Species-Specific Associations between Soil-Transmitted Helminths and Micronutrients in Vietnamese Schoolchildren

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Abstract. Soil-transmitted helminth (STH) infections and micronutrient deficiencies are closely related and often coexist among low-income populations. We studied the association between infections with specific STH species and micronutrient status in rural Vietnamese schoolchildren. Children (N = 510) aged 6–9 years were recruited from two primary schools. STH infections were determined in stool samples. Hemoglobin, ferritin, retinol, and zinc were measured in blood samples, as well as C-reactive protein to control for inflammation. Iodine excretion was measured in urine. Associations of single and multiple infections with Ascaris lumbricoides, Trichuris trichiura, and hookworm with micronutrient status (hemoglobin, plasma ferritin, retinol, zinc, and urinary iodine) were estimated by multiple regression analysis. Ascaris infections showed a specific and intensity-dependent negative association with vitamin A. Trichuris and hookworm infections were associated with lower hemoglobin concentration, but not with plasma ferritin. Trichuris-infected children had zinc deficiency less often than uninfected children. In conclusion, our study shows species-specific associations between STH infections and micronutrient status in children. The different life cycles of STH species might have specific effects on the absorption or loss of specific micronutrients. Tailor-made combinations of deworming and nutritional interventions may be needed to improve child health and nutrition.

INTRODUCTION

Soil-transmitted helminths (STHs) Ascaris lumbricoides, Trichuris trichiura, and hookworm and micronutrient deficiencies such as iron, zinc, and vitamin A pose major health issues in the developing world. Both STH infections and micronutrient deficiencies are highly prevalent in tropical and subtropical regions and are strongly related to poverty.1,2 As school-age children are most often infected with STH and micronutrient deficiencies are essential for growth and development, these children are particularly vulnerable to both conditions.

Various studies have reported associations between STH infections and micronutrient status in children.3,4,5 However, the reported associations differed between studies performed in different populations, harboring different STH species and measuring different markers of micronutrient status.6 Although STH species are often studied as a group, they represent distinct organisms with different life cycles and transmission patterns. Adult A. lumbricoides worms reside in the small intestine. They are the largest of the STH species, reaching up to 40 cm in length, and can obstruct the gut lumen in high-intensity infection.7 Ascaris feeds on intestinal content, not on host tissue.8 Trichuris trichiura inhabits the colon and feeds on the gut mucosa. Hookworm worms feed on host blood in the upper small intestine and is therefore often indicated as an important cause of anemia.9 As the different STHs inhabit different parts of the gut and use distinct feeding mechanisms, they may have specific effects on absorption or loss of certain micronutrients. In addition, the extent of this effect may be dependent on the infection intensities of these intestinal worms. Using baseline data from a micronutrient fortification and deworming trial in rural Vietnamese schoolchildren,7 we examined the species-specific associations of STH infections with iron, hemoglobin, vitamin A, zinc, and iodine status.

METHODS

Study population. The study was conducted in Hung Yen Province in northern Vietnam, a region with a humid, subtropical climate. Hung Yen Province is located just southeast of Hanoi, northern Vietnam, and while urbanization is on the rise, the province can be classified as poor and rural, with most families dependent on agriculture. Hung Yen Province was chosen because of the relative close distance to Hanoi, enabling daily transport of blood, urine, and stool samples, while the socioeconomic circumstances in Hung Yen are typical for most of northern Vietnam. A sample of 510 children (ages 6–9 years old) was randomly selected from a total of 642 children. Exclusion criteria were severe malnutrition, obesity, hemoglobin concentration below 80 g/L (referred to health center) chronic illness, congenital abnormalities, mental or severe physical handicap, or having received deworming treatment in the last 6 months. Baseline data were collected in January 2007. Written informed consent was obtained from parents or caregivers. Ethical approval was obtained from the Ethical Committee on Human Research of the National Institute of Nutrition (NIN), Hanoi, Vietnam, and the Human Ethics Committee of Mahidol University, Bangkok, Thailand. This study population has been described in detail elsewhere.7

