The effect of anthelmintic treatment on helminth infection and anaemia

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SUMMARY

A 24-week randomized double blind intervention trial was conducted on adult female tea pluckers from an estate in Bangladesh to investigate the impact of iron supplementation and anthelmintic treatment on changes in ferritin and haemoglobin levels as well as on prevalence and intensity of helminth infections. A total of 553 women were randomly assigned to 1 of 4 intervention groups: group 1 received iron supplementation on a weekly basis, group 2 received anthelmintic treatment at the beginning and half way through the trial, group 3 received both iron supplementation as group 1 and anthelmintic treatment as group 2, and group 4 was a control group and received placebos for both iron supplementation and anthelmintic treatment. Prevalence and intensity of helminth infections (egg counts/g stool) of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms significantly fell in the 2 groups receiving anthelmintic treatment and there were some reductions in the 2 groups not receiving anthelminthic treatment. Haemoglobin and haematocrit concentrations increased significantly in the iron supplemented groups with smaller increases in the anthelmintic only group. All women showed a decrease in serum ferritin levels post-trial with greater losses in the 2 dewormed groups. Significant negative associations were found between hookworm egg counts and ferritin levels and *Trichuris trichiura* egg counts and haemoglobin concentration.

Key words: intestinal helminth infections, iron deficiency anaemia, hookworms and ferritin, Trichuris trichiura , haemoglobin.

INTRODUCTION

Chronic gut infections in humans commonly result from nematode infection, particularly with *Ascaris lumbricoides*, *Trichuris trichiura* and the 2 hookworm species *Ancylostoma duodenale* and *Necator americanus*. Hookworms feed on jejunal mucosa and ingest blood; each *Ancylostoma* worm results in an estimated 0·15 ml blood loss per day and each *Necator* in an estimated blood loss of 0·03 ml. As a result iron deficiency anaemia and hypoalbuminaemia may occur. The extent of anaemia depends on the intensity and duration of infection, the iron content of the diet, and the state of the iron stores (Gilles, 1990).

Severe trichuriasis has also been associated with anaemia probably due to chronic inflammation and to iron deficiency. *Trichuris* is not known to ingest blood but fibreoptic colonoscopy has shown bleeding and ulcerated mucosa. A Jamaican study (Ramdath *et al*. 1995) found that children with *Trichuris* intensities of greater than 1200 eggs/g had significantly lower haemoglobin levels than uninfected controls while in heavily infected children (defined as > 5000 eggs/g) from Jamaica (Ramdath *et al*. 1995) and Panama (Robertson *et al*. 1992) haemoglobin was reduced to anaemic levels.

A study of over 3500 children in Zanzibar showed that infections with malaria, *Ascaris*, *Trichuris* and hookworms were all associated with poorer iron status (Stoltzfus *et al*. 1997). Variation in hookworm infections was the strongest explanatory variable for haemoglobin, erythrocyte protoporphyrine and serum ferritin levels. Hookworm infections were responsible for 25% of all anaemia, 35% of iron deficiency and 73% of severe anaemia. Less than 10% of anaemia was attributable to *Ascaris* or malaria infection.

This study used a randomized clinical trial designed to test the extent of change in haemoglobin and serum ferritin levels and prevalence and intensity of worm infestation following anthelminthic treatment alone, weekly iron supplementation alone, or a combined regimen with both anthelmintic and iron supplementation.

MATERIALS AND METHODS

Intervention

A 24-week intervention trial involving 553 female tea pluckers was carried out in an estate, in north-east Bangladesh. The pluckers were randomly assigned

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to 1 of 4 intervention groups; group 1 received iron supplementation on a weekly basis (1 capsule containing 200 mg ferrous fumurate and 200 mg folic acid, Aristopharma Ltd, Dhaka Bangladesh [Owned by Ciba AG Switzerland, now Novartis]), group 2 were dewormed at the beginning of the trial and half way (week 12) through the trial using a single dose of albendazole 400 mg, (Aristopharma Ltd, Dhaka Bangladesh), group 3 received both iron supplementation as group 1 and anthelminthic treatment as group 2, while group 4 received placebos for both iron supplementation and anthelmintics.

