The effect of iron and multi-micronutrient supplementation on *Ascaris lumbricoides* reinfection among Zambian schoolchildren

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Summary A randomised, placebo-controlled, double-blind trial was conducted among schoolchildren in Chawama, Lusaka, Zambia, to determine the effect of iron and multi-micronutrients on reinfection with *Ascaris lumbricoides*. Supplementation was given on every school day for 10 months. Baseline *A. lumbricoides* prevalence and geometric mean intensity among positives were 43.4% and 2526 eggs per gram (epg) faeces, respectively. Serum ferritin <12 μg/l was associated with higher egg counts than serum ferritin $\geq$12 μg/l (4728 vs. 2036 epg, $P = 0.033$). Of 406 children recruited, 378 (93.1%) were examined at baseline and all infected children were treated and cure ascertained. The mean number of tablets taken per week was 2.5, giving 50% compliance. At six months 283 (74.9%) children complied, and reinfection intensities in those receiving iron were lower than in those receiving placebo (1600 vs. 3085 epg, $P = 0.056$). This effect disappeared at 10 months, where 215 (56.9%) complied. Iron had no effect on *A. lumbricoides* reinfection rates and multi-micronutrients had no effect on reinfection rates or intensities. Iron appears to affect reinfection intensity with *A. lumbricoides*, but further investigations are required to confirm this effect and elucidate the mechanisms involved.
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1. Introduction

Micronutrient deficiencies damage mucosal barriers and weaken the immune system, thus rendering the host more vulnerable to infection. For children who live in overcrowded and unsanitary conditions, the risk of infection is much increased and micronutrient deficiencies may lead to more severe infections of longer duration.[1]

Ascaris lumbricoides infection is the most common parasitic infection in the world, affecting approximately 1.2 billion people, with 350 million people showing morbidity.[2,3] The infection seems to reduce food intake, with a possible effect on iron status, to impair fat digestion and reduce vitamin absorption, which may lead to reduced growth.[2] *Ascaris lumbricoides* infections are prevalent in tropical and sub-tropical countries in poor communities with unsanitary conditions; areas where micronutrient deficiencies are also often prevalent. In combination, *A. lumbricoides* infection and malnutrition exert a considerable toll on the health of large sectors of the population. It would thus be of significant public health importance if micronutrient supplementation had an effect on the severity and/or duration of helminth infection apart from the direct effect on malnutrition.

Iron supplementation decreased the rate of reinfection with *A. lumbricoides*, *Trichuris trichiura* and *Schistosoma mansoni* in Kenyan adults, while there was no effect on reinfection intensities. Iron supplementation did not affect reinfection rates or intensities in children.[4] Full multi-micronutrient supplementation lowered *S. mansoni* reinfection intensities in Kenyan schoolchildren, leaving reinfection rates unaffected. Full supplementation had no effect on the reinfection rates or intensities of *A. lumbricoides* and *T. trichiura*.[5] Both studies were thus difficult to interpret, and in order to gain more information we conducted a randomised, placebo-controlled, double-blind, two-by-two factorial trial on the effects of iron and multi-micronutrient supplementation (without iron) on reinfection with *A. lumbricoides* among schoolchildren.

2. Subjects and methods

2.1. Study area and population

The study was carried out in Chawama from February 2001 to December 2001. Chawama is a shanty town a few kilometres away from the city centre of Lusaka, with an estimated population of 56 300.[6] The area lies approximately 1300 m above sea level and receives 800 mm of rainfall between November and April. It is a high density area with a mixture of planned and unplanned housing facilities ranging from modern housing units with electricity and water closet toilets to bare rooms made of mud or unplastered cement blocks, with corrugated iron sheets or bare asbestos for a roof. Unauthorised building in response to increased demand for housing has resulted in overcrowding. The rented single rooms are sometimes occupied by families and these often have to share a pit latrine with three or four other families in the same compound. Water is mostly drawn from communal taps scattered around the township. As in most poor communities, *A. lumbricoides* infection is most likely transmitted through poor hygienic practices. In some parts of Lusaka vegetables are fertilized with 'night soil'; and if not properly treated this could be a source of infection. The main occupation in the area is small-scale trading. The study population consisted of schoolchildren aged 7–15 years from two schools.