Measurements. Demographic characteristics, such as age and sex, were determined by interviewing parents or caregivers of the children by trained research assistants. The day before the survey, children were given containers and detailed instructions for stool sample collection. The next day, laboratory technicians retrieved and preserved the...
samples. STH infections were determined on the day of collection in stool samples by Kato-Katz (duplicate 25 mg smears of one stool) at the Medicine Laboratory Technology Co. Ltd., Hanoi, Vietnam, and were expressed as eggs per gram of stool. Hemoglobin concentrations were measured in a 2-mL nonfasting blood sample, drawn in an EDTA vacutainer, by an electronic Coulter counter (Beckman Coulter, Brea, CA) at the Medicine Laboratory Technology co. For biochemical measurements of micronutrients, 4-mL blood samples were drawn in a heparinized trace element-free tube. All samples were transported in coolboxes with icepacks. Plasma ferritin concentration was measured by enzyme-linked immunosorbent assay (ELISA) according to the kit manufacturer’s protocol (Ramco Laboratories, NY) at the NIN. Plasma retinol concentration was determined by reverse-phase high-performance liquid chromatography (LC-10 ADVP, Shimadzu, Kyoto, Japan) and plasma zinc was analyzed using a flame atomic absorption spectrophotometer (GBC, Avanta+, IL) using trace element free procedures at NIN. Results were verified against reference materials for zinc (Merck, Darmstadt, Germany) and iodine (BDH Laboratory Supplies, PA). Plasma C-reactive protein (CRP) was measured by ELISA according to the kit manufacturer’s protocol (Diagnostic Systems Laboratories, Webster, TX). Iodine concentration was measured in urine samples, using spectrophotometric methods and was performed at the Provincial Preventive Medicine Center, Thai Nguyen, Vietnam. For quality control, 10% of the samples were measured in duplicate for each biochemical parameter. The within-assay variability was < 6% for all parameters and between-assay variability was < 10%.

**Definitions.** STH infection intensities were categorized according to World Health Organization references. Ascaris infections with < 5,000 eggs/gram feces (eggs) were categorized as light, 5,000–50,000 eggs as moderate, and > 50,000 eggs as heavy. Trichuris infections below 1,000 eggs were categorized as light, 1,000–10,000 eggs as moderate, and > 10,000 eggs as heavy. Hookworm infections below 2,000 eggs were defined as light, 2,000–4,000 eggs as moderate, and > 4,000 eggs as heavy. Vitamin A deficiency was defined as plasma retinol below 0.70 μmol/L; marginal vitamin A status was defined as retinol below 1.05 μmol/L. Iron deficiency was defined as plasma ferritin below 15 μg/L and anemia as hemoglobin below 115 g/L. Zinc deficiency was defined as plasma zinc < 9.9 μmol/L, and low urinary iodine excretion was defined as < 100 μg/L. CRP was considered elevated when concentrations exceeded 5 mg/L.

**Statistical analysis.** Statistical analyses were done using SPSS version 21 (IBM, New York, NY). Double and triple infections were combined into a “multiple STH infections” group. Single and multiple infections were compared with the uninfected group in all analyses. Associations with micronutrient concentrations were estimated by linear regression with the micronutrient concentration as dependent variable. Variables ferritin and iodine were log-transformed to achieve a Gaussian distribution. Associations with deficiencies of micronutrients (as dichotomous outcome) were analyzed by logistic regression. For estimations of associations between infection intensities and micronutrient status, intensities (light, moderate, and heavy) were analyzed as categorical independent variables. In all regression analyses, the primary independent variable, STH infection, was accompanied by covariates sex, age (in months), and elevated CRP (yes/no). Interaction between two STH species was studied by adding interaction terms to the full model including the two separate species. Statistical significance was defined as \( P < 0.05 \) whereas an interaction was regarded as significant if \( P < 0.10 \).

