The effect of iron fortification and de-worming on anaemia and iron status of Vietnamese schoolchildren

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Previous data from Vietnam show that anaemia is highly prevalent among schoolchildren, who are considered not to be iron deficient. *Trichuris* infection doubled the risk of anaemia. The present study aimed to evaluate the hypothesis that de-worming is more effective than iron fortification in an anaemic, infection-prone population. In a trial with a 2 × 2 factorial design, 425 anaemic children aged 6–8 years were randomly assigned to receive either iron-fortified noodles or placebo, and mebendazole or placebo. Outcomes considered were change in haematological indicators of iron status (Hb, serum ferritin (SF), serum transferrin receptor (TfR) and haemoglobinopathies analysis); inflammations (C-reactive protein (CRP)); parasite infection status (hookworm, *Trichuris* and *Ascaris* infection); and IgE. ANOVA and logistic regression were used to assess the effects of iron fortification and de-worming on Hb, SF, TfR, body iron and anaemia. Hb improved in all groups after 6 months of intervention. Iron fortification significantly improved Hb, SF and body iron (2·6 g/l, 16·3 μg/l and 1 mg/kg, respectively). Prevalence of elevated IgE was very high at baseline (99 %) and significantly reduced to about 75 % in all groups after intervention. De-worming unexpectedly showed no effect on Hb, iron status and IgE level. It is concluded that iron fortification slightly improved anaemia and iron status in anaemic schoolchildren in rural Vietnam that were not considered iron deficient. Chronic infection or other unidentified factors may play an important role in the seasonal reduction of anaemia seen in all treatment groups.


Anaemia is a significant public health problem in Vietnam. The 2000 National Nutrition Risk Factor Survey in Vietnam showed an anaemia prevalence of 34 % in children under 5 and 25 % in women (Ninh et al. 2001). No nationally representative data are available on the prevalence of anaemia among primary schoolchildren in Vietnam; however, a few local studies showed an anaemia prevalence of around 30 % (Le, 1999; Hoa, 2002). Although various nutrients and cofactors are involved in maintaining the normal synthesis of Hb, iron deficiency is the most frequent cause of anaemia on a worldwide basis (Osório, 2002). However, infection and inflammation (Yip & Dallman, 1988; Means, 2000), malaria (Fleming, 1981), intestinal parasite infection (Stoltzfus et al. 1997, 2000), as well as haemoglobinopathies (Dugdale, 2001; INACG, 2002) may play a role. Their relative importance in Vietnam is still unclear. A previous cross-sectional study conducted in Tam Nong district, Phu Tho province, a poor rural area of North Vietnam, showed a high prevalence of anaemia among primary schoolchildren with low iron deficiency as measured by serum transferrin receptor (TfR) and serum ferritin (SF) with 2 % of children with TfR > 8·5 mg/l and 0·5 % of children with SF < 12 μg/l (Le et al. 2005). Further, *Trichuris* infection was associated with a doubled risk of anaemia, probably not mediated through iron deficiency. Food fortification is often suggested as one of the most effective and sustainable strategies for increasing iron intake in the general population (Hurrell, 1997). Also in the nutritional strategies for prevention and control of micronutrient deficiencies in Vietnam, food fortification is considered as a sustainable solution to combat iron deficiency anaemia (Ninh et al. 2001). However, based on the previous study, it is hypothesized that de-worming is more effective than iron fortification in an anaemic, infection-prone population that was not considered iron deficient. Using a trial with iron-fortified noodles and de-worming, we assessed the changes in iron and anaemia status among anaemic schoolchildren, and tested whether the present data are consistent with this hypothesis.

