## Helminth infections and micronutrients in children

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CHAPTER 2

# Helminth infections and micronutrients in school-age children: a systematic review and meta-analysis

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## Abstract

Helminth infections and micronutrient deficiencies are both highly prevalent in developing countries. Neither condition typically causes overt disease but they do lead to indirect morbidity such as impaired physical and cognitive development. We aimed to systematically review current evidence on the relationship of helminth infections with micronutrient status in school-age children worldwide. We included both observational studies and RCTs. We applied random effects meta-analysis to estimate 1) cross-sectional associations between helminths and micronutrient status; 2) effects of anthelminthic treatment on micronutrient status, and 3) effects of micronutrient supplementation on helminth infection and reinfection. Meta-analyses of observational studies showed an association between helminth infections and serum retinol (SMD (standardized mean difference) -0.30 [-0.48,-0.13]) but not serum ferritin (SMD 0.00 [-0.7,0.7]). Conversely, meta-analyses of anthelminthic treatment RCTs showed a positive effect on ferritin (SMD 0.16 [0.09,0.22]) but not on retinol (SMD 0.04 [-0.06,0.14]). The number of studies on micronutrients other than ferritin and retinol was not sufficient for pooling. Meta-analyses of micronutrient supplementation RCTs only showed a modest protective effect for multi-micronutrient interventions on helminth infection and reinfection rates (OR 0.77 [0.61, 0.97]). Using a non-Cochrane approach, this review shows evidence of distinct associations between helminth infections and micronutrients in school-age children. More studies are needed on micronutrients other than iron and vitamin A, and on possible helminth species-specific effects. A thorough comprehension of the interplay between helminth infections and micronutrients will help guide integrated and sustainable intervention strategies in affected children worldwide.

## Introduction

Helminth infections and micronutrient deficiencies are both major health problems of the developing world[1,2]. Both phenomena are highly prevalent and often occur in the same individuals[3]. Although helminth infections and micronutrient deficiencies often go unnoticed, both are known to impair children's cognitive development and physical growth[4-6]. Helminth infections have often been linked to malnutrition (reduced height and weight for age) and anemia (reduced hemoglobin concentration) in children[7-10]. Human and animal studies have suggested that helminth infection also influences host micronutrient status[11]. While helminth infections are thought to contribute to malnutrition, malnutrition can also predispose for infections[11,12]. However, much remains to be elucidated on the mutual impacts of both entities. Specifically poor vitamin A, iron and zinc intakes seem to predispose for helminth infections, which in turn can exacerbate nutritional deficiencies and prolong helminth survival in human and rodent models[11]. Vitamin A status can be diminished in the case of *Ascaris* infection through malabsorption, while impaired iron status can be caused through direct blood loss in the intestine by hookworm infection[11]. In addition, deficiencies of these micronutrients seem to suppress the immune response[11].

To our knowledge, no systematic review exists to date on the relationship between helminth infections and micronutrient status in children. Several systematic reviews and meta-analyses have addressed the effects of anthelminthic treatment on anthropometric indices and hemoglobin (not on iron per se) in school-age children, but effects on the status of other micronutrients have thus far largely been ignored in these reviews [9,10,13]. Our aim was to review the current evidence on possible associations between helminth infections and (markers of) micronutrient status in school-age children. While existing systematic reviews on helminth infections and child nutrition focus on randomized controlled trials (RCTs) only, we chose a non-Cochrane approach, including not only experimental evidence but also evidence from observational studies.

## Methods

The aim of the literature search was to locate all published studies on helminth infections (*Ascaris*, *Trichuris*, hookworm, *Schistosoma* and/or *Strongyloides*) and markers of micronutrient status (retinol, iron (ferritin), iodine, zinc, folate and vitamin B12) in school-age children. We performed a comprehensive systematic search to find:

- observational studies in order to estimate cross-sectional associations between helminth infection and micronutrient status
- experimental studies in order to estimate effects of anthelminthic treatment on micronutrient status
- experimental studies in order to estimate effects of micronutrient supplementation on helminth infection and reinfection.

*Search strategy*

Searches were performed in May 2012 and repeated in October 2012 with help of a librarian in MEDLINE, EMBASE and the Cochrane Library. Search terms included (several variations on) helminth, intestinal worm/parasite, Ascaris, Trichuris, Ancylostoma, Necator, hookworm, whipworm, roundworm, pinworm, deworm, Schistosoma, Strongyloides, (multi)micronutrients, folate, vitamin A, retinol, vitamin B12, iron, ferritin, transferrin, zinc, iodine and anemia in titles and abstracts of studies, and where possible as MeSH terms or Emtree terms. Reference lists of papers included in the meta-analysis and of key reviews were used for handsearches. No restrictions were set on language or year of publication.