**RESULTS**

The STH infection prevalence was high (80.2%) in this study population (Table 1). Ascaris lumbricoides was the most common STH species (64.1%), followed by T. trichiura (53.7%) and hookworm (5.3%). Trichuris and hookworm infection intensities were generally light, whereas most Ascaris infections were of moderate intensity. Figure 1 shows the distribution of single, double, and triple STH infections among the Vietnamese schoolchildren. In this study population, children were most often found to be infected by both Ascaris and Trichuris (\( N = 197, 38.6% \)).

The study population’s micronutrient status (concentrations as well as prevalences of deficiency and marginal status) by STH infection is shown in Table 2. Ascaris single infections were significantly associated with lower plasma retinol (\( aB = −0.10 \mu mol/L, 95\% \text{ confidence interval } [CI] = −0.17, −0.02 \)) and higher prevalence of marginal vitamin A status (adjusted odds ratio \( a\text{OR} = 2.31, 95\% \text{ CI} = 1.23, 4.31 \)). Vitamin A deficiency occurred almost exclusively in Ascaris-infected children; only two out of 52 vitamin A deficient children did not harbor an Ascaris infection. Multiple infections were also significantly associated with all three measures of vitamin A status. This was most likely attributable to the Ascaris infection, as most cases of multiple infections included Ascaris and there was no significant interaction effect on plasma retinol between Ascaris and Trichuris (\( aB = −0.03 \mu mol/L, \ P = 0.51 \)) or Ascaris and hookworm (\( aB = 0.00 \mu mol/L, \ P = 0.98 \)).

Plasma zinc concentrations were higher in children with STH infection than in those uninfected, but this difference was not significant. Zinc deficiency was found to be significantly and negatively associated with Trichuris infection (\( a\text{OR} = 0.39, 95\% \text{ CI} = 0.16, −0.94 \)), although the associations of zinc deficiency with Ascaris and multiple infections

| Table 1 Characteristics of the study population (\( N = 510 \)) |
|-----------------|-----------------|-----------------|-----------------|
| **N (%)**       | **Male**        | 243 (47.6)      |
| **Age**         | 7.5 ± 0.9       |
| **Any STH infection** | 409 (80.2)   |
| **Ascaris lumbricoides** | 327 (64.1)   |
| **Light (< 5,000 epg)** | 116 (22.7)   |
| **Moderate (5,000–50,000 epg)** | 198 (38.8)  |
| **Heavy (> 50,000 epg)** | 13 (2.5)     |
| **Trichuris trichiura** | 274 (53.7)   |
| **Light (< 1,000 epg)** | 241 (47.3)   |
| **Moderate (1,000–10,000 epg)** | 33 (6.5)    |
| **Heavy (> 10,000 epg)** | 0 (0.0)      |
| **Hookworm**    | 25 (4.9)        |
| **Light (< 2,000 epg)** | 2 (0.4)       |
| **Moderate (2,000–4,000 epg)** | 2 (0.4)      |
| **Heavy (> 4,000 epg)** | 0 (0.0)       |
| **C-reactive protein > 5 mg/L** | 78 (16.7)   |

\( \text{egpg = eggs per gram; STH = soil-transmitted helminth.} \)

*Mean ± standard deviation.*
were also borderline significant (aOR = 0.46, 95% CI = 0.21, 1.01 and aOR = 0.49, 95% CI = 0.24, 1.01, respectively).

Hemoglobin concentrations were significantly lower in children with Trichuris (aB = −5.32 g/L, 95% CI = −7.46, −3.17), hookworm (aB = −6.42 g/L, 95% CI = −11.20, −1.64) and multiple infections (aB = −4.20 g/L, 95% CI = −6.02, −2.37) than in those uninfected. No interaction effect was observed between Trichuris and hookworm infections on hemoglobin (aB = 2.72 g/L, P = 0.36). Anemia occurred significantly more often in children with Trichuris (aOR = 11.74, 95% CI = 4.17, 33.04) and multiple (aOR = 4.91, 95% CI = 2.11, 11.39) infections. However, none of the STH infections were associated with iron status as measured by plasma ferritin concentrations. No associations between urinary iodine excretion and STH infections were observed.

