Helminth infections and micronutrients in children

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2015

document version
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CHAPTER 1

General introduction

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Helminth infections and micronutrient deficiencies present two major global poverty-associated health problems. Children are especially vulnerable to both conditions, as they are most often infected with helminths and have specific nutritional requirements for their growth and development. These common but hidden diseases in a particularly vulnerable group are estimated to cause the combined yearly loss of 57.8 million disability-adjusted life years (DALYs); 2.3% of the total global disease burden[1, 2].

Soil-transmitted helminths

Soil-transmitted helminths (STH) are intestinal parasitic worms that are transmitted via eggs shed in fecal matter, hatching in soil and infecting humans through larval skin penetration or ingestion of food or water contaminated with eggs. While infection can occur at any age, school-age children are most often infected by STH. Children playing outdoors come into close contact with soil. The most common STH are roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworm (*Ancylostoma duodenale* and *Necator americanus*). Co-infections by two or more STH are common, and a combined prevalence exceeding 50% is not unusual in tropical regions (Figure 1.1)[3, 4]. It was recently estimated that over 1.45 billion people were infected worldwide with one or more STH in 2010[1]. Anthelminthic drugs against STH are available and cheap, and regular mass treatment via schools is widespread[3].

STH belong to the 'neglected tropical diseases', which mainly affect the poorest people in low-income countries. A tropical climate combined with a lack of sanitation and hygiene promotes survival and transmission of STH. A recent estimation attributes 5.18 million disability-adjusted life years lost to STH[1, 5]. This loss is mainly due to morbidity, since STH infections cause relatively low mortality[3, 6]. Helminths are very 'old' parasites; they have co-evolved with their human and animal hosts for millennia[7]. This has resulted in adaptations of both the host and the parasite, allowing co-existence without much host mortality and thus increasing transmission chances for the parasite, which cannot survive without the host[7]. Many STH infections remain unnoticed, but subtle or indirect morbidity can be substantial. This involves, among others, impairment of nutritional status, immune function and possibly cognitive development[6, 8, 9]. Even small health effects could, given the high prevalence of STH infections in children, greatly hamper the development of a population or country.

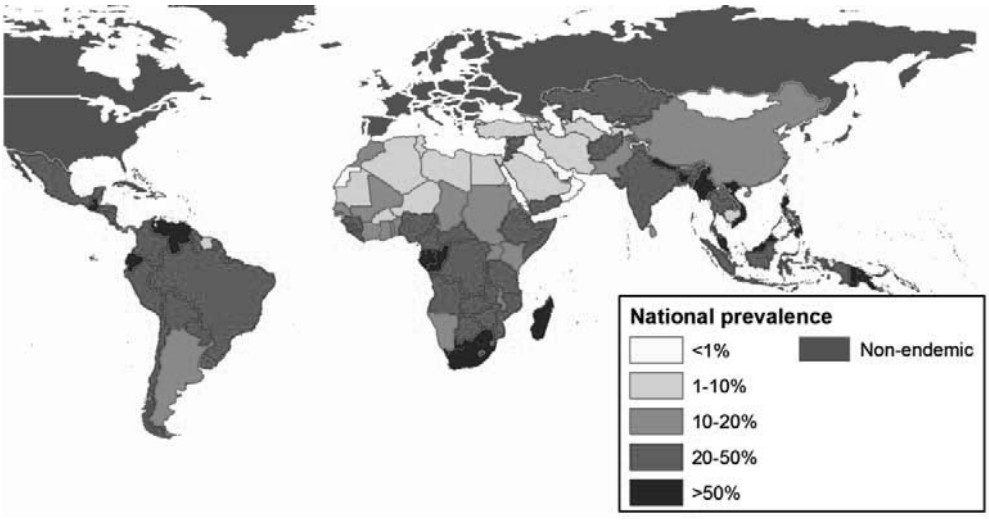

Figure 1.1. Worldwide prevalence of STH infections *Ascaris, Trichuris* and hookworm combined, estimates for 2010. From ref. 5.

