately. Our analyses revealed that the most important factor for regular hand washing was the type of preceding (e.g. field work or defecation) or subsequent activity (e.g. food consumption). “Before a meal” was mentioned by almost all interviewees and indeed with a high proportion of spontaneous responses, which is important for the prevention of diarrheal diseases (Ejemot et al., 2008). Although hand washing after defecation was reported by 85.3% of the interviewees, it was reported spontaneously only by a small proportion of study participants. This could indicate that people only answered yes to please the interviewer, but in reality, they do not wash their hands regularly after defecation. Failing to wash hands after defecation favours the transmission of faecal-orally transmitted diseases (Curtis & Cairncross, 2003).

In conclusion, our results show that the use of latrines is associated with lower odds of hookworm infection. The study also indicates that morbidity due to soil-transmitted helminthiasis and schistosomiasis has been greatly reduced in the Taabo HDSS and preventive chemotherapy certainly played a key role (Fürst et al., 2012, 2013). However, there is rapid re-infection after deworming, and hence integrated control approaches are necessary to keep the prevalence and intensity of infection – and thus morbidity – low. The parasitological and questionnaire data reported here will serve as a benchmark to monitor the effect of CLTS and hygiene education with the goal to reduce and interrupt the transmission of helminth and intestinal protozoa infections.

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6. Health-related quality of life among schoolchildren with parasitic diseases: findings from a national cross-sectional survey in Côte d'Ivoire

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6.1 Abstract

Parasitic infections are still of considerable public health relevance, notably among children in low- and middle income countries. Measures to assess the magnitude of ill-health in infected individuals, however, are debated and patient-based proxies through generic health-related quality of life (HrQoL) instruments are among the proposed strategies. Disability estimates based on HrQoL are still scarce and conflicting and hence, there is a need to strengthen the current evidence-base.

Between November 2011 and February 2012, a national school-based cross-sectional survey was conducted in Côte d'Ivoire. Children underwent parasitological and clinical examination to assess infection status with *Plasmodium* and helminth species and clinical parameters and responded to a questionnaire interview incorporating sociodemographic characteristics, self-reported morbidity and HrQoL. Validity analysis of the HrQoL instrument was performed, assessing floor and ceiling effects, internal consistency and correlation with morbidity scores. Multivariate regression models were applied to identify significant associations between HrQoL and children's parasitic infection and clinical status.

Parasitological examination of 4,848 children aged 5-16 years revealed *Plasmodium* spp., hookworm, *Schistosoma haematobium*, *Schistosoma mansoni*, *Ascaris lumbricoides* and *Trichuris trichiura* prevalences of 75.0%, 17.2%, 5.7%, 3.7%, 1.8% and 1.3%, respectively. Anaemic children showed a significant 1-point reduction in self-rated HrQoL on a scale from 0 to 100, whereas no significant negative association between HrQoL and parasite infection was observed. The 12-item HrQoL questionnaire proofed useful, as floor and ceiling effects were negligible, internally consistent (Cronbach's alpha=0.71) and valid, as revealed by significant negative correlations and associations with children's self-reported and clinically assessed morbidity.

Our results suggest that HrQoL tools are not sufficiently sensitive to assess subtle morbidities due to parasitic infection in Ivorian school-aged children. However, more advanced morbid sequelae (e.g. anaemia), were measurable by the instrument's health construct. Further investigations on health impacts of parasitic

infection among school-aged children and refinement of generic HrQoL questionnaires are warranted.

Running title: HrQoL in parasitized children, Côte d'Ivoire

6.2 Author summary

Infectious diseases like malaria and parasitic worms affect hundreds of millions of people and impact on physical and cognitive development of children in Africa, Asia and the Americas. Over the past 20 years it was debated how the magnitude of ill-health due to these conditions should be assessed. One proposed strategy was to include patient-based ratings of wellbeing by administration of health-related quality of life (HrQoL) questionnaires. In order to provide new evidence on disability from parasitic infections, we conducted HrQoL interviews with children aged 5-16 years from 92 schools across Côte d'Ivoire. Children were examined for parasitic infections and clinical signs like anaemia, malnutrition and organ enlargement. We compared the self-rated HrQoL of infected and non-infected children and also considered their sociodemographic background. We could not identify lowered HrQoL in infected children, but we found that children with anaemia reported a 1-point lower score on a 100-point HrQoL scale in comparison with their non-anaemic counterparts. We consider our HrQoL questionnaire as useful and valid, but would recommend its further testing and development in few purposefully selected settings. Further investigation of disability induced by malaria and parasitic worm infections is warranted.

6.3 Introduction

Malaria and the neglected tropical diseases (NTDs) are still of considerable public health relevance in the tropics and subtropics and their successful control is a key issue toward progress of the millennium development goals (MDGs) and the post-2015 agenda of sustainable development (Murray et al., 2012a; Utzinger, 2012; WHO, 2013; Pullan et al., 2014). Preschool-aged children are considered at highest risk of malaria, whereas school-aged children are the most affected by parasitic worm infections (helminthiases) (Woolhouse, 1998; Brooker et al., 2007; WHO, 2011). The assessment of the precise burden attributable to parasitic infections, however, is a difficult issue and there is ongoing discussion and debate (Hotez et al., 2014; King, 2014). Over the past 20 years, the magnitude of health loss due to diseases, injuries and risk factors has been increasingly expressed in disability-adjusted life years (DALYs). This metric is a combined measure of premature death and years of life lived with disability. For measuring the burden of helminthiases and other NTDs, specific disability weights (DWs) of morbid sequelae are considered and, by convention, scaled on an axis from 0 (no health loss) to 1 (health loss equivalent to death) (Mathers et al., 2007). Former estimates were often criticized for underestimating the true burden of infectious diseases, due to separating out morbidity (e.g. anaemia), although such morbidity is partially associated with infection (e.g. hookworm and *Plasmodium*). Additionally, cultural and socioeconomic contexts are insufficiently taken into account and DWs were usually based on expert opinion; thus, ignoring community- or patient-based appraisal (Reidpath et al., 2003; King & Bertino, 2008; Payne et al., 2009). Meanwhile, the Global Burden of Disease (GBD) consortium presented estimates for the year 2010 by incorporating different sequelae to capture direct consequences of infections and judgments on health losses from the general public in culturally and socioeconomic diverse settings (Murray et al., 2012b; Salomon et al., 2012). Nonetheless, the use of generic health status measurement instruments to expand the GBD approach has been discussed by the lead authors of the GBD 2010 study (Salomon et al., 2012), thus partially

addressing concerns that have been articulated a decade ago (King et al., 2005; Voigt & King, 2014).

The discussed generic health status measurement instruments evaluate health burden in a comprehensive way based on health-related quality of life (HrQoL) and typically include domains on physical, mental and social wellbeing and a visual analogue scale (VAS) for subjective health rating (Skevington et al., 2004; King & Hinds, 2011; Rabin et al., 2014). Thus far only few studies have assessed HrQoL and derived DWs in individuals with parasitic diseases, indicating the early stage of this approach in the field of parasitology. This issue is further underscored by conflicting results; while negative associations between HrQoL measures and *Trichuris trichiura*, *Schistosoma mansoni*, *Schistosoma haematobium* and advanced *Schistosoma japonicum* infections were observed (Jia et al., 2011; Fürst et al., 2012; Terer et al., 2013), other studies failed to show significant differences in HrQoL and DWs between infected children and their non-infected counterparts (Ziegelbauer et al., 2010; Fürst et al., 2011; Samuels et al., 2012). A weaker explanatory power in previous studies may partly be explained by a lack of cross-cultural validity of the questionnaires. HrQoL instruments have been developed and broadly validated in Europe and the United States of America and were originally designed for adult respondents. Child-friendly versions meanwhile exist (Varni et al., 1999; Wille et al., 2010), but application in different cultural settings imply careful adaptations in language and scoring, thorough pre-testing and validity analysis.

Considering the scarcity of empirical data on HrQoL assessments in school-aged children with single and multiple species infections, the aim of the present study is to strengthen the current evidence-base of disability due to parasitic diseases among pupils in Côte d'Ivoire. Hence, a cross-sectional school-based survey was carried out using standardized, quality-controlled parasitological and questionnaire tools. Furthermore, we discuss the utility and validity of a HrQoL questionnaire tailored to a given setting with basic elements from readily available tools.

6.4 Materials and methods

Ethics statement

The study protocol was approved by the institutional research commissions of the Swiss Tropical and Public Health Institute (Basel, Switzerland) and the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (Abidjan, Côte d'Ivoire). Ethical approval was obtained from the ethics committees in Basel (EKBB; reference no. 30/11) and Côte d'Ivoire (CNER; reference no. 09-2011/MSHP/CNER-P). Additionally, permission to carry out the study was sought from the Ministry of National Education in Côte d'Ivoire. Directors and teachers of the selected schools, district and local health and education authorities were informed about the purpose and procedures of the study. Written informed consent was obtained from parents and legal guardians of children, whilst children assented orally. Participation was voluntary and hence, children could withdraw from the study at any time without further obligations. All collected data were coded and kept confidential. Participating children benefited from free of charge deworming with albendazole (single oral dose of 400 mg). Children identified to harbour *Schistosoma* spp. were given praziquantel (single oral dose of 40 mg/kg). In schools where the prevalence of *Schistosoma* infection was above 25%, the entire study sample was treated with praziquantel. Symptomatic malaria cases, defined as having a positive rapid diagnostic test (RDT) and fever, were offered artemisinin-based combination therapy (ACT; using artesunate-amodiaquine) and paracetamol against fever.

Study design and subjects

Between November 2011 and February 2012 (i.e. dry season) we conducted a national cross-sectional, school-based study, including parasitological and clinical examinations and administered a questionnaire. Our aim was to select approximately 100 schools across Côte d'Ivoire, which we considered as a maximum number of locations that we would be able to visit within a 3-month period and our financial and human resources would allow to cover. A lattice plus close pairs design (Diggle & Lophaven, 2006; Yapi et al., 2014) was applied for the sampling of the schools. In brief, a grid indicating latitude and longitude at a unit of

0.5° was overlaid on a map of Côte d'Ivoire that divides the country into two major ecological zones (Raso et al., 2012). The southern ecozone is characterized by abundant rainfall (> 1,000 mm per annum) and dense forest vegetation cover, whereas the northern ecozone corresponds to a savannah-type profile with markedly less precipitation. In order to achieve a representative sample of the country, 58 and 42 possible survey locations were retained after randomly drawing from each or every second grid cell of ecozones 1 and 2, respectively, taking into account population density from the last available census in 1998. About 27% of the population was estimated to live in the major urban centres in 2007 (Institut Nationale de la Statistique, 2014). We aimed at including at least one fifth of all schools from urban areas. In total, 94 schools were selected and we double-checked that the schools comprised a minimum of 60 children attending grades 3 and 4, using a recent school inventory from a national UNICEF education program (UNICEF 2010; personal communication). Children attending grades 3 and 4 were considered as capable to express themselves and give reliable answers to questionnaire items on household assets, experienced symptoms and diseases and HrQoL and may be retrievable in case of followed-up studies. The sample size per school was delimited to 60 children due to financial and operational constraints, considering the high number of schools to be surveyed and the maximum number of children that a survey team could sample in a single day, including questionnaire interviews and detailed laboratory work-up of blood, stool and urine specimens. This sample size exceeds the minimum of 50 children to be surveyed in a school, as recommended by the World Health Organization (WHO) for collection of baseline information on helminth prevalence and intensity in the school-aged population within large-scale surveys (WHO, 2011).

Two schools were omitted in the final analysis. One school refused to participate, while another school was subjected to recent deworming. The latter would have biased the results, since signs and symptoms due to chronic helminth infections and HrQoL are likely to change after anthelmintic treatment. The remaining 92 schools are mapped by ecozone and stratified by rural and urban setting characteristics (Figure 6.1).

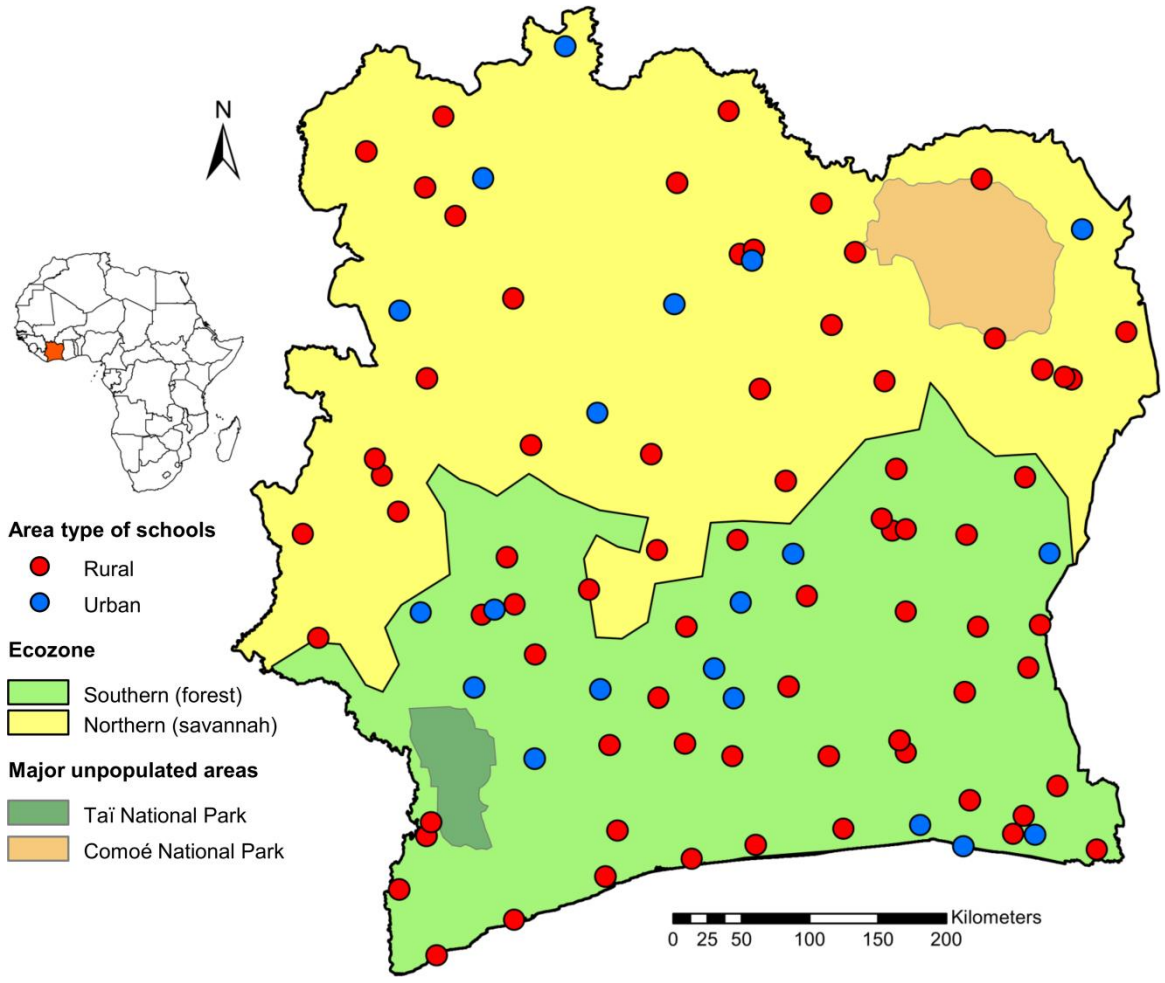

Figure 6.1 Map of Côte d'Ivoire showing the included schools (n=92), stratified by rural and urban residential area

The study was conducted between November 2011 and February 2012 among school children aged 5-16 years. The majority (60%) of the enrolled schools were situated in the more densely populated southern ecozone.

Field and laboratory procedures

In advance of the study conduct, directors and teachers of the selected schools were contacted and they were invited to inform parents or legal guardians of 60 children attending grades 3 and 4. Whenever necessary, children from grade 5 were invited to complement sampling to reach the targeted number of 60 children. Children whose parents/guardians had provided written informed consent were invited for participation. The objectives and procedures of the study were explained on the day of the visit. Children were then asked to provide fresh urine and stool samples in plastic containers distributed upon arrival at school. Additionally, a finger-prick blood sample was taken for preparation of an RDT of malaria (ICT ML01 malaria Pf kit; ICT Diagnostics, Cape Town, South Africa) and thick and thin blood films on microscope slides for subsequent analysis of *Plasmodium* infection. All biological samples were transferred to nearby laboratories and processed the same day. In brief, urine reagent strips (Hemastix; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) were used to assess microhematuria in urine samples, as a proxy for *S. haematobium* infection (Savioli et al., 1990). Of note, reagent strips show a high specificity for indirect diagnosis of *S. haematobium* among school-aged children in endemic areas (King & Bertsch, 2013). Duplicate Kato-Katz thick smears (Katz et al., 1972), using 41.7 mg templates, were prepared from each stool sample. Kato-Katz thick smears were allowed to clear for 30-45 min prior to microscopic examination by experienced laboratory technicians. The number of helminth eggs was counted and recorded for each species separately (i.e. *S. mansoni*, *A. lumbricoides*, *T. trichiura*, hookworm and other helminths). Blood films were stained with a 10% Giemsa solution and examined under a microscope for *Plasmodium* species identification and quantification of parasitemia (parasites/µl of blood) (N'Goran et al., 2003). For quality control, 10% of the Kato-Katz thick smears and stained blood film slides were re-examined by a senior microscopist. In case of discrepancies (e.g. positive *versus* negative results or counts of parasitic elements differing by more than 10%), slides were read by a third technician and findings discussed until agreement was achieved.

All participating children underwent a clinical examination, conducted by experienced medical staff, which included haemoglobin (Hb) measurement using a HemoCue analyser (Hemocue Hb 301 system; Angelholm, Sweden) to assess anemia, palpation for liver and spleen enlargement and measurement of body temperature using an ear thermometer (Braun ThermoScan IRT 4520; Kronberg, Germany) for identification of fever cases (≥ 38.0 °C). Two anthropometric measurements were taken (i.e. height in cm and body weight in kg, precision 0.5 kg) for subsequent calculation of children's nutritional status.

Questionnaire study

A questionnaire assessing the socioeconomic status, self-reported symptoms and diseases and HrQoL was administered to all children. Questions on household asset ownership, diseases and disease-related symptoms were adapted from an instrument previously used in school-based surveys conducted in Côte d'Ivoire (Raso et al., 2005). Children were asked for 11 different symptoms (i.e. abdominal pain, blood in stool, blood in urine, diarrhoea, dysentery, fatigue, fever, headache, loss of appetite, respiratory problems and vomiting/nausea) and eight diseases (i.e. cold, cough, eye disease, malaria, malnutrition, schistosomiasis, skin disease and worms) using a recall period of 2 weeks. To evaluate self-rated HrQoL, the French version of the WHOQOL-BREF tool (Skevington et al., 2004) served as template. Specific questions were dropped and some questions were slightly rephrased to be more specific for the current context, interviewing school-aged children in Côte d'Ivoire. In addition to specific questions focusing on HrQoL, children were asked to rate their general health status using an adapted VAS (Oppe et al., 2008). This single-item measure basically consists of a thermometer-like scale, in which the anchors are 'best imaginable health' and 'worst imaginable health', in our case defined as a maximum and minimum value of 10 and 0, respectively. The complete questionnaire instrument was further refined in several rounds of pre-testing in a primary school that was not otherwise involved in the current study. In this pre-testing, children attending grades 2-5 with different cultural backgrounds were included. We determined interview duration using a stopwatch and comprehensibility

and appropriateness of the HrQoL part, which was not yet validated from earlier studies, with the goal to achieve a compact, understandable and locally valid instrument. Questionnaire interviews in the field were conducted by members of the study team and teachers from the selected schools, who were trained beforehand.

Statistical analysis

Data were double-entered and cross-checked using EpiInfo version 3.5.3 (Centers for Disease Control and Prevention; Atlanta, United States of America) and analysed in Stata version 10.1 (Stata Corp.; College Station, United States of America). Only data from children with written informed consent, completed questionnaire, valid parasitological results and clinical assessments were considered for further analysis.

Socioeconomic data were utilized to calculate a wealth index following an asset-based approach as adopted and explained elsewhere (Raso et al., 2005; Schmidlin et al., 2013). According to their index score, children were stratified into five economic groups according to wealth quintiles (i.e. most poor, very poor, poor, less poor and least poor). Data on helminth infections were classified into light, moderate and heavy, following WHO guidelines (WHO, 2011). Anaemia was defined as having a Hb level below 115 g/l in children aged 5-11 years and 120 g/l in children aged12-15 years (WHO, 2008). The presence of organ enlargement was defined as having a palpable liver or spleen; the latter of grade 1 or higher using a Hackett's scale (Ruzagira et al., 2010). Indicators for malnutrition were calculated according to WHO child growth standards for children aged 5-19 years (Duggan, 2010). They included stunting (height-for-age), wasting (body mass index (BMI)-for-age) and underweight (weight-for-age). The latter is considered a valid measure for nutritional status in children up to 10 years only and was incorporated in a summary measure for malnutrition, defined as Z-score < -2 for any of the three nutritional indicators.

HrQoL questionnaire answers were coded as 1, 2 or 3 (in question 1 up to five codes; Appendix 10.2.3) with higher scores indicating fewer problems for a certain issue or activity. HrQoL questionnaire scores were summarized into three main domains on (i) physical, (ii) psychosocial and (iii) environmental wellbeing. The first

comprised the sum of scores from questions 2-6, the second from questions 7-9 and the third from questions 10-12. Each child's overall score on HrQoL was built by summing up individual scores from questions 1-12. Domain and overall raw scores were further converted to a 100-point scale (formula: [(raw score – lowest possible score) / raw score range] x 100) (Bradley, 2013). Cronbach's alpha coefficient was used to assess for internal consistency of the HrQoL scores. Overall HrQoL, domain and VAS scores were subjected to analysis on floor and ceiling effects. Floor or ceiling effects (> 15% of respondents achieved lowest or highest possible score) can indicate limited content validity and reduced reliability, whilst responsiveness may be limited since changes in respondents with lowest or highest possible scores cannot be measured (Terwee et al., 2007). The validity of the HrQoL instrument was further evaluated by assessing relationships of domain, overall HrQoL and VAS scores with symptoms reporting and clinical signs using Spearman rank correlation and linear regression analysis, as appropriate. In order to relate the questionnaire measures with self-reported and clinically assessed morbidity, additional summary variables providing the total number of self-reported symptoms and diseases (n = 19) and clinical signs (n = 7) for each child was generated, with possible ranges of 0 to 19 and 0 to 7, respectively.

Chi square (χ^2), Fisher's exact, Student-*t*, Kruskal-Wallis and Wilcoxon rank sum tests were applied, as appropriate, to investigate significant univariate differences between groups for sociodemographic, parasitological, clinical and HrQoL indicators. Associations between the HrQoL outcome and parasitic infection, infection intensity and clinical status were assessed using multivariate linear regression analysis with random effects to account for clustering within schools. In case of censored data, we additionally applied tobit regression models. Particular emphasis was placed on total HrQoL and physical wellbeing domain scores as outcome in order to make explicit the physical and non-physical impacts of the health conditions assessed. Explanatories of regression models included sociodemographic, parasitological and clinical variables. The final models were built, following a stepwise backward elimination approach. Covariates were excluded from the model at a significance level of 0.20 or higher. Relationships between the

outcome and remaining explanatory variables were expressed as adjusted mean differences with corresponding 95% confidence intervals (CIs).