*Eligibility criteria*

The inclusion criteria were: helminth infections determined microscopically in feces/ urine or by PCR, markers of micronutrient status measured biochemically in patient material (not by clinical observations such as xeropthalmia) and mean age of study population between 4 and 18 years old. Exclusion criteria were pregnancy and clinical illness. In the case of experimental studies, only randomized controlled trials were included, with the only difference between experimental and control groups being the intervention of interest (anthelminthic treatment or micronutrient supplementation). We excluded hemoglobin as an outcome reflecting iron status; while hemoglobin has been extensively addressed in previous systematic reviews serum ferritin is in fact the key measure of iron depletion[9,13-15]. Similarly, we also did not include studies in which iron deficiency anemia defined by combined hemoglobin and ferritin concentration was reported, but focussed on studies that reported ferritin concentrations separately. Studies were eligible for the meta-analysis when data were reported in a format from which we could extract or

calculate (logarithmic) means and standard deviation (for continuous outcomes) or odds ratios with 95% confidence intervals (for dichotomous outcomes).

Figure 2.1 shows the process of in- and exclusion as a flow chart. Results of the searches in MEDLINE, EMBASE and Cochrane Library were imported into EndNote X3.0.1 (Thomson Reuters, New York, NY, USA). Duplicates were searched by the EndNote software, whereas their removal was done by hand by the author (BG). Assessment for eligibility was done by two researchers independently (BG and MCP) and any discrepancies were solved by consensus.



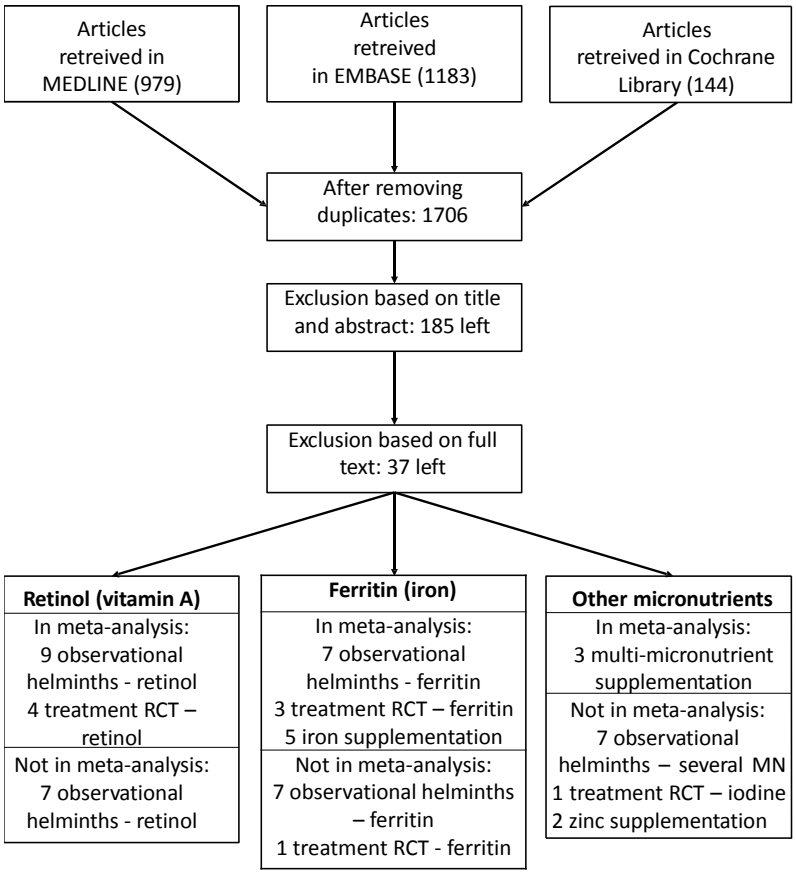



Figure 2.1. Search and selection of studies for review.

*Risk of bias assessment*

The methodological quality of the included papers was assessed. For quality assessment of experimental studies, the criteria list by Verhagen *et al* was used[16]. In short, this list encompasses the categories treatment allocation, baseline similarity, eligibility criteria, blinding, reporting of outcomes and intention-to-treat analysis. For each criterion, 'yes' was assigned one point, and 'no' or 'don't know' zero points. Sum scores were used as a measure of quality for subsequent sensitivity analyses. For the cross-sectional studies, the Newcastle-Ottawa Scale was used to assess quality[17]. Criteria from this scale (case definition, case representativeness, control selection and definition, exposure ascertainment and non-response rate) were used as inclusion criteria for the review. Risk of publication bias was assessed by visual inspection of funnel plots[18].

*Data extraction and analysis*

When a study was eligible for meta-analysis but not all data necessary for pooling were presented in the article, corresponding authors were contacted. Meta-analyses were performed using Review Manager 5.1 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Since ferritin followed a skewed distribution, ferritin data were analysed on a natural logarithmic scale. The data on retinol followed a normal distribution. In meta-analyses with micronutrient status as continuous outcome, mean differences were calculated as standard deviation-units (standardised mean difference) using random effects models. Standardised mean differences were chosen to be able to pool data measured on different scales and in different populations. In the meta-analyses with helminth infection and reinfection as dichotomous outcome, odds ratios with 95% confidence intervals were calculated using random effects models.