Ascaris lumbricoides, or the large common roundworm, is by far the largest of the STH species, reaching up to 40cm in length[3]. An individual *Ascaris* worm can live for a year or two in the lumen of the small intestine. Transmission of *Ascaris* occurs through the feco-oral route; large quantities of eggs are shed via feces (up to 200 000 per day per female worm)[3]. When eggs are ingested, larvae hatch in the gastrointestinal tract. The larvae invade the mucosa and travel via the portal vein into the systemic circulation. Subsequently, the larvae migrate to the lungs, where they enter the alveoli. Through coughing and swallowing, the larvae are transported via the throat back into the gastrointestinal tract. Upon arrival in the small intestine, they develop into adult worms. This process, from egg ingestion to egg-producing worms, takes two to three months[10]. Clinical symptoms include intestinal obstruction and malabsorption[6].

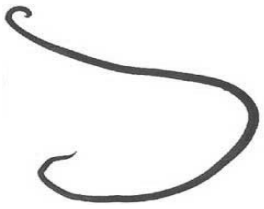

Figure 1.2. Ascaris lumbricoides, adapted from ref 3.

Trichuris trichiura, or whipworm, is a small thin worm (30-50 mm in length) which infects the colon[3]. Its life cycle is more straightforward than that of *Ascaris* and does not involve stages outside of the gastrointestinal tract. When eggs (which have entered the environment by fecal contamination) are ingested, the larvae hatch in the small intestine and develop into adults in the caecum and colon[3]. The adults are burrowed into the mucosal epithelium with their anterior parts and protrude into the lumen. The resulting mucosal damage may lead to inflammation of the bowel (colitis)[3]. Another complication of heavy infection is Trichuris dysentery syndrome, characterized by chronic diarrhea and rectal prolapse[6, 11]

Figure 1.3. Trichuris trichiura, adapted from ref 3.

Hookworm refers to two genera of helminths: *Necator* and *Ancylostoma*, of which the eggs are visually indistinguishable. Not the eggs, but the larvae, which hatch and moult in soil, form the infective stage of hookworm. The infective larvae penetrate the skin, most often of the foot, which is why wearing shoes can protect against hookworm infection[12]. Via the lymphatic system and the blood, the larvae enter the lungs and subsequently the gastrointestinal tract (through coughing and swallowing). Adult hookworms, which are approximately 1 cm long, reside in the upper small intestine[3]. Hookworms have sharp 'teeth' with which they latch onto the mucosa, causing tissue damage and blood loss. Because of the blood loss, which is proportional to the infection intensity, hookworm infection is associated with anemia and iron deficiency[13]. Since women of reproductive age and children have smaller iron stores than men, they are at higher risk of iron deficiency.

Figure 1.4. Hookworm, adapted from ref 3.

The estimated worldwide prevalences in 2010 of hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* are depicted in Figure 1.5 A, B, and C respectively [1].

Several techniques have been developed to diagnose STH infection, including Kato-Katz, McMaster and FLOTAC[14]. These techniques all have in common that fecal samples are studied microscopically to detect helminth eggs. The appearance of the eggs differs between the types of helminths, enabling differentiation between *Ascaris*, *Trichuris* and hookworm. The number of eggs per gram of feces indicates severity of infection (number of worms inhabiting the gut).

