# IMPACT OF HOOKWORM DEWORMING ON ANEMIA AND NUTRITIONAL STATUS AMONG CHILDREN IN THAILAND

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**Abstract.** Hookworm infection is associated with anemia, especially among children and deworming can improve anemic status; however, little information is available about the degree to which anemia improves after deworming. We chose hookworm-endemic rural areas of Thailand, Nan Province in the north, Kanchanaburi Province in the west and Nakhon Si Thammarat Province in the south, to evaluate this problem. Subjects were selected by primary school-based stool egg examinations. Blood tests of 182 hookworm-positive primary school children, composed of 22 heavy, 65 moderate and 95 light infections, were compared with a control group of 57 children who were helminth-free both before and after receiving deworming medicine. Before deworming , the red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and albumin levels of the hookworm-infected groups were significantly lower than the helminth-free control group. The Hb and Hct levels showed an inverse relationship with intensity of hookworm infection. After deworming, the Hb, Hct, total protein and albumin levels of the hookworm-infected children improved within 2 months to become comparable with the helminth-free control group. One year after deworming, the mean blood test results in the 2 groups were not significantly different from each other.

**Keywords:** hookworm, deworming impact, anemia, nutritional status, children, Thailand

## INTRODUCTION

Soil-transmitted helminths (STHs) are widely distributed in tropical and sub-tropical areas. Globally, more than 1,500 million people suffer from these geohelminths. Approximately 300 million people are estimated to be suffering from severe morbidity due to malnutrition and anemia (Montresor *et al*, 2002; Kung'u *et al*, 2009). Among STHs, severe chronic infection with hookworms and/or *Trichuris* infections is known to cause malnutrition and blood loss, leading to hypochromic microcytic, iron-deficiency anemia (Beaver *et al*, 1984). School-aged

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children are at high risk for STH infections because of their habit of playing in the dirt which may be contaminated with the infective stages of STH eggs/larvae and their lacks of good personal hygiene (Montresor *et al*, 2002; Tomono *et al*, 2003). Children in this age group are in a period of intense learning, but helminthic infections hinder their performance, and may retard physical and mental growth and development (Montresor *et al*, 2002). The reduction of worm burden with drug treatment is widely believed to improve nutritional status (Stephenson *et al*, 1993; Beasley *et al*, 1999) and the cognitive ability of infected children (Drake *et al*, 2000).

In Thailand, STHs are commonly found in mountainous areas in the south. Muennoo *et al* (1993, 2000) reported over 50% of STH-infected children had 2-3 kinds of worms, including hookworms. In their study, 6.7% of children with hookworm infection had a heavy infection without obvious signs of malnutrition or anemia. Srinophakun and Jeradit (1978) reported hookworm-infected adults felt debilitated, rendering them unable to work, but infected school-aged children appeared normal. During previous studies in Thailand, anemia was evaluated by general physical examination, including evaluation of skin color, conjunctiva and nails; but the degree of anemia was not determined quantitatively. In this study, we compared a variety of hematological parameters to evaluate hookworm-positive and helminth-free children. Changes in pre- and post-deworming hematological parameters among hookworm-infected children were determined and analyzed. The suitability of hematological indicator(s) to diagnose anemia among hookworm-infected children was also assessed.

## MATERIALS AND METHODS

### Ethical clearance

This study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (TM-IRB 019/2003). Written informed consent for participation in the study was obtained from the children themselves and their parents prior to being enrolled in the study.

### Study areas

The hookworm endemic areas selected were in southern, northern and western Thailand based on previous studies (Jongsuksantikul *et al*, 1992; Muennoo *et al*, 1998; Waikagul *et al*, 2002; Anantaphruti *et al*, 2004). Schoolchildren from 2 primary schools were examined, in the villages of Ban Nam-sot and Ban Pang Kae, Thung Chang District, Nan Province, northern Thailand. Schoolchildren from 5 primary schools were examined in Ban Pak Lum Pilok, Ban Huay Kayeng, Ban Pracham Mai, Ban Rai Pa and Ban E-tong, Thong Pha Phum District, and 2 primary schools in Ban Huay Malai and Ban Huay Kob, Sangkhla Buri District, western Thailand. Schoolchildren from 3 primary schools were examined in villages Ban Nai Thung, Ban Sa Bua and Ban Pung Ping, Tha Sala District, Nakhon Si Thammarat Province southern Thailand.

### Stool examination

Stool samples were collected and examined by Katz's modified thick-smear technique as reported previously (Katz *et al*, 1972). The species and numbers of eggs/worms were identified and counted to determine intensity. Among the egg/worm-positive cases, hookworm-infected children were selected as study subjects. The first priority was to select those having only hookworm infection. In cases of

mixed-infection with hookworm and other parasites, only those having light concurrent infections with *Ascaris* or *Trichuris* were included in the hookworm-positive group. Hookworm-only children were classified into heavy, moderate and light infections, according to the criteria set by the WHO (1981). After stool examination, helminth-free children were selected to serve as the control group.