2.2. Study design

The study was a randomised, placebo-controlled, double-blind, two-by-two factorial intervention trial on the effects of iron and multi-micronutrient supplementation (without iron) on geophagy, *A. lumbricoides* reinfection, iron status, growth, intestinal permeability, illness episodes and school absenteeism.

The effect on *A. lumbricoides* reinfection is reported in this paper. After baseline examinations as described below (interviews, stool and blood collection), the children were independently randomised to iron or identical-looking placebo and to multi-micronutrients or identical-looking placebo. The simple randomisation was repeated until baseline equivalence was obtained between the resulting four groups with respect to age, gender, *A. lumbricoides* infection, haemoglobin level, geophagy status and group size.

Multi-micronutrient tablets, containing approximately 200% of recommended dietary allowances (RDA[7]), were given by field assistants on every school day for 10 months. The composition of the multi-micronutrient tablets was: vitamin A 2000 μg, vitamin B_1 2.6 mg, vitamin B_2 3 mg, vitamin B_6 3.4 mg, vitamin B_{12} 4 μg, vitamin C 100 mg, vitamin D 20 μg, vitamin E 20 mg, niacin 34 mg, folate 300 μg, zinc 30 mg, iodine 300 μg, copper 4 mg and selenium 80 μg (Almega, Ringsted, Denmark). Ferrous dextran tablets contained iron corresponding to 60 mg elemental iron (Almega). Each child was allocated two containers, one for iron/placebo and one for multi-micronutrient/placebo tablets, labelled with name and study number. Each child thus swallowed two tablets a day under observation of the field assistants. The containers were entrusted to the field assistant and held a number of tablets in known excess of the tablets needed for each term. During each survey, the containers were collected and the remaining number of tablets registered. The children were followed up after 6 and 10 months.

Blinding was assured by ensuring that none of the research or field staff, or the pupils, knew which tablets were which. The code was kept at DBL-Centre for Health Research and Development, Copenhagen, and broken only after the study.

As the interventions employed were nutrient supplements rather than drugs, the study was not viewed as a clinical trial in the strict sense and was therefore not registered.

2.3. Sample size calculation

With a power of 90%, a significance level of 5%, an assumed reinfection rate of 50% per year, a reduction of infection of 50% in the iron supplemented group and allowing for a 20% loss to follow-up, a sample size of 100 individuals in each of the two main supplementation groups (iron and placebo) was chosen. This would have been sufficient in the absence

of interactions between iron and other micronutrients. As we wished to be able to measure possible interactions, the sample size was doubled and therefore 100 individuals for each of the four supplementation groups were selected

2.4. Interviews

The children were interviewed by the principal investigator, helped by field assistants, at baseline and at the 6 and 10 month follow-ups to determine the presence and frequency of geophagy as well as the amount of earth eaten per day. The methods are described in more detail elsewhere.[8]

2.5. Helminths

Examinations for helminth infections were carried out at baseline and at the 6 and 10 month follow-ups. One stool sample on two consecutive days was collected and examined by duplicate 41.7 mg Kato-Katz cellophane thick smears for counting helminth eggs.[9] Intensity of infection was expressed as eggs per gram faeces (epg). After the baseline examination, all infected children were treated with 400 mg albendazole on two consecutive days. Efficacy of treatment was assessed three weeks later by examination of one stool sample. Children still infected were retreated with albendazole (400 mg in a single dose). Cure rate was defined as the percentage of positive individuals who became negative after the first treatment. The analysis of reinfection was based on individuals who were either initially uninfected or who had no eggs in their stool after the first treatment. The assumption was made that the initial state with regard to infection was not fixed and children initially negative could later become infected. *Ascaris lumbricoides* is believed to be transmitted mainly in the domestic domain, but children move around and play in other households and transmission is likely to occur at school too. Even in the event that the baseline infection status remained static throughout the study, the outcome of the trial for either the infected or non-infected would not be influenced by the other. The equivalence achieved at baseline with regard to a number of variables, particularly *A. lumbricoides* infection, for the four groups removes the possibility of bias.