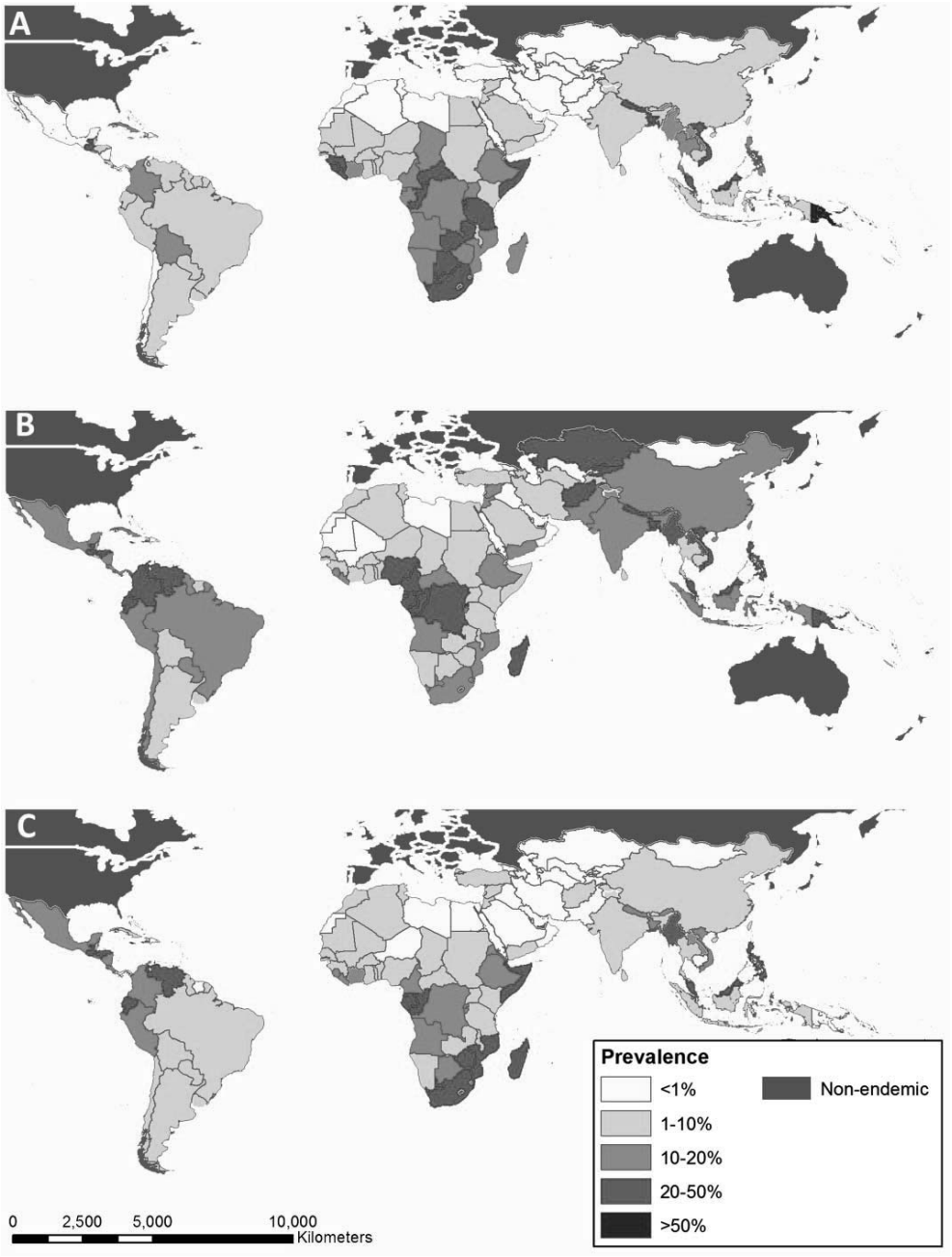

Figure 1.5. Estimated distribution of hookworm (A), *Ascaris lumbricoides* (B) and *Trichuris trichiura* (C) in 2010. From ref.5.

The compounds mostly used for STH treatment are the benzimidazoles: albendazole and mebendazole. The efficacy of single doses of these compounds has recently been estimated based on data from six (mebendazole) and seven (albendazole) countries, respectively, across all endemic continents. The benzimidazoles were shown to be effective in reducing fecal egg counts of hookworm (80-96%) and *Ascaris* (98-100%) but less so for *Trichuris* (62-64%)[15]. However, the persistence of eggs or larvae in soil facilitates high reinfection rates[16]. In addition, the widespread periodic mass deworming which is now commonplace in many endemic countries might be inducing drug resistance, as has occurred with veterinary anthelminthics[17]. For more sustainable solutions against STH infections, improvements in access to clean water, sanitation and hygiene are essential[12].

STH infections have often been linked to malnutrition[6]. Since poverty and lack of access to clean water and healthy food are important causes of both STH infection and malnutrition, this is not surprising. Many observational studies have reported associations between STH infections and reduced height and weight of children, which are important markers of poor nutritional status[18-21]. However, it is still unclear whether STH infections have a direct effect on nutritional status or vice versa. Several systematic reviews have tried to answer the question whether deworming improves growth in children[9, 22, 23]. Unfortunately, possibly due to several methodological differences, the reviews are not in agreement and this matter remains unresolved thus far.