In case (logarithmic) mean and standard deviations were not directly reported, these were calculated from other data formats provided (e.g. confidence intervals), or data were extracted from graphic representations of mean and standard errors[19,20].

When observational studies reported on independent subgroups (different populations, e.g. villages), these subgroups were added separately in meta-analysis[18]. When data were presented by helminth species and not by 'any helminth infection', we used the data of only one species, since species-specific infections are not independent of each other within the same population [13]. We chose to extract data of the one species for which the prevalence was closest to 50%. This relatively arbitrary criterion was chosen to obtain the most precise effect estimate, since infected and control groups would then differ the least in size, thus resulting in the most power. In 2x2 trials, data were pooled according to the

comparison of interest: e.g. anthelminthic treatment plus or minus micronutrient supplementation, as long as the groups were of equal size and randomised.

## Results

*Search results*

The combined searches in Medline, Embase and the Cochrane Library resulted in 1706 unique references. After title and abstract review, 185 full text papers were assessed for eligibility. Contact with authors resulted in the retrieval of additional data for three studies[21-23].Thirty-seven papers fulfilled our review eligibility criteria, of which 24 papers were also found eligible for meta-analysis. A flow chart of the search is shown in figure 2.1. We mainly found studies reporting on serum retinol (vitamin A) and serum ferritin (iron). Hence, we were able to perform meta-analyses on only these two markers of micronutrient status. The few identified studies on other micronutrients and studies that did not present data in a format suitable for pooling are discussed in narrative. Characteristics of observational studies, anthelminthic treatment RCTs and micronutrient supplementation RCTs are found in tables 2.1, 2.2 and 2.3, respectively.

*Sensitivity analysis*

We examined whether excluding any one helminth species or including data from another species from the same study would significantly change estimates or heterogeneity between observational studies, which it did not. For experimental studies, the sum scores of the methodological quality assessment were used to perform sensitivity analyses. Two of the included studies had scores below 7 out of 9[20,24]. Removing these studies from their respective meta-analyses did moderately reduce effect size, but did not result in different conclusions or changes in statistical significance.

Table 2.1. Characteristics of observational studies included in meta-analysis.

| First author + year of publication | Study setting + year | Age range (years) | n | Helminths measured | Prevalence (%) | Micronutrients measured | Ref. |
|---|---|---|---|---|---|---|---|
| Awadalla 1979 | Egypt[1] | 6-14 | 24 | *Ascaris* | 11.1 | Serum retinol<br>Serum carotene | 25 |
| Atukorala 1999 | Sri Lanka[1] | 14-18 | 320[2]; 201[3] | *Ascaris*<br>*Trichuris*<br>Hookworm | 16.7 [2], 2.2 [3]<br>46.2 [2], 4.3 [3]<br>5.7 [2], 4.8 [3] | Serum vitamin A | 28 |
| Berhe 2007 | Ethiopia, 2003-'04 | Mean 12.6, SD 3.6 | 333 | *S.mansoni*<br>Other helminths | 73.4<br>15.6 | Serum retinol<br>Serum alpha-tocopherol | 31 |
| Borel 1988 | Mauretania, 1985 | 1-15 | 174 | *S.haematobium* | 34.5 | Serum retinol | 26 |
| Brooker 2007 | Brazil, 2004 | (subgroup) 5-19 | 185 | Hookworm | 27.0 | Serum ferritin | 23 |
| Curtale 1994 | Nepal, 1991 | (subgroup) 5-10 | 592 | *Ascaris*<br>Hookworm | 50.8<br>3.7 | Serum retinol | 27 |
| Kongsbak 2006 | Bangladesh, 2002 | 3-7 | 579 | *Ascaris*<br>*Trichuris*<br>Hookworm | 57.9<br>54.6<br>1.2 | Serum zinc<br>Serum retinol | 30 |

Table 2.1. Continued

| First author + year of publication | Study setting + year | Age range (years) | n | Helminths measured | Prevalence (%) | Micronutrients measured | Ref. |
|---|---|---|---|---|---|---|---|
| Leenstra 2006 | Philippines, 2002-'03 | 7-30 | 738 | *Ascaris*<br>*Trichuris*<br>Hookworm<br>*S. japonicum* | 74.0<br>91.9<br>57.7<br>87.7 | Serum ferritin<br>Serum transferrin receptor | 45 |
| Nchito 2009 | Zambia, 2001 | 7-15 | 378 | *Ascaris*<br>*Trichuris*<br>*S. mansoni* | 43.4<br>0.8<br>0.5 | Serum ferritin<br>Serum transferrin receptor | 46 |
| Osei 2010 | India[1] | 6-10 | 437 | *Ascaris*<br>*Trichuris*<br>Hookworm<br>*Tenia saginata* | 9.4<br>1.6<br>7.6<br>1.6 | Serum ferritin<br>Serum transferrin receptor<br>Serum folate<br>Serum zinc<br>Serum retinol<br>Serum vitamin B12 | 21 |
| Quihui-Cota 2010 | Mexico[1] | 6-10 | 73 | *Trichuris* | 45.2 | Serum iron<br>Serum ferritin<br>Total iron binding capacity | 47 |