Subjects and methods

Study design and population

The study was implemented from November 2004 to May 2005 in six primary schools in Tam Nong district, Phu Tho province, situated 90 km from Hanoi.Selection was based
on high prevalence of anaemia and absence of interventions to control iron-deficiency anaemia in schoolchildren. Children recruited into the study were in Grades 1–3 with Hb concentrations <110 g/l but not <70 g/l in an initial Hb screening study. We excluded children with Hb level less than 70 g/l because these children were considered to be severely anaemic and received treatment immediately. The study concerns a randomized, placebo-controlled double-blind parallel trial with a 2 × 2 factorial design plus standard treatment (iron supplementation and de-worming) and an intervention period of 6 months. A total of 425 eligible children were randomly assigned to one of five groups receiving: (1) iron-fortified noodles and mebendazole (Fe + MEB); (2) noodles without iron fortificant and mebendazole (MEB); (3) iron-fortified noodles and placebo (Fe); (4) noodles without iron fortificant and placebo (placebo); and (5) iron supplementation and mebendazole (Fe tablet + MEB) (Fig. 1). Randomization was carried out by a researcher from the Division of Human Nutrition, Wageningen University, The Netherlands, who did not know the children and could not introduce bias in the randomization. Stratified randomization was applied based on the Hb levels and age of the children to ensure equal distribution of Hb and age across groups. Sample size was assessed to achieve a statistical power of 95 %, based on an α error of 0·05, a between-group difference in treatment effect of 5 g Hb/l in Hb concentration being clinically relevant, a standard deviation of 11 g/l (Hoa, 2002) and accounting for 10 % of children being lost in the course of the intervention. In this paper we focus on the placebo-controlled parallel trial with a 2 × 2 factorial design to assess the effect of iron-fortified noodle and de-worming on iron and anaemia status of schoolchildren. The extent of the effect of the iron-fortified noodles compared to the standard treatment and its policy relevance is discussed in another paper (Le, 2006).

Children were invited for the study and a written informed consent was obtained from their parents. The study was approved by the Scientific Committee of the National Institute of Nutrition and the Ethics Committee of Hanoi Medical University – Ministry of Health.

**Products and field procedures**

Fortified instant noodles were produced at the Hanoi Food Company. Noodles were fortified with a water-soluble, highly bioavailable iron compound (NaFeEDTA; Ferrazone®; Akzo Nobel Chemicals Pte Ltd, Arnhem, The Netherlands) to a fortified level of 10·7 mg iron/52 g noodles calculated based on the JECFA recommendation of an acceptable daily intake of 2·5 mg EDTA/kg body weight (JECFA, 1974) and an average body weight of 29 kg (Food & Agriculture Organization & World Health Organization, 1998). Before intervention retention of iron after production and preparation of fortified instant noodles was checked in laboratories at the National Institute of Nutrition Hanoi, Wageningen University and Akzo Nobel Chemicals Pte Ltd. Capillary zone electrophoresis analysis (Sheppard & Henion, 1997) showed that 70 % of the NaFeEDTA dissolves within 5 min into the soup independent of extraction time. No degradation products of NaFeEDTA were found.

Noodles were prepared in school by caretakers trained by field staff. The whole content of a package of instant noodles (52 g) was broken into smaller pieces and put into a plastic heat-resistant bowl together with seasoning. Hot boiled water from a thermos flask (200 ml) was added to the noodles and given to children at break time (09.00 hours) 5 d/week for 6 months under the supervision of teachers and field staff. Children were encouraged to consume all the noodles and water. During the intervention, 100 % of the study population consumed the given noodles or iron tablet. More than 95 % of

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![Fig. 1. Study profile: initial screening to enrol anaemic children in the study, followed by a 6-month intervention of: Fe, iron-fortified noodles and placebo; Fe + MEB, iron-fortified noodles and mebendazole; MEB, noodles without iron fortificant and mebendazole; Placebo, noodles without iron fortificant and placebo; Fe tablet + MEB, iron supplementation and mebendazole.](image-url)
children were present every school day (5 d/week) and more than 95 % finished the whole portion of the noodles given to them daily under the supervision of caretakers and field staff (based on notes taken by caretakers in a monitoring book). MEB (500 mg) and an identical placebo were given to children at the beginning of the intervention and after 3 months. Children, teachers and researchers were blinded to the treatment.