2.6. Iron status

Haemoglobin concentration (Hb), serum ferritin concentration and soluble transferrin receptors (sTfR) were measured at baseline and at the 10 month follow-up. Blood samples were taken from the antecubital vein. Methods of determination are described in detail elsewhere.[8] Anaemia was defined as an Hb value <115 g/l for children aged 5—11 years, <120 g/l for boys aged 12—13 years and for girls ≥12 years of age, and <130 g/l for boys aged >13 years.[10] Serum ferritin values <12 μg/l were defined as depleted iron stores,[11,12] while tissue iron deficiency was defined as soluble transferrin receptor (sTfR) values >3.0 mg/l for children aged 4—10 years and >2.7 mg/l for those aged 11—16 years. The concentration of sTfR in serum was determined from a particle-enhanced immunoturbidimetric kit according to

the kit manual (Cat. no. 67968, Orion Diagnostica, Espoo, Finland). The assay was fully automated and analyzed with Cobas Mira Plus (Roche, USA).[13]

2.7. Data analysis

The *A. lumbricoides* epg were $\log_{10}$ transformed to obtain a normal distribution. χ^2 tests were used to test for differences in proportions, while Student's *t* test and ANOVA were used to assess differences in means between groups. Multiple logistic and linear regression analyses, respectively, were used to identify predictors for the presence and intensity of *A. lumbricoides* reinfection. Interactions were assessed between the effect of iron and multi-micronutrient supplementation on *A. lumbricoides* reinfection prevalence and intensity. If no interaction was found, the effect of one intervention could be assessed without considering the other. Iron and multi-micronutrient supplementation were forced into all models. A significance level of 0.05 was used for all tests.

2.8. Ethical considerations

Ethical approval was obtained (see below). At the school, the children in Grades 3 and 4 were sent home with a consent form in both English and Nyanja for their principal caretaker to sign. Among other things, the form contained information that the study design involved a placebo-controlled component. If the form was unsigned or parents did not consent, the child, even if willing, was excluded from the study. Similarly, the child who was unwilling but whose parents consented was also excluded. It was made clear to both parents and children that inclusion into the study was voluntary and they could withdraw at any time without negative consequences.

Children with Hb lower than 80 g/l were excluded from the study and offered iron treatment. All children who were helminth-positive at baseline were treated, even if they did not continue in the study. Any children who were ill were referred to the clinic.