Micronutrient deficiencies

Aside from growth and body composition, which can be described by (among others) height, weight, and skinfolds (anthropometric measurements), other forms of malnutrition exist which are much less visible and therefore often overlooked. One of these are the micronutrient deficiencies: a lack of specific nutrients other than protein, carbohydrates and fats. Micronutrients are vitamins and minerals that are of vital importance to a wide array of body functions. Hereafter, we discuss four well-known micronutrients. Deficiencies of these four micronutrients are highly prevalent in tropical regions and associated with poverty and lack of access to healthy and diverse foods.

Iron

The most prevalent human micronutrient deficiency is iron deficiency. Iron is a critical factor in many cellular processes, such as DNA replication and cellular respiration[24]. As part of the heme protein, iron serves as the carrier of oxygen within hemoglobin. Iron in its free form is toxic, therefore it is continuously bound to enzymes in human iron metabolism. Iron deficiency can result from blood loss, but is also caused by the low bioavailability of dietary iron[25]. Since the majority of iron is in erythrocytes, anemia is an

important consequence of depleted iron stores. The main symptom of iron-deficiency anemia is fatigue[25]. Anemia is defined as low blood hemoglobin concentration, while iron deficiency is diagnosed via the measurement of plasma ferritin, an iron-storing protein of which the concentration in serum correlates with intracellular stores. Different cut-offs defining anemia or iron deficiency are recommended by the World Health Organization per sex and age[26]. Anemia is in itself not a measure of iron deficiency, since it can have other causes such as genetic disorders, infections or deficiencies in folate or vitamin B12[27].

Zinc

As part of many cellular, endocrine and metabolic pathways, zinc has a wide array of functions in the human body[28]. Zinc is also an elusive micronutrient; there are no known tissue reserves and no gold standards for its measurement in humans. Zinc plays a role in all major biochemical pathways, as a vast array of enzymes is dependent on zinc. Zinc is therefore an important micronutrient for child growth and immunity[28]. Zinc supplementation has been shown to improve growth and reduce pneumonia and diarrhoea in children, which are the two most important causes of death in under-five year olds[28-30]. Zinc deficiency may present itself in a population as high rates of growth stunting, diarrhoea, and pneumonia in children[31]. Plasma zinc measurements, dietary intakes and stunting are currently the best known indicators of zinc status[28].

Vitamin A

Vitamin A refers to retinol and related compounds such as beta-carotene[32]. It has functions in vision, immunity, growth, and erythropoeisis[33]. Deficiency of vitamin A results primarily in eye problems such as dry eyes or night blindness. Supplementation of vitamin A to apparently healthy children under the age of 5 reduces their mortality risk[33]. Vitamin A is stored in the liver. Plasma retinol concentration is usually kept relatively stable, and starts to decline only when liver stores are nearly depleted[32]. Therefore, plasma retinol can be used to determine vitamin A deficiency (severe or marginal) but not absolute stores.

Iodine

In contrast to iron, zinc and vitamin A, iodine has only one known biological function, as a component of thyroid hormones[34]. Deficiency of iodine in childhood can cause neurodevelopmental deficits such as lower IQ score, as thyroid hormones are necessary for brain development[35]. Iodine can be measured in urine, wherein most dietary iodine is excreted[36]. Urinary iodine excretion below 100 µg/L is considered a sign of deficiency[34].

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Micronutrient deficiencies seldom occur isolated; often, an individual suffers from a combination of nutritional deficiencies[37]. Children are especially vulnerable to the effects of micronutrient deficiency, as their diets are often insufficient in providing the micronutrients required for optimal growth, development and immunity to infections[38]. Micronutrient deficiencies contribute greatly to child mortality, as it is an important risk factor for the many infectious diseases that cause child deaths[39]. Figure 1.6 shows a world map with regions estimated to be at high risk for iron, vitamin A and/or iodine deficiency. Figure 1.7 shows estimated prevalence of insufficient zinc intake at country level[40]. Because of the co-existence of micronutrient deficiencies in individuals and populations, most strategies to combat these deficiencies are multi-micronutrient-based. Supplementation of micronutrients has been common practice for many years, while fortification of foods is a more recent strategy[41]. Fortification means adding nutrients to staple foods or condiments and can be driven by industry or governments, such as salt iodization or iron-fortified wheat flour[42]. Alternatively, families can practice home fortification by adding powdered micronutrients to prepared meals[38]. Some promising results have been achieved by fortification and supplementation of micronutrients. The currently available evidence supports positive effects on linear growth and anemia, but there is still insufficient evidence regarding other health outcomes[37, 38].