6.5 Results

Operational results

A total of 94 schools across Côte d'Ivoire were visited during the study and 5,491 children invited to participate. Figure 6.2 depicts the study compliance and participation in the various assessments undertaken. The final sample used for in-depth analysis consisted of 4,848 children from 92 schools with a mean age of 9.8 years (range: 5 to 16 years). These children had complete questionnaire, parasitological and clinical data and had not received deworming drugs within the past 4 weeks prior to the survey. There were slightly more boys than girls (2579 *versus* 2269). 72 schools were considered rural, whilst the remaining 20 (21.7%) were based in urban settings. 4101 (84.6%) of the children belonged to the two targeted school grades, 3 and 4.

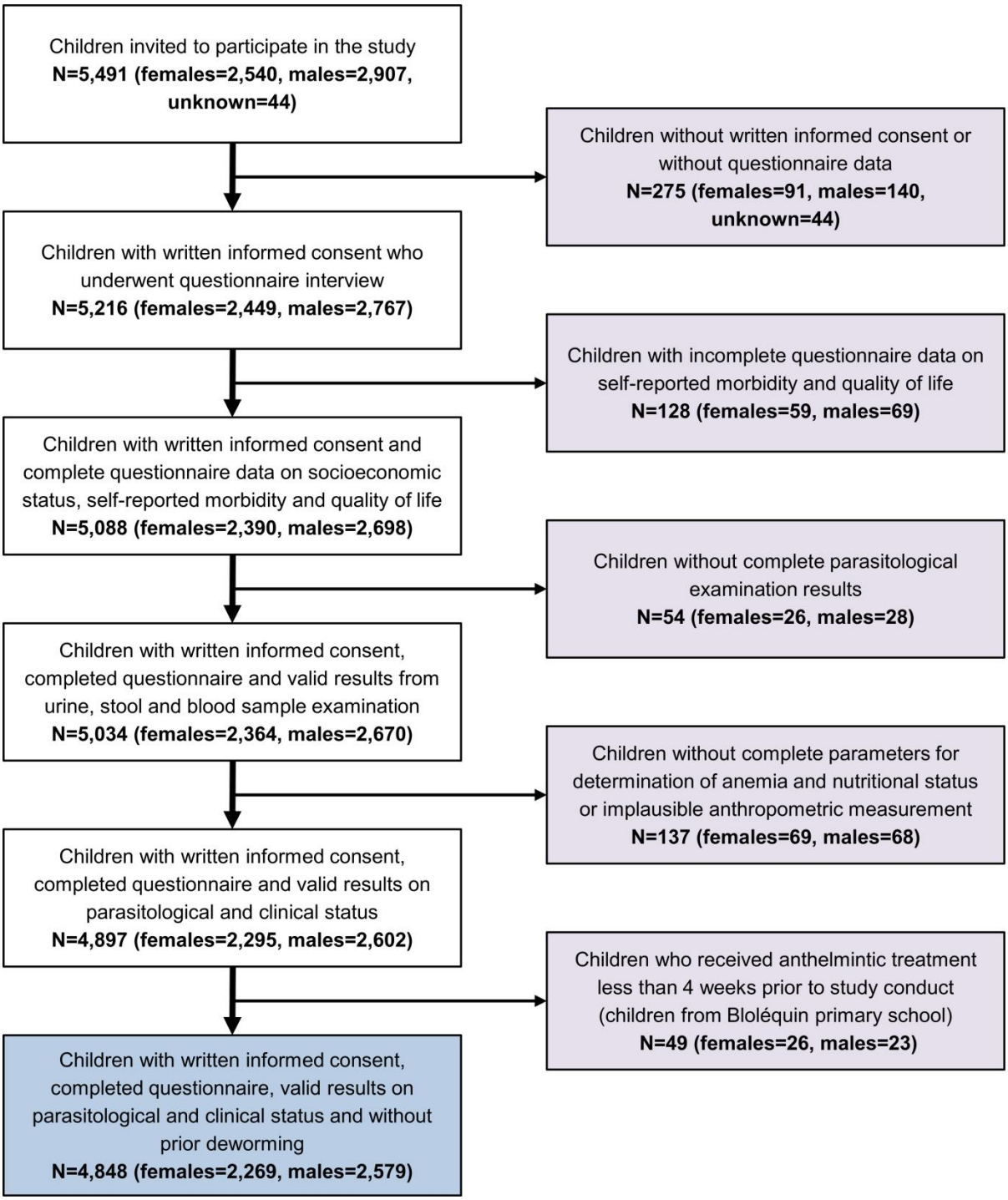

Figure 6.2 Flow chart, detailing study participation and compliance

The cross-sectional, school-based, national survey was carried between November 2011 and February 2012 in Côte d'Ivoire.

Parasite infection and clinical status

Table 6.1 summarizes overall prevalence and intensity of parasitic infections, clinical signs and self-reported symptoms and diseases. Overall 3,635 of the 4,848 children (75.0%) harboured any malaria parasite. *P. falciparum* was the predominant species (74.1%), followed by *P. malariae* (3.9%) and *P. ovale* (0.3%). The latter two *Plasmodium* species occurred mainly as co-infections with *P. falciparum*. Helminth infections; namely, hookworm, *S. mansoni*, *A. lumbricoides* and *T. trichiura* were observed in 17.2%, 3.7%, 1.8% and 1.3% of the children, respectively. Microhematuria was found in 5.7% of the children. The majority (95.6%) of soil-transmitted helminth infections were of light intensity, whereas about half of the *S. mansoni*-infected children had moderate- to heavy-intensity infections (≥ 100 eggs per gram of stool). More than a fourth of all children were found to be anaemic (28.7%) or malnourished (28.4%) and a mean number of 6.1 experienced symptoms or diseases were reported.

Detailed information on parasitic infections and clinically assessed and self-reported morbidity stratified by sex, age group, residential area and ecozone are provided in the Appendix (Appendix 10.2.1 and 10.2.2). Boys showed significantly higher infection rates for *P. falciparum*, hookworm and *S. mansoni* (Appendix 10.2.1). Prevalence rates differed between age groups; while *P. malariae* was more often found in younger children, infections with *Schistosoma* and soil-transmitted helminths were more prevalent in children aged 11-16 years than in their younger counterparts. *Plasmodium* spp. and soil-transmitted helminth infections were most prevalent among the poorest and rural households (all $p < 0.001$). *Plasmodium* spp. was more common in children living in the northern ecozone. Clinical morbidity, such as anaemia and indicators for malnutrition, was more pronounced in boys than girls and in older children compared to their younger counterparts (Appendix 10.2.2). Splenomegaly was found to be more common in the younger age group ($p = 0.014$) and in children from rural and northern settings compared to children living in urban and southern environments (both $p < 0.001$). Anaemia ($p = 0.049$), splenomegaly ($p < 0.001$) and stunting ($p < 0.001$) were significantly lower in children from wealthier households. Furthermore, helminth (OR = 1.69, $p < 0.001$) and *Plasmodium* (OR =

1.44, $p < 0.05$) mono-infected as well as co-infected (OR = 2.0, $p < 0.001$) children showed significantly higher odds ratios (ORs) for anaemia than their non-infected peers in multivariable logistic regression analysis. Symptom and disease reporting was higher in girls compared to boys, in older compared to younger individuals, in children from northern regions compared to their counterparts living in the southern ecozone (all $p < 0.001$) and in children from poorer households ($p = 0.025$).

Table 6.1 Prevalence and intensity of parasitic infections, clinical signs and self-reported symptoms and diseases among 4,848 schoolchildren in Côte d'Ivoire

Parasitic infection	N	%	Morbidity	N	%
P. falciparum	3,593	74.1	Observed clinical signs		
P. malariae	190	3.9	Anemia[b]	1,391	28.7
P. ovale	13	0.3	Any form of malnutrition[d]	1,375	28.4
Plasmodium spp.	3,635	75	Stunting[c]	875	18.1
Parasitemia ≥ 1,000 parasites/µl of blood	1,134	23.4	Wasting[c]	574	11.8
S. haematobium	276	5.7	Spleen enlargement[e]	559	11.5
S. mansoni	177	3.7	Liver enlargement[e]	126	2.6
Light infection[a]	85	48	Fever (≥ 38 °C)	90	1.9
Moderate infection[a]	60	33.9	Clinical malaria[f]	69	1.4
Heavy infection[a]	32	18.1	Self-reported symptoms		
Hookworm	835	17.2	Headache	2,633	54.3
Light infection[a]	808	96.8	Abdominal pain	2,477	51.1
Moderate infection[a]	16	1.9	Fatigue	2,356	48.6
Heavy infection[a]	11	1.3	Fever	2,335	48.2
A. lumbricoides	89	1.8	Vomiting/nausea	1,696	35.0
Light infection[a]	75	84.3	Diarrhea	1,525	31.5
Moderate infection[a]	14	15.7	Blood in stool	1,452	30.0
Heavy infection[a]	0	0	Loss of appetite	1,399	28.9
T. trichiura	61	1.3	Respiratory problems	1,301	26.8
Light infection[a]	61	100	Dysentery	1,170	24.1
Moderate infection[a]	0	0	Blood in urine	491	10.1
Heavy infection[a]	0	0	Self-reported diseases		
Soil-transmitted helminths	926	19.1	Cough	2,777	57.3
Light infection[a]	885	95.6	Cold	2,237	46.1
Moderate infection[a]	30	3.2	Malaria	1,472	30.4
Heavy infection[a]	11	1.2	Malnutrition	1,038	21.4
			Eye disease	928	19.1
			Worms	812	16.8
			Schistosomiasis	686	14.2
			Skin disease	635	13.1

Parasite prevalences are provided in % of all included school children. Data on infection intensities are provided as % of all positive cases. Prevalences of clinical or self-reported morbidities are given in % of all included school children.

[a]Intensities of intestinal helminth infections are categorized according to WHO guidelines (WHO, 2011).

[b]Defined as haemoglobin levels below 115 g/l and below 120 g/l in children aged 5-11 years and 12-16 years, respectively.

[c]Calculated according to WHO child growth standards (Duggan, 2010); defined as BMI-for-age (wasting) and height-for-age (stunting) resulting in a Z-score < -2.

[d]Defined as any of the assessed nutritional indicators resulting in a Z-score < -2; this includes wasting, stunting and weight-for-age (underweight).

[e]Defined as palpable liver and spleen (≥ grade 1 by Hackett's classification), respectively.
[f]Clinical malaria is defined as being *Plasmodium*-positive and having fever (≥ 38° C)

Validity of HrQoL instrument

Table 6.2 shows the results from the utility and validity analysis of the HrQoL measures. For the summary scores, floor and ceiling effects were negligible. In contrast, relevant ceiling effects were observed for single HrQoL domains and the VAS scores. Internal consistency of the 12-item HrQoL questionnaire was above the recommended threshold of 0.7 for Cronbach's alpha needed for comparison between groups. The item-rest correlations were all above 0.25, indicating that single items measured the same construct as the remaining ones and removal of a specific item would not have increased Cronbach's alpha.

Self-reported symptoms and diseases were reflected in the HrQoL. All HrQoL measures showed significant negative correlations and associations with increasing number of self-reported morbidities. For an incremental increase of 1 self-reported morbidity, the overall HrQoL decreased by 1.4 points ($p < 0.001$). Clinical signs were mainly captured by the physical domain of the HrQoL tool, showing a decreased domain score of 1.2 points ($p = 0.001$) by each supplemental clinical morbidity observed. VAS scores showed a statistically significant correlation and association with self-reported symptoms and diseases (Table 6.2) and also a statistically significant correlation with overall HrQoL (all $p < 0.001$), but the correlations were only weak ($\rho = -0.22$ and $\rho = 0.30$, respectively). The VAS results were not considered for in-depth analysis and calculation of DWs due to deviance between actual data collected and the original concept of the scale.

Table 6.2 Utility and validity measures of HrQoL instrument from 4,848 schoolchildren with complete questionnaire data

	Scale				
	Domain 1 (physical)	Domain 2 (psychosocial)	Domain 3 (environmental)	Total HrQoL	VAS score
Number of items	5	3	3	12	-
Utility					
Floor (%)[a]	0.5	0.4	0.7	0.0	0.0
Ceiling (%)[a]	22.8	38.0	54.0	2.1	26.2
Internal consistency					
Cronbach α[b]	0.65	0.43	0.48	0.71	-
Relationship with symptom reporting					
Spearman rank sum correlation	-0.28	-0.22	-0.18	-0.37	-0.22
p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Association (95% CI)	-1.5 (-1.7, -1.4)	-1.1 (-1.3, -1.0)	-0.9 (-1.0, -0.7)	-1.4 (-1.5, -1.3)	-1.3 (-1.4, -1.1)
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
Relationship with clinical signs					
Association (95% CI)	-1.2 (-1.8, -0.5)	-0.3 (-0.9, 0.3)	0.3 (-0.2, 0.9)	-0.5 (-1.0, -0.1)	-0.5 (-1.2, 0.1)
p-value	0.001*	0.322	0.250	0.012*	0.113

This study was conducted between November 2011 and February 2012 in 92 schools all over Côte d'Ivoire.

[a]Floor and ceiling correspond to the percentage of scores at the minimum (0) and maximum (100) of the scaling range. Floor or ceiling effects ≤ 15% are considered acceptable and providing reliable estimates (Terwee et al., 2007).

[b]All items of the HrQoL instrument added up to the Cronbach α values indicating measurement of the same concept. Values of α ≥ 0.7 are recommended for comparison between groups (Nunnally & Bernstein, 1994).

To assess the relationship between HrQoL and VAS scores with symptom and disease reporting, a variable providing the total number of self-reported symptoms (n = 11) and diseases (n = 8) for each child was generated first, with a possible range of 0 to 19. Subsequently, Spearman rank correlation and linear regression analysis was performed with instrument scores in relation to the number of self-reported morbidities. All correlations and associations were of negative direction indicating decreasing HrQoL scores for increasing numbers of self-reported symptoms and diseases.

Similarly, a summary variable for 7 examined clinical signs (i.e. anaemia, fever, hepatomegaly, splenomegaly, stunting, underweight and wasting) was generated, with a possible range of 0 to 7 and relationship with HrQoL and VAS scores assessed using linear regression analysis. Particularly the physical health domain showed strong negative association with increased number of clinical signs.

*Statistically significant ($p < 0.05$).

Self-Reported QoL

Univariate analysis showed several differences in overall HrQoL among groups with different sociodemographic factors and observed clinical signs (Table 6.3). Boys reported higher overall HrQoL scores, which were mainly driven by higher self-rated environmental wellbeing. Children from the most poor wealth quintile showed significantly lower scores for all three HrQoL domains. Lower scores for psychosocial and environmental wellbeing and thus lower overall HrQoL scores, were observed in older children and children living in urban areas. Children from the northern regions reported higher physical but lower environmental wellbeing than their peers from the southern zone. Children's HrQoL with regard to parasitic infections mainly showed differences for the physical domain. Microhematuria negatively affected physical wellbeing, while light-intensity soil-transmitted helminth infections and low *Plasmodium* parasitemia were associated with fewer problems in this domain compared to non-infected counterparts. Comparison for *Plasmodium*-helminth co-infection categories and the number of concurrent parasitic infections (including malaria parasites) showed that children harbouring two or more concurrent infections reported the highest physical wellbeing scores compared to their mono- or non-infected counterparts. Anaemic children's HrQoL was considerably compromised compared to non-anaemic children. A similar but less pronounced decrease in HrQoL was found in children with splenomegaly. Other observed clinical signs showed no significant effects on children's overall HrQoL, but wasted children reported a significantly increased psychosocial wellbeing, while generally malnourished children reported not only higher psychosocial but also higher environmental wellbeing.

Table 6.4 provides an overview on significant associations between sociodemographic, parasitological and clinical variables on one hand and self-reported HrQoL on the other hand, placing emphasis on summary and physical wellbeing scores, derived from multivariate linear regression with a stepwise backward elimination procedure. Sex, socioeconomic status, anaemia, *Plasmodium* spp. infection, *Plasmodium*-helminth co-infection and number of concurrent parasitic infections remained significant predictors for overall HrQoL. If only physical

wellbeing was considered, negative associations of clinical manifestations such as anaemia and malnutrition were more pronounced. Interestingly, several single species parasitic infections (i.e. *Plasmodium* spp. and soil-transmitted helminths) and multiple species parasitic infections (i.e. *Plasmodium*-helminth and number of concurrent infections ≥ 2) showed a significant positive association with self-reported physical wellbeing.

Table 6.3 Mean overall and domain HrQoL scores grouped by sociodemographic, parasitological and clinical variables from 4,848 schoolchildren (2,269 females, 2,579 males) in Côte d'Ivoire

Parameter		Mean scores							
		Total HrQoL	p-value	Domain 1	p-value	Domain 2	p-value	Domain 3	p-value
Sex	Female	76.0		72.9		80.4		84.3	
	Male	77.0	0.004*	74.0	0.071	79.8	0.431	86.4	<0.001*
Age group (years)	5-10	76.7		73.0		80.5		86.5	
	11-16	76.1	0.088	74.5	0.040*	79.0	0.006*	83.0	<0.001*
Socioeconomic status	Most poor	75.1		71.4		79.3		83.9	
	Very poor	77.7		75.6		81.4		85.6	
	Poor	76.0		73.7		79.2		84.4	
	Less poor	76.4		73.4		79.4		86.3	
	Least poor	77.5	<0.001*	73.5	0.003*	81.2	0.020*	86.8	0.030*
Residential area	Rural	76.9		73.6		80.8		86.1	
	Urban	75.1	0.003*	73.2	0.426	77.3	<0.001*	82.9	<0.001*
Ecozone	South	76.5		72.1		80.4		86.4	
	North	76.5	0.429	75.6	<0.001*	79.6	0.220	83.9	<0.001*
Plasmodium spp.	Not infected	75.5		71.9		79.4		84.8	
	Infected with < 1,000 parasites/µl of blood	77.4		74.8		80.5		85.8	
	Infected with ≥ 1,000 parasites/µl of blood	75.5	<0.001*	72.2	<0.001*	79.0	0.131	85.1	0.308
S. haematobium	No microhematuria	76.7		73.8		80.2		85.4	
	Microhematuria positive	73.5	0.005*	68.9	0.002*	77.9	0.286	84.8	0.245
S. mansoni	Not infected	76.4		73.4		80.0		85.3	
	Light-intensity	77.9		75.5		81.0		87.3	
	Moderate- to heavy-intensity	80.4	0.019*	77.9	0.034*	84.2	0.314	87.9	0.584
Soil-transmitted helminths	Not infected	76.2		73.0		79.8		85.4	
	Light-intensity	77.6		75.5		81.1		85.1	
	Moderate- to heavy-intensity	77.9	0.160	74.4	0.019*	80.1	0.605	86.2	0.854
Plasmodium-helminth co-infection	Neither of the two infections	75.5		71.9		79.3		84.5	
	Plasmodium only	76.7		73.7		80.0		85.8	

	Helminth only	75.8		72.0		79.7		86.2	
	Co-infected	77.2	0.023*	74.8	0.017*	80.9	0.385	85.0	0.127
Number of concurrent parasitic infections	0	75.5		71.9		79.3		84.5	
	1	76.5		73.6		79.9		85.7	
	≥ 2	77.3	0.013*	74.6	0.015*	81.1	0.211	85.5	0.244
Number of concurrent helminth infections	0	76.3		73.2		79.8		85.5	
	1	77.0		74.2		81.0		84.9	
	≥ 2	76.8	0.412	74.5	0.291	79.3	0.262	87.4	0.301
Anaemia	Not anaemic	77.1		74.5		80.7		85.5	
	Anaemic	75.0	<0.001*	71.0	<0.001*	78.4	0.001*	85.2	0.413
Wasting	Not wasted	76.3		73.3		79.8		85.2	
	Wasted (Z-score < -2)	77.6	0.120	74.9	0.152	82.1	0.008*	86.4	0.187
Stunting	Not stunted	76.4		73.5		79.9		85.3	
	Stunted (Z-score < -2)	76.8	0.742	73.5	0.862	81.0	0.227	85.8	0.408
Any malnutrition	Not malnourished	76.3		73.5		79.5		85.0	
	Malnourished (Z-score <- 2)	77.0	0.326	73.5	0.808	81.5	0.004*	86.4	0.040*
Fever	No fever (< 38 °C)	76.5		73.5		80.0		85.4	
	Fever (≥ 38 °C)	75.6	0.797	71.2	0.632	83.1	0.273	86.9	0.756
Liver enlargement	Normal	76.5		73.5		80.1		85.4	
	Enlarged	75.8	0.806	73.7	0.853	77,9	0.646	84.4	0.430
Spleen enlargement	Normal	76.7		73.7		80.3		85.4	
	Enlarged (Hackett's scale ≥ 1)	74.8	0.011*	71.6	0.027*	78.2	0.039*	85.2	0.626

Domain 1 = physical wellbeing; domain 2 = psychosocial wellbeing; domain 3 = environmental wellbeing.

*Statistically significant ($p < 0.05$) based on Wilcoxon rank sum (for variables with 2 categories) and Kruskal-Wallis test (for variables with more than 2 categories).

Table 6.4 Associations between health-related quality of life and physical domain scores with sociodemographic, parasitological and clinical variables from multivariate regression analysis

	Health-related quality of life (summary)			Domain 1 (physical wellbeing)[b]		
Variable[a]	**Coeff.**	**95% CI**	**p-value**	**Coeff.**	**95% CI**	**p-value**
Sex (male)	1.0	(0.2, 1.8)	0.015*	1.0	(-0.2, 2.3)	0.113
Age group (11-16 years)	-0.6	(-1.5, 0.2)	0.154	1.6	(0.2, 2.9)	0.025*
Wealth quintile (Most poor)	-1.7	(-2.8, -0.6)	0.002*	-1.7	(-3.3, -0.0)	0.048*
Ecozone (North)	-	-	-	3.7	(0.2, 7.1)	0.036*
Plasmodium spp. infected	1.0	(0.0, 2.0)	0.046*	1.6	(0.2, 3.1)	0.029*
Anemia	-1.2	(-2.1, -0.2)	0.013*	-2.0	(-3.4, -0.6)	0.006*
Splenomegaly	-1.1	(-2.4, 0.2)	0.099	-	-	-
Soil-transmitted helminths	-	-	-	1.9	(0.2, 3.5)	0.030*
Any form of malnutrition (Z-score < -2)	-	-	-	-1.5	(-2.9, -0.1)	0.037*
Plasmodium-helminth co-infected	1.4	(0.0, 2.7)	0.043*	2.8	(0.7, 4.8)	0.009*
Number of concurrent parasitic infections (≥ 2)	1.7	(0.4, 3.0)	0.009*	2.9	(0.9, 4.9)	0.004*

Multivariate regression models with random effects to account for clustering and a stepwise backward elimination approach were utilized to identify explanatory variables, which most significantly influence the children's overall quality of life and physical domain scores. Initial models included sociodemographic (e.g. sex, age group, socioeconomic status, residential area (rural or urban) and ecozone), parasitological (by infection intensity for each species investigated) and clinical (anaemia, wasting, stunting, fever, hepatomegaly and splenomegaly) variables. During stepwise removal, variable categories were combined, based on expert knowledge and logical deduction, before eventually eliminating the respective variable. Remaining explanatories were included at a significance level of $p < 0.2$. Quality of life and domain scores were pre-transformed into a scale from 0 to 100, thus coefficients correspond to percentages of change. For variables on concurrent infections (*Plasmodium*-helminth co-infection and number of concurrent parasitic infections), the single parasite variables were exchanged by the concurrent infections variables but the same additional explanatories as for the single species models were used.