3. Results

3.1. *Ascaris lumbricoides* infection and iron status

Of the 406 children recruited, 378 (93.1%) submitted a stool specimen at baseline (Figure 1). The mean age of the children was 10.4 (range 7—15) years and 198 (52.4%) were girls. Parasitological examination of the stool revealed that *A. lumbricoides* was the only major parasitic infection in the study population, with 164 (43.4%) infected. The geometric mean intensity of infection for positives only was 2526 epg. The prevalence and intensity of *A. lumbricoides* was similar in girls and boys (41.4 vs. 45.6%, P = 0.48 and 2339 vs. 2727 epg, P = 0.60, respectively). The presence of other helminth infections was negligible: two (0.5%) had *Schistosoma mansoni*, six (1.6%) had *Taenia* spp. and three (0.8%) had *Trichuris trichiura* infections. The 28 children from whom baseline data were missing were comparable to the retained ones with respect to age and gender.
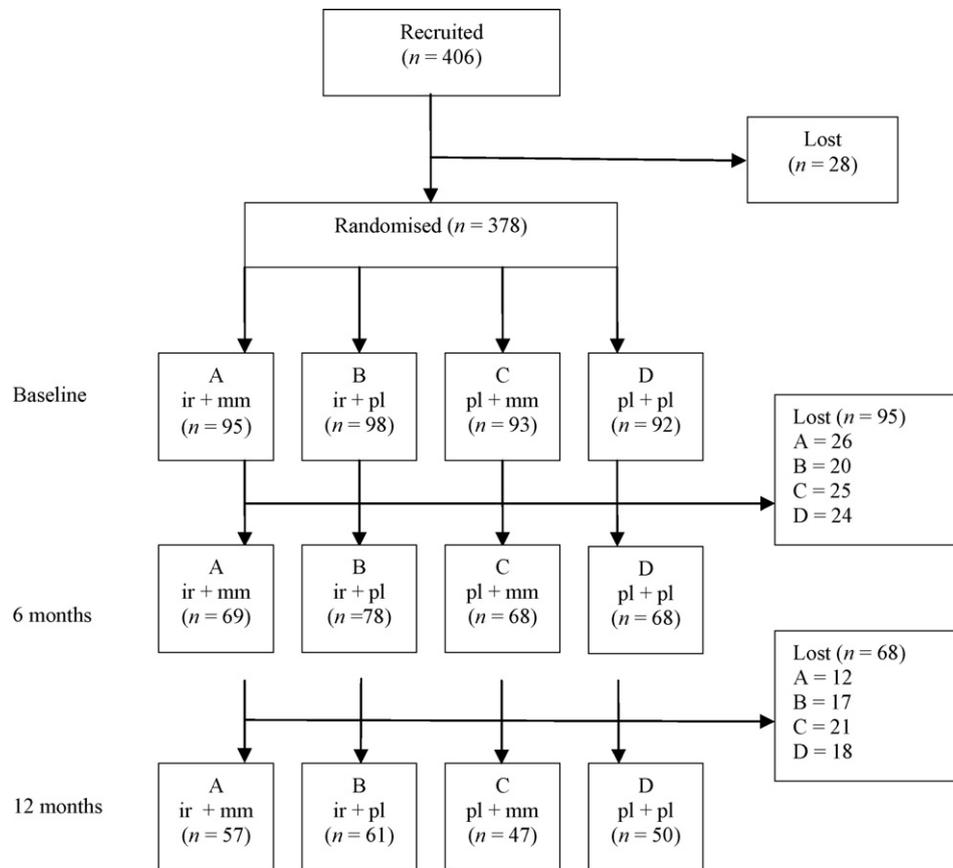
Figure 1 Trial diagram showing randomisation of Zambian schoolchildren to iron (ir), multi-micronutrients (mm) and placebo (pl), and retention in the study.

Only ten children were excluded due to Hb lower than 80 g/l.

At baseline there was no significant difference in the level of markers of iron status between *A. lumbricoides*-infected and -uninfected children as shown in Table 1. However, children with depleted iron stores (serum ferritin <12 μg/l) had higher egg counts than those with adequate iron stores (4728 vs. 2036 epg, *P* = 0.033). Similarly, children with high transferrin receptor values had higher egg counts than those with low values, although this difference was not significant at the 5% level (5278 vs. 2090 epg, *P* = 0.06).

3.2. Supplementation and *Ascaris lumbricoides* infection

The simple randomization resulted in baseline equivalence among the four groups concerning age, gender, Hb level, and prevalence and intensity of *A. lumbricoides* infection (Table 2). The cure rate for *A. lumbricoides* infection three weeks after administering albendazole was 89% and there was no significant difference among the groups.

Of the 378 children examined at baseline, 95 (25.1%) were lost to follow-up at 6 months and a further 68 (24.0%) were lost to follow-up at 10 months. The children lost to follow-up were equally distributed among the four supplementation groups. Furthermore, there were no significant

differences in the proportion of children lost at any of the two follow-ups with respect to age, gender and *A. lumbricoides* infection status.