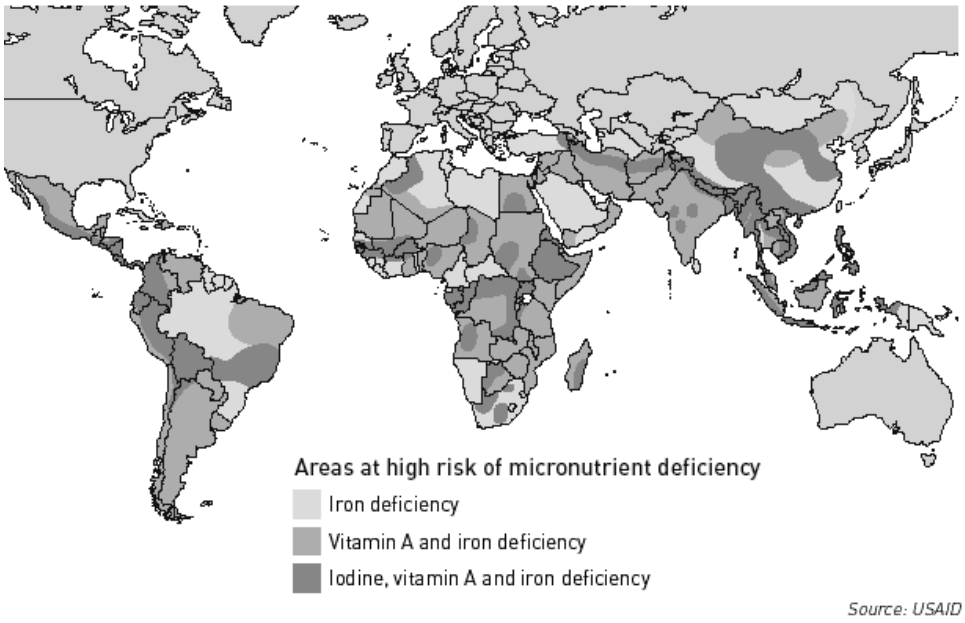

Figure 1.6: USAID estimates of regions at high risk for iron, vitamin A and/or iodine deficiency, from ref.41.

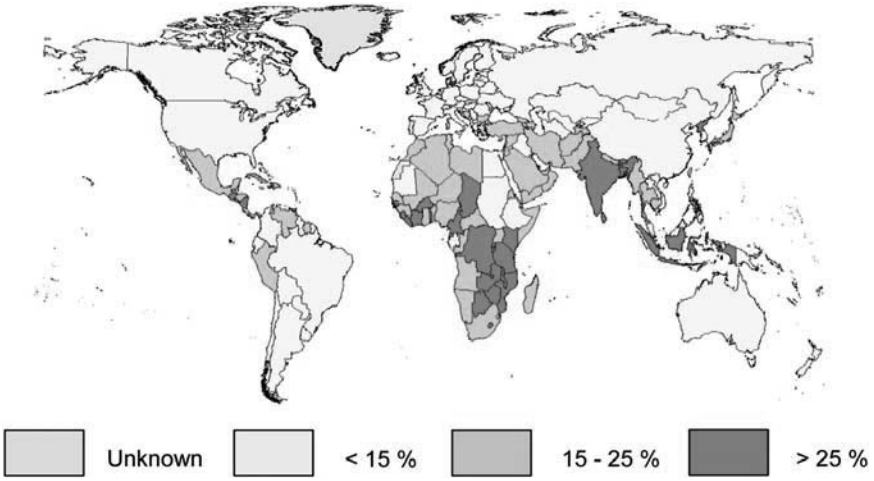

Figure 1.7: Estimated country prevalence of inadequate zinc intake, from ref 40.