Ascaris infection intensity showed a dose-dependent association with plasma retinol concentrations (aB = −0.08 μmol/L, 95% CI = −0.1, −0.06) and with the prevalence of marginal vitamin A status (Wald 26.06, P < 0.001) (Figure 2). The same relationship was found for vitamin A deficiency (Wald 30.34, P < 0.001) (Figure 2). No intensity-dependent relationships were found for Trichuris or hookworm infections and hemoglobin concentration or for Trichuris infection and zinc deficiency.

**DISCUSSION**

This study in rural Vietnamese schoolchildren shows that associations between STH infections and micronutrient status are STH species specific. Ascaris single infections and multiple infections were significantly associated with lower retinol concentrations. Ascaris infection intensity showed an intensity-dependent relationship with plasma retinol and with prevalences of marginal vitamin A status and vitamin A deficiency.

Associations have been reported between Ascaris and xerophthalmia (dry eyes), a consequence of vitamin A deficiency. However, epidemiological studies addressing associations between Ascaris infection and vitamin A (retinol) itself have shown conflicting results. A recent study conducted in Kenya reported higher prevalence of vitamin A deficiency (defined as low retinol-binding protein) in Ascaris-infected preschool children. In accordance with our findings, moderate intensity infections had a stronger association with vitamin A deficiency than light infections did.

Although we cannot infer causality from our cross-sectional data, we speculate that Ascaris infection might impair vitamin A absorption from the diet. Vitamin A is absorbed after emulsification with fatty acids and bile salts in the jejunum. However, a 1993 study in rural Vietnamese schoolchildren showed an intensity-dependent relationship with plasma retinol concentration.

**Table 2**

Micronutrient concentrations and prevalence of (marginal) micronutrient deficiencies among uninfected children and children with single and multiple STH infections, respectively

<table>
<thead>
<tr>
<th></th>
<th>STH uninfected</th>
<th>Ascaris single infection</th>
<th>Trichuris single infection</th>
<th>Hookworm single infection</th>
<th>Multiple STH infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>101</td>
<td>118</td>
<td>67</td>
<td>9</td>
<td>215</td>
</tr>
<tr>
<td>Retinol, μmol/L</td>
<td>1.13 ± 0.21</td>
<td>1.03 ± 0.30*</td>
<td>1.13 ± 0.2</td>
<td>1.08 ± 0.13</td>
<td>0.98 ± 0.26*</td>
</tr>
<tr>
<td>Marginal vitamin A status (&lt; 1.05 μmol/L)</td>
<td>28 (34.1)</td>
<td>60 (56.6)*</td>
<td>21 (33.3)</td>
<td>1 (11.1)</td>
<td>128 (61.5)*</td>
</tr>
<tr>
<td>Vitamin A deficiency (&lt; 0.70 μmol/L)</td>
<td>1 (1.2)</td>
<td>17 (16.0)*</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>34 (16.3)*</td>
</tr>
<tr>
<td>Zinc, μmol/L</td>
<td>8.30 ± 1.93</td>
<td>8.53 ± 2.14</td>
<td>8.71 ± 2.36</td>
<td>8.45 ± 2.60</td>
<td>8.67 ± 2.19</td>
</tr>
<tr>
<td>Zinc deficiency (&lt; 9.9 μmol/L)</td>
<td>70 (86.4)</td>
<td>79 (75.2)</td>
<td>46 (73.0)*</td>
<td>6 (66.7)</td>
<td>159 (76.8)</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>122.00 ± 5.87</td>
<td>121.08 ± 7.32</td>
<td>116.88 ± 7.51*</td>
<td>117.44 ± 8.41*</td>
<td>118.13 ± 7.67*</td>
</tr>
<tr>
<td>Anemia (Hb &lt; 115 g/L)</td>
<td>12 (11.9)</td>
<td>14 (11.9)</td>
<td>30 (44.8)*</td>
<td>3 (33.3)</td>
<td>68 (31.6)*</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>61.68 (43.09–82.73)</td>
<td>63.99 (41.37–88.09)</td>
<td>59.89 (47.00–75.48)</td>
<td>69.21 (48.00–86.55)</td>
<td>65.11 (42.53–84.38)</td>
</tr>
<tr>
<td>Iron deficiency (ferritin &lt; 15 μg/L)</td>
<td>0 (0.0)</td>
<td>3 (2.9)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Urinary iodine, μg/L</td>
<td>108 (77.00–167.00)</td>
<td>112.50 (79–149)</td>
<td>106 (83.00–174.00)</td>
<td>96.00 (77.00–255.00)</td>
<td>115.00 (77.00–167.00)</td>
</tr>
<tr>
<td>Low urinary iodine excretion (&lt; 100 μg/L)</td>
<td>46 (45.5)</td>
<td>50 (42.4)</td>
<td>27 (40.3)</td>
<td>5 (55.6)</td>
<td>91 (42.3)</td>
</tr>
</tbody>
</table>