The number of school days in the intervention period and, hence, the optimal number of tablets to be taken was 100. Twenty-five percent of the children took less than 20% of the optimal number of tablets, while half took 45% or more. The mean number of tablets taken per week was 2.5, which is 50% of the optimal. There was a decline in the weekly tablet intake between the 6 and 10 month follow-ups, from 2.9 tablets per week to 2.0 tablets per week. The mean number of tablets taken in the four supplementation groups during the intervention period was not significantly different (46, 42, 44 and 43, respectively, *P* = 0.68).

At the 6 month follow-up, 31.0% of the children were reinfected, while 45.0% were reinfected at 10 months, reflecting 71% and 104% of pretreatment levels, respectively. There were no differences in reinfection rates among the four groups at either 6 or 10 months. Of the four supplementation regimes, the iron with placebo multi-micronutrient group had the lowest reinfection intensity at both follow-ups, but this was not significant (Table 3). At 6 months, prevalence was lower in those supplemented with only iron compared to those receiving placebo, but this difference was not significant (21.8 vs. 32.4%, *P* = 0.21).

There was an interaction between iron and multi-micronutrient supplementation on *A. lumbricoides* reinfection rates at 6 months (*P* = 0.045) as assessed in multiple

Table 1 Baseline characteristics of 378 schoolchildren by *Ascaris lumbricoides* infection

	Ascaris lumbricoides		P-value
	Infected	Non-infected	
Mean age (years) (95% CI)	10.3 (10.1—10.5)	10.2 (10.0—10.4)	0.45
Female (%) (95% CI)	50.0 (42.2—57.8)	54.2 (47.4—61.0)	0.48
Mean haemoglobin (g/l) (95%CI)	128.8 (126.0—131.6)	130.1 (127.3—132.9)	0.56
Anaemic children (%) (95% CI)	11.3 (6.2—16.5)	12.6 (7.8—17.4)	0.84
Geometric mean serum ferritin (μg/l) (95% CI)	21.5 (19.3—23.8)	21.2 (19.1—24.0)	0.85
Serum ferritin <12.0 μg/l (%) (95% CI)	19.6 (13.0—26.4)	17.6 (12.0—23.2)	0.77
Geometric mean serum transferrin receptors (mg/l) (95% CI)	2.4 (2.2—2.5)	2.4 (2.3—2.6)	0.59
Tissue iron deficiency[a] (%) (95% CI)	20.2 (11.9—28.5)	24.6 (16.9—32.3)	0.54

[a] Defined as soluble transferrin receptors >3.0 mg/l for children ≤10 years of age and >2.7 mg/l for children >10 years of age.

Table 2 Baseline characteristics of the 378 schoolchildren in the four supplementation groups

	Placebo/placebo (n = 92)	Placebo/multi-micronutrients (n = 93)	Iron/placebo (n = 98)	Iron/multi-micronutrients (n = 95)
Mean age (years)	10.1	10.3	10.3	10.2
Female (%)	51.1	54.3	51.5	52.1
Mean haemoglobin (g/l)	132.4	130.3	128.5	127.3
Anaemic children (%)	6.5	11.6	11.6	17.4
Geometric mean serum ferritin (μg/l)	20.8	22.0	21.0	21.8
Serum ferritin <12.0 μg/l (%)	14.1	16.9	22.2	19.5
Geometric mean serum transferrin receptors (mg/l)	2.2	2.4	2.5	2.4
Tissue iron deficiency (%)[a]	20.8	25.9	27.0	27.0
Ascaris lumbricoides				
Prevalence (%)	44.6	44.2	42.4	43.8
Intensity (geometric mean epg among positives)	2655	1645	3135	2972

epg: eggs per gram faeces.
[a] Defined as soluble transferrin receptors >3.0 mg/l for children ≤10 years of age and >2.7 mg/l for children >10 years of age.