Table 2.1. Continued

| First author + year of publication | Study setting + year | Age range (years) | n | Helminths measured | Prevalence (%) | Micronutrients measured | Ref. |
|---|---|---|---|---|---|---|---|
| Rai 2000 | Nepal, 1993 -96 | 5-12 | 145 [4]; 79 [5] | *Ascaris*<br>*Trichuris*<br>Hookworm | 63.3 [4], 79.1 [5]<br>24.7 [4], 7.2 [5]<br>12.0 [4], 15.0 [5] | Serum retinol<br>Serum β-carotene | 29 |
| Ramdath 1995 | Jamaica[1] | 7-11 | 421 | *Trichuris* case-control design | | Serum ferritin<br>Free erythrocyte protoporphyrin | 43 |
| Stoltzfus 1997 | Zanzibar, 1994 | 90,5% between 7-13 | 3427 | *Ascaris*<br>*Trichuris*<br>Hookworm | 72.0<br>96.0<br>93.7 | Serum ferritin | 44 |
| Taren 1987 | Panama, 1983 | 3-6 | 121 | *Ascaris* | 46.3 | Plasma vitamin A | 38 |

[1] year of study not stated in article.

[2] urban subgroup

[3] rural subgroup

[4] Okharpauwa village subgroup

[5] Boya village subgroup

Table 2.2. Characteristics of anthelminthic treatment studies included in meta-analysis.

| First author + year of publication | Study setting + year | Age range (years) | n | Helminths measured | Prevalence (%) | Anthelminthics | Micro-nutrients measured | Follow-up timepoint (no. of treatment rounds) | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Jalal 1998 | Indonesia, 1989 | 3-6 | 156 | *Ascaris*<br>*Trichuris*<br>Hookworm | 49.4<br>18<br>8 | Levamisole | Serum retinol | 3 weeks (1) | 20 |
| Jinabhai 2001 | South Africa, 1995 | 8-10 | 302 | *Ascaris*<br>*Trichuris*<br>Hookworm<br>*S.haemato-bium* | 27.5<br>54.1<br>3.3<br>24.6 | Albendazole Praziquantel | Serum retinol | 16 weeks (1) | 39 |
| Mwaniki 2002 | Kenya[1] | 9-18 | 496 | *S. mansoni*<br>Hookworm<br>*Trichuris*<br>*Ascaris* | 73<br>51<br>49<br>14 | Albenzadole Praziquantel | Serum retinol | 8 months (1) | 40 |

Table 2.2. Continued

| First author + year of publication | Study setting + year | Age range (y) | n | Helminths measured | Prevalence (%) | Anthelminthics | Micro-nutrients measured | Follow-up timepoint (no. of treatment rounds) | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Nga 2009 | Vietnam, 2007 | 6-8 | 467 | *Ascaris*<br>*Trichuris*<br>Hookworm | 65.2<br>55.2<br>5.5 | Albendazole | Plasma ferritin<br>Plasma transferrin receptor<br>Plasma retinol<br>Plasma zinc<br>Urinary iodine | 4 months (1) | 41 |
| Rohner 2010 | Côte d'Ivoire, 2006-'07 | 6-14 | 551 | Hookworm | 52.6 | Albendazole<br>Praziquantel | Plasma ferritin<br>Transferrin receptor<br>Zinc proto-porphyrin | 6 months (2) | 22 |
| Stolzfus 1998 | Zanzibar, 1994 | Mean 10.5, sd 1.6 | 2924 | *Ascaris*<br>*Trichuris*<br>Hookworm | 71.8<br>96.1<br>93.7 | Mebendazole | Ferritin<br>Protoporphyrin | 12 months (2 or 3) | 24 |

[1] year of study not stated in article.

Table 2.3. Characteristics of micronutrient supplementation studies included in meta-analysis.

| First author + year of publication | Study setting + year | Age range (years) | n | Supplementation/ fortification equivalent | Outcomes | Follow-up time | Ref. |
|---|---|---|---|---|---|---|---|
| Le 2007 | Vietnam, 2004-'05 | 6-8 | 326 | Iron, 10.7 mg per school day | *Ascaris, Trichuris,* hookworm infection | 6 months | 52 |
| Nchito 2008 | Zambia, 2001 | 7-15 | 215 | Iron, 60 mg per day or MMN[2] each school day | *Ascaris* reinfection rate | 10 months | 46 |
| Nga 2009 | Vietnam, 2007 | 6-8 | 489 | MMN each school day | *Ascaris, Trichuris* and hookworm infection rates and intensity | 4 months | 41 |
| Olsen 2000 | Kenya, 1994-'96 | Subgroup 4-15 | 170 | Iron, 60 mg twice weekly | *Ascaris, Trichuris*, hookworm and *S. mansoni* reinfection rates and intensities | 12 months | 19 |
| Olsen 2003 | Kenya, 1995-'96 | 8-18 | 476 | MMN each school day | *Ascaris, Trichuris* and hookworm reinfection rates and intensities | 11 months | 59 |
| Palupi 1997 | Indonesia[1] | 2-5 | 187 | Iron, 30 mg once weekly | Ascaris, Trichuris, hookworm infection | 9 weeks | 53 |
| Rohner 2010 | Côte d'Ivoire, 2006-'07 | 6-14 | 139 | Iron, 20 mg, 4 days per week | Hookworm reinfection rate | 6 months | 22 |

[1] year of study not stated in article.