Possible mechanisms underlying relationships between STH infections and micronutrients

The relationship between STH infections and micronutrients has been studied far less than associations between STH infections and height and/or weight. It is as yet unknown whether there is an association between STH infections and micronutrients, let alone how these two conditions might influence each other. Several possible mechanisms are discussed below and summarized in figure 1.8.

Impaired immunity

Micronutrient deficiencies can decrease immunity to infections and thereby make a child more susceptible to STH. Deficiencies in several micronutrients can induce oxidative stress and have different impacts on both humoral and cellular immunity[43]. Vitamin A deficiency can result in damage to the mucosal barrier and in impaired innate and adaptive immunity[43, 44]. Iron deficiency and iron overload both impair innate and T cell responses to infection[45]. Another well-known micronutrient essential for immune function is zinc. Prolonged zinc deficiency has several detrimental effects on the immune system[43].

Malabsorption and blood loss

The presence of STH in the intestine can influence the absorption of nutrients, by blocking or damaging the mucosa: the top tissue layer of the intestine which absorbs nutrients and has an important role in immune function. Mucosal damage could result in diarrhoea, hampering nutrient absorption, and in intestinal inflammation, impairing mucosal

function. Repeated or continued exposure to pathogens can result in remodelling of the mucosal tissue. This phenomenon has been described as 'environmental enteropathy'; a reversible intestinal condition commonly observed in people living in rural tropical areas and thought to be induced by continuous or repeated exposure to pathogens and/or toxins[46]. This condition hampers absorption of both macro- and micronutrients. It is unknown whether STH infections play a role in the development of environmental enteropathy. Recently, it was shown that treatment with the anthelminthic drug albendazole delayed the progression of environmental enteropathy in Malawian children[47]. Aside from the loss of dietary nutrients through malabsorption, infection with STH (most importantly hookworm) can result in blood loss[13]. This can, in turn, lead to anemia.

Intestinal microbiota composition

When considering possible mechanisms of action concerning the intestinal environment, the intestinal microbiota cannot be overlooked. It seems quite plausible that the mere presence of STH in the intestinal lumen, the niche of both commensal and possibly pathogenic bacteria, can disturb the delicate balance of colonization by and tolerance of these microbes. The possibility of such an effect has been addressed in animal studies. Indeed, experimental helminth infection resulted in changes in microbiome in both pigs and mice[48]. These changes had consequences for microbiota metabolism, on which humans are dependent for the production of certain micronutrients such as vitamins B_{12} and K. Composition of the intestinal microbiota has consequences for both the production and absorption of micronutrients[49].

Decreased appetite

STH infections might decrease appetite. This possibility is often mentioned but has so far not been thoroughly studied. In general, acute infections are associated with a decrease in appetite, mediated by acute phase cytokines[50]. Albendazole treatment has been associated with increases in appetite[51, 52]. However, the increased appetite in these reports coincided with faster growth; therefore it remains unclear whether the anthelminthic treatment had an independent positive effect on appetite. Also, it has been proposed that the immune response against helminths takes energy, thereby resulting in higher nutritional requirements[22].

Competition for nutrients

It is possible that there is direct competition between host and parasite for certain nutrients. These nutrients could be acquired from the content of the intestinal lumen,

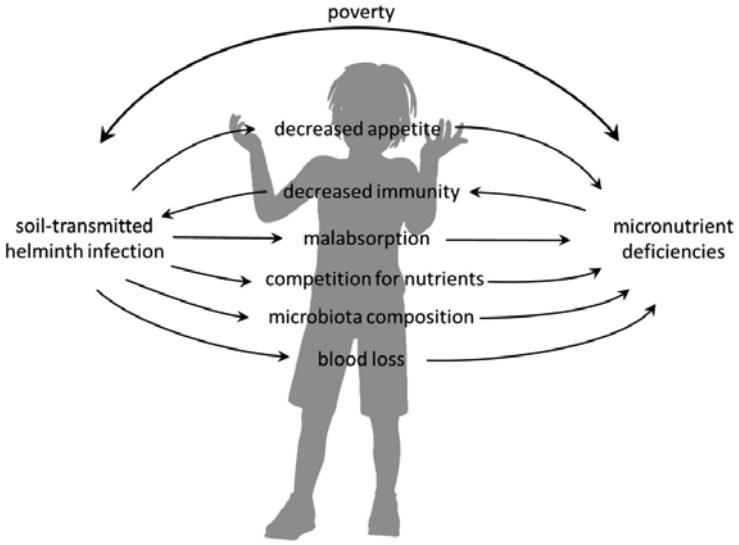

Figure 1.8. Possible mechanisms of interaction between soil-transmitted helminth infections and micronutrient deficiencies