Data collection

Capillary blood samples were taken from children’s fingers during screening for Hb measurement by a cyanmethaemoglobin method. Children’s weight was measured to the nearest 100 g using an electronic scale (Seca 890; Seca, Birmingham, UK) and standing height was measured to the nearest 1 mm with a microtoise (Stanley Mabo no. 191; Stanley Mabo, Poissy, France) during baseline and after intervention. Venous blood (5 ml) was collected in the morning at baseline (T0) and after intervention (T6); 20 μl whole blood was pipetted immediately before coagulating into a tube containing 5.0 ml Drabkin’s reagent with a Sali pipette for Hb measurement. An aliquot of whole blood was taken for analysing haemoglobinopathies. The remaining blood was allowed to clot for 30 min at room temperature, centrifuged at 3000 g until SF, TfR, C-reactive protein (CRP) and IgE analysis at the end of the intervention.

For assessment of intestinal parasite infection before and after intervention, containers for collection of stools were distributed to each class and children were asked to collect and deliver a sample of their faeces to school the next day. In cases where children were unable to return a sample, one of the field workers returned the next day to collect the rest of the samples.

Laboratory analysis

Hb concentration was measured in whole blood within 12 h of sampling by a cyanmethaemoglobin method using a Sigma Kit (Sigma Chemical Co., St Louis, MO, USA) in the Tamnong District Health Centre. The CV of intra-assays and inter-assays was 4.0 ± 1.2 and 5.0 ± 2.0 %, respectively. SF, TfR, CRP and IgE analysis was carried out at the same time for both samples of baseline and after intervention at the National Institute of Nutrition and the laboratory of Hanoi Medical University in May and June 2005. Concentrations of SF and TfR were analysed by an ELISA method (catalogue numbers S-22 and TF-94; Ramco Laboratories Inc., Houston, TX, USA), with inter-assay variability of 4–7 % and 4–8 %, respectively. Serum CRP was measured by nephelometry using Epress plus, with an inter-assay variability of 4–8 %. Serum IgE was determined by ELISA using the Kallestad Total IgE Microplate Kit from Kallestad GmbH (Mannheim, Germany), with an inter-assay variability of 4–6 %. A 10 % subsample was re-examined for quality control. Haemoglobinopathies analysis was performed by using the Variant Beta-Thalassemia Short Program (Bio-Rad Laboratories Inc., Hercules, CA, USA) within 24 h of sampling in the Children’s Hospital, Hanoi, Vietnam. Stools samples were examined using the Kato-Katz Technique – a cellophane faecal thick smear method (World Health Organization, 1991). Hookworm, Trichuris and Ascaris eggs were counted. A 10 % subsample of smears was re-examined for quality control.

Data analysis

Anthropometric indices were calculated using WHO/NCHS reference data (World Health Organization, 1995). Being wasted, stunted and underweight was defined by z-scores $< -2$sd for weight-for-height, height-for-age and weight-for-age, respectively. Anaemia was defined as Hb concentrations <115 g/l (World Health Organization, 2000). Iron deficiency was defined as SF concentrations $<12$ μg/l (World Health Organization, 2000), and tissue iron deficiency was defined as TIR concentration $>8.5$ mg/l (Skikne et al. 1990). Body iron content was calculated using the following formula: body iron (mg/kg) = $-((\log(TfR/SF)\text{ ratio}) - 2.8229)/0.1207$ (Cook et al. 2003).

CRP and IgE concentrations were considered to be elevated when ≥8 mg/l (Hoffbrand & Pettit, 1993) and >90 IU/ml (Heil et al. 2000), respectively. Hb type was determined in each subject on the basis of haematological indexes: HbAA (normal haemoglobin type), HbF (haemoglobin F); HbA2 (haemoglobin A2); HbAE (trait for haemoglobin E disease) or HbEE (haemoglobin E disease). Severity of intestinal worm infections was expressed as the number of eggs/g faeces using the WHO classification system (World Health Organization, 1987).