CI = confidence interval.

[a]Reference categories for explanatory variables: sex = female; age group = 5-10 years; wealth quintile = wealthier quintiles (top 80%); ecozone = South; *Plasmodium* = no or low parasitemia (< 1,000 parasites/µl of blood); anaemia = not anaemic; *S. haematobium* = no microhematuria; soil-transmitted helminths = not infected; any form of malnutrition = neither stunted, nor wasted, nor underweight (Z-score > -2); *Plasmodium*-helminth co-infected = neither infected with any of the two; number of concurrent infections = not infected with any investigated parasite species.

[b]Domain 1 showed ceiling effects > 15%. Tobit regression models were therefore built additionally for comparison. Except for ecozone and wealth quintile, the same significant relationships were identified in the tobit regression models as in the linear regression analysis presented above.

*Statistically significant ($p < 0.05$).

6.6 Discussion

We present HrQoL measures among 4,848 school-aged children surveyed during a 3-month cross-sectional survey in the dry season in Côte d'Ivoire and explore associations with parasitic infections and clinical and sociodemographic measures. Parasitological examination revealed a very high prevalence of *Plasmodium* spp. infection (75.0%). Helminth infections were considerably lower; 17.2%, 10.6%, 3.7%, 1.8% and 1.3% for hookworm, *S. haematobium* (microhematuria), *S. mansoni*, *A. lumbricoides* and *T. trichiura*, respectively. More than a quarter of the surveyed children showed clinical signs of anaemia and malnutrition. Findings from multivariate linear regression analysis revealed that the children's self-rated overall HrQoL and physical wellbeing is lower among those affected by anaemia and malnutrition compared to their counterparts without anaemia and malnutrition. Surprisingly, associations between HrQoL and parasitic infection status were of positive rather than negative direction. Sociodemographic variables such as sex, age group, socioeconomic status and setting characteristics had considerable influences on children's perceived HrQoL. The locally adapted HrQoL instrument employed showed acceptable utility considering minimal floor and ceiling effects and a robust internal consistency (Cronbach's $\alpha \geq 0.7$). Significant correlations and associations between HrQoL scales and self-reported and clinically assessed morbidity were found and even though the effect sizes were weak, they may further support the concept of health measured by the HrQoL tool.

Interestingly, we could not identify significantly lower HrQoL scores in *Plasmodium*- and helminth-infected children compared to their non-infected peers. Possible explanations for this finding are offered for consideration. First, in Côte d'Ivoire 100% of the population is at risk of *Plasmodium* infection (WHO, 2013) and previous research concluded that malaria transmission is perennial (Assi et al., 2013; Hürlimann et al., 2014a). Constant exposure from early childhood onwards leads to naturally acquired immunity to malaria at an early age (Doolan et al., 2009). Thus, most of the *Plasmodium* infections we identified in the school-aged population surveyed were asymptomatic (> 98%). Levels of transmission and endemicity of

parasitic infections has been shown to influence children's HrQoL. For example, Kenyan school-aged children infected with *S. haematobium* from a high endemicity setting reported similar HrQoL measures than their non-infected counterparts, whilst infected children in a low prevalence village reported significantly lower HrQoL compared to non-infected children (Terer et al., 2013).

Second, our study focused on children who were present at school the day of the survey. Children experiencing a clinical disease episode, perhaps related to a parasite infection and who might have expressed lowered HrQoL, were more likely to be absent from school than their healthier peers. Helminth infections, additionally, might still be at a less advanced stage with regard to disability in children compared to adolescents or adults. Smaller studies conducted in the People's Republic of China and Kenya also found no evidence of significant differences in self-rated HrQoL between helminth-infected and non-infected school children (Ziegelbauer et al., 2010; Samuels et al., 2012). Those studies that reported negative associations between HrQoL and helminth infections either focused on adult populations (Fürst et al., 2012) or investigated chronic and advanced clinical stages of an infection (Jia et al., 2007, 2011).

Third, polyparasitism, particularly *Plasmodium*-helminth co-infections, is common in Côte d'Ivoire (Raso et al., 2004; Righetti et al., 2012; Hürlimann et al., 2014b). It follows that interactions between multiple species parasitic infections and their influence on ill-health must be considered. Indeed, potentially beneficial effects from light-intensity helminth infections on clinical outcomes (i.e. anaemia) and subtle morbidity (i.e. physical fitness) in school-aged populations from malaria co-endemic settings in Côte d'Ivoire have been indicated (Righetti et al., 2012; Hürlimann et al., 2014a; b). Underlying mechanisms might be seen in the immunomodulatory features of helminth infections that down regulate the pro-inflammatory immune response needed to combat intracellular parasites like *Plasmodium*. Consequently, this may negatively affect resistance but simultaneously promote tolerance to malaria-related pathology by controlling harmful associated inflammation (Salgame et al., 2013). The current study confirms these prior observations, as we observed a positive association between soil-transmitted helminth infections, *Plasmodium*-helminth co-

infections and two or more concurrent infections including malaria parasites and reported physical wellbeing.

We found significantly lower HrQoL among anaemic children compared to non-anaemic children. Parasitic infections, most notably *Plasmodium* and hookworm contribute to the development of anaemia (Balarajan et al., 2011). *Plasmodium* and helminth mono- or co-infected children in our sample showed significantly higher odds ratios for anaemia than their non-infected counterparts (all ORs > 1.4). Consequently, we suggest the attribution of direct disease consequences (sequelae) – such as anaemia due to specific parasitic infections – to the etiological cause in future burden estimates (Murray et al., 2012b). We found a 1-point lower HrQoL score overall and a 2-point lower physical wellbeing score on a 100-point scale. If divided by 100, these findings might translate to DWs of 0.01 and 0.02 on the DW scale that ranges from 0 to 1. Such DWs are within the range of recent DW estimates of the GBD 2010 Study, which were set at 0.005, 0.058 and 0.164 for mild, moderate and severe anaemia (Salomon et al., 2012; Kassebaum et al., 2014).

The HrQoL concept attempts to evaluate the impact of diseases and injuries from a comprehensive point of view, incorporating psychological, social and environmental wellbeing on top of physical health (King & Bertino, 2008; Salomon et al., 2012). We found that particularly psychosocial and environmental measures of wellbeing were significantly associated with sociodemographic variables like sex, age, socioeconomic status and residential area. Associations of the physical component of HrQoL with parasite infections and clinical signs were observed to be more pronounced and indicated that the perceived health status varies between and depends importantly on different sociocultural settings. Our results are in line with previous observations (Fürst et al., 2011, 2012; Terer et al., 2013) and highlight the importance of inclusion of social determinants for more integrative burden of disease assessments.

Our data stem from a large-scale nation-wide survey, which subjected almost 5000 children to detailed clinical and parasitological examinations, coupled with a questionnaire. A major weakness of previous studies was their small sample sizes (Ziegelbauer et al., 2010; Fürst et al., 2011, 2012). Further, we consider the setting-

tailored, applied HrQoL tool as a useful and valid instrument. Its internal consistency was good (Cronbach's $\alpha > 0.7$) and floor and ceiling effects for the overall HrQoL were minimal, despite its shortness, including only 12 items compared to 26 questions in the WHOQOL-Bref, which was used as a template to develop our tool (Skevington et al., 2004). Particularly the ceiling effects were more pronounced when looking at single domain scores, which is, however, not surprising, considering the lower number of items in each domain. The ceiling effects found for the physical domain, were addressed by utilizing tobit regression analysis, which have been shown to provide more reliable estimates in censored data (Austin et al., 2000), in parallel to linear regression models. The negative associations and correlations between HrQoL and symptom and disease reporting followed the logic of lower self-rated HrQoL in simultaneously higher experienced morbidity and supported the construct that our instrument measures.

Data collection on a national scale entails several limitations. To respect the tight time schedule and in view of limited financial and human resources, all parasitological, clinical and questionnaire information had to be collected within a single day at each location by dedicated field teams. Consequently, teachers of the selected schools were trained to administer our questionnaire and they assisted in the conduct of the interview. Given our time constraints and restricted resources, we were not able to assess inter-observer agreement and cannot exclude measurement errors due to variation between interviewers. Another limitation regarding the questionnaire was the difficult implementation of the VAS, as already observed elsewhere (Ziegelbauer et al., 2010). The concept of this scale, the range of 0 to 100 and the fact that children had to point out their respective health status on a sheet was poorly understood. As an adaptation, children were asked to rate and orally express their health status according to a scale they were more familiar with, a scale of school marks (ranging from 0 to 10). However, this procedure resulted in a categorical rather than an interval distribution of scores. Unfortunately, this limitation hindered us to fully exploit these data and derive DWs, which could have been compared with previous research conducted elsewhere focusing on chronic

S. japonicum infection (Jia et al., 2007). Hence, there is a pressing need for a culturally accepted alternative to the VAS.

We conclude that the assessment of HrQoL in school-aged children in areas where parasitic infections are still widespread tends to be difficult and may not be sensitive enough to capture subtle morbidities. Important factors blurring the picture might be the often asymptomatic course due to acquired immunity in malaria and more subtle morbidities at this age for helminth infections, which therefore may not be perceived as disabling by infected children. School absenteeism adds bias, as non-inclusion of children who might experience more measurable disability will not be part of the analysis. Importantly though, the applied instrument showed acceptable utility and validity and was able to identify significant disability of more chronic sequelae such as anaemia. Further refinement and more rigorous reliability measurements of the tool are needed. Surveys in settings targeting specific parasite endemicity levels and efforts to include non-enrolled and otherwise absent school-aged children might resolve some of the limitations highlighted here. The aim of developing, validating and applying setting-specific HrQoL tools that will allow comparison between areas and measuring changes over time remains – particularly as large-scale control efforts targeting malaria and the NTDs are underway.

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7. Effect of deworming on school-aged children's physical fitness, cognition and clinical parameters in a malaria-helminth co-endemic area of Côte d'Ivoire

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7.1 Abstract

Malaria and helminth infections are thought to negatively affect children's nutritional status and to impair their physical and cognitive development. Yet, the current evidence-base is weak. The purpose of this study was to determine the effect of deworming against soil-transmitted helminthiasis and schistosomiasis on children's physical fitness, cognition and clinical parameters in a malaria-helminth co-endemic setting of Côte d'Ivoire. We designed an intervention study with a 5-month follow-up among schoolchildren aged 5–14 years from Niablé, eastern Côte d'Ivoire. In late 2012, a baseline cross-sectional survey was conducted. Finger-prick blood, stool and urine samples were subjected to standardised, quality-controlled techniques for the diagnosis of *Plasmodium* spp., *Schistosoma* spp., soil-transmitted helminths and intestinal protozoa infections. Haemoglobin level was determined and anthropometric measurements were taken for appraisal of anaemia and nutritional status. Children underwent memory (digit span) and attention (code transmission) cognitive testing, and their physical fitness and strength were determined (20 m shuttle run, standing broad jump and grip strength test). All children were treated with albendazole (against soil-transmitted helminthiasis) and praziquantel (against schistosomiasis) after the baseline cross-sectional survey and again 2 months later. Five months after the initial deworming, the same battery of clinical, cognitive and physical fitness tests was performed on the same children. Lower scores in strength tests were significantly associated with children with harbouring nutritional deficiencies. Surprisingly, boys infected with *Schistosoma mansoni* achieved longer jumping distances than their non-infected counterparts. Light-intensity infection with *S. mansoni* was associated with slightly better aerobic capacity. Deworming showed no effect on haemoglobin levels and anaemia, but children with moderate- to heavy-intensity *Schistosoma* infection at baseline gained weight more pronouncedly than non-infected children. Interestingly, children with soil-transmitted helminth or *Schistosoma* infection at baseline performed significantly better in the sustained attention test than their non-infected counterparts at the 5-month follow-up. This study revealed conflicting results regarding clinical parameters and cognitive behaviour of children after two rounds of deworming. We speculate that potential

beneficial effects of deworming are likely to be undermined in areas where malaria is co-endemic and nutritional deficiencies are widespread.

Keywords: Anaemia, Cognition, Côte d'Ivoire, Deworming, Helminth, Malaria, Malnutrition, Physical fitness

7.2 Introduction

In developing countries, preschool-aged children are at high risk of malaria, whilst school-aged children carry the highest burden of helminth infections, such as soil-transmitted helminthiasis and schistosomiasis (Woolhouse 1998; Raso et al. 2004; Pullan & Brooker 2008; WHO 2011). Consequences of malaria and helminthiases are manifold, including clinical but also more subtle morbidities. Anaemia and malnutrition are common clinical manifestations and negatively affect childhood mental and physical development (Crompton & Nesheim 2002; Fernando et al. 2010; WHO 2011). In 2010, malaria and the neglected tropical diseases (NTDs), of which helminth infections are of particular importance in terms of number of infections and global burden (Hotez et al. 2008; Lustigman et al. 2012), accounted for an estimated 6.4 million disability-adjusted life years (DALYs) among the school-aged population in sub- Saharan Africa, representing 16.5% of the total DALYs (IHME 2013). These burden estimates result from a complex construct to quantify the comparative magnitude of health loss due to diseases, injuries and risk factors. Efforts have been made for improving the assessment of the 'true' burden of disease by incorporating different sequelae to capture direct consequences of disease (e.g. anaemia due to hookworm infection) (Murray et al. 2012). However, these sequelae mainly describe clinical conditions and do not sufficiently take into account subtle morbidities (King et al. 2005; King 2010).

Recent studies assessed not only clinical consequences of parasitic infections, but also included measurements on physical functioning (Bustinduy et al. 2011; Müller et al. 2011; Yap et al. 2012a), school performance and cognitive ability of children (Halliday et al. 2012; Nankabirwa et al. 2013). Yap and colleagues, for

example, showed that *Trichuris trichiura*-infected children in the People's Republic of China had significantly lower age-adjusted aerobic capacity (VO_2 max, expressed in ml kg^{-1} min^{-1}) than their non-infected counterparts, as assessed by a multi-stage 20 m shuttle run test (Yap et al. 2012a). In Côte d'Ivoire, where polyparasitism is still widespread (Raso et al. 2004; Coulibaly et al. 2012; Hürlimann et al. 2014), there was no clear association between children's infection with *Schistosoma* and soil-transmitted helminths and physical fitness (Müller et al. 2011). *Plasmodium falciparum* infection was found to be associated with lower performance in abstract reasoning and sustained attention in Ugandan schoolchildren (Nankabirwa et al. 2013). Ezeamama and colleagues showed that several helminth species (i.e. *Schistosoma japonicum*, *Ascaris lumbricoide*s and *T. trichiura*) were negatively associated with children's cognitive test scores in a study done in the Philippines (Ezeamama et al. 2005).

An important limitation of most previous studies that examined children's physical functioning and cognition in relation to infectious diseases is that the studies pursued a cross-sectional epidemiological design with no detailed follow-up investigations after an intervention. In a recent randomised controlled trial carried out with school-aged children in the People's Republic of China, no significant improvement in physical fitness was observed 6 months after administration of a triple-dose of albendazole against soil-transmitted helminthiasis (Yap et al. 2014). Potential explanations of the lack of a beneficial effect of deworming were the very low cure rates against *T. trichiura* and the high re-infection rates for all soil-transmitted helminth species (Yap et al. 2013). Although some attention has been given on the effects of deworming on school performance, the current evidence-base is weak, with no obvious or consistent effect, as revealed by a systematic review and meta-analysis (Taylor-Robinson et al. 2012).

The purpose of this study was to assess the dynamics of children's physical fitness, cognitive ability and clinical morbidities over a 5-month period after two rounds of deworming. The study was carried out in a malaria-helminth co-endemic area in the eastern part of Côte d'Ivoire.

7.3 Materials and methods

Ethics statement

The study protocol was approved by the institutional research commissions of the Swiss Tropical and Public Health Institute (Basel, Switzerland) and the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (Abidjan, Côte d'Ivoire). Ethical approval was obtained from the ethics committees in Basel (EKBB; reference no. 30/11) and Côte d'Ivoire (CNER; reference no.: 09-2011/MSHP/CNER-P). Our study is registered at Current Controlled Trials (identifier: ISRCTN37143632).

District and village education and health authorities, parents/guardians and schoolchildren were informed about the objectives, procedures and potential risks and benefits of the study. Written informed consent was sought from children's parents/guardians. It was emphasised that participation was voluntary and that children could withdraw anytime without further obligation. All data records were coded and kept confidential. Medical staff performed clinical examinations, supervised physical fitness tests and administered anthelminthic drugs. Children were treated twice with albendazole (400 mg) against soil-transmitted helminthiasis and praziquantel (40 mg/kg) against schistosomiasis at baseline and a 2- month follow-up survey (WHO 2002). At the end of the study, helminth-positive children were again treated with the aforementioned drugs. Children with clinical malaria (i.e. positive rapid diagnostic test (RDT) and tympanic temperature ≥ 38.0°C) were given artemisinin-based combination therapy (i.e. artesunate-amodiaquine) and paracetamol against fever. An anti-anaemic treatment for children with haemoglobin (Hb) levels below 100 g/l was provided in cases where no signs of clinical malaria were present.

Study design and sample size calculation

We designed a 5-month longitudinal study. In December 2012, a baseline cross-sectional survey was conducted to determine children's parasitological, clinical, cognitive and physical fitness status. Children were systematically administered albendazole and praziquantel; after the baseline survey and 2 months later. In May

2013, children were re-examined with the same battery of tests as in the baseline cross-sectional survey.

For sample size calculation, we considered the arithmetic mean and variance of physical fitness, as determined by VO_2 max, in a previous study in a rural setting of south Côte d'Ivoire (Müller et al. 2011). We assumed that a difference of 5% in VO_2 max is of clinical relevance. Results from a recent cross-sectional survey in the current study area revealed a helminth infection prevalence of about 50% (Yapi et al. 2014). To achieve a power of 90% at an alpha error of 5% to obtain a statistical significance in VO_2 max, and allowing for 30% drop-outs for non-compliance and incomplete follow-up assessment, we calculated that 194 children would need to be enrolled (Eng 2003). Additionally, we accounted for unexpected difference in group sizes, which call for larger sample sizes (Rosner 2011). Hence, we aimed for a total of 300 children to participate.

Study area and subjects

The study was conducted in two adjacent primary schools in the village of Niablé (geographical coordinates: 6°39'48.0" N latitude, 3°16'25.1" W longitude). Niablé is located in the Indénie-Djuablin region of eastern Côte d'Ivoire, at the border to Ghana. The village is characterised by a Guinean bio-climate with yearly average precipitation ranging between 1,200 mm and 1,700 mm (Koffi et al. 2013). The rainy season lasts from March to December and is interrupted by a short dry season in August (Grover-Kopec et al. 2005). People are mainly engaged in subsistence farming, while coffee and cocoa serve as cash crops. There are myriad stagnant water bodies (e.g. small multi-purpose dams and fish ponds within and surrounding the village, and a poorly maintained drainage system and open waste disposal sites) that foster in situ transmission of malaria and schistosomiasis. Niablé was selected based on a 23.6% prevalence of *Schistosoma mansoni* infection among school-aged children identified during a national cross-sectional survey conducted in November 2011 (Yapi et al. 2014). To achieve the intended sample size of 300 children aged 8–14 years, all children attending grades 4–6 in the two primary schools were invited to participate.

Field and laboratory procedures

For parasitological examination, each child was asked to provide a fresh urine and stool sample and a finger-prick blood sample. Urine and stool samples were collected in separate plastic containers distributed to children in the early morning hours. Sample collection was done between 10:00 and 12:00 hours. Finger-prick samples were subjected to an RDT (ICT ML01 Malaria Pf kit; ICT Diagnostics, Cape Town, South Africa). Thick and thin blood films were prepared on microscope slides and air-dried.

All biological samples were transferred to a nearby laboratory and processed as follows. A small portion of stool (1–2 g) was put in Falcon tubes, filled with 10 ml of sodium acetate-acetic acid-formalin (SAF) (Marti & Escher 1990). Duplicate Kato-Katz thick smears were prepared from each stool sample, using 41.7 mg templates (Katz et al. 1972). Urine samples were subjected to a filtration method (Savioli et al. 1990) for detection of *S. haematobium* eggs. Kato-Katz thick smears and filter slides were examined under a microscope by experienced laboratory technicians. The number of helminth eggs was counted and recorded for each species separately. Thick and thin blood films were stained with a 10% Giemsa solution and examined under a microscope for *Plasmodium* species identification and quantification of parasitaemia (parasites/µl of blood) (N'Goran et al. 2003). SAF-fixed stool samples were subjected to an ether-concentration technique and examined under a microscope for intestinal protozoa (Utzinger et al. 2010). Helminth eggs were also recorded. Ten percent of all slides were randomly selected and re-examined by a senior microscopist for quality control.

Clinical examination was conducted by experienced medical staff. It included palpation for liver and spleen enlargement, Hb measurement using a HemoCue analyser (Hemocue Hb 301 system; Angelholm, Sweden) to assess anaemia, and measurement of body temperature using an ear thermometer (Braun ThermoScan IRT 4520; Kronberg, Germany) to identify fever cases (≥ 38.0°C). Anthropometric measurements such as height (to the nearest cm) and body weight (to the nearest 0.5 kg) were recorded to determine nutritional status.

The same day children provided biological samples and were examined clinically, they were invited for a questionnaire interview. Questions pertaining to household asset ownership, adapted from instruments previously used elsewhere in Côte d'Ivoire, were employed for calculating socioeconomic status (Raso et al. 2005; Fürst et al. 2010).

Physical fitness testing

To assess children's physical fitness, three tests from the Eurofit physical fitness test battery were employed (EUROFIT 1993); namely (i) the standing broad jump; (ii) the grip strength; and (iii) the 20 m shuttle run tests. The first two tests measure strength, whereas the 20 m shuttle run test is designed to assess the aerobic capacity and cardio-respiratory endurance (Léger et al. 1988). Test procedures were explained and demonstrated to the children before the actual test was conducted. The 20 m shuttle run test was executed on a flat ground without vegetation cover on the school yard either in the morning (between 8:00 and 10:00 hours) or in the late afternoon (between 16:00 and 18:00 hours) to avoid high ambient air temperature. Detailed instructions on how the tests were implemented are given as supporting information (see Appendix 10.3.1). Children identified with health problems by medical staff (e.g. Hb < 80 g/l, clinical malaria or respiratory tract infection) were excluded from physical fitness testing.

Cognitive function testing

Two cognitive tests were chosen; one focussing on sustained attention and the other on working memory. The tests were first explained in class and specific training sessions were conducted for a deeper understanding of the tasks to be performed. The code transmission test is part of the 'Tests of Everyday Attention for Children' (TEA-Ch) (Manly et al. 2001) and has been used before in Africa (Halliday et al. 2012; Nankabirwa et al. 2013). During the test, a list of digits was read out aloud with a speed of one digit/sec to the child. The task of the child was to identify a 'code' of two consecutive 5's, to interrupt the tester and to indicate the number preceding this code. Before the actual start of the test, each child had the possibility

to familiarise with the test through four warm-up digit sequences. Those children who did not understand the principle of the task during the warm-up period were invited for a more basic attention test; the pencil tapping test (Diamond & Taylor 1996). Additionally, the forward digit span test, a subtest of the 'Wechsler Intelligence Scale for Children - Fourth Edition' (WISCIV) (Kirkwood et al. 2011) was conducted to assess children's working memory. Children were asked to correctly recall and repeat a sequence of digits of increasing length in the given order.