but also from feeding on host tissues and blood, most notably by hookworm. The notion that helminths might 'steal our food' has been refuted by Hall et al by calculating the relatively small biomass and energy needs of helminths compared to that of a child[22]. However, in the case of micronutrients, this argument might not apply. Certain scarce or poorly bioavailable micronutrients can be highly coveted by pathogens. Especially by causing blood loss, significant amounts of micronutrients can be lost, as is the case in hookworm-related anemia[13]. Not only helminths but almost all pathogens are dependent on essential micronutrients derived from their host[53]. This battle for nutrients has resulted in elegant and intricate mechanisms of 'nutritional immunity': the sequestering of micronutrients in compartments less available to pathogens[54]. The most well-known example is the human iron withholding strategy: upon infection, the acute phase proteins are produced by the liver, among which the peptide hormone hepcidin. Hepcidin inhibits the transport of iron out of cells, thereby ensuring its intracellular sequestration within the storage protein ferritin[53]. This mechanism results in hypoferremia during infections, which serves as a growth-limiting defense against pathogens, for which iron is also essential and scarce. The role of iron in infections has been emphasized by studies finding increased risk of certain infections (such as tuberculosis or malaria) or complications thereof after iron supplementation[55, 56]. Similarly, zinc and copper are both found in lower concentrations in plasma during acute phase responses, although the mechanisms behind these declines are not yet elucidated[32, 50].

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Cuba, Cambodia and Vietnam

Most studies in this thesis are based on data from studies previously performed in three populations of schoolchildren: in Cuba, Cambodia and Vietnam. Below, we describe the main relevant features of each country, and provide a short outline of the studies that the results presented in this thesis originate from.

Cuba is an upper middle income country with a high development index[57]. The Cuban health system is well organized and highly accessible, resulting in some remarkable achievements in public health such as high life expectancy and vaccine coverage[58]. Overweight and obesity occur more frequently in Cuba than undernutrition[59, 60]. However, STH infections remain endemic[61]. Figure 1.9A shows a map of Cuba, indicating the provinces where STH prevalence surveys have been undertaken and their results. In 2009, a study was undertaken to elucidate the relationships between STH infection, nutrition and atopy and asthma in school-age children. This was done at 13 primary schools in San Juan y Martínez in the province of Pinar del Río, located in the West of Cuba. We used data from this study to explore the associations between height, zinc and STH and between local and systemic inflammation and STH.

Cambodia, like Cuba, has made much progress in public health in the past decades. Nonetheless, Cambodia is still classified as a lower income country, with a high prevalence of malnutrition[60]. In 2010, the child stunting prevalence was 40%[62]. STH surveys have found over 50% of the population infected in several provinces (figure 1.9B), despite school-based deworming programs. The United Nations World Food Program provides school meals containing rice to around half a million schoolchildren in Cambodia. In 2012, a randomized controlled trial was held to assess the impact of introducing micronutrient-fortified rice in these school meals on child growth, cognition, nutritional status, STH infection and intestinal microbiota[63]. In this trial, children were included from 20 schools across Kampong Speu province, just West of Phnom Penh (figure 1.9B). In this thesis, the effect of the micronutrient-fortified rice on hookworm infection risk is described. In addition, baseline data from this trial was used to study associations of STH with height, zinc and local and systemic inflammation.

Since the 1986 ‘doi moi’ reform, a switch from a centralized system to an open market economy, Vietnam has seen fast economic growth. It has progressed from one of the poorest countries in the world to a lower middle income country with a medium human development index[64]. Child stunting prevalence was 22.7% in 2011, and the use of improved drinking water and sanitation was high[65, 66]. Very high prevalences of STH infections (>50%) have been reported throughout the country (figure 1.9C). In 2009, a randomized controlled trial was conducted in Hung Yen province just east of Hanoi, to

evaluate effects of deworming and micronutrient fortified biscuits on micronutrient status and STH infections[67]. This thesis includes an analysis of baseline data from this trial to estimate species-specific associations between STH and micronutrient status in children.