[2] MMN: multi-micronutrient

*Association between helminth infection and retinol (vitamin A)*

We identified 15 observational studies in which associations between helminth infections and serum retinol measurements were reported[21,25-38]. Of these studies, 9 presented data eligible for pooling in the meta-analysis shown in figure 2.2. The analysis included data on 1498 infected and 1507 uninfected individuals. The meta-analysis showed a negative association between helminth infection and retinol. There was a large and significant heterogeneity between these estimates (74%, p<0.001).

The studies included in the meta-analysis concerned different helminth species, i.e. *Ascaris*[25,27,38], *Trichuris*[30], *Schistosoma mansoni*[31] and *Schistosoma haematobium*[26]. From three studies, data were pooled on infection by 'any helminth'[21,28,29]. In the study by Atukorala *et al.*, *Trichuris*, but not *Ascaris*, was significantly associated with retinol concentrations[28]. Among the six studies not included in our meta-analysis, four reported a negative association[32-35], and two reported to have found no association between helminth infections and serum retinol in children but did not provide effect estimates[36,37].

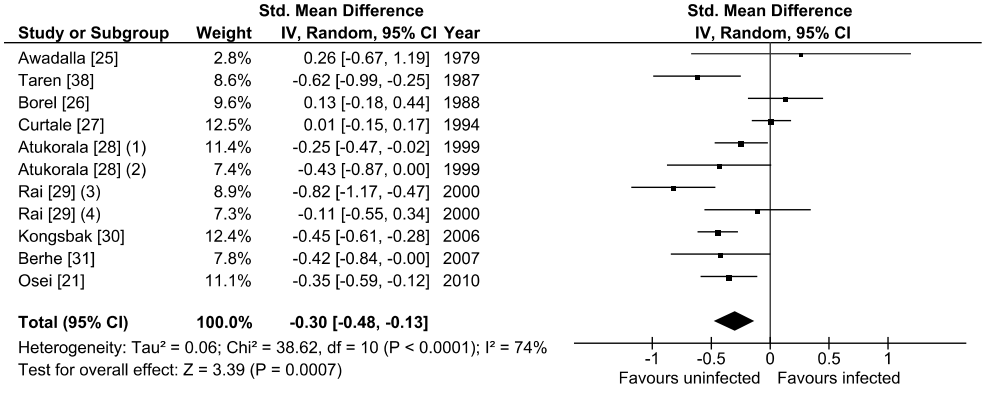



Figure 2.2. Random effects meta-analysis of pooled cross-sectional data on retinol in helminth infected and uninfected children (n=3005). Characteristics of these studies can be found in table 2.1.

*Effect of anthelminthic treatment on retinol*

We identified 4 anthelminthic treatment RCTs in which retinol concentrations were measured, all of which were included in the meta-analysis shown in Figure 2.3 [20,39-41]. A total of 1421 children were included in this analysis, of which 691 received treatment. None of the studies observed a significant difference in retinol status between treatment and control groups. No heterogeneity between study estimates was observed.

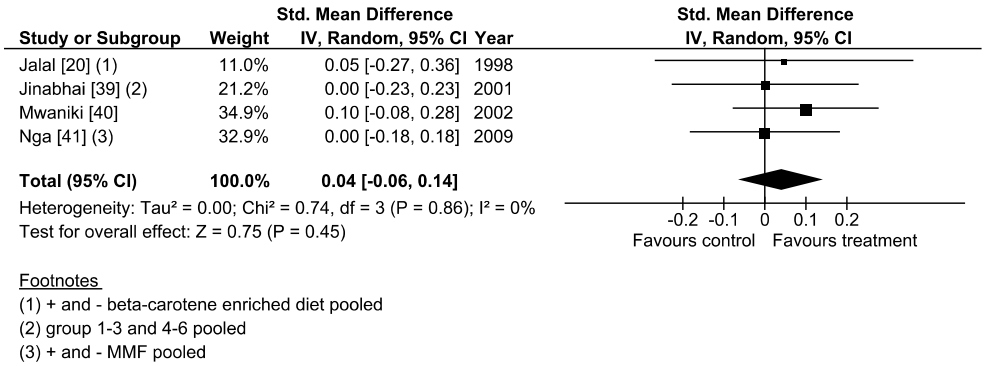



Figure 2.3. Random effects meta-analysis of data on effects of anthelminthic treatment on serum retinol (n=1421). Characteristics of these studies can be found in table 2.2.