Blood analyses

Capillary blood samples were taken at baseline and post-trial to determine haemoglobin, haematocrit and ferritin concentration. Haemoglobin was analysed using a battery powered HemoCue photometer. For haematocrit determination, blood was placed into a heparinized haematocrit tube and spun in a micro-haematocrit centrifuge. Mean corpuscular haemoglobin concentration (MCHC) was calculated using the haemoglobin and haematocrit values. MCHC g/l = [(haemoglobin in g/l)/(haematocrit in $\% \times 10)] \times 100$. All blood samples were analysed within 1 h of collection by a fully qualified and experienced medical laboratory assistant. The blood samples for ferritin analysis were spun and the serum stored at -20 °C for 4 weeks and subsequently analysed with the NovaPath Ferritin Enzyme Immonassay Kit.

Stool analyses

Stool samples were collected pre-trial, 2 weeks after deworming and post-trial. The samples were collected and processed the same day using the formalin–ether concentration method and egg counts were calculated per g faeces. Egg counts were not adjusted for stool consistency as less than 1 % of the samples consisted of loose stools.

Data management and statistical analyses

All information was transferred onto a database using Epi Info 6·01 (double entry). After cleaning, the files were imported into the statistical package SPSS 7·5 for statistical analyses. All continuous variables were first checked for normality and those showing significant skewness were, in the main, normalized using either log (10) for egg counts and haematocrit or square root transformation for ferritin values. Where normalization was not possible both parametric and non-parametric tests were used. Statistical tests used included χ^2 test for categorical variables, paired samples *t*-test, one-way analyses of variance with *a posteriori* Scheffé test, bivariate and partial correlations, linear and multiple regressions for normalized continuous variables and non-parametric tests such as Mann–Whitney, Kruskall–Wallis, Wilcoxon matched pairs signed rank test, McNemars matched pairs test and Spearman rank correlation. All the participants were volunteers. Full details of the project were provided and a signed consent form was obtained from each plucker. Ethical clearance for the study was obtained by WHO.

RESULTS

Baseline

The average age of the pluckers was 39·6 years (minimum 14, maximum 66) and they had a mean of 17·9 years plucking experience (minimum 0, maximum 51). At baseline the 4 groups were found to be homogenous in haemoglobin and ferritin concentrations, prevalence and intensity of infection with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms, and years of age and plucking experience.

No significant associations occurred between haemoglobin values and worm egg counts (separate analyses for each worm). A significant positive association was found between ferritin and age. No significant associations occurred between ferritin levels and egg counts of *Ascaris* or *Trichuris*. However, ferritin levels negatively correlated with hookworms egg count ($P < 0{\cdot}02$) after removing the age effect. The association with hookworm egg counts was still apparent when the effects of all 3 worms were simultaneously tested ($P = 0{\cdot}002$, Table 1). When analyses were restricted to infected (positive) individuals only the significant negative relationship of ferritin with hookworms remained just significant ($P = 0{\cdot}04$).

In addition a strong positive correlation ($r = 0{\cdot}57$) between haemoglobin and ferritin values ($P < 0{\cdot}001$) was found and increase in *Trichuris* egg counts showed a significant negative association with haemoglobin ($P = 0{\cdot}04$) in a multiple regression of haemoglobin with all 3 worms (infected individuals only) and ferritin (Table 2).

The effect of deworming on prevalence and intensity of helminth infection

Significant reductions in prevalences of all 3 worms were found in the anthelmintic groups pre- and post-trial. After a single dose of 400 mg albendazole the prevalence of *Ascaris* fell from 45·5 % pre-trial to 0·8 % and rose to 10·0 % in the de-wormed groups at the end of the trial following a second treatment with albendazole at 12 weeks. The prevalence of *Trichuris* was 56·1 % pre-trial and 39·8 % post-trial. The

Table 1. Pre-trial: multiple regression of ferritin on worm eggs/g

Variable	Step	*B*	*t*	*P*	R^2
Age	1	0·04	3·11	< 0·02	
Ascaris	2	0·15	1·74	N.S.	0·04
Trichuris	2	0·19	1·67	N.S.	
Hookworm	2	−0·46	−3·05	0·002	

N.S., Not significant.