regression analysis. The interaction indicates that multi-micronutrients were important for the effect of iron. Thus, iron without multi-micronutrients resulted in a significantly lower reinfection rate than iron with multi-micronutrients (21.8 vs. 37.7%, P = 0.035). Thus, iron increased reinfection among those receiving multi-micronutrients (37.7 vs. 26.5), but reduced reinfection among those receiving placebo (21.8 vs. 32.4). However, in a multiple logistic regression analysis none of the four possible supplementation regimes were predictors. At the 10 month follow-up, *A. lumbricoides* reinfection prevalence was very similar in all groups (Table 3) and neither iron nor multi-micronutrient supple-

Table 3 *Ascaris lumbricoides* infection rates and intensities in the four supplementation groups at 6 and 10 months

A. lumbricoides infection	Treatment group				P-value
	Placebo/placebo	Placebo/multi-micronutrients	Iron/placebo	Iron/multi-micronutrients	
At 6 months (n = 283)					
Prevalence (%)	32.4	26.5	21.8	37.7	0.17
Intensity (epg)[a]	3035	3146	1409	1603	0.28
At 10 months (n = 215)					
Prevalence (%)	46.0	42.6	47.5	47.4	0.96
Intensity (epg)[a]	2646	2628	1902	3110	0.73

epg: eggs per gram faeces.
[a] Geometric mean among positives.

Table 4   *Ascaris lumbricoides* reinfection intensities among positives (geometric mean and 95% CI) after 6 and 10 months of iron and non-iron supplementation

|	Treatment group		P-value
	Iron	Non-iron	
A. lumbricoides reinfection intensity (epg)			
At 6 months (*n* = 88)	1600 (1022—2532)	3085 (1877—5071)	0.056
At 10 months (*n* = 97)	2421 (1553—3776)	2638 (1605—4337)	0.79

epg: eggs per gram faeces.

mentation were predictors in a multiple logistic regression analysis (data not shown).

There was no interaction between iron and multi-micronutrient supplementation on *A. lumbricoides* reinfection intensity at 6 months ($P = 0.81$) or at 10 months ($P = 0.46$). The effect of iron was therefore assessed without considering the multi-micronutrient supplementation. Children allocated to iron had a lower reinfection intensity at six months compared to children not receiving iron although not significant at the 5% level. At 10 months, *A. lumbricoides* egg loads were still lower in the iron group compared to the non-iron group, but the difference was not significant (Table 4).

The effect of iron supplementation at six months was related to the number of tablets taken (Figure 2). There was a significant difference in the intensity of infection between the iron and non-iron supplemented children who took 25 (half the optimal for the six months) or more tablets ($P = 0.018$). This difference was absent in those who took less than 25 tablets.

Multiple linear regression analysis was performed to identify predictors of *A. lumbricoides* intensity and to control for baseline differences. The analysis showed that iron supplementation, baseline Hb and number of tablets taken were predictors of *A. lumbricoides* intensity after six months of supplementation (Table 5). There was no interaction between iron supplementation and the number of tablets taken at six months ($P = 0.21$). The antilog of the regression coefficient of iron supplementation was 0.5, which means that children allocated to iron were reinfected with 0.5 or 50% of the intensity found in children allocated to placebo. If, however, children who took more than 50 tablets (more than 50% of the optimal number) during the whole intervention period were selected, a similar model had an antilog coefficient of iron supplementation of 0.2 ($P < 0.001$) and a
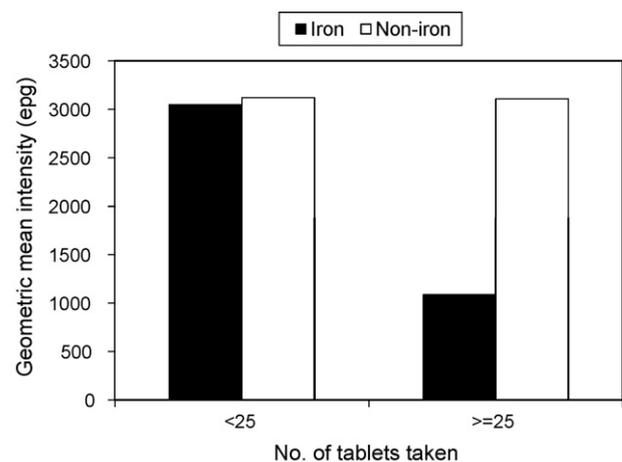
Figure 2   The effect of iron supplementation on the intensity of *Ascaris lumbricoides* infection [eggs per gram (epg)] among positive children at the six month follow-up and the relationship to the number of tablets taken.