*Association between helminth infection and ferritin*

We identified 14 observational studies in which associations between helminth infections and serum ferritin were determined[21,23,34,36,42-51]. Of these studies, seven presented data suitable for our meta-analysis[21,23,43-47]. Figure 2.4 shows the meta-analysis on pooled cross-sectional data on ferritin by helminth infection. This analysis included data from 3799 infected and 1860 uninfected children. Only one study found a difference; *Schistosoma* infected children had higher serum ferritin concentrations than those uninfected[45]. Heterogeneity between these pooled estimates was low at 11% (not significant, p= 0.35). The pooled effect estimate was zero: there was no trend towards a difference in ferritin between helminth infected and uninfected children in these data. Among the seven studies which did not present data in a format suitable for pooling, three did not find an association between helminth infection and serum ferritin[34,48,49], while four studies did report a significant negative association [36,42,50,51].

*Effect of anthelminthic treatment on ferritin*

We identified 4 RCTs in which the effect of anthelminthic treatment on serum ferritin concentration was measured[22,24,41]. Three of these studies are shown in figure 2.5, with a total of 3942 participating children, of which 2424 received treatment. The analysis showed no statistical heterogeneity between studies and the estimated pooled effect was positive. In the one study excluded from our meta-analysis, no significant effect of anthelminthic treatment on serum ferritin was found[52].



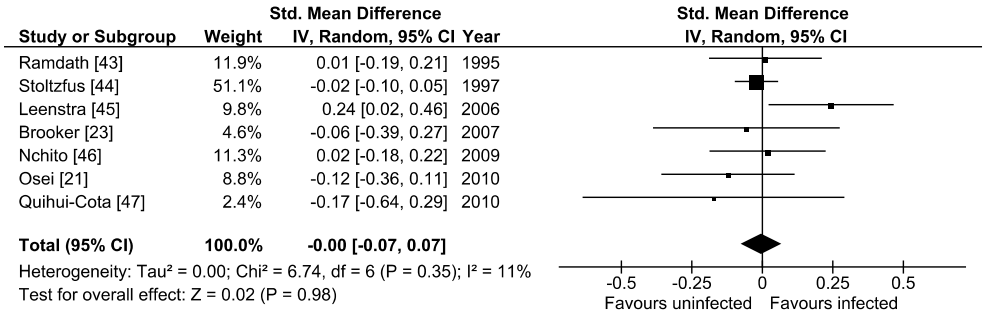
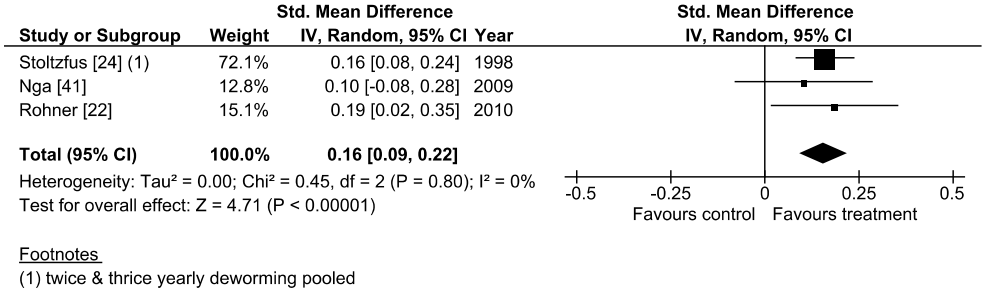



Figure 2.4. Random effects meta-analysis of pooled cross-sectional data on ferritin in helminth infected and uninfected children (n=5659) (analysis is on natural log scale). Characteristics of these studies can be found in table 2.1.




Footnotes
(1) twice & thrice yearly deworming pooled

Figure 2.5. Random effects meta-analysis of data on effects of anthelminthic treatment on serum ferritin (n=3942). Characteristics of these studies can be found in table 2.2.

*Effect of iron supplementation on helminth infection and reinfection*

We identified 5 iron supplementation studies in which helminth infection and reinfection was reported[19,22,46,52,53]. All five studies are shown in figure 2.6, including a total of 1037 children of which 541 received supplementation. The meta-analysis shows no significant effect of iron supplementation on helminth infection and reinfection and no significant heterogeneity between studies.

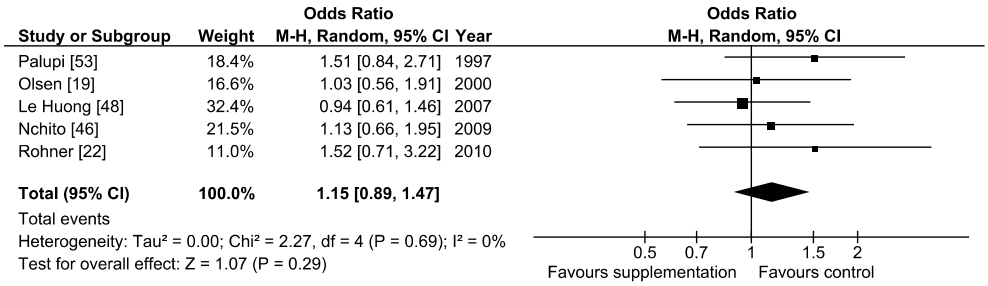



Figure 2.6. Random effects meta-analysis of data on effects of iron supplementation on helminth infection and reinfection (n=1037). Characteristics of these studies can be found in table 2.3.