Data were entered into the computer, cleaned and managed using Epi Info version 6 (Dean et al. 1995) and analysed using SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA; Field, 2005). Data were checked for normality by visual inspection. One-way ANOVA was used to determine differences in Hb concentration and other biochemical indicators between groups. Paired t test was used to compare Hb and other biomarkers before and after intervention. χ² test and McNemar test were used to assess the differences in proportions between and within groups. To assess the effect of iron fortification and de-worming on indicators of iron status, we calculated dummy variables for each treatment (iron fortification and de-worming), as well as their interaction. The effect on change in Hb concentration, SF, TfR and body iron, respectively, was then assessed by including them in a general linear model, so as to simulate a two-way ANOVA. In all regression analyses, we adjusted for concentration at baseline, sex and age, and sometimes CRP or thalassemia. Logistic regression was used similarly to study the effect of iron fortification and de-worming on anaemia prevalence, adjusting for baseline characteristics.

Results

At baseline, the mean age of children was 87.4 (sd 10.2) months. The four groups did not significantly differ in age. Hb concentration, iron status (SF, TIR and body iron) and nutritional status (Table 1) nor in CRP, IgE status and parasite infection (Table 2). The prevalence of iron deficiency was very low as only 1.2 % of children showed SF concentration below 12 μg/l and 5.5 % of children showed TIR above 8.5 mg/l. Mean body iron was around 6.3 mg/kg body weight (Table 1). Two-thirds of the children were infected with
Ascaris and/or Trichuris, and about 8-6% were infected with hookworm. However, the intensity of Ascaris and Trichuris infection was mainly 'light' or 'average', and only 27% and 2% among infected children showed severe infection with Ascaris or Trichuris, respectively. The hookworm infection was 'light' for most of the cases (data not show). Very few children showed elevated CRP levels (1.8%) and 99% of the children showed an elevated IgE level (Table 2). The prevalence of thalassaemia (HbA2, HbAE, HbF) was around 7% (Table 1).

Prevalence of hookworm infection decreased in all four groups, except the placebo group, where the number of children with elevated CRP level even increased. Prevalence of elevated IgE significantly decreased in all four groups, with no significant differences between groups (one-way ANOVA): **P < 0.001. Values were significantly different within group before and after intervention (McNemar): †P < 0.01; ††P < 0.01; †††P < 0.001.

Table 1. Baseline characteristics by treatment group in rural Vietnamese schoolchildren (n 409)

<table>
<thead>
<tr>
<th>Treatment groups§</th>
<th>Fe (n 86)</th>
<th>Fe + MEB (n 79)</th>
<th>MEB (n 79)</th>
<th>Placebo (n 82)</th>
<th>Fe tablet + MEB (n 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male sex (%)</strong></td>
<td>51.2</td>
<td>50.6</td>
<td>46.8</td>
<td>48.8</td>
<td>53.0</td>
</tr>
<tr>
<td><strong>Age (months)</strong></td>
<td>88.0</td>
<td>10.1</td>
<td>87.6</td>
<td>11.4</td>
<td>87.7</td>
</tr>
<tr>
<td><strong>Hb (g/l)</strong></td>
<td>107.5</td>
<td>8.3</td>
<td>107.3</td>
<td>6.8</td>
<td>108.5</td>
</tr>
<tr>
<td>Serum ferritin (µg/l)†††</td>
<td>55.0</td>
<td>33.0, 78.8</td>
<td>47.4</td>
<td>33.7, 67.5</td>
<td>58.9</td>
</tr>
<tr>
<td>Transferrin receptor (mg/l)‡</td>
<td>5.9</td>
<td>3.0</td>
<td>6.0</td>
<td>1.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Body iron (µg/kg)</td>
<td>6.5</td>
<td>2.3</td>
<td>6.1</td>
<td>2.9</td>
<td>6.5</td>
</tr>
<tr>
<td>Serum ferritin &lt;12 µg/l (%)</td>
<td>1.2</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Transferrin receptor &gt;8.5 mg/l (%)</td>
<td>3.5</td>
<td>3.8</td>
<td>6.3</td>
<td>8.5</td>
<td>3.6</td>
</tr>
<tr>
<td>WAZ</td>
<td>1.8 ± 0.8</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>HAZ</td>
<td>1.6 ± 0.7</td>
<td>1.6 ± 0.8</td>
<td>1.7 ± 0.8</td>
<td>1.7 ± 0.8</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>WHZ</td>
<td>1.2 ± 0.8</td>
<td>1.2 ± 0.7</td>
<td>1.2 ± 0.7</td>
<td>1.2 ± 0.6</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>Thalassaemia (%)</td>
<td>9.3</td>
<td>6.3</td>
<td>6.3</td>
<td>8.5</td>
<td>8.4</td>
</tr>
</tbody>
</table>