R^2 of 0.30. This means that the effect of iron was even more pronounced as the intensity of infection in those receiving iron was only 20% of the intensity found in those receiving placebo. *Ascaris lumbricoides* intensities at baseline were the only predictors of reinfection intensities at the 10 month follow-up. Neither iron nor multi-micronutrient supplementation was a predictor (data not shown).

4. Discussion

Typical of a densely populated peri-urban area, *A. lumbricoides* was the most important helminth infection in our study population. The lack of hookworm can be attributed to

Table 5   The antilog of the regression coefficients (10^B), 95% CI and corresponding *P*-values of predictors of *Ascaris lumbricoides* intensity ($\log_{10}$ transformed) among positives at six months in a multiple linear regression model controlling for baseline infection and geophagy (*n* = 80, R^2 = 0.17)

	10^B	95% CI	P-value
Iron supplementation[a]	0.5	0.2—0.9	0.032
Multi-micronutrient supplementation[b]	1.2	0.6—2.5	0.55
Haemoglobin at baseline[c]	0.4	0.2—0.8	0.012
Number of tablets taken[d]	0.5	0.2—0.9	0.033

[a] Coded as 0 = placebo, 1 = iron.
[b] Coded as 0 = placebo, 1 = multi-micronutrient.
[c] Coded as 0 <130 g/l, 1 ≥130 g/l.
[d] Coded as 0 <25 tablets, 1 ≥25 tablets.

the fact that this is an urban area and children wear shoes, at least in school as part of the school uniform.

Schoolchildren allocated to iron supplementation had slightly lower *A. lumbricoides* reinfection intensities at six months compared to schoolchildren allocated to placebo. This effect was most pronounced in those receiving iron without multi-micronutrients. The difference, however, disappeared at 10 months, which probably can be attributed to the general decline in tablet intake between the first six months and the last four months. Although not supported in a multiple regression analysis, a similar trend was seen in reinfection rates, as iron without multi-micronutrients resulted in a significantly lower *A. lumbricoides* reinfection rate than iron with multi-micronutrients. A similar study in adults in Kenya found a strong effect of iron supplementation on *A. lumbricoides*, *T. trichiura* and *S. mansoni* reinfection rates, but not intensities.[4] It was postulated that the effect on *A. lumbricoides* and *T. trichiura* reinfection rates could be due to changes in behaviour like geophagy, which is a source of infection. Although there was a general decline in geophagy between baseline and the six month follow-up,[8] this was not restricted to a particular supplementation group. In fact, the iron supplemented group had the smallest decrease in geophagy rates and amount of earth eaten. Thus, if differences in geophagy practices were the explanation, *A. lumbricoides* intensities should be higher in the iron supplemented group compared to the placebo group, not lower.