**Deworming and blood sampling**

A single dose of albendazole 400 mg (2 x 200 mg tablets) was given to children with hookworm only or hookworm and *Ascaris* infections. Mebendazole, 100 mg twice daily for 3 consecutive days was administered to children with mixed hookworm/*Trichuris* infections. Each treated child was observed to ensure they took the medicine properly.

Blood samples were obtained from both subjects and controls prior to treatment (MO). The day after the blood test all subjects were treated; treatment was repeated at 2, 6 and 12 months. Treatment was repeated to prevent reinfection. Blood samples were drawn 10 days after each treatment (M2, M6 and M12) to minimize the effect of the drugs on the blood test results. The control children were also given a single dose of albendazole 400 mg on the same schedule as the hookworm-positive group, and M2, M6 and M12 blood samples were taken at the same intervals as the subjects (Fig 1). To reduce the risk of reinfection we provided health education about preventing infection.

**Hematological examinations**

Approximately 8-10 ml of blood was drawn from the vein of each subject, 1 ml was put in an ethylenediamine-tetra-acetic acid (EDTA) tube to check the complete blood count (CBC), which included the red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The rest of the blood was kept for serum separation. The blood specimens were kept in an icebox and sent to the laboratory of the district hospital for analysis. Individual serum samples were collected in a microcentrifuge tube and kept frozen in the field until transportation in an icebox to the Faculty of Tropical Medicine in Bangkok where they were kept at -10˚C until used. The serum samples were tested for total protein and albumin levels. Ferritin and transferrin levels were measured in moderately and heavily infected patients.

**Statistical evaluation**

The lab results of the subjects and controls were compared using the non-parametric Mann-Whitney *U*/Wilcoxon rank-sum test, with median ± 95% confidence interval ($p < 0.05$). The percentages of children with improved blood profiles among the subjects and controls were compared with the chi-square test.

## RESULTS

**Stool examination**

Similar to previous reports (Maipanich *et al*, 2004), the prevalences of hookworm-positive schoolchildren in Nan, Kanchanaburi and Nakhon Si Thammarat Provinces were 97/181(53.6%), 153/855 (17.9%), and 382/884 (43.2%), respectively. Of the 632 hookworm-positive children, 499 (79%) had a light infection, 95 (15%) had a moderate infection and 38 (6%) had a heavy infection. Most (33/38) of the heavily-infected cases were from Nakhon Si Thammarat Province. Of the hookworm-positive children, 182 (30 from Nan, 53 from Kanchanaburi and 99 from Nakhon Si Thammarat) agreed to join

Table 1
Epidemiological profile of hookworm patients [number examined (males : females)] in Nakhon Si Thammarat, Nan and Kanchanaburi Provinces, Thailand.

| Province | Hw +ve | L | M | H | Hw -ve | Total |
|---|---|---|---|---|---|---|
| Nan | 30 (18:12) | 14 (6:8) | 14 (10:4) | 2 (2:0) | 30 (13:17) | 60 |
| Kanchanaburi | 53 (41:12) | 40 (30:10) | 10 (8:2) | 3 (3:2) | 0 | 53 |
| Nakhon Si Thammarat | 99 (52:47) | 41 (21:20) | 41 (22:19) | 17 (9:8) | 27 (10:17) | 126 |
| Total | 182 (111:71) | 95 (57:38) | 65 (40:25) | 22 (14:10) | 57 (23:34) | 239 |

H, heavy infection group; M, moderate infection group; L, light infection group
Hw +ve, hookworm positive; Hw -ve, hookworm negative

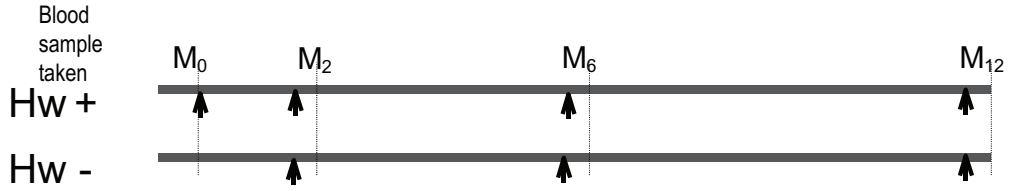
Table 2
Biographical data of the participants.

| Variable | Hw +ve | Hw -ve | Total average (range) |
|---|---|---|---|
| No. examined (male : female) | 182 | 57 | 239 (134:105) |
| Age (years) | 9.9 | 9.6 | 9.93 (5.08-17.0) |
| Weight (kg) | $M_0$ = 25.7<br>$M_{12}$ = 29.1 | $M_0$ = 24.6<br>$M_{12}$ = 29.0 | 25.4 (15.1-50.5) |
| Height (cm) | $M_0$ = 129.6<br>$M_{12}$ = 134.8 | $M_0$ = 128.0<br>$M_{12}$ = 132.2 | 129.2 (104-158.5) |

Hw +ve, hookworm positive; Hw -ve, hookworm negative

the follow-up study (Table 1). Fifty-seven hookwom-negative children (controls), 30 from Nan and 27 from Nakhon Si Thammarat, agreed to join this study. The total study sample consisted of 239 schoolchildren, 182 were hookworm-positive and 57 were hookworm-negative. In terms of severity of infection, 95 (52.2%) had a light infection, 65 (35.7%) had a moderate infection and 22 (12.1%) had a heavy infection (Table 1). Compared with the original population, the proportions of moderately and heavily infected groups were higher in the follow-up study.