Statistical analysis

Data were double-entered and cross-checked using EpiInfo version 3.5.3 (Centers for Disease Control and Prevention; Atlanta, USA). Statistical analyses were performed in STATA version 10.1 (STATA Corp.; College Station, USA). For the main analysis, a two-sample strategy was applied. Data from participants with complete baseline records served as the first sample, while the second sample consisted of those children who additionally had complete data records at the 5-month follow-up survey. Data from code transmission testing were analysed only for those children who fully understood the test, while treatment efficacy was evaluated for individuals from sample 2 with complete stool and urine examinations. Children with incomplete follow-up records were excluded from the second sample and were subjected to an attrition analysis.

Helminth infections were classified into light, moderate and heavy intensity categories, according to WHO guidelines (WHO 2011). Anaemia was determined using the cut-offs of Hb <115 g/l for children aged 5–11 years and Hb <120 g/l for children aged 12–15 years (WHO 2008). *Plasmodium* spp. parasitaemia was transformed and expressed as log (parasitaemia +1) to normalise the distribution for subsequent descriptive statistics and comparison of means. Nutritional status was calculated using STATA macros from WHO child growth standards for children aged 5–19 years (Duggan 2010). Indicators for malnutrition included stunting (height-for-age), wasting (body mass index (BMI)-for-age) and underweight (weight-for-age), whereof the latter was used as a valid reference measure for malnutrition in children younger than 10 years of age only.

Socioeconomic status was calculated using a household asset-based approach. Subsequently, children were stratified according to their asset index into three economic groups (wealth tertiles; e.g. most poor, poor and least poor) as done elsewhere (Schmidlin et al. 2013). VO_2 max was used as the main outcome measure from the 20 m shuttle run test and was calculated for each child according to an equation provided by Léger and colleagues (Léger et al. 1988).

Chi-square and Fisher's exact test, as appropriate, were applied for comparison of infection, morbidity and low performance in digit span test proportions between different groups in the main analysis and the attrition analysis. McNemar's test was applied for comparison of baseline *vs.* follow-up differences. To compare continuous outcomes such as fitness scores, weight gain, *Plasmodium* parasitaemia and mean differences in cognition scores between baseline and follow-up by group, t-test statistics, Wilcoxon signed rank sum, one-way ANOVA and Kruskal- Wallis tests were used, respectively. A binary variable for low performance in the digit span test was defined using a cut-off for the longest spans forward (LSF) raw score, at the level of LSF ≤ 4 according to Iverson and Tulsky (Iverson & Tulsky 2003). Depending on whether outcome variables were continuous, binary or overdispersed and censored count data, bivariate and multivariate linear, logistic, negative binomial and tobit regression models were utilised, as appropriate, to assess relationships with covariates. A population-averaged generalised estimating equation (GEE) and a random effects tobit regression model approach was adopted for analysis of the repeated outcome measurements of individuals with complete baseline and follow-up records. Sociodemographic, baseline parasite infection and morbidity variables served as explanatories in the regression analysis on baseline outcomes and changes after the 5-month follow-up. In addition to the covariates mentioned above, dynamic explanatories (i.e. change in anaemia status, nutritional status or *P. falciparum* parasitaemia) were introduced in the GEE and random effects models to assess associations with changes over time in the outcome measures. All cross-sectional and longitudinal models were fitted following a stepwise elimination process, excluding explanatory variables at significance level of 0.2 and considering the Akaike information criterion (AIC) and quasi-likelihood information criterion

(QIC), respectively. Relationships between an outcome and explanatory variables were expressed as adjusted odds ratios (ORs) in case of logistic regression, incidence rate ratios (IRRs) for negative binomial and mean differences for linear or tobit models, respectively, with corresponding 95% confidence intervals (CIs). Presented results from GEE analysis focused on main effects (time trend) and interaction terms with time to highlight within-subject effects between groups.

Treatment efficacy 3 months after the second round of deworming was assessed by calculating cure rate (CR, defined as the proportion of baseline helminth-positive children who became egg-negative after treatment) and egg reduction rate (ERR; formula: 1 – [egg counts after treatment/egg counts at baseline] × 100 based on population geometric mean eggs counts).

7.4 Results

Compliance and study samples

Figure 7.1 gives a flow chart, summarising study participation. In brief, study sample 1 consisted of 257 children (134 girls; 123 boys) who had complete baseline data. Among them, 219 children (112 girls, 107 boys) also had complete clinical, physical fitness and cognitive data at the 5-month follow-up assessment, and hence served as sample 2. The mean age was 10.6 and 10.7 years in sample 1 and 2, respectively, with a range of 5–14 years. The main reason why children were excluded from sample 2 is that they missed at least one of the follow-up assessments (clinical examination, physical fitness and cognition; n = 38). 213 children from sample 2 provided stool samples at the end of the study for treatment efficacy evaluation against helminth infections, whilst 217 provided finger-prick blood samples at follow-up and were considered for analysis of changes of *Plasmodium* parasitaemia. Children not understanding the code transmission test were kept in the two samples, but only 146 children with valid results at baseline and the 5-month follow-up were considered for evaluation of dynamics in test scores.

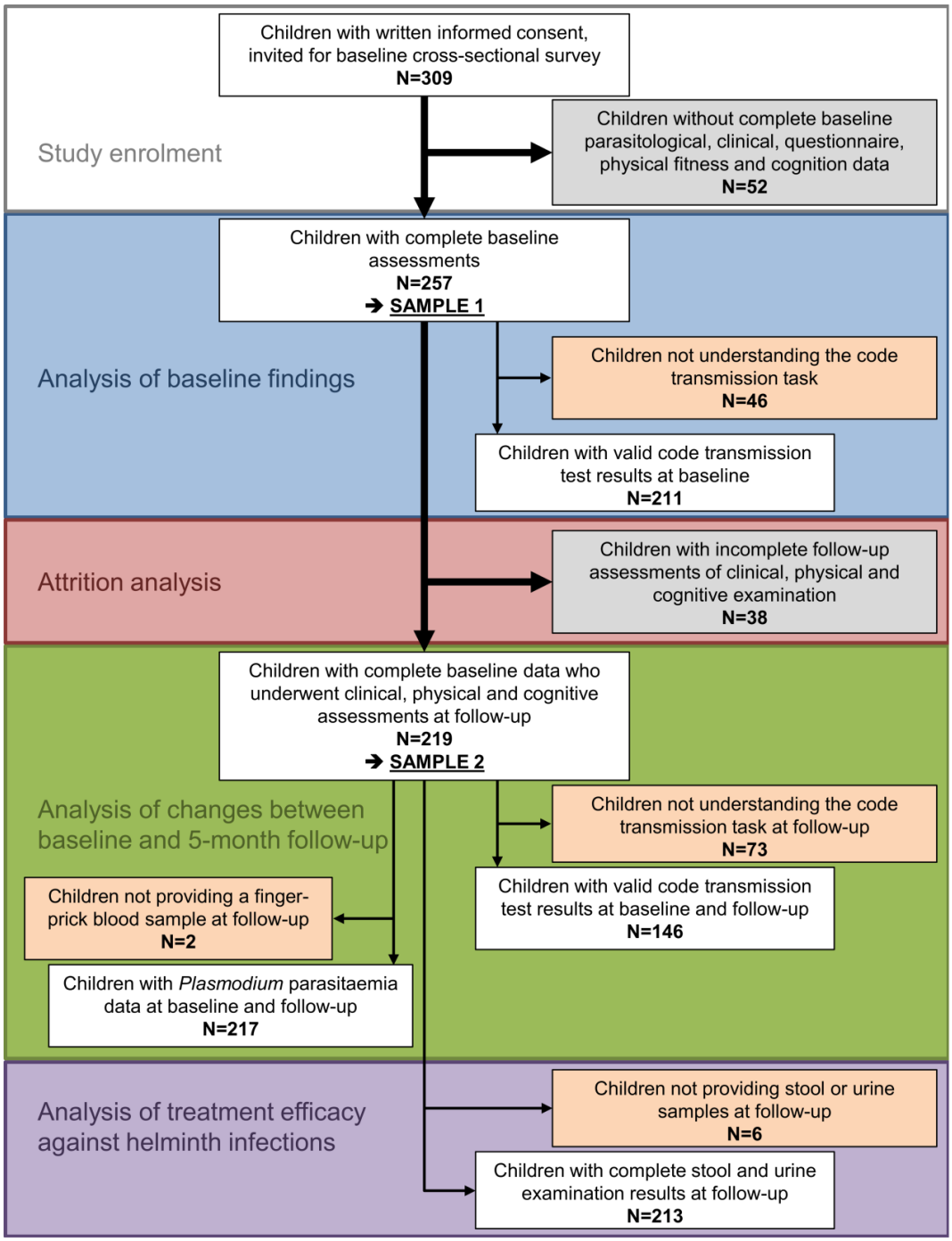

Figure 7.1 Flow chart illustrating study participation, compliance and respective analysis grouping

The baseline cross-sectional survey was carried out in December 2012, while the end-of-study survey took place in May 2013 in the village of Niablé, eastern part of Côte d'Ivoire.

Results from attrition analysis

The attrition analysis of the 38 children excluded from sample 2 revealed no statistically significant differences in terms of age, sex and socioeconomic status, baseline prevalence of parasitic infections and clinical morbidity, such as anaemia and nutritional status. Moreover, children excluded from sample 2 showed comparable baseline characteristics of physical fitness and cognition than their non-excluded counterparts. Mean *Plasmodium* parasitaemia at baseline was the only parameter with a statistically significant difference; mean parasitaemia 1,872 parasites/µl of blood in the excluded children compared to 1,031 parasites/µl of blood in sample 2 ($p = 0.022$).

Baseline situation

Baseline characteristics of study sample 1, including parasitic infections and clinical signs, are summarised in Table 7.1. *P. falciparum* was the predominant species (91.1%). *S. mansoni* was the most prevalent helminth species (35.4%), followed by hookworm (9.7%). With regard to intestinal protozoa, *Giardia intestinalis* was the predominant pathogenic species with a prevalence of 14.8%. Males were significantly more often infected with intestinal helminths, such as *S. mansoni* and hookworm. Concurrently, males were significantly more often found to be co-infected with *Plasmodium* and helminths compared to females. Anaemia was identified in 34.6% of the 257 children with a slightly higher prevalence in males than females. Boys had significantly lower Hb levels than girls. About one third of the children showed moderate or severe signs of malnutrition. Moderate to severe stunting was exclusively observed in children aged 10–14 years. There was no statistically significant ($p < 0.05$) difference in infection prevalence and clinical outcomes between the three wealth groups. The findings from the multivariate logistic regression analysis underline the association of clinical outcomes with age and highlight the relationship between anaemia and malnutrition (Table 7.2). Apart from a significant negative relationship between Hb level and *Plasmodium* parasitaemia there was no statistically significant association of clinical morbidity with parasite infections.

Table 7.1 Baseline demographic, parasitological and clinical characteristics of study sample 1 (257 schoolchildren) in Niablé, eastern Côte d'Ivoire in December 2012

Characteristic	Total (n=257)	Females (n=134)	Males (n=123)
Age (years)			
Mean (range)	10.6 (5-14)	10.6 (5-14)	10.7 (7-14)
Age group 5-9, no. of children (%)	65 (25.3)	37 (27.6)	28 (22.8)
Age group 10-14, no. of children (%)	192 (74.7)	97 (72.4)	95 (77.2)
School grade			
4	48 (18.7)	26 (19.4)	22 (17.9)
5	115 (44.8)	63 (47.0)	52 (42.3)
6	94 (36.6)	45 (33.6)	49 (39.8)
Infection with *P. falciparum*			
No. of children infected (%)	234 (91.1)	119 (88.8)	115 (93.5)
Parasitaemia, mean no. of parasites/µl of blood (log-transformed)	1,254 (6.2)	1,085 (6.2)	1,429 (6.2)
Infection with *P. malariae*			
No. of children infected (%)	21 (8.2)	14 (10.5)	7 (5.7)
Parasitaemia, mean no. of parasites/µl of blood (log-transformed)	1,037 (6.5)	972 (6.6)	1,167 (6.3)
Infection with *S. mansoni*			
No. of infected (%)**	**91 (35.4)**	**25 (18.7)**	**66 (53.7)**
Infection intensity, no. of children infected (%)			
Light (1-99 EPG)	49 (53.9)	15 (60.0)	34 (51.5)
Moderate (100-399 EPG)	33 (36.3)	9 (36.0)	24 (36.4)
Heavy (≥ 400 EPG)	9 (9.9)	1 (4.0)	8 (12.1)
Infection with soil-transmitted helminths[#]			
Hookworm, no. of children infected (%)**	**25 (9.7)**	**2 (1.5)**	**23 (18.7)**
T. trichiura, no. of children infected (%)	2 (0.8)	1 (0.8)	1 (0.8)
A. lumbricoides, no. of children infected (%)	1 (0.4)	1 (0.8)	0 (0.0)
Infection with pathogenic intestinal protozoa			
G. intestinalis, no. of children infected (%)	38 (14.8)	16 (11.9)	22 (17.9)
E. histolytica/E. dispar, no. of children infected (%)	11 (4.3)	5 (3.7)	6 (4.9)
Major co-infections			
Plasmodium spp. + helminth, no. of children co-infected (%)**	**97 (37.7)**	**27 (20.2)**	**70 (56.9)**
Plasmodium spp. + pathogenic protozoa, no. of children co-infected (%)	44 (17.1)	19 (14.2)	25 (20.3)
P. falciparum + *S. mansoni*, no. of children co-infected (%)**	**86 (33.5)**	**23 (17.2)**	**63 (51.2)**
Haemoglobin levels and anaemia			
Hb, mean (SD), g/l*	**120.2 (13.6)**	**122.3 (13.0)**	**118.0 (13.9)**
Anaemia, no. of children (%)	89 (34.6)	40 (29.9)	49 (39.8)
Malnutrition			

Any form of malnutrition (Z-score ≤ -2), no. of children (%)	84 (32.7)	45 (33.6)	39 (31.7)
Stunting H/A, no. of children (%)			
Moderate to severe (Z-score ≤ -2)	35 (13.6)	20 (14.9)	15 (12.2)
Wasting BMI/A, no. of children (%)			
Moderate to severe (Z-score ≤ -2)	59 (23.0)	30 (22.4)	29 (23.6)
Underweight W/A[§], no. of children (%)			
Moderate to severe (Z-score ≤ -2)*	**9 (13.9)**	**8 (21.6)**	**1 (3.6)**

[#]All soil-transmitted helminth infections were of light intensity
[§]Assessed for children under the age of 10 years; n=65 (37 females; 28 males)
*/**Statistically significant difference between males and females (*p < 0.05; **p < 0.001)
Statistically significant differences are highlighted in bold.

Statistics from *t*-test and one-way ANOVA showed significant differences between sex, socioeconomic status and helminth infection status for specific fitness outcomes. Males had higher test scores for the standing broad jump test (141 cm *vs.* 129 cm) and for the 20 m shuttle run test (VO_2 max: 50.6 ml kg^{-1} min^{-1} *vs.* 47.7 ml kg^{-1} min^{-1}) (both $p < 0.001$) compared to females, while no significant sex difference could be found for the grip strength test. Children from the lowest wealth tertile were found to have a significantly lower performance in the 20 m shuttle run test than children from wealthier tertiles (VO_2 max: 48.2 ml kg^{-1} min^{-1} *vs.* 49.5 ml kg^{-1} min^{-1}, $p < 0.05$). Children with hookworm and *S. mansoni* infections were found to have higher VO_2 max estimates and standing broad jump scores than their non-infected counterparts. These results were strongly sex confounded, since males scored better in these tests and were significantly more often infected with helminths than females. After stratification by sex, males infected with *S. mansoni* still showed a significantly better performance in the jump task than non-infected boys (Figure 7.2). Scores of the strength tests were positively associated with age, whilst VO_2 max values decreased for each incremental increase of one year of age (Table 7.2).

Table 7.2 Results from regression analysis highlighting significant associations between explanatories and children's clinical status, physical fitness and cognitive capacity

Logistic models (binary outcomes)	**Association**	**Adjusted OR (95% CI)**
Clinical status[§]:		
Anaemia	Age group (10-14 years)	0.50 (0.27, 0.92)
	Any form of malnutrition	1.83 (1.04, 3.21)
Stunting (HAZ-scores < -2)	Age (years)	2.34 (1.71, 3.20)
Wasting (BMIZ-scores < -2)	Any severity of stunting	2.28 (1.18, 4.40)
Any form of malnutrition (WAZ|HAZ|BMIZ-scores < -2)	Age (years)	1.21 (1.02, 1.44)
	Anaemia	1.96 (1.10, 3.47)
Cognition[†]:		
Low digit span test performance (LSF ≤ 4)	Grade (6)	0.42 (0.20, 0.90)
Negative binomial model (count outcome)	**Association**	**Adjusted IRR (95% CI)**
Plasmodium parasitaemia[§]	Haemoglobin level (g/l)	0.98 (0.97, 0.99)
Linear/tobit models (continous outcomes)	**Association**	**Adjusted mean difference (95% CI)**
Physical fitness[§]:		
VO_2 max (ml kg^{-1} min^{-1})	Age (years)	-0.97 (-1.25, -0.70)
	Sex (female)	-2.72 (-3.70, -1.74)
	S. mansoni intensity (light)	1.24 (0.05, 2.43)
Hand grip strength (kg)	Age (years)	1.41 (1.01, 1.80)
	Anaemia	-1.99 (-3.30, -0.68)
	Stunting (Z-score < -2)	-4.79 (-6.70, -2.89)
Standing broad jump (cm)	Age (years)	3.45 (2.06, 4.84)
	Sex (female)	−9.95 (−14.46, −5.44)
	Wasting (Z-score < -2)	−9.82 (−14.86, −4.78)
	S. mansoni	6.62 (1.81, 11.43)
Cognition[†]:		
Code transmission test (score range: 0-20)	Grade (6)	2.73 (0.93, 4.55)
	Sex (female)	-1.49 (-2.76, -0.23)
	Wealth tertile (least poor)	1.85 (0.44, 3.27)

Reference groups of explanatories: intestinal parasites (status or intensity) = non-infected with the particular species; clinical status = not affected by particular indicator; wealth tertile = most poor, age group = 5–9 years, grade = 4th grade.

[§]Fixed explanatories for adjustment in clinical and physical fitness outcomes: age, sex, socioeconomic status and anaemia or Hb.

[†]Fixed explanatories for adjustment in cognition outcomes: sex, grade and socioeconomic status. Not predetermined covariates were kept at a significance level of 0.20.

The different types of models used according to the outcome variables are highlighted in bold.

The study was carried out among 257 schoolchildren (134 females, 123 males) in December 2012 in Niablé, eastern Côte d'Ivoire.

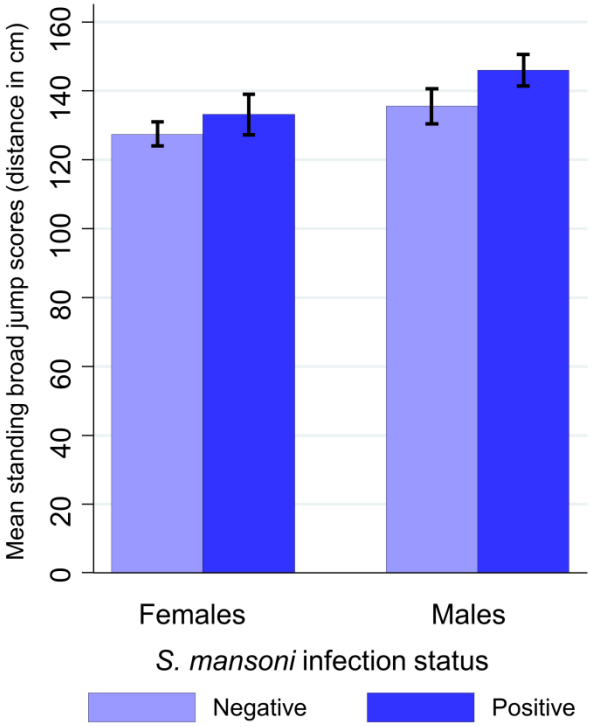

Figure 7.2 Mean difference in standing broad jump test scores (distance in cm) for *S. mansoni* infection categories among 134 girls and 123 boys

The positive association of *S. mansoni* light intensity and infection of any intensity remained significant with 20 m shuttle run and standing broad jump test scores, respectively, after adjusting for other covariates. Clinical morbidities, such as anaemia and malnutrition, were important predictors for physical fitness outcome measures. Particularly strength test scores seemed to be affected by malnutrition and anaemia, whilst for the 20 m shuttle run test, none of these explanatories were significant.

At baseline 117 out of 219 children (53.4%) showed low performance in the memory test, as defined by the cut-off for the LSF raw score. Low performance was higher among anaemic compared to non-anaemic children (60.7% *vs.* 47.6%, $p < 0.05$) and decreased with school grade (64.6%, 53.9% and 43.6% in children from grades 4, 5 and 6, respectively). After adjusting for explanatories in the regression analysis, school grade remained the only significant association for poor performance in the digit span test (Table 7.2). A higher school grade, wealth tertile

and sex were the only significant predictors found for test performance in the code transmission task identified from multivariate tobit regression. Female sex was associated with lower attention scores, while children belonging to the highest wealth tertile performed significantly better than the most poor. Children from grade 6 achieved significantly higher scores in this test compared to 4th graders. The drop-out rate for non-understanding of the test was highest among the 4th grade (43.8% compared to 16.5% and 6.4% among all participants from grades 5 and 6, respectively, $p < 0.001$). None of the parasite infections and clinical outcomes were significantly associated with memory and sustained attention test performances in the multivariate regression analyses.

Effect of deworming on parasitic infections, clinical status, physical fitness and cognition

The infection prevalence of *S. mansoni*, hookworm and *T. trichiura* were 8.9%, 0.9% and 0.5% at the end-of-study survey. CRs for *S. mansoni*, *S. haematobium* and soil-transmitted helminths (mainly hookworm), were 79% (60 out of 76), 100% (7 out of 7) and 91% (21 out of 23), respectively. For *S. mansoni* and hookworm, we found ERRs of 98.6% and 99.6%, respectively. Although not directly targeted by anthelminthic drugs, a clearance of all baseline positive *Entamoeba histolytica*/*E. dispar* cases (n = 9) was observed, whereas 19 out of 31 (61.3%) children with *G. intestinalis* infection at baseline were found to be free of infection at the 5-month follow-up. Both *Plasmodium* spp. infection prevalence and log-parasitaemia were significantly lower at follow-up compared to baseline (92.2% *vs.* 78.0% and 5.7 *vs.* 4.1, both $p < 0.001$) according to Mc Nemar's and Wilcoxon signed rank sum tests and showed similar changes in baseline helminth-infected and non-infected girls and boys (Table 7.3). If observation dependence was considered, however, significant differences in parasitaemia over time were observed in children with baseline helminth infections and changed anaemia status (Table 7.4). Children cured from hookworm infection and recovering from anaemia showed a significant decrease in *Plasmodium* parasitaemia, whilst baseline *S. mansoni*-infected children showed higher *Plasmodium* parasitaemia at follow-up.

The mean Hb levels did not differ significantly between surveys (baseline, 120.4 g/l; follow-up, 119.9 g/l; p = 0.631). The overall prevalence of anaemia remained unchanged (baseline, 34.3%; follow-up, 34.3%); the anaemia status of 88 children, however, changed. 44 became non-anaemic, while 44 children were identified as newly anaemic cases. Changes in Hb showed no significant relationship with baseline helminth infection (Table 7.3). Hb levels of children found with high *Plasmodium* parasitaemia at baseline showed a significant increase over time (Table 7.4). Overall children did not catch up in growth; on the contrary, z-scores for chronic malnutrition (i.e. stunting), further decreased compared to reference populations by −0.07 ($p < 0.05$). Older aged children, however, showed a significant increase in height-for- age z-scores compared to their younger peers in the population-averaged model (Table 7.4). On average, the children gained 1.5 kg (95% CI: 1.2, 1.8 kg) of weight over the 5-month study period. Weight gain and reduction of acute malnutrition (i.e. wasting, z-score < −2) was highest among girls, particularly those with moderate- to heavy-intensity *S. mansoni* infection at baseline (Tables 7.3 and 7.4).