Several studies have shown that supplementation with miscellaneous micronutrients has an effect on infectious diseases. For example, vitamin A has effects on diarrhoea, malaria and post-measles pneumonia,[14,15] and zinc has effects on diarrhoea and respiratory tract infections,[16–18] while evidence of an effect of micronutrients on helminth infection is more sparse.[4,5,19,20] It was expected that combined iron and multi-micronutrient supplementation would exhibit a greater effect than iron supplementation alone. This was because supplementation with vitamin A in combination with iron has been shown to be more efficient in improving iron status than iron alone[21,22] and because minerals and vitamins with antioxidant properties should work in concert to have an optimal effect.[23] Furthermore, micronutrients other than iron are known to be of importance to immune function.[24–26] However, there is a possibility that interactions between micronutrients are not always synergistic. As an example, iron has been shown to interfere with zinc absorption and vice versa.[27,28] Our study, however, suggests that iron and the other micronutrients worked together, as the greatest increase in Hb was seen in the combined group.[29]

Iron is important for the proper functioning of the immune system, as iron deficiency has been associated with impaired T lymphocyte function,[30,31] which laboratory studies have shown affects the expulsion of gastrointestinal nematodes.[32,33] Iron is also necessary for disease-causing micro-organisms. The sequestration of iron by the host is a well-known defence mechanism in infection, denying invading organisms access to the valuable element. It is therefore reasonable to suppose that iron is influential in the outcome of disease and could have a protective effect against reinfection with *A. lumbricoides*. However, in the absence of a significant increase in Hb in the group receiving iron with-out other micronutrients,[29] it would appear that the effect of iron on infection might be mediated through other means than increased iron status. The effect of malaria on Hb in this study was negligible as there was very little malaria in the study area. At baseline, only 2% of the subjects were positive for malaria. The possibilities are that the pathway of iron metabolism in the presence or absence of other micronutrients is different, or that the presence of unabsorbed iron in the intestinal tract increases the production of free radicals and renders the gut unsuitable for the establishment of infection.[4] This is supported by the fact that the effect of iron was most pronounced in those receiving iron without other micronutrients, indicating that micronutrients with antioxidant properties, e.g. vitamins A, C and E, are able to neutralize the free radicals generated by the iron.

Because worm burden is determined indirectly as eggs in faeces, there are two other possible explanations for the decrease in intensity as a consequence of iron supplementation. One is a decrease in worm fecundity and the other is an increase in the volume of stool. The fecundity of *Trichuris suis* was increased in pigs fed a low iron diet compared to pigs on a normal diet, while there was no difference in the fecundity of *A. suum*.[34] In the same study the authors did not observe a protective effect of iron on either infection intensities or rates, but this was not conclusive as the sample size was very small. Stool volume could be increased if children supplemented with iron had an increased appetite, as was shown in a study from Kenya.[35] This is a possibility, as iron supplementation had a significant positive effect on weight in our study population. Furthermore, the effect of iron on the change in weight between baseline and six months was related to tablet intake, as was the decrease in infection intensity.[29] However, since there was no correlation between change in weight and *A. lumbricoides* egg intensity at six months, and since gain in weight neither predicted *A. lumbricoides* reinfection intensity nor influenced the effect of iron in the regression model, iron seems to affect weight and infection intensity independently.

The calculated sample size for this study was doubled to be able to measure possible interactions between the effect of iron and other micronutrients. In the absence of any interaction, the power of the study was retained despite the high drop-out rates. This study adds to a tiny but seemingly growing body of evidence suggesting that iron supplementation might have some effect on helminth infections. It is unclear whether the effect is immunologically mediated or brought about by some other means. However, although not conclusive the most likely interpretation of our results is that oral iron contributes to the regulation of *A. lumbricoides* infection through changes in intra-luminal factors. More studies are needed to clarify the role of micronutrients in helminth infection and to shed more light on the mechanisms involved.

Authors' contributions: All authors planned the study and designed the protocol; MN conducted the field study and managed and collected the data, carried out the analysis and interpretation of the data and prepared the first draft of the manuscript. All authors were involved in preparing subsequent drafts and read and approved the final manuscript. MN is guarantor of the paper.

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