*Helminth infection and other micronutrients*

Few studies exist on micronutrients other than vitamin A and iron in association with helminth infections in schoolchildren. While one study found *Trichuris* infection to be a negative predictor of serum zinc concentration[54], other studies addressing zinc, copper, magnesium, vitamin E, folate, vitamin B12 or iodine did not find any association between these markers of micronutrient status and helminth infections[21,31,34,55,56] (Osei A, Houser R, Bulusu S, Joshi T, Hamer D, 2010, unpublished data). As for experimental studies, we found one study in which treatment for *Ascaris* and hookworm improved efficacy of iodine supplementation[56]. We also identified two zinc supplementation studies[57,58], neither of which found an effect of zinc supplementation on helminth infection and reinfection, nor did the pooled data (data not shown).

We identified 3 studies in which multi-micronutrient supplementation was provided and helminth infection and reinfection was reported[41,46,59] (figure 2.7). A total of 1180 participants were included in this analysis, of which 586 received supplementation. The individual studies did not find a significant effect, but when combining the data a significant reduction in helminth infection and reinfection was observed among children who received multi-micronutrients (OR 0.77, 95%CI 0.61,0.97). No heterogeneity was observed between the study estimates.

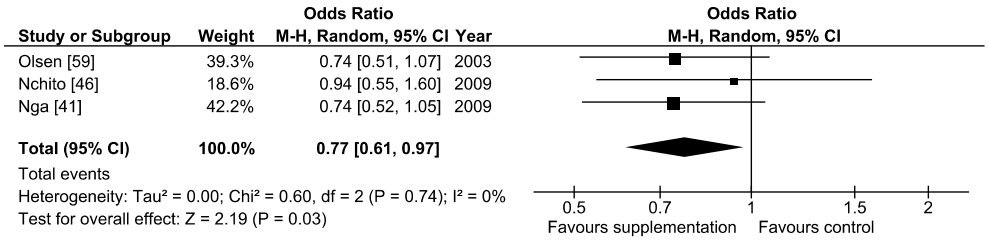



Figure 2.7. Random effects meta-analysis of data on effects of multi-micronutrient supplementation on helminth infection and reinfection (n=1180). Characteristics of these studies can be found in table 2.3.

## Discussion

To our knowledge, this is the first systematic review and meta-analysis on helminth infections and micronutrient status. We used a non-Cochrane approach to review evidence from observational studies, anthelminthic treatment and micronutrient supplementation studies.

*Retinol (vitamin A) and helminth infections*

Based on pooled cross-sectional data we found a negative association between helminth infection and serum retinol status of schoolchildren (figure 2.2). This was supported by additional studies from which we were not able to extract data for meta-analysis. In contrast, the results from our meta-analysis of RCTs showed that anthelminthic treatment does not significantly increase retinol concentrations (figure 2.3).

Reduced serum retinol in helminth infected children is a phenomenon which has been attributed to *Ascaris* infection in the 1970's[60-62]. When *Ascaris*-induced intestinal obstruction extends to the bile duct, malabsorption of fat-soluble vitamins such as vitamin A could be impaired. Direct effects of ascariasis on the intestinal mucosa cannot be excluded either, as has been suggested for hookworm before[63]. Clinical observations of xeropthalmia support the reports of *Ascaris* infection reducing vitamin A status[64-66]. Retinol deficiency may also predispose for helminth infections. Indeed, in a study in Mexican infants, vitamin A supplementation reduced *Ascaris* reinfection rates[67]. In addition, animal studies found reduced intestinal Th2 immune responses against nematode infections in vitamin A deficient mice[3]. We could not further test this hypothesis due to a lack of vitamin A supplementation studies addressing helminth infection and reinfection risk in school-age children.

We observed a large heterogeneity between study estimates in our meta-analysis of observational studies on associations between helminth infections and serum retinol.

Given current WHO recommendations which have resulted in the implementation of periodic supplementation of vitamin A in many low and middle income contexts, as well as the observed large heterogeneity between study estimates in our meta-analysis of observational studies on associations between helminth infections and serum retinol, there is a clear need for new large, well-designed studies on this topic in order to draw reliable conclusions.



*Ferritin (iron) and helminth infections*

Serum ferritin concentration was not associated with helminth infection (figure 2.4). On the other hand, anthelminthic treatment did increase serum ferritin in schoolchildren (figure 2.5). Studies supporting the notion that helminth infections, in particular hookworm, cause iron deficiency anemia, are mainly based on hemoglobin data[5,44]. However, hemoglobin and serum ferritin concentrations may show distinct responses to infection. Hence, while hookworm infection is known to cause blood loss, as reflected by reduced hemoglobin concentration, it may not necessarily decrease serum ferritin concentration[68,69]. According to our meta-analysis, iron supplementation studies did not have an effect on helminth infection and reinfection (figure 2.6). Similarly, a previous systematic review and meta-analysis by Gera and Sachdev did not find a protective effect of iron supplementation on infectious illness[70].