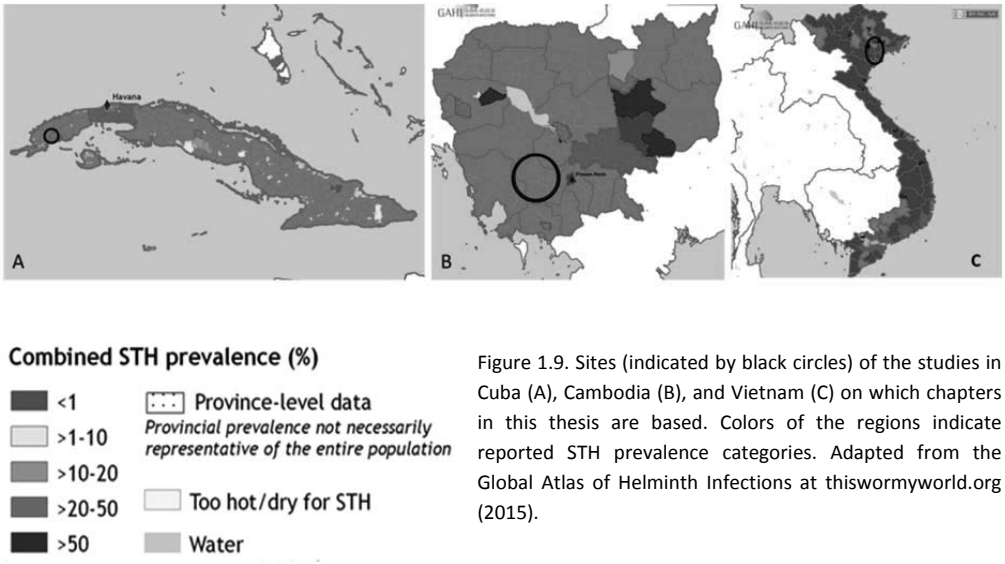

Figure 1.9. Sites (indicated by black circles) of the studies in Cuba (A), Cambodia (B), and Vietnam (C) on which chapters in this thesis are based. Colors of the regions indicate reported STH prevalence categories. Adapted from the Global Atlas of Helminth Infections at thiswormyworld.org (2015).

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Thesis outline

Soil-transmitted helminths and micronutrient deficiencies are both recognized as public health priorities that must be addressed to improve global child health. Any type of interrelationship between these two health problems may have important implications for public health policy. The aim of this thesis is to study possible associations between soil-transmitted helminth infections and micronutrients and to shed light on the directions of these associations. By studying both cross-sectional and experimental data, we aim to contribute to disentangling the complex interplay between micronutrient status and soil-transmitted helminth infections in schoolchildren. The main research questions of this thesis are:

- Are STH infections associated with micronutrient status in school-age children? If so, are these associations STH species-specific and/or micronutrient-specific?
- Does deworming have an effect on micronutrient status?
- Does micronutrient supplementation or fortification have an effect on STH infection risk?
- Are STH infections associated with local intestinal and systemic inflammation?

Chapter 2 describes a systematic review of the available literature from studies across the globe on associations between helminth infections and micronutrient status. We review and meta-analyse cross-sectional studies, in addition to deworming trials where micronutrient status was measured as outcome and micronutrient supplementation trials wherein helminth (re)infection was monitored. In chapter 3, we explore STH species-specific associations with markers of iron, vitamin A, zinc, and iodine status of Vietnamese schoolchildren. In chapter 4, we study the associations between STH infection, plasma zinc concentrations and height in Cuban and Cambodian schoolchildren. Chapter 5 explores whether markers of local intestinal and systemic inflammation are elevated in STH infected Cuban and Cambodian schoolchildren compared to uninfected children. In Chapter 6, the effects of consumption of micronutrient-fortified rice on hookworm infection risk and intestinal inflammation in Cambodian children are presented. Chapter 7 summarizes and discusses the results of the studies described in chapters 2 to 6 in light of the thesis research questions. The implications of the study results for public health and remaining knowledge gaps will be addressed.

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