HB = hemoglobin; STH = soil-transmitted helminth.
*Significantly different from uninfected (P < 0.05).
†Mean ± standard deviation.
‡Median (interquartile range).
deficiency may predispose to *Ascaris* infection by impairing gut mucosal immunity and integrity. However, this would increase susceptibility to STH infections in general, not specifically to *Ascaris*. An observational prospective study in Panama reported less *Ascaris* reinfection in children receiving vitamin A supplements. However, two randomized placebo-controlled trials found no protective effect of vitamin A supplementation on *Ascaris* infection. In the children in this study, both *Trichuris* and *Ascaris* reinfection rates were significantly decreased by the combination of multi-micronutrient fortified biscuits and albendazole treatment \((P \text{ for interaction} < 0.05)\). Only for *Ascaris*, significantly lower egg counts were observed after consumption of fortified biscuits combined with albendazole treatment, compared with albendazole alone.

Hemoglobin concentrations were lower in *Trichuris*, hookworm, and in children infected with multiple STH species than in those uninfected. Surprisingly, these infections were not associated with ferritin concentrations. Plasma ferritin reflects the amount of intracellular iron stores. During inflammation, iron is redistributed in the body by intracellular sequestering, reducing its availability to pathogens. This phenomenon has been termed nutritional immunity, as it can reduce susceptibility to infections. In this study, only CRP was available as an indicator for inflammatory status, perhaps leading to incomplete adjustment of ferritin concentrations because AGP data were not available. Furthermore, our study does not include assessment of micronutrient intake, which could have confounded the associations between STH infections and micronutrient status. However, if the differences in micronutrient status between STH infected and uninfected children were because of differences in intake, an overall effect on susceptibility to infection would be more likely and the observed species-specific associations would be unexpected. Another possible source of confounding are coinfections by protozoa such as *Giardia*, which have also been implicated as cause of vitamin A deficiency and malnutrition in general. A limitation of the Kato Katz technique for diagnosis of STH is that light infections may remain undiagnosed, especially for hookworm. The use of single stool samples further reduces the sensitivity of STH diagnosis. Therefore, possible misclassification may constrain the validity of our findings.
In conclusion, our study demonstrates STH species-specific associations with micronutrient status in schoolchildren. Moreover, this association was clearly related to infection intensity for *Ascaris* and vitamin A status. Hence, both prevalence and intensity of specific helminth infection species in a population may predispose to specific nutritional deficiencies. If so, then some micronutrient deficiencies, such as vitamin A deficiency, are best combated by a combination of deworming and nutritional interventions. Tailor-made public health strategies according to the STH species and micronutrient deficiencies present in a specific population may need to be designed to improve child health and nutrition in the most effective way.

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