*Strengths and limitations*

Although helminth species did not explain heterogeneity in sensitivity analysis, the pooling of data on different species is not ideal. Helminth infections are often studied together, but they represent different species with quite distinct life cycles and pathogenesis. Especially the blood-dwelling *Schistosoma* is quite different from intestinal, soil-transmitted helminths. In our meta-analysis on associations between helminth infections and serum ferritin, the only study which reported a significant association concerned *Schistosoma* infection, although soil-transmitted helminths were also highly prevalent in this study population[45]. Hence, additional species-specific studies would be welcome, but are complicated: co-infection by multiple helminth species is very common and infection by one helminth species is a risk factor for becoming infected by another[9]. For that reason, we could not pool data on different species within the same population in our meta-analysis and chose to extract data from only one helminth species in case data on more species were presented.

In our meta-analyses of RCTs, we pooled data on different anthelminthic treatments, micronutrient supplementation strategies and follow-up timepoints (tables 2.2 and 2.3). Yet, no heterogeneity was found between these studies. The data that were extracted

from experimental studies on treatment and supplementation partly come from 2x2 trials in which both interventions were implemented simultaneously in the same population. Hence, data were pooled from different study groups, e.g. anthelminthic treatment effects were compared between groups of which half received supplementation and half did not. This might have confounded our estimates. On the other hand, interaction effects between the two interventions were not found in any of these trials[22,40,41,46,52].

None of the studies in our meta-analyses adjusted for acute phase markers. Nevertheless, it is known that acute phase inflammatory responses can affect the validity of markers of micronutrient status[71,72], and not adjusting for inflammation could thus be considered a limitation of this review. On the other hand, if inflammatory responses are intermediate factors on the causal pathway between helminth infections (or anthelminthic treatment) and micronutrient status, then adjustment for inflammation would not be appropriate. Future studies on the relation between (helminth) infections and markers of micronutrient status should attempt to address the role of inflammation. More research is needed on the mutual impacts of both entities and the exact underlying mechanisms.

An examination of funnel plots for the observational studies did not reveal a risk of publication bias for the outcome retinol. The funnel plot for observational studies with the outcome ferritin was slightly asymmetrical, hinting toward a small bias in the direction of positive associations between helminth infection and higher ferritin concentration. The additional data we received from contact with authors could have introduced a source of bias in our estimates, since not all of the contacted authors responded to our request. Furthermore, it is possible that selective reporting played a role in studies that were included in our review: e.g. when one of the helminth species was associated with an outcome, data for only this species might have been presented, while data for other species were omitted.

In previous meta-analyses on helminth infections and nutritional status, only anthelminthic treatment RCTs have been reviewed[5,9,11]. Recently, the debate surrounding the evidence base of deworming has been fueled by the results of the 'DEVTA' (Deworming and Enhanced Vitamin A) trial and the recently updated Cochrane review on anthelminthic treatment effects on height, weight and hemoglobin[9,73]. Both studies did not show convincing beneficial effects of deworming on nutritional indicators. However, we must be careful not to interpret this as a lack of association between helminth infections and nutritional status. For example, anthelminthic RCTs are usually characterized by short follow-up periods, and long-term effects of deworming on nutritional status may therefore be missed, such as the restoration of the intestinal mucosa followed by enhanced micronutrient status or catch-up growth. We argue that anthelminthic treatment RCTs only do not suffice for thoroughly building the evidence

base regarding the interplay between helminth infections and micronutrients. In this review, we chose to include not only experimental evidence, but also evidence from observational studies. While observational studies are often considered inferior to randomized trials, we believe that they play an important complementary role. The degree in which these two major public health issues are associated in a 'real life' setting can be very different to the effects of treatment in experimental conditions which are contrived. Both types of information are of essence in order to complete the complex puzzle of the bidirectional helminth-micronutrient relationship. Evidence from observational studies should therefore not be overlooked when reviewing literature and designing policy.

In conclusion, although helminth infection was associated with lower serum retinol but not ferritin status, anthelminthic treatment elevated ferritin concentrations but not retinol in school-age children. Studies on associations of other micronutrients with helminth infections are lacking. Multi-micronutrient supplementation tends to decrease the risk of helminth infection and reinfection.

Our review shows distinct interrelationships between helminth infections and micronutrients. Assuming a non-Cochrane approach we showed that effects found in experimental studies do not necessarily reflect cross-sectional associations and vice versa. More evidence is needed on micronutrients other than iron and retinol, and on possible helminth species-specific effects. Our finding that multi-micronutrient supplementation might reduce helminth infection risk is also an important point for further investigation and provides an interesting public health perspective for reducing infectious disease burden. Gaining more insight into the interrelationship between helminth infections and micronutrients is crucial for designing adequate public health interventions to improve health and development of children.

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