WAZ, weight-for-age z-score; HAZ, height-for-age z-score; WHZ, weight-for-height z-score.
§ Values are means and standard deviations unless otherwise indicated.
††† Values are medians with 25th and 75th percentiles.
‡ Values are means and standard deviations unless otherwise indicated.
† Values are medians with 25th and 75th percentiles.

Table 2. Parasite infection, inflammation and nutritional status before and after intervention in rural Vietnamese schoolchildren

<table>
<thead>
<tr>
<th>Treatment groups§</th>
<th>Fe (n 86)</th>
<th>Fe + MEB (n 79)</th>
<th>MEB (n 79)</th>
<th>Placebo (n 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascaris (%)</strong></td>
<td>69.8</td>
<td>65.8</td>
<td>67.1</td>
<td>69.5</td>
</tr>
<tr>
<td>At the end***</td>
<td>68.6</td>
<td>44.3†††</td>
<td>41.8†††</td>
<td>74.4</td>
</tr>
<tr>
<td><strong>Trichuris (%)</strong></td>
<td>76.7</td>
<td>78.5</td>
<td>63.3</td>
<td>73.2</td>
</tr>
<tr>
<td>At the end***</td>
<td>47.7†††</td>
<td>15.2†††</td>
<td>48.1†</td>
<td>72.0</td>
</tr>
<tr>
<td><strong>Hookworm (%)</strong></td>
<td>7.0</td>
<td>10.1</td>
<td>7.6</td>
<td>11.0</td>
</tr>
<tr>
<td>At the end†</td>
<td>4.7</td>
<td>1.3§</td>
<td>2.5</td>
<td>3.7</td>
</tr>
<tr>
<td><strong>CRP elevated ≥8 mg/l (%)</strong></td>
<td>1.2</td>
<td>2.5</td>
<td>3.8</td>
<td>0.0</td>
</tr>
<tr>
<td>At the end</td>
<td>0</td>
<td>1.3</td>
<td>0</td>
<td>3.7</td>
</tr>
<tr>
<td>IgE elevated &gt;90 IU/ml (%)</td>
<td>97.7</td>
<td>98.7</td>
<td>100</td>
<td>98.8</td>
</tr>
<tr>
<td>At the end</td>
<td>76.5†††</td>
<td>74.3†††</td>
<td>78.5†††</td>
<td>72.0†††</td>
</tr>
<tr>
<td><strong>Underweight (%)</strong></td>
<td>41.9</td>
<td>51.9</td>
<td>50.6</td>
<td>45.1</td>
</tr>
<tr>
<td>At the end</td>
<td>33.7†††</td>
<td>46.8†††</td>
<td>38.1†††</td>
<td>35.4†††</td>
</tr>
<tr>
<td><strong>Stunting (%)</strong></td>
<td>30.2</td>
<td>31.6</td>
<td>41.8</td>
<td>31.7</td>
</tr>
<tr>
<td>At the end</td>
<td>29.1</td>
<td>27.8</td>
<td>29.1†††</td>
<td>29.3</td>
</tr>
<tr>
<td><strong>Wasting (%)</strong></td>
<td>9.3</td>
<td>16.5</td>
<td>13.9</td>
<td>12.2</td>
</tr>
<tr>
<td>At the end</td>
<td>5.8†††</td>
<td>17.7</td>
<td>13.9</td>
<td>13.4</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein.
Values were significantly different between groups (one-way ANOVA): ***P < 0.001.
Values were significantly different within group before and after intervention (McNemar): †P < 0.05; ††P < 0.01; †††P < 0.001.
§ Fe, iron-fortified noodles and placebo; Fe + MEB, iron-fortified noodles and mebendazole; MEB, noodles without iron fortificant and mebendazole; Placebo, noodles without iron fortificant and placebo; Fe tablet + MEB, iron supplementation and mebendazole.
Anaemia and infection in Vietnam