The children's performance in the standing broad jump and the grip strength tests improved significantly over the 5-month study period with a mean increase of 12 cm (95% CI: 10, 14 cm) and 1.07 kg (95% CI: 0.49, 1.65 kg), respectively (Figure 7.3). The 20 m shuttle run test revealed similar VO_2 max estimates at baseline and end-of-study surveys, but boys performed better in the follow-up assessment with a mean increase of 0.83 ml kg^{-1} min^{-1}, while VO_2 max estimates for girls decreased by −0.77 ml kg^{-1} min^{-1} (one-way ANOVA, p = 0.005). Children with moderate- to heavy-intensity *S. mansoni* infection at baseline showed a higher increase in VO_2 max estimates at the 5-month follow-up assessment compared to their non-infected counterparts (Table 7.3). Especially in boys, the change in VO_2 max estimates varied substantially between different intensities of infection. Figure 7.4 depicts the dynamics of VO_2 max estimates in boys with different *S. mansoni* infection intensities at baseline. While boys without a *S. mansoni* infection and those with moderate- or heavy-intensity infection showed an improvement in aerobic capacity,

lightly infected boys showed a decreasing trend with lower VO_2 max estimates at follow-up. In girls, this effect was not observed.

Table 7.4 illustrates development over time and significant within-subject effects on physical fitness outcomes derived from GEE regression analyses. Status or changes of nutritional indicators remained most important predictors for changes in physical fitness outcomes. In contrast to the strength tests, significant relationships with parasitic infections and changes in VO_2 max estimates were observed. Light-intensity *S. mansoni* infection at baseline showed a negative relationship with changes in VO_2 max estimates.

Figure 7.5 illustrates the dynamics in performance for the two cognition tests. Children performed significantly better in the digit span test at the 5-month follow-up compared to baseline ($p < 0.001$). None of the predictors used in the basic univariate comparison and the more sophisticated GEE regression analysis showed any significant association with improved digit span test performance. Female participants performed much better in the code transmission test at follow-up compared to baseline. Furthermore, comparing for the difference in code transmission test scores between baseline and follow-up, it was found that sex and helminth infections at baseline were associated with an improvement in this test (Table 7.3). The random effects tobit model further revealed that, apart from female sex, particularly children with light-intensity helminth infections at baseline had a higher positive change in score at follow-up compared to their baseline non-infected counterparts (Table 7.4).

Table 7.3 Comparison of means for clinical, *Plasmodium* parasitaemia, physical and cognitive parameters in helminth-infected and non-infected schoolchildren at baseline and the 5-month follow-up surveys, stratified by sex

Parameter			Baseline, mean			Follow-up, mean			Mean change (95% CI)		
	Helminth infection	N	All	F	M	All	F	M	All	F	M
Weight (kg)[§]	Not infected	141	31.7	32.4	30.4	33.0	33.9	31.4	1.4 (1.0, 1.8)	1.6 (1.0, 2.1)	1.0 (0.3, 1.7)
	S. mansoni (+)	44	31.8	33.0	31.2	33.4	34.9	32.6	1.6 (0.9, 2.2)	2.0 (1.0, 3.0)	1.4 (0.5, 2.3)
	S. mansoni (++/+++)	34	33.1	35.5	32.5	35.2	39.1	34.2	2.1 (1.3, 2.8)	3.6 (1.2, 6.1)*	1.7 (1.0, 2.4)
Haemoglobin (g/l)	Not infected	130	121.4	123.7	116.8	120.9	121.3	120.1	-0.5 (-3.0, 2.1)	-2.3 (-5.4, 0.7)	3.4 (-1.2, 7.9)
	Helminth[#]	89	119.0	119.7	118.8	118.6	122.1	117.3	-0.4 (-3.7, 2.8)	2.4 (-2.4, 7.2)	-1.5 (-5.6, 2.6)
Plasmodium parasitaemia (log(p/µl+1))	Not infected	129	5.5	5.4	5.7	4.0	3.9	4.2	-1.5 (-2.1, -0.9)[Δ]	-1.4 (-2.6, -0.3)[Δ]	-1.5 (-2.3, -0.7)[Δ]
	Helminth[#]	88	5.9	5.9	5.9	4.4	4.6	4.3	-1.5 (-2.3, -0.8)[Δ]	-1.6 (-2.4, -0.8)[Δ]	-1.3 (-3.1, 0.5)
VO_2 max (ml kg^{-1} min^{-1})	Not infected	141	48.8	47.7	50.9	48.7	46.9	52.0	-0.1 (-0.8, -0.6)	-0.8 (-1.7, 0.1)	1.2 (0.0, 2.3)†
	S. mansoni (+)	44	50.3	48.1	51.3	49.4	48.0	50.0	-0.9 (-2.1, 0.3)	-0.1 (-1.8, 1.6)	-1.3 (-2.9, 0.4)*
	S. mansoni (++/+++)	34	49.4	47.1	50.0	51.1	45.5	52.5	1.7 (0.2, 3.2)†*	-1.6 (-4.9, 1.7)	2.5 (0.9, 4.1)†
Standing broad jump (cm)	Not infected	141	130	128	135	143	141	146	13 (10, 15)†	13 (10, 17)†	12 (8, 15)†
	S. mansoni (+)	44	140	133	144	151	140	157	11 (7, 15)†	7 (-0, 14)	13 (7, 18)†
	S. mansoni (++/+++)	34	146	134	149	158	148	161	12 (6, 19)†	13 (-2, 29)	12 (5, 19)†
Hand grip strength (kg)	Not infected	130	16.8	16.7	16.8	17.9	17.6	18.5	1.1 (0.4, 1.8)†	0.8 (-0.0, 1.7)	1.7 (0.3, 3.0)†
	Helminth[#]	89	17.7	17.1	18.0	18.8	17.9	19.1	1.0 (0.4, 2.0)†	0.8 (-1.0, 2.7)	1.1 (-0.1, 2.3)
Digit span test (score range: 2-8)[§]	Not infected	130	4.5	4.5	4.4	5.2	5.1	5.4	0.8 (0.6, 0.9)	0.7 (0.5, 0.9)	0.9 (0.6, 1.3)
	Helminth[#]	89	4.4	4.4	4.5	5.2	5.3	5.1	0.8 (0.5, 1.0)	1.0 (0.5, 1.4)	0.7 (0.3, 1.0)
Code transmission test (score range: 0-20)	Not infected	81	16.2	16.0	16.7	16.2	16.4	15.6	-0.1 (-0.8, 0.7)	0.4 (-0.5, 1.2)	-1.1 (-2.6, 0.4)
	Helminth[#]	65	15.2	12.5	16.1	15.7	15.1	15.9	0.5 (-0.4, 1.3)	2.6 (0.7, 4.5)†*	-0.2 (-1.1, 0.7)

F=females, M=males;

(+) = light intensity infection, (++/+++) = moderate or heavy intensity infection.

[#]Infected with any soil-transmitted helminth or *Schistosoma* species of any intensity.

[§]Significant change ($p < 0.05$) between baseline and 5-month follow-up in all groups from paired *t*-test analysis.

†Significant change ($p < 0.05$) between baseline and 5-month follow-up for this sub-group from paired t-test analysis.

[Δ]Significant change ($p < 0.05$) between baseline and 5-month follow-up for this sub-group from Wilcoxon signed rank sum test analysis.
*Significant difference in change between infected and non-infected in this sub-group from bivariate linear regression analysis.
The study was carried out in Niablé, eastern Côte d'Ivoire between December 2012 and May 2013. Data from 219 children (112 girls, 107 boys) with complete baseline and end-of-study follow-up were considered.

Table 7.4 Main effects (time) and significant predictors for changes over time (within-subjects effects) in *Plasmodium* parasitaemia, clinical, physical and cognitive outcomes after deworming from population-averaged GEE and random effects tobit analysis

Model by outcome	Predictor	Change
Logistic models (binary outcomes)		**OR (95% CI)**
Cognition†		
Change in low performance in digit span test	Time	0.27 (0.16, 0.46)*
Negative binomial model (count data)	**Predictor**	**IRR (95% CI)**
Plasmodium parasitaemia	Time	0.84 (0.55, 1.29)
	S. mansoni	6.37 (1.94, 20.88)
	Hookworm	0.19 (0.05, 0.79)
	Anaemia status (no longer anaemic)	0.18 (0.07, 0.51)
Linear models (continuous outcomes)	**Predictor**	**Coefficient (95% CI)**
Clinical status§		
Change in Hb level (g/l)	Time	0.76 (-2.91, 4.43)
	Plasmodium parasitaemia (1000+)	5.16 (0.87, 9.44)
Change in H/A Z-score (stunting)	Time	-0.19 (-0.32, -0.06)*
	Age group (10-14 years)	0.14 (0.02, 0.26)
Change in BMI/A Z-score (wasting)	Time	0.30 (-0.01, 0.60)
Weight gain (kg)	Time	0.88 (0.31, 1.45)*
	Sex (female)	0.73 (0.07, 1.39)
	S. mansoni intensity (++/+++)	1.06 (0.19, 1.92)
Physical fitness§		
Change in VO_2 max (ml kg^{-1} min^{-1})	Time	1.01 (-0.31, 2.33)
	Sex (female)	-1.40 (-2.56, -0.24)
	S. mansoni intensity (+)	-1.58 (-2.99, -0.16)
	Stunting severity (↑)	-2.65 (-4.52, -0.79)
Change in hand grip strength (kg)	Time	1.45 (0.71, 2.18)*
	Anaemia status (new anaemia case)	-1.59 (-3.05, -0.13)
Change in standing broad jump (cm)	Time	13.84 (10.67, 17.01)*
	Anaemia status (constantly anaemic)	-5.71 (-11.42, -0.00)
Tobit model (censored count data)	**Predictor**	**Coefficient (95% CI)**
Cognition†		
Change in code transmission score	Time	-0.87 (-2.25, 0.50)
	Sex (female)	2.07 (0.73, 3.41)
	Helminth infection intensity (+)	2.02 (0.52, 3.52)

(+) = light intensity infection, (++/+++) = moderate or heavy intensity infection, (↑) = higher severity at follow-up.
Reference groups of explanatories: age group = 5–9 years; sex = male; intestinal parasites at baseline (status or intensity) = non-infected with a particular species; *Plasmodium* parasitaemia at baseline = parasitaemia below 1,000 parasites/μl of blood; anaemia status = constantly not anaemic; stunting severity = unchanged severity.
§Fixed explanatories for clinical and physical fitness outcomes: age, sex, socioeconomic status and anaemia.
†Fixed explanatories for cognition outcomes: sex, school grade and socioeconomic status. Not predetermined predictors were kept at a significance level of 0.20.
*Significant change over time ($p < 0.05$).
The different types of models used according to the outcome variables are highlighted in bold.
The study was carried out in Niablé, eastern Côte d'Ivoire between December 2012 and May 2013. Data from 219 children (112 girls, 107 boys) with complete baseline and end-of-study follow-up were considered.

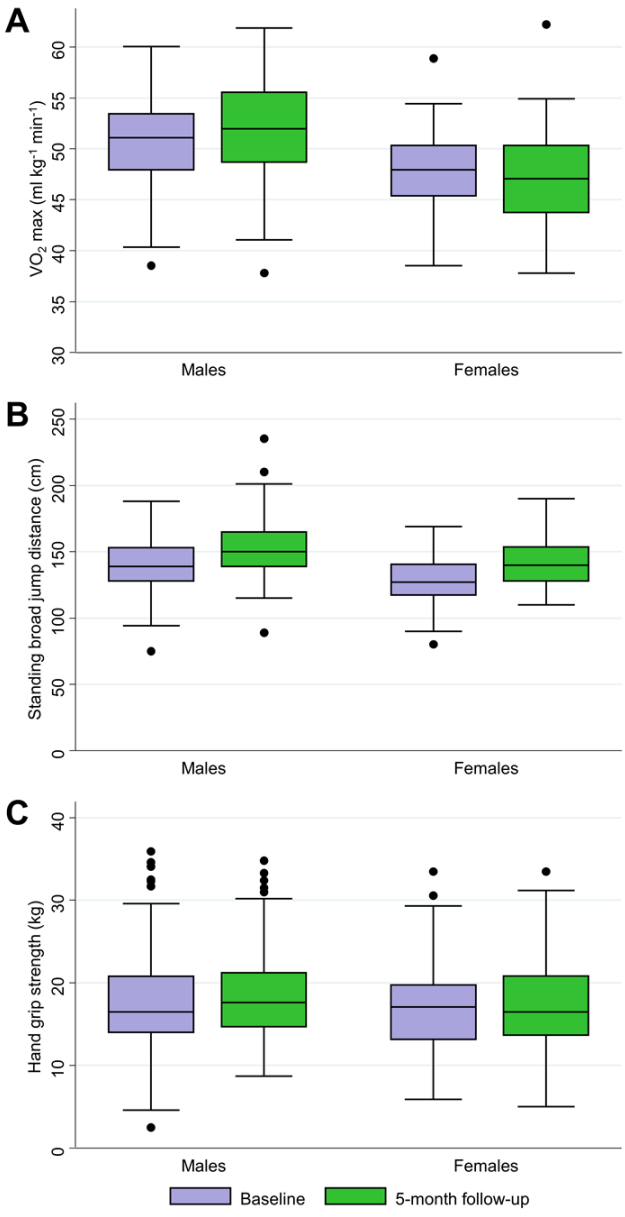

Figure 7.3 Physical fitness performance at baseline and end-of-study survey among 219 schoolchildren, stratified by sex

A: VO_2 max estimates from the 20 m shuttle run test. **B:** Jumping distance from the standing broad jump test. **C:** Hand grip strength. Included are performances of 219 children (112 girls, 107 boys) who had complete baseline data (December 2012) and end-of-study data (May 2013). Box plot: boxes illustrate the 25th and 75th percentiles (ptile), while the whiskers indicate the adjacent lower and upper values (most extreme values which are within 25th ptile −1.5*(75th-25th ptile) and 75th ptile + 1.5*(75th-25th ptile), respectively). The median is shown by the line within the boxes and outliers are indicated with dots.

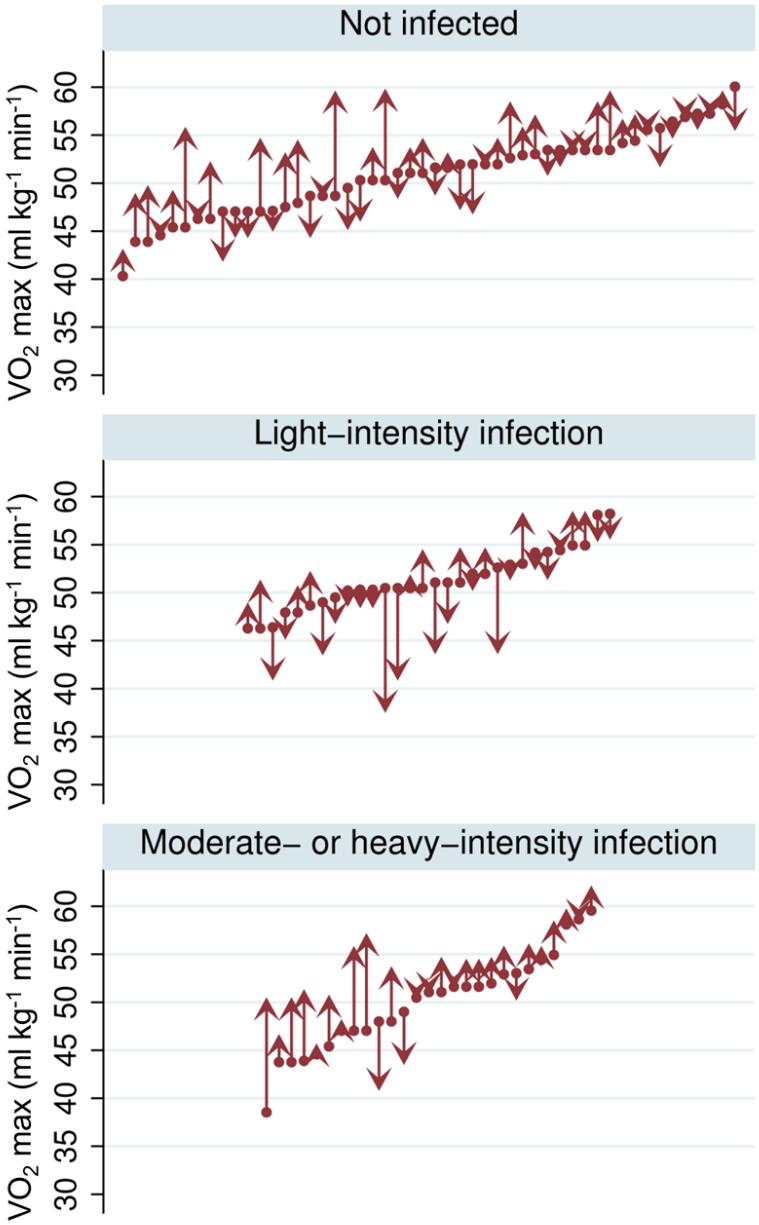

Figure 7.4 Dynamics of VO_2 max estimates in boys (n = 107) with different intensities of *S. mansoni* infection

Upward-pointing arrows indicate improved performance at the end-of-study survey, while downward-pointing arrows stand for decreased VO_2 max estimates at the end-of-study survey compared to baseline. Each point with arrow represents a separate individual in the respective infection intensity category (x-axis).

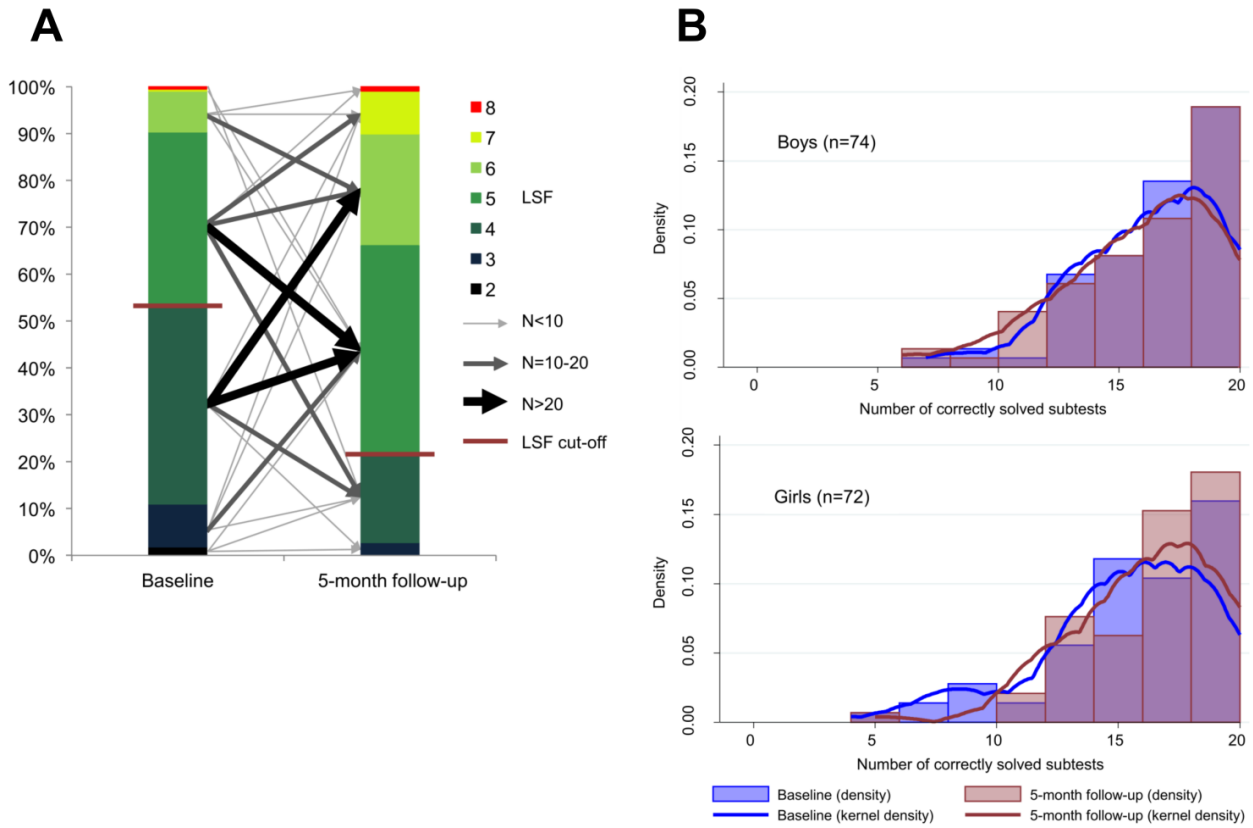

Figure 7.5 Dynamics of cognition test scores from the forward digit span test (A) and the code transmission test (B)

A: Proportions of digit span test scores expressed as longest span forward (LSF) at baseline and end-of-study survey from 219 participants with complete data for both assessments. The arrows depict the directions of change in performance over time, whereas the width of the arrow indicates the number of children in each category of change. The cut-off to define low performance was set at LSF ≤ 4 (LSF cut-off). **B:** Relative frequency (density) of code transmission test scores (number of correctly solved subtests out of 20 subtests) at baseline and follow-up from 146 participants, stratified by sex. Girls showed a significantly higher improvement in test performance at follow-up than boys (mean difference in change between the sexes assessed by *t*-test: 1.31, p-value < 0.05).

7.5 Discussion

Baseline

We present findings from an intervention study with a 5-month longitudinal follow-up among school-aged children living in a malaria-helminth co-endemic area of eastern Côte d'Ivoire. Children were subjected to a battery of parasitological, clinical, physical fitness and cognition tests at baseline and the 5-month follow-up cross-sectional surveys. The intervention consisted of two rounds of deworming (albendazole plus praziquantel) immediately after the baseline survey and 2 months later. At baseline in December 2012, we found that 91.1% of the children harboured *P. falciparum* parasites in their blood. As we only collected a single finger-prick blood sample, the 'true' *Plasmodium* infection rate might reach 100%. Using duplicate Kato-Katz thick smears from a single stool sample, we found *S. mansoni* and hookworm prevalences of 35.4% and 9.7%, respectively. Interestingly, boys had much higher helminth prevalences than girls (*S. mansoni*, 53.7% *vs.* 18.7%; hookworm, 18.7% *vs.* 1.5%). These observations are somewhat in line with findings from a recent national cross-sectional school-based survey conducted between November 2011 and February 2012 in Côte d'Ivoire, as boys showed significantly higher prevalences than girls, but not as marked as in the current study (Yapi et al., 2014). Given the very high *Plasmodium* infection rate, our findings focusing on helminth infections should thus be interpreted as *Plasmodium*-helminth co-infections.