Table 2. Pre-trial: multiple regression of haemoglobin on worm eggs/g (+ve only) and ferritin

Variable	Step	*B*	*t*	*P*	R^2
Ascaris	1	−2·20	−0·78	N.S.	
Trichuris	1	−8·23	−2·05	0·04	0·33
Hookworm	1	0·19	0·05	N.S.	
Ferritin	2	4·61	7·06	< 0·001	

N.S., Not significant.

Table 3. Pre- and post-trial prevalence of helminth infection, albendazole treated

Infection	Pre-trial positive		Positive after first treatment	Post-trial positive
	n	%	%	%
Ascaris	233	45·5	0·8	10·0
Trichuris	233	56·1	37·3	39·8
Hookworm	233	73·7	8·8	16·8

Table 4. Pre- and post-trial, geometric means of pairs, albendazole treated

Infection	Pre-trial	Post-trial	D.F.	*t*	*P*
Ascaris	19·2	1·6	239	11·33	< 0·001
Trichuris	14·3	5·2	239	5·66	< 0·001
Hookworm	57·2	1·9	239	18·65	< 0·001

Table 5. Pre- and post-trial, geometric means of pairs, not albendazole treated

Infection	Pre-trial	Post-trial	D.F.	*t*	*P*
Ascaris	20·5	19·3	223	0·25	N.S.
Trichuris	16·1	9·0	223	3·28	< 0·001
Hookworm	62·0	27·2	223	4·15	< 0·001

Table 6. Pre- and post-trial prevalence of helminth infection, not albendazole treated

Infection	Pre-trial positive		Post-trial positive
	n	%	%
Ascaris	270	49·6	49·8
Trichuris	270	57·4	52·8
Hookworm	270	75·2	69·5

Table 7. Pre- and post-trial means of pairs, haemoglobin (g/l)

Group	Pre-	Post-	*n*	*t*	*P*
Iron	99·50	105·02	133	−4·29	< 0·001
Albendazole	98·65	100·59	133	−1·25	N.S.
Iron + Albendazole	99·12	106·90	121	−6·03	< 0·001
Control	96·90	96·66	125	0·17	N.S.
Total	98·55	102·27	512	−5·31	< 0·001

N.S., Not significant.

prevalence of hookworms was also significantly reduced in the albendazole-treated groups from 73·7 % pre-trial to 16·8 % post-trial (Table 3).

Pre-trial geometric mean egg counts were 15·6 eggs/g stool for *Ascaris*, 13·5 eggs/g for *Trichuris* and 49·7 egg/g for hookworms. The geometric mean egg counts post-trial, 12 weeks after the second dose of albendazole for the 3 worms was still significantly lower than post-trial with 1·6 eggs/g, 5·2 eggs/g and 1·9 eggs/g respectively (Table 4).

Individuals who did not receive any anthelmintic treatment experienced also some reduction in egg counts of all 3 helminths post-trial. The mean egg counts per gram stool were 20·5 pre- and 19·3 post-trial of *Ascaris*, 16·1 pre- and 9·0 post-trial for *Trichuris* and 62·0 pre- and 27·2 post-trial for hookworms (Table 5). There was no change in prevalence of *A. lumbricoides* infection whereas prevalence of both *T. trichiura* and hookworms were reduced between pre- and post-trial from 57·4 to 52·8 % and from 75·2 to 69·5 % respectively (Table 6).

The effect of deworming on the degree of anaemia

There were significant differences in both haemoglobin and ferritin values between the group which received anthelmintic treatment only and the group which received both anthelmintic treatment and iron supplementation. The albendazole only group showed a mean rise in haemoglobin of 1·94 g/l between pre- and post-trial whereas in the group receiving both albendazole and 24 weekly doses of iron supplementation a rise of 7·78 g/l was seen in

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Table 8. Pre- and post-trial means of pairs, MCHC (g/l)

Group	Pre-	Post-	*n*	*t*	*P*
Iron	307·8	306·7	51	0·23	N.S.
Albendazole	301·4	296·9	49	0·58	N.S.
Iron + Albendazole	304·4	306·0	55	−0·26	N.S.
Control	299·2	295·4	37	0·76	N.S.
Total	303·5	301·8	192	0·55	N.S.

N.S., Not significant.