Table 3. Change in Hb, anaemia and iron status during intervention in rural Vietnamese schoolchildren

<table>
<thead>
<tr>
<th>Treatment groups§</th>
<th>Fe (n 86)</th>
<th>Fe + MEB (n 79)</th>
<th>MEB (n 79)</th>
<th>Placebo (n 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Hb (g/l)*</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>Change in serum ferritin (μg/l)**</td>
<td>17-83 0.97+++</td>
<td>17-54 0.85+++</td>
<td>14-60 1.00+++</td>
<td>15-40 0.92+++</td>
</tr>
<tr>
<td>Change in TfR (mg/l)***</td>
<td>15-05 3.28+++</td>
<td>17-86 3.61+++</td>
<td>17-00 3.05+++</td>
<td>5-37 4.31</td>
</tr>
<tr>
<td>Change in body iron (mg/kg)++</td>
<td>−0-35 0.10+++</td>
<td>−0-38 0.10+++</td>
<td>−0-39 0.10+++</td>
<td>−0-31 0.13‡</td>
</tr>
<tr>
<td>Anaemia (%)</td>
<td>89-5±±</td>
<td>84-8±±</td>
<td>83-5±±</td>
<td>91-5±±</td>
</tr>
<tr>
<td>Serum ferritin &lt;12 μg/l (%)</td>
<td>10-5 11-4</td>
<td>15-2 19-5</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>TIR &gt;8-5 mg/l (%)</td>
<td>3-5 3-8</td>
<td>6-3 8-5</td>
<td>5-2 6-1</td>
<td>6-1 6-1</td>
</tr>
</tbody>
</table>

TIR, serum transferrin receptor.

Values were significantly different between groups (one-way ANOVA): *P<0.001; **P<0.01; ***P<0.001.

Values were significantly different within group before and after intervention (McNemar): †††P<0.001.

§ Fe, iron-fortified noodles and placebo; Fe + MEB, iron-fortified noodles and mebendazole; MEB, noodles without iron fortificant and mebendazole; Placebo, noodles without iron fortificant and placebo; Fe tablet + MEB, iron supplementation and mebendazole.

Table 4. Differential change in Hb, serum ferritin (SF), serum transferrin receptor (TfR) and body iron during the intervention period by treatment, compared to no treatment, from four different models (n 320)§

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iron fortification</th>
<th>De-worming</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome variables</td>
<td>Estimated effects 95 % CI P</td>
<td>Estimated effects 95 % CI P</td>
</tr>
<tr>
<td>Change in Hb (g/l)</td>
<td></td>
<td>2-68</td>
</tr>
<tr>
<td>Change in SF (μg/l)</td>
<td></td>
<td>16-32</td>
</tr>
<tr>
<td>Change in TfR (mg/l)</td>
<td></td>
<td>−0-03</td>
</tr>
<tr>
<td>Change in body iron (mg/kg)</td>
<td></td>
<td>1-02</td>
</tr>
</tbody>
</table>