About one third of the children surveyed had clinical manifestations, most importantly anaemia (34.6%) and moderate to severe malnutrition (32.7%). These clinical parameters were negatively associated with children's physical fitness, as determined by three standard tests (Léger et al., 1988; EUROFIT, 1993). Helminth infections, on the other hand, showed no clear association with clinical outcomes. Hence, our study highlights the importance of a deeper mechanistic understanding of how helminths and other parasites mediate pathways to ill-health and impaired physical fitness (King et al., 2005; Hall et al., 2008; Taylor-Robinson et al., 2012). Our findings are in agreement with a recent study assessing physical fitness in

Kenyan schoolchildren, which identified nutritional deficiencies as major predictors for impaired physical fitness performance, whilst there was no robust association with parasitic diseases (Bustinduy et al., 2011). These observations might also explain why the VO_2 max estimates from the current and a previous study in Côte d'Ivoire (Müller et al., 2011) were comparable with findings from the Kenyan study, where the extent of malnutrition and anaemia was similar. In contrast, a cohort of school-aged children in Yunnan province, People's Republic of China, had a strikingly high rate of stunting and helminth infections (Yap et al., 2012a, 2014), and VO_2 max estimates and strength test scores were substantially lower than in age-matched African children (Bustinduy et al., 2011; Müller et al., 2011). Although we were not able to identify clear relationships between parasitic infections and baseline performance in physical fitness and cognition tests, possible interactions between intestinal schistosomiasis and malaria should be considered. Indeed, boys concurrently infected with *Plasmodium* and *S. mansoni* showed significantly higher broad jump scores and in the multivariate regression analysis, VO_2 max estimates were positively associated with light-intensity *S. mansoni* infection. Anaemia was found to be associated with high *Plasmodium* parasite densities and negatively impacted on fitness outcomes. Our results from the longitudinal models revealed lower *Plasmodium* parasitaemia in *S. mansoni*-infected children (data not shown) and thus may indicate an indirect beneficial effect on clinical and physical condition from co-infection. However, it remains to be determined whether this positive association is related to a potential protective effect of chronic schistosomiasis on malaria pathology, as suggested by different groups (Lyke et al., 2005; Hartgers & Yazdanbakhsh, 2006), or whether causality goes the other way around. It is also conceivable that more physically fit children show higher activity patterns, which include water-contact activities, and thus are at higher risk of schistosomiasis (Rudge et al., 2008).

Effects of deworming

The deworming with two rounds of albendazole (400 mg) and praziquantel (40 mg/kg) spaced by 2 months was highly efficacious against soil-transmitted

helminths, while 16 children (21.1%) continued to excrete *S. mansoni* egg in their stool, although at drastically reduced egg loads. *Plasmodium* spp. prevalence was lower at the 5-month follow-up than baseline. Malaria incidence depends on rainfall patterns. Considering 2-week intervals before the baseline (December 2012) and follow-up surveys (May 2013) in our study area, rainfall patterns were comparable (Grover-Kopec et al., 2005). The clearance of helminth infections and the much reduced intensities among those who remained helminth-positive showed conflicting effects on *Plasmodium* parasitaemia depending on the respective helminth species. While hookworm-infected children showed reduced IRRs for parasitaemia at follow-up, the opposite was found in children infected with *S. mansoni*. Enhancing effects on antimalarial immune responses favoured by schistosomiasis co-infection and reduced *Plasmodium* parasitaemia in individuals with light-intensity *Schistosoma* infections in other West African co-endemic settings have been highlighted in previous studies (Lyke et al., 2006; Diallo et al., 2010; Lemaitre et al., 2014). Similarly to our findings with regard to hookworm, repeated anthelminthic treatment positively impacted on *Plasmodium* infection and parasitaemia among *Ascaris*-infected children in Nigeria (Kirwan et al., 2010).

Given the lack of measurable benefit of deworming on anaemia, Hb levels and stunting over the 5-month observation period, clinical morbidity is likely to be malaria-driven or induced by other non-investigated issues (e.g. other infectious diseases, non-communicable diseases, nutritional deficiencies and genetic Hb disorders) (Balarajan et al., 2011). We found a significant weight gain among children within the 5-month study period, which conforms to the weight gain in a healthy reference population of similar age range (Stock et al., 2007). We identified a higher weight gain in girls compared to boys, and in moderate- to heavy-intensity *S. mansoni*-infected individuals. Several other studies showed benefits on growth catch-up after deworming in chronic schistosomiasis (Parraga et al., 1996; Zhou et al., 2007). Yet, interpretation of effects on growth can be complex considering that there are two types of catch-up growth. First, recovery of a chronic disease after an intervention. Second, catch-up growth is linked to hormonal changes, also referred

to as growth spurt in puberty, which usually starts earlier in girls (Parraga et al., 1996; Gurarie et al., 2011).

We observed substantial improvement in the two strength tests after deworming. Since both test outcomes were positively associated with age, we would expect better performance over time; to identify potential benefits from deworming we thus have to compare between baseline helminth infected, which were cured from infection or showed considerably reduced egg output at follow-up, respectively, *vs.* baseline non-infected children. For the standing broad jump test, where we found significantly better performances in *S. mansoni*-infected individuals at baseline, no difference in change of test achievement between infected and non-infected individuals was observed at the 5-month treatment follow-up. It is conceivable that 5 months is too short of a time period to observe any measurable change in explosive leg power after deworming in a malaria hyper-endemic area. In contrast, changes in VO_2 max estimates after deworming showed an interesting pattern in *S. mansoni*-infected children. Children with heavy infections seemed to benefit from deworming, while children with light-intensity infections showed a significant decrease. This finding underlines the importance of considering helminth infection intensity when determining potential effects from *Plasmodium*-helminth interactions. Previous studies highlighted possible protective effects from light-intensity helminth infections on clinical consequences in malaria co-endemic settings (Lyke et al., 2005; Righetti et al., 2012; Hürlimann et al., 2014). Unanticipated negative health effects from deworming campaigns in areas where malaria co-exists are conceivable and must be investigated in the future (Nacher, 2006; Fenton, 2013).

Working memory, as assessed by a digit span test, improved dramatically in our child cohort. Nonetheless, we were unable to directly attribute this improvement to the deworming intervention, since we found no significant relationship between helminth infection and test outcomes neither in the baseline survey nor considering changes over the 5-month study period. In attention scores, however, we observed a significant increase at follow-up for individuals with baseline helminth infections (*Schistosoma* or soil-transmitted helminths) and substantially decreased worm loads and cleared infection at follow-up, respectively. Ezeamama and colleagues also

found significantly increased cognition scores after praziquantel treatment against *S. japonicum* infection in participants free of infection during a 12-month follow-up period (Ezeamama et al., 2012). There is a paucity of high-quality studies investigating the effect of deworming on children's cognitive ability. Moreover, we lack setting-specific information regarding the effect of *Plasmodium* infection on children's cognition, which may have altered potential benefit from deworming. In a highly malarious area in Uganda, a significant negative association between *P. falciparum* parasitaemia and code transmission test scores was found in schoolchildren (Nankabirwa et al., 2013). A randomised controlled trial of intermittent preventive treatment (IPT) against malaria showed improved cognition test results in Kenyan children (Clarke et al., 2008).

Strengths and limitations

We consider our results from the physical and cognitive tests as valid, as we adhered to standard protocols (España-Romero et al., 2008; Kriemler et al., 2010; Yap et al., 2012b). Importantly, the tests are sufficiently flexible to allow for local adaptions, so that they can be implemented in resource-constrained settings. Although external influences (i.e. climate, test ground) could not be completely excluded, the physical fitness outcomes showed expected relationships with age and sex, as documented in previous studies in developed countries (Léger et al., 1988; Cohen et al., 2010; Morales et al., 2013), with the exception of sex differences in grip strength. Regarding the latter issue, the same observation was made in rural Kenyan pupils (Adamo et al., 2011). It is conceivable that children's daily activities in rural Africa involve physical exertion, as part of livelihood strategies, which are shared by both sexes (Otto et al., 1996). The two selected cognition tests were comprehensible. For future studies, however, we would recommend the code transmission task to be done with children attending grade 5 or above or to replace it with a more appropriate test in lower grades, as we had considerable drop-outs in 4th graders. Cognition results were coherent with regard to the link between a better performance and a higher education grade (Otto et al., 1996). Both cognitive and physical functioning does not only depend on capacity, but also on factors such as

concentration, mood and motivation on the day of the tests (Léger & Lambert, 1982; Strauss et al., 2006). Ideally, several rounds of testing should be performed for each child, with averages taken for subsequent analysis. Due to time constraints and in order to minimise disruptions of day-to-day activities in school, repeated testing was not feasible. Efforts were made to enhance test-retest reliability and to minimise effects due to repeated testing for physical and cognitive ability by a number of familiarising procedures (e.g. careful explanations, exercise sessions and warmup tasks).

We administered deworming drugs twice keeping children worm-free or achieve low worm loads until the end-of-study follow-up, since reinfection has been shown to damp potential beneficial effects from deworming on physical fitness (Ezeamama et al., 2012; Yap et al., 2014). We did not consider different treatment groups, which would have allowed comparison between differences in children who received drugs and placebo, respectively. To which extent drug administration contributed to improvements in physical fitness and cognition is therefore not straightforward. As most children were infected with *Plasmodium*, the evaluation of *Plasmodium*- related effects on outcomes is impossible due to the lack of a 'healthy' reference group. We addressed this point by discriminating between different levels of parasitaemia. A further limitation of our study is the relatively short follow-up period (5 months). Some beneficial effects might only become observable if interventions continue over a longer period and follow-up assessments at a later stage are considered. More robustness and more clear trends in cognitive ability could be achieved by administering a more comprehensive battery of learning, memory and attention domains as done for previous studies looking at schistosomiasis and school performance (Jukes et al., 2002; Ezeamama et al., 2012). Our study sample was above the calculated minimum number of participants needed to show effects of clinical relevance in physical fitness, but sex-specific helminth prevalence differed and thus group sizes were not equal among males and females. Relationships between helminth infection and test outcomes which are associated with sex (e.g. VO_2 max, standing broad jump distance) may therefore lack power. However, effects on VO_2 max estimates of males still maintain a power

of 80% at an alpha error of 5% considering that the ratio of helminth-infected *vs*. non-infected males was roughly 1:1.

7.6 Conclusions

Taken together, our findings show limited effects of deworming on helminth-related morbidity, physical functioning and cognitive ability in school-aged children in a highly malaria-endemic setting of Côte d'Ivoire. Hence, the potential benefit of deworming in hyper-endemic malaria settings with stable transmission are likely to be tempered by overshadowing consequences due to *P. falciparum* infection, though reduced *Plasmodium* parasitaemia after deworming was observed. Nevertheless, we reported negative associations between helminth infection and physical and cognitive ability among those children who had growth and haematological deficits, which include as underlying causes helminth infections. The conflicting findings and potential beneficial effects from light-intensity helminth infections identified urge for a deeper mechanistic understanding of *Plasmodium*-helminth interactions and how such interactions influence disease-related morbidity. Our study could not identify major detrimental effects on *Plasmodium*-related pathology in helminth co-infected individuals, a potential increase of *Plasmodium* parasitaemia in *S. mansoni*-infected children, however, was indicated after two rounds of deworming. For future intervention studies assessing effects on clinical and subtle morbidity in helminth-malaria co-endemic settings different interventions and study designs could be an option to consider. These may include the use of supplementary treatment besides deworming, which also impact on *Plasmodium* spp. infection and directly on clinical outcomes (e.g. IPT or administration of iron-fortified food products), implemented as a longitudinal cohort study with a follow-up period of at least 2 years. We regard the measures used to assess physical and cognitive impairment as appropriate, but future studies may profit from a repeated measures approach and a prolonged follow-up period to strengthen findings and to assess not only potential short-term effects. With regard to control and morbidity reduction, our findings highlight the need of combined strategies for achieving greater impact and forestalling potential exacerbating effects in settings with stable malaria transmission.

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8. Discussion

The current Ph.D. study was embedded in a Swiss National Science Foundation (SNSF)-funded project, along with another two Ph.D. studies pursued by my Ivorian colleagues, Ms. Clarrisse A. Houngbedji and Mr. Richard B. Yapi. The overarching goal of this 3-year SNSF project was to generate new knowledge on the spatial distribution and burden of polyparasitism in Côte d'Ivoire, placing particular emphasis on malaria and intestinal helminth infections at different spatial scales, to investigate interactions between parasites and how these interactions affect morbidity and self-rated quality of life, including the assessment of age-specific disability weights. The objectives of the Ph.D. study presented here were (i) to assess the extent and spatial distribution of polyparasitic infections within selected rural communities and on regional scale in different parts of Côte d'Ivoire; (ii) to evaluate determinants which govern the presence of polyparasitism; and (iii) to investigate implications of single and multiple species parasite infections on clinical and perceived morbidities. The community-based surveys conducted in the Taabo HDSS were part of the baseline assessment of an UBS Optimus Foundation-funded pilot project on community-led total sanitation (CLTS), which aims at determining the impact of integrated control strategies, combining preventive chemotherapy and community-led approaches to total sanitation on intestinal parasitic infections and reinfection.

The findings from our cross-sectional community-based surveys confirmed that polyparasitic infections with *Plasmodium*, schistosomes, soil-transmitted helminths and intestinal protozoa are still widespread in rural areas of Côte d'Ivoire, thus confirming previous studies in the western part of Côte d'Ivoire (Utzinger et al., 1999; Keiser et al., 2002a; Raso et al., 2004). Further, we revealed important sociodemographic and behavioural factors that govern the presence and severity of parasitic diseases. We found significant associations between parasite species and between infection status and clinical and perceived morbidity that are partly in line with earlier observations (Raso et al., 2004; Coulibaly et al., 2012). Moreover, we identified significant interactions between *Plasmodium* and light-intensity helminth

infections and clinical morbidity, and thus underline findings from previous studies in Côte d'Ivoire and other West African rural areas (Lyke et al., 2005; Righetti et al., 2012).

The cross-sectional school-based survey conducted in 92 schools across Côte d'Ivoire represents the first attempt to determine the extent and distribution of *Plasmodium* and helminth infections on a national scale. This survey also allowed determining the extent of anaemia, as measured by Hb levels in finger-prick blood samples. We found that every third school-aged child suffered from anaemia or was malnourished (Figure 8.1). Finally, we administered a pre-tested questionnaire and present the most extensive HrQoL estimates thus far for the school-aged population in Côte d'Ivoire, in connection with parasitic infections and clinical manifestations. Indeed, while we present data from a representative sample of almost 5,000 children aged 5-16 years, earlier evidence stemmed from studies with much smaller sample sizes (Fürst et al., 2011, 2012). We measured a 1-point lower HrQoL score overall and a 2-point lower physical wellbeing score on a 100-point scale among anaemic children. However, our findings suggested that HrQoL tools are not sensitive enough to capture disability due to subtle morbidities from helminth and asymptomatic *P. falciparum* infections in school-aged children.

Our 5-month prospective intervention study conducted in a malaria-helminth co-endemic setting of eastern Côte d'Ivoire allowed for assessment of a comprehensive range of well-established and recently applied health status measures in schoolchildren with regard to parasitic infections. The baseline findings revealed significantly lower physical strength scores in children with growth and haematological deficits compared to their well-nourished and non-anaemic counterparts. Further analysis on dynamics in helminth-related morbidity, physical functioning and cognitive ability showed only limited effects of deworming in the 5-month follow-up. Noteworthy, we identified complex interactions of *Plasmodium-S. mansoni* co-infection, which showed effects on physical fitness at baseline and changes in aerobe capacity at follow-up depending on *S. mansoni* infection intensity. These findings urge for a better understanding of *Plasmodium*-helminth interactions

and how treatment against helminth infections might alter disease outcomes due to *Plasmodium* infection.

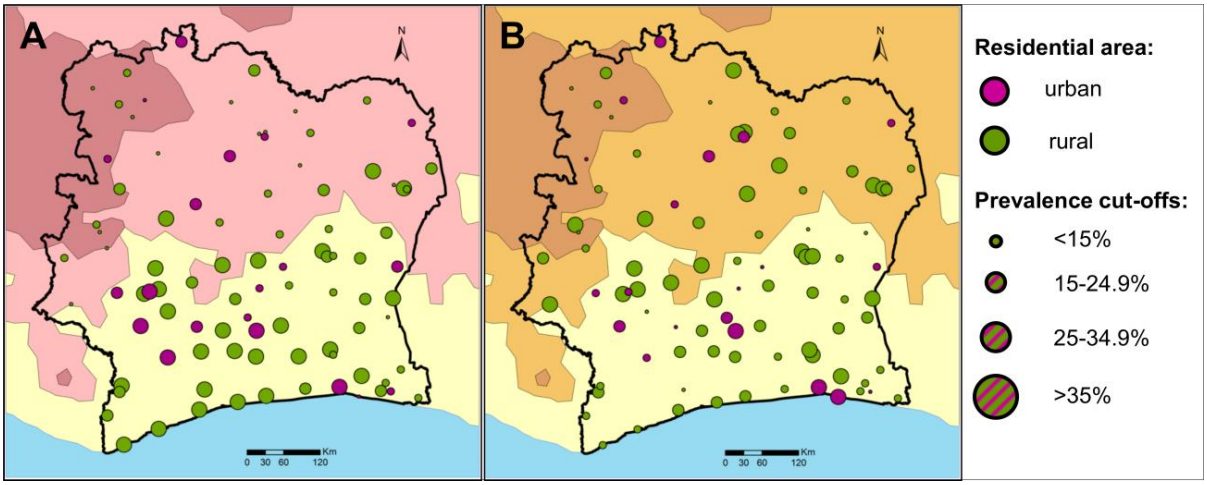

Figure 8.1 Spatial distribution of anaemia (A) and malnutrition (wasting or stunting z-score < -2) (B) among 5,040 children from 92 schools across Côte d'Ivoire

This Ph.D. thesis contributes to the Swiss TPH nexus built on three main pillars, namely, innovation, validation and application (Table 8.1). In brief, it provides new evidence and insights that are of significance from public health and disease control points of view for Côte d'Ivoire and may find application elsewhere. In the following sections, gaps and future research needs are highlighted and the limitations of the current Ph.D. study are discussed.

Table 8.1 Contribution of the different chapters of this Ph.D. thesis to the Swiss TPH nexus of innovation, validation and application

Chapter	Title	Innovation	Validation	Application
4	The epidemiology of polyparasitism and implications for morbidity in two rural communities of Côte d'Ivoire	Major risk factors for multiple species parasite infections identified and implications for morbidity and control among rural communities of southern Côte d'Ivoire discussed.		
5	Effects of hygiene and defecation behaviour on helminths and intestinal protozoa infections in Taabo, Côte d'Ivoire	Local concepts and risk factors of intestinal parasitic diseases determined for nine rural settlements of the Taabo HDSS.	The links between intestinal parasitic infections and sanitation, hygiene and drinking water source were confirmed. Complementary control measures to preventive chemotherapy, addressing hygiene and sanitation, are necessary to keep reinfection rates low.	
6	Health-related quality of life in schoolchildren with parasitic diseases: findings from a national cross-sectional survey in Côte d'Ivoire	First cross-sectional survey assessing multiple species infections, clinical manifestations (e.g. anaemia and malnutrition) and quality of life among school-aged children at national scale in Côte d'Ivoire.	The utility and validity of a setting-specific HrQoL tool determined, including strengths and limitations.	
7	Effect of deworming on school-aged children's physical fitness, cognition and clinical parameters in a malaria-helminth co-endemic area of Côte d'Ivoire	The first investigation of effects of deworming on a multitude of health measures in school-aged children in a malaria-helminth co-endemic setting of Côte d'Ivoire.	Two rounds of deworming were highly efficacious against schistosomiasis and soil-transmitted helminthiasis, but had only limited impact on clinical morbidity.	

8.1 Epidemiology of multiple species infections and implications for morbidity

Our findings from parasitological examinations within rural communities showed that polyparasitism is still widespread in Côte d'Ivoire, although infection prevalence and intensities were lower than in studies conducted in the country more than a decade ago (Utzinger et al., 1999; Keiser et al., 2002a; Raso et al., 2004). The fact that most helminth infections detected were of light intensity among community members of settlements situated in the Taabo HDSS might be attributed to repeated deworming campaigns that started in 2009, including yearly preventive chemotherapy with anthelminthic drugs (single oral dose of albendazole) administered to a population of approximately 40,000 individuals (Fürst et al., 2013). The hookworm prevalence nevertheless was considerable (33.5%) and slightly higher compared to results from the same study area obtained a few months before the current investigation (Righetti et al., 2013), indicating frequent reinfection and the need for complementary control measures on top of preventive chemotherapy (Jia et al., 2012). The frequently reported practice of open defecation and the low level of awareness about the disease transmission stated in the household-based questionnaire interviews may partly explain the persisting problem of hookworm disease (Schmidlin et al., 2013). Availability and use of toilets have been found to be associated with lower odds of infection with soil-transmitted helminths in the present study and elsewhere, emphasising the need for awareness raising from health education that has been shown to decrease reinfection rates (Asaolu & Ofoezie, 2003; Ziegelbauer et al., 2012; Strunz et al., 2014). Intestinal parasitic infections such as *S. mansoni*, hookworm and several intestinal protozoa species showed particularly high prevalences among the most poor (Schmidlin et al., 2013; Hürlimann et al., 2014), supporting the general concept that NTDs are poverty-related (Engels & Savioli, 2006; Steinmann et al., 2010; Lustigman et al., 2012). Several of these parasitic infections showed positive associations between each other such as *S. mansoni* and hookworm and intestinal protozoa species confirming results from previous studies conducted in Côte d'Ivoire (Keiser et al., 2002b; Raso et al., 2006).

The parasitological results from the community surveys and the national cross-sectional survey among school-aged children identified *P. falciparum* and hookworm as the predominant species throughout Côte d'Ivoire, whilst *Schistosoma* infections showed a highly focal distribution with high prevalences in some areas (e.g. *S. mansoni* is highly endemic in the Man region in western Côte d'Ivoire). The rate of *Plasmodium* infection was found to be alarming; indeed, three-quarter of school-aged children harboured *Plasmodium* parasites in their blood and infection was most prevalent in young children, which is typically seen in high-transmission areas (Cotter et al., 2013; WHO, 2013a). This calls for urgent action and implementation of the new strategies put forth by the national control programme, namely to reach universal coverage with LLINs, to apply IRS as a supplementary vector control measure and to provide IPT in pregnancy (RBM, 2008; Koffi et al., 2013; WHO, 2013a).

Infection with *P. falciparum*, *Schistosoma* and hookworm are known causes of anaemia (Crompton, 2000; Bethony et al., 2006; Gryseels et al., 2006; White et al., 2014). We have observed strong associations between *Plasmodium* parasitaemia and clinical manifestations, such as fever, anaemia and splenomegaly; the latter is a common sign of malaria and is related to sequestration of parasitized red blood cells in the spleen (del Portillo et al., 2012; WHO, 2013a). Light-intensity hookworm and *S. mansoni* infection, by contrast, were negatively associated with anaemia. Further analysis on *Plasmodium*-helminth co-infection revealed significant interactions on morbidity. Children with a hookworm-*Plasmodium* co-infection and adults with a *S. mansoni*-*Plasmodium* co-infection showed lower odds for anaemia and support earlier findings from Côte d'Ivoire (Righetti et al., 2012). There has been a growing number of studies investigating for *Plasmodium*-helminth interactions in recent years (Brooker et al., 2007; Nacher, 2011; Naing et al., 2013). Of major interest are how helminths affect *Plasmodium* parasitaemia and how they alter malaria pathology (i.e. anaemia, severe clinical malaria). Underlying mechanisms are mainly seen in the immunomodulatory activities of helminths which are still insufficiently understood (Hartgers & Yazdanbakhsh, 2006; Courtin et al., 2011). Interactions, particularly if they are of protective nature against malaria, are of public health importance in view

of ongoing and planned helminth control programmes and potential exacerbation of malaria pathology. Antibody response to vaccines for *Plasmodium* further has been shown to be decreased in helminth co-infected individuals and thus may affect efficacy testing and future implementation of vaccines (Hartgers & Yazdanbakhsh, 2006; Esen et al., 2012). Our findings, in addition, suggest that children with a nutritional deficiency show a higher susceptibility to clinical morbidity due to *Plasmodium* infection, adding up to the burden of polyparasitism as emphasised earlier (Scrimshaw & SanGiovanni, 1997; Pullan & Brooker, 2008). Future research on the species-specific immunological nature within the human host, significance of co-infection and co-morbidities for population-based morbidity patterns and implications from and on treatment strategies are warranted for a deeper understanding of mechanisms of interactions in polyparasitic infections.