Table 9. Pre- and post-trial means of pairs, ferritin (μg/l)

Group	Pre-	Post-	*n*	*t*	*P*
Iron	27·24	26·91	66	−0·74	N.S.
Albendazole	27·93	21·08	83	2·62	0·01
Iron + Albendazole	29·76	27·40	80	0·56	N.S.
Control	25·22	19·91	68	2·79	< 0·01
Total	27·65	23·81	297	2·81	< 0·01

N.S., Not significant.

the 24 weeks of the trial (Table 7). Furthermore mean corpuscular haemoglobin concentration (MCHC) increased only in the group which received both interventions, anthelmintic treatment and iron supplementation (+1·6 g/l) whereas MCHC decreased in the group which received anthelmintic treatment only (−4·5 g/l) (Table 8).

All groups experienced a decrease in serum ferritin values between pre- and post-trial. The largest decrease was found in the group which received albendazole only (−6·85 μg/l), followed by the placebo group (−5·32 μg/l). The decrease was smallest in the group which received iron only (−0·33 μg/l) (Table 9).

DISCUSSION

The association between helminth infection and haemoglobin and ferritin concentration

The finding of a negative association between hookworm egg counts and ferritin levels, and a positive correlation between ferritin levels and haemoglobin is in agreement with previous research findings. For example, Crompton & Whitehead (1993) estimated that worm burdens of 100–200 were associated with reduced iron stores. Other studies confirmed a correlation between hookworm burden and ferritin (Pritchard *et al*. 1991) but not between hookworm burden and haemoglobin (Bakta, Widjana & Sutisna, 1993; Pritchard *et al*. 1991). Adult hookworms live in the jejunum where they are attached to the mucosa and suck blood. Crompton & Whitehead (1993) suggested that up to 42% of the haemoglobin iron ingested by hookworms is excreted again and re-absorbed in the gut. This is unlikely since iron absorption takes place in the duodenum (Despopoulos & Silbernagl, 1991) whereas hookworms reside lower in the gut in the jejunum. Thus iron, ingested by hookworms, even if excreted again cannot be re-absorbed and is therefore lost. High intensity hookworm infections may cause inflammation to the gut mucosa and this protein-losing entheropathy may aggravate hypoalbuminaemia (Warren *et al*. 1993) which is particularly important in a population with a diet deficient in both iron and protein. Demarchi (1958) observed that farmers and mud workers in Iraq who had been given portein supplements recovered much more quickly from iron deficiency anaemia than did those on their usual diet.

Stoltzfus *et al*. (1996) assessed faecal haemoglobin of children with hookworm infection, using HemoQuant to measure intact heme and degraded heme in the form of porphyrin. They found that 56% of haemoglobin was converted to porphyrin indicating that the blood lost mostly originated from the upper gastrointestinal tract. Hookworm infection therefore affects mainly ferritin levels, while the protein part of haemoglobin may be re-utilized.

In this Bangladeshi sample, mean egg counts of *Trichuris* were 120·3 (geometric mean 14·82), egg counts classified as light infection (Montresor *et al*. 1998). Even so, there was a significant negative association between *Trichuris* egg counts (infected individuals only) and haemoglobin values. Adult *Trichuris* live in the large intestine where the anterior, narrow portion of the worm is partially embedded in the mucosa of the intestine. It is the mouth part which enters the mucosa and the worm obtains nutrition from the host tissue. Blood loss caused by *Trichuris* cannot be re-absorbed since *Trichuris* resides in the gut beyond absorption of iron or protein (Despopoulus & Silbernagl, 1991). Thus, even though blood may not be ingested by the worms, any leakage of red blood cells or blood plasma cannot be absorbed and will be excreted and lost. Visible blood in stools indicates bleeding occurring in the large intestine and Simeon & Grantham-McGregor (1990) reported chronic, mucoid bloody diarrhoea and linear growth retardation and anaemia in children with a severe form of Trichuriasis.