§ Six children with CRP ≥ 8 mg/l were excluded.
| Adjusted for Hb baseline, thalassaemia, sex and age.
| Adjusted for SF baseline, C-reactive protein (CRP), sex and age.
| Adjusted for TIR baseline, sex and age.
| Adjusted for body iron baseline, CRP, sex and age.
due to infection or inflammation masking iron deficiency during infection. Malope et al. (2001) supposed that SF values between 12 and 100 μg/l can still indicate iron deficiency especially in the presence of infection and inflammation. However, considering the low prevalence of elevated CRP in the present study population, it seems unlikely that acute infection is affecting the SF levels. TIR has an advantage over SF as it is unaffected by the acute-phase response (Ritchie et al. 2004) and is therefore considered to be a sensitive indicator of iron status in children (Asobayire et al. 2001). However, the diagnostic cut-offs for TIR used to identify iron deficiency were derived from studies in adults (Anonymous, 2000). These cut-offs may not apply to children due to their increased erythropoiesis during growth (Kling et al. 1998). Based on a study conducted in Morocco and Côte d’Ivoire, Zimmerman et al. (2005) suggest a lower cut-off point for white compared to black Africans although the differences between suggested cut-off points are small. In addition, researchers indicate, regardless of diagnostic cut-offs, modest sensitivity and specificity of TIR in identifying iron deficiency in children (Ritchie et al. 2004; Zimmermann et al. 2005).

Based on the previous study indicating that Trichuris infection was associated with a doubled risk of anaemia, we expected an improved Hb level after de-worming. The results of the present study, however, did not support this hypothesis as de-worming showed no effect on Hb levels and iron status. The Trichuris infection in the present study population was classified as mild. Therefore, the absence of effect is in line with the previous studies showing that blood loss can occur in Trichuris infection, but probably becomes significant only in severe infection (Layrisse et al. 1967; Stephenson, 1987; Stephenson et al. 2000). MEB treatment was given directly to children at school by researchers and field staff, and the two groups receiving MEB showed a larger reduction in prevalence of all three types of worms. Surprisingly, the effects on Trichuris in both groups receiving iron were remarkable and indicate interplay between certain helminth infections and iron. More data are becoming available suggesting that iron (and other micronutrients) has important effects on the natural resistance to parasites and that iron supplementation may reduce parasite infection. Studying Trichuris suis, Pedersen et al. (2001) found that iron deficiency increased the severity of T. suis in pigs. It has recently been documented that adults given supplemental iron have significantly lower reinfection rates of Trichuris trichiura, Ascaris lumbricoides and Schistosoma mansoni infection (Olsen et al. 2000). The effect was suggested to be due to reduced risk behaviour, to improved immune function or to unfavourable host gut conditions caused by an increased oxidative stress.

Remarkably, Hb increased and anaemia reduced in all groups, including the placebo group, during the intervention period. Hb levels are mainly a function of red blood cell production and turnover, affected by factors other than iron deficiency in the present study population. A study in school-children in north-east Thailand found a high prevalence of anaemia without iron deficiency, with haemoglobinopathies, suboptimal vitamin A status and age as the major predictors of Hb concentration (Thurlow et al. 2005). Another study in school-age children in Alaska, with 15% anaemia and 8% being iron deficient, showed an association between a bacterial infection (Helicobacter pylori) and anaemia (Baggett et al. 2006). Data from the present study indicated that the prevalence of thalassaemia was very low and therefore could not explain the high prevalence of anaemia in the study population. Vitamin A status is considered to be a major determinant of anaemia (Gamble et al. 2004), but sub-samples (n=81) analysed for serum retinol levels showed a very low prevalence of suboptimal vitamin A deficiency (8 and 6% with serum retinol concentration <0.70 μmol/l at baseline and after intervention, respectively). Other nutrient deficiencies associated with anaemia include deficiencies of vitamins B6, B12, riboflavin and folic acid (Broek & Letsky, 2000), although not all of the causal pathways are yet clearly understood. Vitamin B12 and folic acid deficiency are associated with an increased TIR (Gibson, 2005), but we did not observe elevated TIR levels in the present study population. The role of other nutrients could not be verified in the present study. Malaria remains another important cause of anaemia in tropical countries (Phillips & Pasvol, 1992; Cardoso et al. 1994), however, malaria was not present in the study area.