Although the data collected in this study represent an important evidence-base and allowed to depict the extent and implications of polyparasitism in Côte d'Ivoire on different scales, the study suffers from several limitations with regard to multiple species infections and related morbidity that are offered for consideration. It has to be pointed out that the extent of polyparasitic infections at community and national level is likely to be higher than reported here, considering the diagnostic approach we applied. Indeed, our results are based on single blood, urine and stool samples, while more intensive sampling efforts and, particularly for the Kato-Katz method, analysis of multiple stool samples from several consecutive days have been shown to increase sensitivity for helminth detection (Knopp et al., 2008; Bergquist et al., 2009; Utzinger et al., 2011). The FLOTAC method, a recently developed and tested floatation technique that showed higher sensitivity for detection of light-intensity helminth infections compared to the Kato-Katz technique, might be a promising application in future field studies (Knopp et al., 2008, 2014; Utzinger et al., 2008).

In our study we were particularly interested in assessing how parasitic infections impact on health. Taking into account that morbidity is likely to be more pronouncedly measurable and perceived in high-intensity infections (Ezeamama et al., 2008; WHO, 2013b), we consider our findings related to morbidity nevertheless

as valid. Future studies focussing on interactions with light-intensity helminth infections should use more rigorous diagnostic approaches.

8.2 Re-assessing disability due to parasitic infections

Decisions on priority setting in public health, implementation of policies and resource allocation to address health conditions are generally taken based on burden estimates assessing the magnitude of health loss due to disease and injuries. The most referred evidence-base of burden estimates is the global burden of disease study, which is based on the DALY metric (Murray et al., 2012a; b). The DALY construct has been criticised for having a narrow focus, strictly separating “health” from welfare, well-being and social context, and thus likely to provide insensitive estimates for poor areas (Byass et al., 2013; Voigt & King, 2014). Consequently, this pertains also to parasitic diseases which most frequently occur among the poorest of the poor in resource-constrained areas. Furthermore, DALYs are considered inaccurate in capturing disability weights for infections with low mortality but more chronic morbid sequelae, i.e. NTDs and in particular helminth infections. Thus, the actual burden for NTDs, including helminth infections, might be underestimated (King et al., 2005; Hotez et al., 2014; King, 2014).

In the frame of this Ph.D. study, we applied and provide new evidence on several alternative approaches and measures proposed to re-assess the burden due to parasitic diseases, namely HrQoL, physical fitness and cognitive functioning testing. The major findings and also potential limitations shall be discussed here.

Our results on HrQoL among 4,848 school-aged children showed significant difference in self-reported QoL for several sociodemographic determinants; the most poor showed a 1.6-point lower HrQoL on a scale from 0 to 100 than children from wealthier quintiles. HrQoL reporting further differed between sex, and showed tendencies between different age groups and between children from rural and urban residential areas. This exemplifies that health status is differently rated between different social-ecological contexts and thus should be considered in health rating estimates. The recent estimates of the Global Burden of Disease (GBD) 2010 study made an attempt to account for socio-cultural context by leading surveys in five

culturally different countries (Bangladesh, Indonesia, Peru, Tanzania and the United States of America) and let respondents rate between two health conditions which one they considered healthier in order to derive disability weights from the general public (Salomon et al., 2012). By additionally including survey results from a web-based survey, the 'Western world' tended finally to be overrepresented, somewhat dampening the intention to render the estimates more culturally valid.

We were able to measure a significant 1-point lower HrQoL, which can be translated in a DW of 0.01 (0.006 for mild and 0.015 for moderate anaemia if GBD cut-offs are used), in anaemic children compared to their non-anaemic counterparts, whilst the HrQoL tool seemed not to be sensitive enough to reveal a significant disability in children with parasitic infections compared to non-infected children. Anaemia, however, was significantly associated with helminth-mono-, *Plasmodium*-mono- and co-infection in our sample of school-aged children. The new approach applied by the GBD 2010 to include sequelae, which may be related to infection (e.g. hookworm-induced anaemia) seems therefore reasonable (Kassebaum et al., 2014).

The findings from the physical fitness testing showed expected lower performance in children with nutritional and haematological deficits as already observed elsewhere (Bustinduy et al., 2011; Nhantumbo et al., 2013). Unexpected were, however, the results indicating better fitness in *S. mansoni*-infected children compared to their non-infected counterparts. This observation may indicate a possible interaction with *Plasmodium* infection. In our study setting the prevalence of *Plasmodium* spp. was strikingly high and virtually every child was infected.

Cognition tests applied did not show any significant relationship between cognitive performance and parasitic diseases or infection intensities. This raises the question about the choice of the tests and their sensitivity to measure cognitive deficits in parasitic diseases as exemplified by a study done among Filipino children with *S. japonicum* and soil-transmitted helminth (co-)infections. Some tests of the battery applied could capture deficits in infected children while others could not (Ezeamama et al., 2005). Future research investigating cognitive functioning in school-aged children ideally should apply a battery of different tests. A repeated

measures approach additionally would strengthen findings since both children's cognition and physical functioning may vary from one day to another.

8.3 Impact of deworming and challenges and opportunities for integrated control strategies

We conducted a 5-month prospective intervention study involving two rounds of treatment against schistosomiasis and soil-transmitted helminthiasis in a *Plasmodium*-helminth co-endemic setting. We found baseline prevalences of *Plasmodium*, *S. mansoni* and hookworm of 92%, 35% and 10%, respectively. The study aimed at identifying effects of deworming on children's clinical status of anaemia and malnutrition, physical fitness and cognition.

We found no beneficial effects on children's clinical status 5 months after deworming. Anaemia remained constantly high and affected a third of all children. The missing effect on anaemia might be explained by the high malaria burden present in the setting, which was not addressed by our deworming intervention. Anaemia and malnutrition are multifactorial thus deworming alone is unlikely to significantly improve these clinical manifestations in areas of high-malaria transmission and where micro-nutrient deficiencies may play a role as well (Hall et al., 2008). It should also be noted that we used Hb measurement as a proxy for anaemia, which did not allow assessing to which extent parasitic diseases, haematological deficits or nutritional deficiencies contributed to anaemia. Knowledge of the extent of iron-deficiency and parasitic disease-related anaemia would allow to plan the most effective intervention strategy (i.e. iron supplementation, IPT, anthelminthic treatment or a combination thereof) (Rohner et al., 2010).

Nevertheless, we could find moderate beneficial effects on cognition and weight gain in baseline helminth-positive children. Deworming negatively impacted on aerobe capacity, as assessed by a multi-stage 20 m shuttle run test in light-intensity *S. mansoni*-infected children supporting the finding from baseline that light-intensity *S. mansoni* infection might show interaction with *Plasmodium*.

Concerted efforts of helminthiasis and malaria control programmes promise a higher impact on morbidity and would also avoid conceivable harmful effects from

deworming alone taking into account potential interactions on malaria pathology. Worth considering is further the application of ACTs in schistosomiasis-*Plasmodium* co-endemic settings. ACTs have an effect on both *S. haematobium* and *S. mansoni*, as shown in recent randomised controlled trials (Keiser et al., 2010; Utzinger et al., 2010). The implemented national schistosomiasis, soil-transmitted helminthiasis and lymphatic filariasis control programmes may further profit from complementing preventive chemotherapy with school-based health education that proved to be valid to decrease reinfection rates of soil-transmitted helminths in the People's Republic of China (Bieri et al., 2013). Many promising complementary approaches targeting awareness (community- and school-based health education), improved sanitation and access to clean water (e.g. CLTS) have been proven or are currently in an evaluation phase to show enhanced impact on control of NTDs (King et al., 2013; Schmidlin et al., 2013). To achieve a substantial change and for implementation on regional and national scale, however, a strong collaboration between Ministries of Health, Ministries of Water and Sanitation and control programmes and other stakeholders are essential.

8.4 Conclusions

The overarching goal of this Ph.D. thesis was to provide new insight into multiple species parasite infections and to determine implications for morbidity at local and regional scales in Côte d'Ivoire. Several community-based surveys allowed exploring the epidemiology of polyparasitism, sociodemographic and behavioural factors driving its transmission and the extent it affects health in rural populations. Additionally, self-rated quality of life with an emphasis on *Plasmodium* and helminth infections among a representative sample of school-aged children across Côte d'Ivoire was determined. Finally a 5-month prospective intervention study was conducted to investigate associations between *Plasmodium* and helminth infections and schoolchildren's physical and cognitive performance as well as to highlight potential benefits from deworming. Based on the work presented in this thesis a number of conclusions can be drawn.

- Malaria is a major public health issue; in young age groups in rural communities, every third school-aged child was found to be anaemic or malnourished. Effective prevention and control of *Plasmodium* infection, including universal coverage with LLINs and early diagnosis and prompt treatment with ACTs are urgently needed.

- Interactions between helminth infections and *P. falciparum* showed beneficial effects on malaria parasitaemia and clinical morbidity. Potential exacerbation of malaria-related pathology from helminthiasis control interventions should thus be investigated.

- KAPB studies revealed a very low awareness of intestinal parasitic infections and transmission. Since open defecation is still widespread in rural parts of Côte d'Ivoire, there is rapid reinfection with these parasites. These findings call for integrated control approaches. CLTS - as a complementary measure to preventive chemotherapy - promises a lasting impact on disease transmission in rural settings with poor sanitation and should thus be considered for implementation.

- HrQoL instruments are likely to miss disability of subtle morbidities due to uncomplicated *Plasmodium* and to helminth infections in school-aged children but proved to be sensitive to measure burden due to more advanced morbid sequelae such as anaemia. This result calls for further development or refinement of tools which capture these health conditions.

- Two rounds of deworming showed no beneficial effect on clinical signs in a malaria high-transmission setting. To enhance impact and to tackle the underlying factors of anaemia and malnutrition, a complementary approach is needed, including concurrent helminth and malaria prevention and control as well as nutritional interventions.

8.5 Research needs and recommendations

8.5.1 Identified research needs

- A deeper understanding of the mechanism involved in *Plasmodium*-helminth infections are needed (e.g. immunology, morbidity and metabolism) and why it differs between sex and different age groups.
- Assess the potential, applicability and risks of ACTs for use as antimalarial and antischistosomal treatment in co-endemic settings.
- Compare the sensitivity of HrQoL tools to measure disability of parasitic infections in targeted communities with different endemicity levels.
- Investigate effects on health status (measured and perceived) from different combinations of interventions (anthelminthic, IPT, ACT, iron supplementation (in combination with antimalarials only)) against *Plasmodium* and helminths following randomised controlled trials with different intervention groups. This would further allow to assess potential adverse effects from anthelminthic treatment in malaria high-transmission areas.
- Assess the relative contribution of parasitic infections and poor diet on anaemia and nutritional deficiencies in children.
- Assess the impact of CLTS on reinfection with intestinal parasitic infections and hygiene behaviour. A pilot study has been conducted in the Taabo HDSS between 2011 and 2012. Of further interest would be its implementation in culturally diverse settings of Côte d'Ivoire to allow for comparison of impact and acceptance between regions.
- Evaluate cognitive impairment from parasitic diseases using a battery of cognition tests and use repeated measurements.
- Assess the effect on helminth reinfection rates of a school-based health education programme. A pilot study investigating different control strategies including also the application of an educational Cartoon targeting helminth infections among school-aged children has been designed and is currently being implemented in more than 20 schools in Côte d'Ivoire.

8.5.2 Recommendations

- Based on the evidence gained from a pilot project implemented in 2011 and 2012 in the Taabo HDSS, CLTS has been considered for implementation on a broader scale.
- Incorporate nutritional education addressed to women.
- LLIN coverage needs to be scaled up.
- Implement IPTp and IPTi.
- Improve access to health care and strengthen health systems.
- Improve access to safe water (build community pumps).
- Provide accurate diagnosis of diseases at health centres. RDTs for malaria case management have been implemented. A similar strategy using existing point-of-care tests should be adopted for schistosomiasis case management. This calls for the addressing of the issue of frequent praziquantel stock-outs and poor access to treatment.
- Apply integrated control approaches to enhance cost-effectiveness:
    - Combine school-based treatment campaigns (e.g. the national schistosomiasis, soil-transmitted helminthiasis and lymphatic filariasis control programme) with health education targeting disease transmission and prevention. Use drugs which affect a range of different species (e.g. in known schistosomiasis-*Plasmodium* co-endemic areas administration of ACTs should be considered).
    - Couple LLIN distribution campaigns with education programmes focussing on malaria transmission, emphasis for protection of the most vulnerable (infants, pregnant women and children) and larval source management.
    - Promote IPTp and IPTi in health centres for pregnant women and mothers and offer education on malaria, diet diversification and healthy food preparation techniques.

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9. Curriculum Vitae

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Education/Training:

1. 2011-3.2014	PhD in Epidemiology Swiss Tropical and Public Health Institute, Basel, Switzerland Epidemiology and Public Health department Ecosystem Health Sciences unit Thesis title: Effects of parasitic infections on clinical outcomes, self-rated quality of life and physical fitness in Côte d'Ivoire
10. 2006 – 6. 2008	MSc Master of Science in Epidemiology and Infection Biology with Major in Epidemiology Swiss Tropical and Public Health Institute, Basel, Switzerland Epidemiology and Public Health department Society, Gender and Health unit Thesis title: Local concepts of hypertension and implications for cardiovascular disease control in rural Tamil Nadu, India
9. 2002 – 9. 2006	BSc Bachelor of Science in Biology Specialisation in Nature-Landscape-Environment (NLU) University of Basel, Basel, Switzerland
8. 1998 – 6. 2002	Matura Typus D (Neusprachen), Kantonsschule Zofingen (AG)

Presentation activies and field work:

10. 2013	• Attendance with oral and poster presentation at the Africa 2013 Ecohealth conference in Abidjan, Côte d'Ivoire
2011-2013	• Implementation of scientific studies in Côte d'Ivoire with supervision in situ and from Switzerland. The studies were conducted in the frame of a PhD at the Swiss Tropical and Public Health Institute (Swiss TPH)
10. 2009	• Attendance and oral presentation at the fourth annual meeting of the CONTRAST meeting in Kilifi, Kenya • Visit of partner institution, National Institute for Medical Research (NIMR), in Mwanza. Tanzania for data collection
3. – 6. 2007	Implementation of a scientific pilot study in the frame of my MSc thesis in Epidemiology in Chennai, India: • Assistance in planning, implementation and evaluation of a pilot study among hypertensive patients in rural Tamil Nadu • Collaboration with local partners from the National Institute of Epidemiology (NIE)

Further work experience:

10. 2008 – 12. 2010	Research assistant in the frame of the CONTRAST project Swiss Tropical and Public Health Institute, Basel, Switzerland Epidemiology and Public Health department Biostatistics unit
7. 2007 – 9. 2010	Part-time employment at Offene Jugendarbeit Zofingen in the frame of the "Heitere Box" project

Professional skills:

Languages	• German: mother tongue/first language • English: business fluent • French: fluent • Italian: conversational • Spanish: elementary
Computer	• Data management: MS Office (Word, Excel, PowerPoint, Access), Reference Manager, MAXQDA • Statistics: STATA, Epi Info, OpenBUGS • Mapping: ArcGIS, Google Earth

Publications:

1. **Hürlimann E**, Houngbedji CA, Yapi RB, Ndri PB, Silué KD, Soro G, Kouamé FN, Fürst T, Utzinger J, N'Goran EK, Raso G (2014) Health-related quality of life among school children with parasitic infections: findings from a national cross-sectional survey in Côte d'Ivoire. *PLoS Neglected Tropical Diseases* 8: e3287.
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3. **Hürlimann E**, Yapi RB, Houngbedji CA, Schmidlin T, Kouadio BA, Silué KD, Ouattara M, N'Goran EK, Utzinger J, Raso G (2014) The epidemiology of polyparasitism and implications for morbidity in two rural communities of Côte d'Ivoire. *Parasites & Vectors* 7: 81.
4. Koné S, Baikoro N, N'Guessan Y, Jaeger FN, Silué KD, Fürst T, **Hürlimann E**, Ouattara M, Séka M-CY, N'Guessan NA, Esso ELJC, Zouzou F, Boti LI, Gonety PT, Adiossan LG, Dao D, Tschannen AB, von Stamm T, et al. (2014) Health & demographic surveillance system profile: the Taabo health and demographic surveillance system, Côte d'Ivoire. *International Journal of Epidemiology* (in press).
5. Yapi RB, **Hürlimann E**, Houngbedji CA, N'Dri PB, Silué KD, Soro G, Kouamé FN, Vounatsou P, Fürst T, N'Goran EK, Utzinger J, Raso G (2014) Infection and co-infection of helminths and *Plasmodium* among school children in Côte d'Ivoire: results from a national cross-sectional survey. *PLoS Neglected Tropical Diseases* 8: e2913.
6. Chammartin F, **Hürlimann E**, Raso G, N'Goran EK, Utzinger J, Vounatsou P (2013) Statistical methodological issues in mapping historical schistosomiasis survey data. *Acta Tropica* 128: 345–352.
7. Ekpo UF, **Hürlimann E**, Schur N, Oluwole AS, Abe EM, Mafe MA, Nebe OJ, Isiyaku S, Olamiju F, Kadiri M, Poopola TOS, Braide EI, Saka Y, Mafiana CF, Kristensen TK, Utzinger J, Vounatsou P (2013) Mapping and prediction of schistosomiasis in Nigeria using compiled survey data and Bayesian geospatial modelling. *Geospatial Health* 7: 355–366.
8. Schmidlin T, **Hürlimann E**, Silué KD, Yapi RB, Houngbedji C, Kouadio BA, Acka-Douabélé CA, Kouassi D, Ouattara M, Zouzou F, Bonfoh B, N'Goran EK, Utzinger J, Raso G (2013) Effects of hygiene and defecation behavior on helminths and intestinal protozoa infections in Taabo, Côte d'Ivoire. *PloS One* 8: e65722.
9. Schur N, **Hürlimann E**, Stensgaard A-S, Chimfwembe K, Mushinge G, Simoonga C, Kabatereine NB, Kristensen TK, Utzinger J, Vounatsou P (2013) Spatially explicit Schistosoma infection risk in eastern Africa using Bayesian geostatistical modelling. *Acta Tropica* 128: 365–377.
10. Stensgaard A-S, Utzinger J, Vounatsou P, **Hürlimann E**, Schur N, Saarnak CFL, Simoonga C, Mubita P, Kabatereine NB, Tchuem Tchuenté L-A, Rahbek C, Kristensen TK (2013) Large-scale determinants of intestinal schistosomiasis and intermediate host snail distribution across Africa: does climate matter? *Acta Tropica* 128: 378–390.
11. **Hürlimann E**, Schur N, Boutsika K, Stensgaard A-S, Laserna de Himpsl M, Ziegelbauer K, Laizer N, Camenzind L, Di Pasquale A, Ekpo UF, Simoonga C, Mushinge G, Saarnak CFL, Utzinger J, Kristensen TK, Vounatsou P (2011) Toward an open-access global database for mapping, control, and surveillance of neglected tropical diseases. *PLoS Neglected Tropical Diseases* 5: e1404.
12. Schur N, **Hürlimann E**, Garba A, Traoré MS, Ndir O, Ratard RC, Tchuem Tchuenté L.A, Kristensen TK, Utzinger J, Vounatsou P (2011) Geostatistical model-based estimates of Schistosomiasis prevalence among individuals aged ≤ 20 years in West Africa. *PLoS Neglected Tropical Diseases* 5: e1194.

10. Appendix

10.1 The epidemiology of polyparasitism and implications for morbidity in two rural communities of Côte d'Ivoire

10.1.1 Additional file 1. Statistically significant associations between parasite species from multivariate regression analysis

Parasite	Association	Adjusted OR (95% CI)
Plasmodium		
P. falciparum[8,12]	Age group (10-18 years)	0.28 (0.16, 0.51)
	Age group (19-39 years)	0.11 (0.06, 0.19)
	Age group (≥40 years)	0.07 (0.04, 0.13)
	S. mansoni	2.03 (1.29, 3.19)
P. malariae[1,5,10]	Age group (10-18 years)	0.08 (0.01, 0.67)
	Age group (19-39 years)	0.13 (0.03, 0.63)
	Age group (≥40 years)	0.11 (0.01, 0.93)
Schistosoma		
S. haematobium[13]	Age group (5-9 years)	6.32 (2.96, 13.51)
	Age group (10-18 years)	9.76 (4.53, 21.04)
	S. mansoni	0.11 (0.03, 0.34)
S. mansoni[10]	Wealth quintile (least poor)	0.21 (0.09, 0.48)
	Age group (5-9 years)	16.10 (2.04, 127.20)
	Age group (10-18 years)	46.69 (6.03, 361.68)
	Age group (19-39 years)	52.42 (6.91, 397.95)
	Age group (≥40 years)	42.07 (5.41, 327.38)
	Sex (male)	1.70 (1.08, 2.66)
	Hookworm	2.78 (1.76, 4.41)
	E. coli	0.33 (0.19, 0.57)
	S. haematobium	0.12 (0.04, 0.41)
	B. hominis	1.66 (1.04, 2.67)
	P. falciparum	2.14 (1.30, 3.50)
Soil-transmitted helminths		
Hookworm[9]	Wealth quintile (poor)	0.34 (0.19, 0.61)
	Age group (5-9 years)	4.26 (2.08, 8.75)
	Age group (10-18 years)	7.05 (3.43, 14.49)
	Age group (19-39 years)	7.53 (3.78, 14.99)
	Age group (≥40 years)	6.48 (3.15, 13.33)
	Sex (male)	1.92 (1.39, 2.64)
	S. mansoni	2.78 (1.79, 4.31)
	B. hominis	0.70 (0.49, 0.99)
Intestinal protozoa		
E. histolytica/E. dispar[12,14]	*E. coli*	2.86 (1.71, 4.77)
	E. nana	1.84 (1.09, 3.08)
E. coli	*E. nana*	4.68 (3.17, 6.89)
	I. bütschlii	5.93 (3.41, 10.30)
	E. histolytica/E. dispar	3.19 (1.84, 5.55)
	S. mansoni	0.31 (0.18, 0.55)

	Age group (19-39 years)	3.63 (2.02, 6.52)
	Age group (≥40 years)	3.73 (2.00, 6.95)
	C. mesnili	3.51 (1.30, 9.48)
	Wealth quintile (very poor)	2.05 (1.16, 3.61)
	Wealth quintile (least poor)	1.91 (1.13, 3.24)
E. nana[1,5,11,15]	*E. coli*	4.88 (3.36, 7.09)
	B. hominis	2.21 (1.50, 3.23)
	E. histolytica/E. dispar	1.90 (1.12, 3.24)
	Age group (5-9 years)	4.83 (1.97, 11.81)
	Age group (10-18 years)	5.66 (2.26, 14.16)
	Age group (19-39 years)	7.52 (3.09, 18.29)
	Age group (≥40 years)	5.56 (2.19, 14.10)
I. bütschlii[5,10,14]	*E. coli*	6.56 (3.93, 10.95)
	B. hominis	2.03 (1.26, 3.28)
G. intestinalis[2,5,12]	Age group (19-39 years)	0.20 (0.08, 0.47)
	Age group (≥40 years)	0.21 (0.08, 0.58)
	I. bütschlii	0.38 (0.14, 0.99)
	S. mansoni	0.36 (0.14, 0.95)
C. mesnili[8,13,14]	*E. coli*	4.63 (1.79, 11.96)
B. hominis[5,10]	*E. nana*	2.00 (1.42, 2.83)
	I. bütschlii	2.06 (1.29, 3.29)
	S. mansoni	1.86 (1.21, 2.86)
	P. falciparum	1.39 (1.00, 1.92)

Reference categories: sex: female, age group: 0-4 years, and wealth quintile: most poor.