The effect of deworming on egg counts of non-dewormed individuals

A number of reasons have been put forward in the literature for variations in egg counts in untreated individuals including variation in egg output of worms, differential survival rate of eggs, seasonal changes in larval development (Bruyning, 1985), seasonal variation in soil contamination (Udonsi & Amabibi, 1992; Sorensen *et al*. 1994; Uga *et al*. 1995;

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Weidong *et al*. 1996), arrested development of hookworm larval (Schad *et al*. 1973; Schad, 1991), and reduction in intensity of infection as a reaction to decreased environmental contamination when a part of the population was dewormed (Bundy *et al*. 1990; Asaolu, Holland & Crompton, 1991; Butterworth *et al*. 1991; Chan *et al*. 1994).

Both hookworm eggs and larvae are short lived and most do not survive more than 1 month in soil (Sorensen *et al*. 1994). Nevertheless, the dense shade of the tea bushes and the high humidity provide ideal conditions for the survival of hookworm eggs until they are either pushed deeper into the soil or are flushed away by the rapid flow of water in the rainy season. *Ascaris* eggs are more resistant to heat and cold than *Trichuris* eggs and remain infective for years deep in the soil. In addition, the very sticky surface of *Ascaris* eggs might prevent them from being flushed away as easily as *Trichuris* and hookworms eggs.

In the present study nearly three-quarters of the labourer population had no access to toilet facilities and usually defecated in the tea bushes. When the numbers of deposited eggs are reduced due to treatment of a part of the population, then infection rate might be expected to decrease for all tea pluckers as each worm is the result of a separate infection event. In traditional Hindu societies, human faeces are repugnant not only for the universally accepted reasons but also are considered defiling in a ritualistic sense. However, when individuals enter a defecation ground for some other purpose, such as tea plucking, they will not be concerned about faeces around them and purification rituals rarely follow (Schad, Nawalinski & Kochar, 1983). Tea pluckers are particularly vulnerable to hookworm infections due to the extended contact of their feet with contaminated soil.

The effect of deworming on haemoglobin and ferritin concentration

Both interventions of iron supplementation and anthelmintic treatment resulted in an increase in haemoglobin concentration. The effect of the anthelmintic treatment on haemoglobin values can be estimated by comparing the haemoglobin changes between the placebo group and the dewormed group and between the iron supplemented group and the dewormed and iron supplemented group. The average differences of 2·17 g/l and 2·27 g/l respectively were due to the anthelmintic treatment which caused a reduction in iron- or haemoglobin-depleting helminth infections. Conversely, the effect of iron supplementation alone was, on average, 5·84 g/l (difference in haemoglobin increase between placebo and iron supplemented group) and 5·75 g/l (difference between the dewormed and the dewormed and iron supplemented group). Therefore, iron supplementation had a much larger impact on improvement in haemoglobin values than anthelmintic treatment. Nevertheless, an increase in functionally improved erythrocytes as measured in an increase in mean corpuscular haemoglobin concentration (MCHC) occurred only in the group which received both anthelmintic treatment and iron supplementation.

Compared to pre-intervention values, all groups showed decreases in mean ferritin post-trial. The 2 groups which did not receive iron supplementation had a greater loss than the supplemented groups. However, when the 2 iron supplemented groups were compared with each other and with the 2 non-supplemented groups, it becomes clear that anthelmintic treatment caused a greater ferritin loss.

In the present study a seasonal nutritional stress was indicated by a decrease in both haemoglobin and ferritin in the control group. The intervention trial took place between July and December, a time-period which included the rainy season, when high quality food is scarcer than usual and more expensive, and prevalence of infectious diseases is high. All study participants had their anthropometric measurements taken twice, pre- and post-trial (height, weight and mid upper-arm circumference) and were found to have lost weight during the 24 weeks of the trial, although not more than 400 g on average. No screening for malaria parasites was performed as malaria is not known to be endemic in this region. The effect of the anthelmintic treatment can be observed in the differences in post-trial ferritin and haemoglobin values between the 4 intervention groups, and the pre- and post-trial changes. Protein deficiency, exacerbated by helminth infections may have been reduced due to the anthelmintic treatment so that protein and iron stores could now be utilized. This resulted in an increase in haemoglobin concentration and in an additional decrease in ferritin levels in individuals in the dewormed groups which were now able to use their iron stores to produce haemoglobin. Anaemia in this study population was probably due to protein deficiency caused by both a protein-deficient diet and intestinal helminth infections rather than iron deficiency.

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