The increase of Hb levels in all groups, including the placebo group, might be due to an increase in iron intake due to a seasonal change in food habits or an increase in energy intake through the noodles. We do not have reason to believe there is a seasonal increase in iron intake. A food consumption study among a sub-sample of fifty-nine children (data not shown) showed no change in iron intake between October (baseline) and January, but we did not measure iron intake at the end of intervention (May). Although the noodles intervention did increase energy intake in the sub-sample, we do not think this caused the improvement of Hb levels, as the iron supplementation with de-worming group (the ‘standard’ treatment who did not receive noodles) also showed a Hb improvement (data not shown).

In the absence of the aforementioned causes of anaemia and the observed improvements in the placebo group, the present data may suggest the presence of anaemia of inflammation. Firstly, SF in the study population was much higher compared to the 50th percentile SF value (28-7 μg/l) for the age group 6–9 years in NHANES III (Gibson, 2005), being close to the 90th percentile level of 55-9 μg/l. This may indicate that inflammation is of a more chronic nature. Secondly, Malope et al. (2001) suggest the use of log TIR:SF ratio to differentiate between iron deficiency anaemia (ratio >2.55) and anaemia of inflammation (ratio <2.55), although the latter ratio was not able to exclude iron deficiency. In the present study the ratio of log TIR:SF was 2.06, indicating the existence of anaemia of inflammation but being unable to exclude iron deficiency. Children from the placebo group not receiving iron showed increased Hb levels, having a log TIR:SF ratio of 2.07, accompanied by absence of increase (in placebo) or even decrease (in de-worming only) in SF. This indicates a shift of iron from storage to Hb and according to Malope et al. (2001) this suggests that besides iron deficiency another likely cause of anaemia in the children was infection which improved during the supplementation trial. Thirdly, the seasonal burden of infections in developing countries has been recognized for many years by agricultural and health professionals (Tomkins, 2005). Mild inflammatory conditions such as upper-respiratory infections and otitis media, which remain common in early childhood, may contribute to anaemia (Yip & Dallman, 1988). In the study population the
proportion of children with elevated CRP is very low. However, CRP is a good measure of acute infection or inflammation but less appropriate when conditions are chronic (Looker et al. 1997). Earlier a relationship between IgE and respiratory disease was found (Hodge et al. 2001). In our previous study, *Trichuris* infection was associated with IgE levels which could have confounded the association of *Trichuris* with anaemia and Hb concentration. De-worming apparently did not affect IgE levels and if the assumption holds true that another unknown infection reflected by IgE plays a role in the anaemia in the study population, this would explain the absence of de-worming effect. In the present study, the high level of IgE and the reduction of IgE levels concurrently with reduction of anaemia independent of treatment may suggest that chronic infection may and intestinal parasite infection may not play a role in inflammation anaemia. Finally, the present data indicated that at the baseline 38·7 % children reported fever or respiratory infection in the previous 2 weeks but at the end survey this prevalence reduced to 13·2 % (data not shown). We did not find an association between fever and respiratory infection with anaemia, however, the reduction in fever and respiratory infection may reflect a general reduction of infection status during the intervention period in the study population. This supports our speculation that anaemia is associated with chronic infection in the present study population and that the anaemia reduction observed in the placebo group may be due to a seasonal reduction of chronic infection. However, the role of chronic infections in anaemia needs to be further investigated.

In conclusion, iron fortification slightly improved anaemia and iron status in anaemic schoolchildren in rural Vietnam that were not considered iron deficient. De-worming reduced prevalence of worm infection but had no effect on anaemia and iron status. A positive seasonal effect was seen in all treatment groups. Chronic infection or other unidentified factors including a limited iron uptake for haem synthesis may play an important role.

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