*After adjusting for: 1 = *P. falciparum*, 2 = *P. malariae*, 3 = *S. haematobium*, 4 = *S. mansoni*, 5 = Hookworm, 6 = *E. histolytica/E. dispar*, 7 = *E. coli*, 8 = *E. nana*, 9 = *I. bütschlii*, 10 = *G. intestinalis*, 11 = *C. mesnili*, 12 = *B. hominis*, 13 = sex, 14 = age group, 15 = wealth quintile

10.1.2 Additional file 2. Prevalence of clinical morbidity and indicators for malnutrition, stratified by sex and age group

Parameter	Overall	Sex			Age group (years)					
	pos/n (%)	Females pos/n (%)	Males pos/n (%)	p-value	< 5 (n = 150)	5-9 (n = 172)	10-18 (n = 144)	19-39 (n = 237)	≥ 40 (n = 149)	p-value
Fever (≥38.0 °C)	43/852 (5.1)	26/431 (6.0)	17/421 (4.0)	0.184	14 (9.3)	14 (8.1)	4 (2.8)	8 (3.4)	3 (2.0)	0.005
Mean hemoglobin level (g/l)	122.4	117.9	126.4	<0.001	100.3	115.8	124.7	132.0	133.1	<0.001
Moderately anaemic	163/852 (19.1)	86/431 (20.0)	77/421 (18.3)	0.537	64 (42.7)	31 (18.0)	22 (15.3)	29 (12.2)	17 (11.4)	<0.001
Severly anaemic	3/852 (0.4)	2/431 (0.5)	1/421 (0.2)	1.000	3 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.015
Splenomegaly	134/852 (15.7)	59/431 (13.7)	75/421 (17.8)	0.098	60 (40.0)	48 (27.9)	13 (9.0)	6 (2.5)	7 (4.7)	<0.001
Hepatomegaly	2/852 (0.2)	0/431 (0.0)	2/421 (0.5)	0.244	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.7)	0.450
Indicators of malnutrition*:										
Moderately stunted (H/A Z-score ≤2)	77/466 (16.5)	28/223 (12.6)	49/243 (20.2)	0.027	33 (22.0)	26 (15.1)	18 (12.5)	N/A	N/A	0.074
Severly stunted (H/A Z-score ≤3)	42/466 (9.0)	13/223 (5.8)	29/243 (11.9)	0.022	27 (18.0)	9 (5.2)	6 (4.2)	N/A	N/A	<0.001
Any form of stunting[#]	235/466 (50.4)	96/223 (43.1)	139/243 (57.2)	0.002	95 (63.3)	82 (47.7)	58 (40.3)	N/A	N/A	<0.001
Moderately wasted (W/H Z-score ≤2)	5/150 (3.3)	3/69 (4.4)	2/81 (2.5)	0.662	5 (3.3)	N/A	N/A	N/A	N/A	N/A
Severly wasted (W/H Z-score ≤3)	4/150 (2.7)	3/69 (4.4)	1/81 (1.2)	0.334	4(2.7)	N/A	N/A	N/A	N/A	N/A
Any form of wasting[§]	9/150 (6.0)	6/69 (8.7)	3 (3.7)	0.303	9(6.0)	N/A	N/A	N/A	N/A	N/A
Moderately underweight (W/A Z-score <2)	31/322 (9.6)	11/157 (7.0)	20/165 (12.1)	0.120	13/150 (8.7)	18/172 (10.5)	N/A	N/A	N/A	0.585
Severly underweight (W/H Z-score <3)	13/322 (4.0)	7/157 (4.5)	6/165 (3.6)	0.708	7/150 (4.7)	6/172 (3.5)	N/A	N/A	N/A	0.592
Any form of underweight[#]	124/322 (38.5)	63/157 (40.1)	61/165 (37.0)	0.561	50/150 (33.3)	74/172 (43.0)	N/A	N/A	N/A	0.075
Moderate thinness (defined by BMI)	36/852 (4.2)	19/431 (4.4)	17/421 (4.0)	0.788	5/150 (3.3)	9/172 (5.2)	9/144 (6.3)	5/237 (2.1)	8/149 (5.4)	0.259
Severe thinness (defined by BMI)	14/852 (1.6)	6/431 (1.4)	8/421 (1.)	0.560	4/150 (2.7)	1/172 (0.6)	5/144 (3.5)	0/237 (0.0)	4/149 (2.7)	0.009
Any form of thinness[#]	177/852 (20.8)	85/431 (19.7)	92/421 (21.9)	0.443	22/150 (14.7)	41/172 (23.8)	61/144 (42.4)	28/237 (11.8)	25/149 (16.8)	<0.001
Moderately malnourished (defined by MUAC)	9/536 (1.7)	4/277 (1.4)	5/259 (1.9)	0.745	8/150 (5.3)	N/A	N/A	1/237 (0.4)	0/149 (0.0)	<0.001
Severly malnourished (defined by MUAC)	1/536 (0.2)	0/277 (0.0)	1/259 (0.4)	0.483	1/150 (0.7)	N/A	N/A	0/237 (0.0)	0/149 (0.0)	0.558
Any form of malnutrition/low MUAC[#]	40/536 (7.5)	16/277 (5.8)	24/259 (9.3)	0.124	39/150 (26.0)	N/A	N/A	1/237 (0.4)	0/149 (0.0)	<0.001
Any indicator for malnutrition[§]	162/852 (19.0)	66/431 (15.3)	96/421 (22.8)	0.005	70/150 (46.7)	45/172 (26.2)	29/144 (20.1)	6/237 (2.5)	12/149 (8.1)	<0.001

852 study participants underwent a clinical examination and had full records for anthropometric measurements for the assessment of their nutritional status.

*Certain nutritional indicators are applicable for specific age classes only: stunting for age group 0-14 years, wasting for children under 5 years, underweight for children up to 10 years, and MUAC is considered as an appropriate indicator for children under 5 and adults (> 18 years)

[#] Of mild, moderate or severe nature

[§] Of moderate or severe nature

10.2 Health-related quality of life among schoolchildren with parasitic diseases: findings from a national cross-sectional survey in Côte d'Ivoire

10.2.1 Supporting information. Table S1. Prevalence and intensity of parasitic infections, stratified by sex, age group, residential area and ecozone among 4,848 schoolchildren in Côte d'Ivoire

Parasitic infection	Total	Age group (years)			Sex			Residential area			Ecozone		
	(n=4,848)	5-10 (n=3,282)	11-16 (n=1,566)	p-value	Females (n=2,269)	Males (n=2,579)	p-value	Rural (n=3,783)	Urban (n=1,065)	p-value	South (n=2,862)	North (n=1,986)	p-value
P. falciparum	74.1	73.6	75.1	0.281	71.6	76.4	<0.001*	77.1	63.4	<0.001*	70.7	79.0	<0.001*
P. malariae	3.9	4.5	2.8	0.004*	3.8	4.1	0.560	4.4	2.4	0.003*	3.8	4.1	0.531
P. ovale	0.3	0.3	0.2	0.476	0.3	0.3	0.963	0.3	0.1	0.213	0.4	0.1	0.060
Plasmodium spp.	75.0	74.5	75.9	0.293	72.2	77.4	<0.001*	78.1	63.9	<0.001*	71.8	79.6	<0.001*
Parasitemia ≥ 1000 parasites/µl of blood	23.4	24.9	20.2	<0.001*	21.8	24.8	0.015	24.0	21.4	0.084	23.4	23.4	0.970
S. haematobium	5.7	4.7	7.7	<0.001*	5.4	5.9	0.443	5.7	5.7	0.956	7.6	3.0	<0.001*
S. mansoni	3.7	2.7	5.7	<0.001*	1.9	5.2	<0.001*	3.4	4.4	0.133	4.2	2.9	0.016*
Light infection[a]	48	54.6	41.6		47.7	48.1		44.6	57.5		45.0	54.4	
Moderate infection[a]	33.9	25.0	42.7		29.6	35.3		35.4	29.8		34.2	33.3	
Heavy infection[a]	18.1	20.5	15.7	0.045	22.7	16.5	0.598	20.0	12.8	0.286	20.8	12.3	0.320
Hookworm	17.2	13.7	24.6	<0.001*	11.1	22.6	<0.001*	20.0	7.4	<0.001*	16.5	18.3	0.090
Light infection[a]	96.8	97.6	95.8		97.6	96.4		96.6	98.7		96.0	97.8	
Moderate infection[a]	1.9	1.1	2.9		0.8	2.4		2.0	1.3		1.9	1.9	
Heavy infection[a]	1.3	1.3	1.3	0.186	1.6	1.2	0.273	1.5	0.0	0.502	2.1	0.3	0.067
A. lumbricoides	1.8	1.5	2.6	0.010*	1.8	1.9	0.723	2.1	0.9	0.006*	2.8	0.5	<0.001*
Light infection[a]	84.3	83.7	85.0		85.0	83.7		83.8	88.9		83.8	88.9	
Moderate infection[a]	15.7	16.3	15.0		15.0	16.3		16.3	11.1		16.3	11.1	
Heavy infection[a]	0.0	0.0	0.0	0.864	0.0	0.0	0.864	0.0	0.0	0.688	0.0	0.0	0.688
T. trichiura	1.3	1.3	1.2	0.639	1.2	1.3	0.689	1.2	1.5	0.418	1.6	0.8	0.019
Light infection[a]	100.0	100.0	100.0		100.0	100.0		100.0	100.0		100.0	100.0	
Moderate infection[a]	0.0	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
Heavy infection[a]	0.0	0.0	0.0	1.000	0.0	0.0	1.000	0.0	0.0	1.000	0.0	0.0	1.000
Soil-transmitted helminths	19.1	15.5	26.8	<0.001*	13.3	24.2	<0.001*	21.9	9.1	<0.001*	19.0	19.2	0.843
Light infection[a]	95.6	96.3	94.8		96	95.4		95.3	97.9		94.1	97.6	
Moderate infection[a]	3.2	2.6		4.1	2.7	3.5		3.4	2.1		4.0	2.1	
Heavy infection[a]	1.2	1.2		1.2	1.3	1.1	0.760	1.3	0.0	0.404	1.8	0.3	0.022*

Parasite prevalences are provided in % of all included school children. Data on infection intensities are provided as % of all positive cases.

[a]Intensities of intestinal helminth infections are categorized according to WHO guidelines (WHO, 2011).

*Statistically significant ($p < 0.05$) based on χ^2statistics.

10.2.2 Supporting information. Table S2. Clinical signs and self-reported symptoms and diseases, stratified by sex, age group, residential area and ecozone among 4,848 schoolchildren in Côte d'Ivoire.

Morbidity	Total	Age group (years)			Sex			Residential area			Ecozone		
	(n=4,848)	5-10 (n=3,282)	11-16 (n=1,566)	p-value	Females (n=2,269)	Males (n=2,579)	p-value	Rural (n=3,783)	Urban (n=1,065)	p-value	South (n=2,862)	North (n=1,986)	p-value
Observed clinical signs													
Mean hemoglobin level (g/l)	121.8	121.1	123.3	<0.001*	122.7	121.1	<0.001*	121.9	121.7	0.796	119.9	124.6	<0.001*
Anemia[a]	28.7	27.3	31.6	0.002*	25.9	31.2	<0.001*	29.2	27.0	0.178	33.4	21.9	<0.001*
Wasting[b]	11.8	10.2	15.3	<0.001*	10.3	13.2	0.001*	12.5	9.6	0.010*	11.9	11.8	0.918
Stunting[b]	18.1	12.6	29.4	<0.001*	15.5	20.3	<0.001*	18.4	16.8	0.233	17.6	18.7	0.303
Any form of malnutrition[c]	28.4	23.4	38.8	<0.001*	24.5	31.8	<0.001*	29.1	25.7	0.031*	28.3	28.5	0.860
Liver enlargement[d]	2.6	2.7	2.5	0.743	1.7	3.4	<0.001*	2.5	3.1	0.246	2.6	2.7	0.800
Spleen enlargement[d]	11.5	12.3	9.9	0.014*	10.7	12.3	0.093	12.4	8.5	0.001*	9.3	14.7	<0.001*
Fever (≥ 38 °C)	1.9	1.9	1.9	0.987	2.2	1.6	0.142	1.8	2.0	0.752	2.0	1.6	0.292
Clinical malaria[e]	1.4	1.4	1.4	0.940	1.5	1.3	0.511	1.5	1.0	0.223	1.6	1.2	0.194
Self-reported symptoms													
Headache	54.3	52.4	58.4	<0.001*	59	50.2	<0.001*	54.6	53.3	0.468	50.5	59.8	<0.001*
Abdominal pain	51.1	49.1	55.2	<0.001*	55.6	47.2	<0.001*	50.7	52.5	0.303	47	57	<0.001*
Fatigue	48.6	46	54	<0.001*	51	46.5	0.002*	48.3	49.6	0.469	46.1	52.2	<0.001*
Fever	48.2	46.7	51.2	0.003	53	43.9	<0.001*	47.9	49.1	0.485	42.3	56.7	<0.001*
Vomiting/nausea	35	34.7	35.5	0.599	36.7	33.5	0.021*	35	34.8	0.909	30.6	41.3	<0.001*
Diarrhea	31.5	30.6	33.3	0.06	32.5	30.5	0.133	31.2	32.3	0.502	28.9	35.2	<0.001*
Blood in stool	30	28.6	32.7	0.004*	31.2	28.9	0.074	30.4	28.4	0.199	29.1	31.2	0.123
Loss of appetite	28.9	27.5	31.8	0.002*	30.9	27.1	0.004*	27.6	33.3	<0.001	26.7	31.9	<0.001*
Respiratory problems	26.8	26	28.6	0.054	28.9	25.1	0.003*	27.7	23.7	0.008*	23.8	31.2	<0.001*
Dysentery	24.1	23.6	25.3	0.195	26.2	22.3	0.002*	24.5	23	0.33	23.2	25.5	0.059
Blood in urine	10.1	11.2	9.6	0.077	9.7	10.5	0.35	10.6	8.6	0.068	11.3	8.5	0.002*
Self-reported diseases													
Cough	57.3	57.7	56.3	0.351	58.4	56.3	0.126	57.5	56.5	0.573	53.2	63.1	<0.001*
Cold	46.1	46.6	45.3	0.402	48	44.5	0.013*	46.2	45.8	0.812	40.3	54.6	<0.001*
Malaria	30.4	29.3	32.5	0.025*	32.4	28.6	0.004*	30.6	29.7	0.578	24.6	38.6	<0.001*
Malnutrition	21.4	21.2	21.9	0.564	20.9	21.9	0.407	21.5	21.2	0.864	17.9	26.5	<0.001*
Eye disease	19.1	18.7	20	0.301	20.8	17.7	0.007*	18.5	21.6	0.021*	17.5	21.5	0.001*
Worms	16.8	16.4	17.5	0.336	17.4	16.2	0.282	17.2	15	0.088	15.3	18.8	0.002*
Schistosomiasis	14.2	13	16.5	0.001*	14.5	13.9	0.567	13.9	15.1	0.305	14.9	13	0.065
Skin disease	13.1	12.9	13.6	0.473	11.7	14.4	0.006*	13	13.4	0.719	13.3	12.8	0.596
Mean no. of reported morbidities	6.1	5.9	6.4	<0.001*	6.4	5.8	<0.001*	6.1	6.1	0.985	5.6	6.8	<0.001*

Prevalences of clinical or self-reported morbidities are provided in % of all included school children with the exception of hemoglobin levels and the number of self-reported morbidities, where mean values are displayed.

[a]Defined as hemoglobin levels below 115 g/l and below 120 g/l in children aged 5-11 years and 12-16 years, respectively.

[b]Calculated according to WHO child growth standards (Duggan, 2010); defined as BMI-for-age (wasting) and height-for-age (stunting) resulting in a Z-score < -2.

[c]Defined as any of the assessed nutritional indicators resulting in a Z-score < -2; this includes wasting, stunting, and weight-for-age (underweight).

[d]Defined as palpable liver and spleen (≥grade I by Hackett's classification), respectively.

[e]Clinical malaria is defined as being *Plasmodium* positive and having fever (≥ 38°C)

*Statistically significant ($p<0.05$) based on t-test (Hb, mean no. of reported morbidities) and chi-square (prevalence of specific clinical and self-reported morbidities) statistics

10.2.3 Supporting information. Appendix S1. Questionnaire for HrQoL assessment and VAS (in French)

Questionnaire en milieu scolaire **Code de l'école……..**

Localité:…… Classe : CP2☐ CE1☐ CE2☐ CM1☐ CM2☐

École:…….. Code de la classe : 0 1 2 3 4

Nom et signature de l'enquêteur :……Date :……

Elève N°		01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
5. Qualité de vie																										
1)	En général, comment tu te sens? Dis-tu ta santé est : (très faible=1, faible=2, ni f ni b=3, bonne=4, très bonne=5)																									
2)	As-tu des problèmes pour marcher sur une distance très longue (comme marcher jusqu' au prochain village ou marcher de la maison jusqu'aux champs plus loin)? (Oui b.d.p.=1, Oui q.p.=2, Non p.d.p.=3)																									
3)	As-tu des problèmes pour monter sur une côte ? (Oui b.d.p.=1, Oui q.p.=2, Non p.d.p.=3)																									
4)	As-tu assez de force pour porter l'eau du marigot/puit à la maison ? (→filles) As-tu assez de force pour porter le fargot du champ à la maison? (→garçons) (Non p.d.t.=1, Parfois oui p. non=2, Oui toujours=3)																									
5)	As-tu des problèmes pour te laver ou t'habiller tout(e) seul(e)? (Oui b.d.p.=1, Oui q.p.=2, Non p.d.p.=3)																									
6)	As-tu des douleurs ou gêne qui t'empêche de faire ce dont tu as envie ? (Oui b.d.d/g=1, Oui q.d/g=2, Non p.d.d/g=3)																									
7)	As-tu des problèmes pour être attentif (ve)/ te concentrer (par exemple à l'école ou faisant tes devoirs) ? (Oui b.d.p.=1, Oui q.p.=2, Non p.d.p.=3)																									
8)	As-tu souvent des soucis ou es-tu triste ou malheureux (se)? (Oui tout le temps=1, Oui parfois=2, Non jamais=3)																									
9)	Parles-tu facilement avec ton papa ou ta maman? (Npn,P.d.t..=1, Oui, quelques fois=2, Oui, facilement=3)																									
10)	As-tu assez de temps pour t'amuser/jouer avec tes camerades ? (Non, p.d.t.=1, Oui, quelques fois=2, Oui, toujours=3)																									
11)	Te sens-tu en sécurité à la maison ou dans le village où tu habites ? (Non jamais=1, Oui parfois=2, Oui toujours=3)																									
12)	Aimes-tu l'endroit où tu habites ? (Non pas du tout=1, Oui un peu=2, Oui beaucoup=3)																									
VAS score (valeur de l'échelle)																										

Meilleur état de santé imaginable

Quel est ton état de santé AUJOURD'HUI?

Nous aimerions savoir si ton état de santé est bon AUJOURD'HUI

L'échelle est graduée de 0 à 10 .
L'échelle représentent les differents notes de santé sur dix. Commes tes differentes notes des devoirs de mathématique par example sur dix.
10 signifie le meilleur état de santé imaginable.
0 signifie le pire état de santé imaginable.

Quelle note sur dix de santé te donnes-tu aujourd'hui?

Pire état de santé imaginable

10.2.4 Supporting information. Checklist S1. STROBE statement - checklist of items that should be included in reports of cross-sectional studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found✓
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported✓
Objectives	3	State specific objectives, including any pre-specified hypotheses✓
Methods		
Study design	4	Present key elements of study design early in the paper✓
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection✓
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants✓
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable✓
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group✓
Bias	9	Describe any efforts to address potential sources of bias✓
Study size	10	Explain how the study size was arrived at✓
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why✓
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding✓
		(*b*) Describe any methods used to examine subgroups and interactions✓
		(*c*) Explain how missing data were addressed✓
		(*d*) If applicable, describe analytical methods taking account of sampling strategy✓
		(*e*) Describe any sensitivity analyses✓
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed✓
		(b) Give reasons for non-participation at each stage✓
		(c) Consider use of a flow diagram✓
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders✓

		(b) Indicate number of participants with missing data for each variable of interest✓
Outcome data	15*	Report numbers of outcome events or summary measures✓
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included✓
		(*b*) Report category boundaries when continuous variables were categorized✓
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period✓
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses✓
Discussion		
Key results	18	Summarise key results with reference to study objectives✓
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias✓
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence✓
Generalizability	21	Discuss the generalizability (external validity) of the study results✓
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based✓

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

10.3 Effect of deworming on school-aged children's physical fitness, cognition and clinical parameters in a malaria-helminth co-endemic area of Côte d'Ivoire

10.3.1 Additional file 1. Detailed procedures of physical fitness testing.

The standing broad jump test was implemented according to adapted standard operating procedures (SOPs) from a study on a school-based physical activity programme (KISS) conducted in Switzerland (Kriemler et al., 2010). In brief, the distance (in cm, precision 1 cm) from the take-off line to the point, where the back of the heel nearest to the take-off line landed on the ground, after jumping with both feet together was measured. The test was repeated until two valid test scores could be achieved, whereof the better then was recorded.

Hand grip strength (in kg, precision of 0.5 kg) was assessed using a digital hand grip dynamometer (T.K.K. 5401 Grip-D; Takey, Tokyo, Japan). For each participant the dynamometer was adjusted for optimal grip span values proposed by Espana-Romero et al. (España-Romero et al., 2008) after measurement of hand span (with a precision of 0.5 cm). For this test as well the higher score of two attempts was recorded.

For the conduction of the 20 m shuttle run test (Yap et al., 2012) children were asked to run in groups of maximal 10 individuals forth and back on a 20 meter course, which had been marked with two lines on the ground. The test started with an initial running pace of 8.0 km/h and a progressive 0.5 km/min raise of the running speed given by a beep signal from a software run on a portable computer (Team beep test software; Bitworks Design, Cheltenham, UK). To assure that all children run with equal speed and according to the pace given by the sound signal they were accompanied by a field assistant. Scores, expressed as current stage and number of laps of the current stage, were recorded for each child, who ended the test either by giving up or by no longer being able to follow the pace and not reaching the 20 m line two consecutive times.

References:

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Kriemler, S., Zahner, L., Schindler, C., Meyer, U., Hartmann, T., Hebestreit, H., Brunner-La Rocca, H.P., van Mechelen, W. & Puder, J.J., 2010. Effect of school based physical activity programme (KISS) on fitness and adiposity in primary schoolchildren: cluster randomised controlled trial. *BMJ*, 340, p.c785.

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