Of the 239 children included in the follow-up study, 134 were males and 105 were females (Table 2). The median age was 9.9 (5.1-17.0) years. The average body weight was 25.4 (15.1-50.5) kg and the average height was 129.2 (104-158.5) cm. All were healthy appearing with no signs of anemia on physical examination. There were no obvious differences in the physical data between the subjects and




Hw +, hookworm positive; Hw -, hookworm negative
M0, month 0, M2 month 2; M6, month 6; M12, month 12
↑, deworming medicine given
M, blood draw

Fig 1–Deworming schedule and blood samplings.

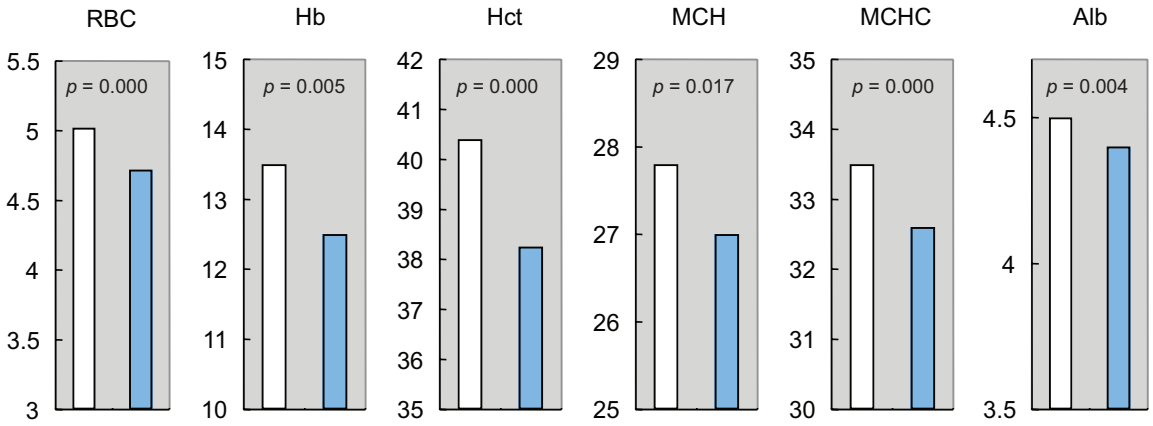
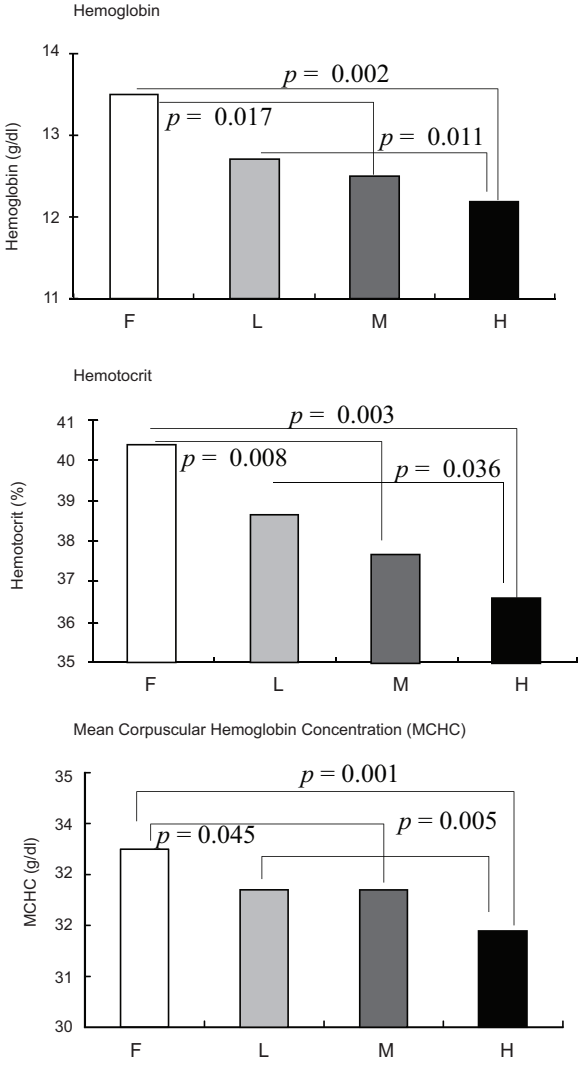



Fig 2–Comparison of the median values of hematological data (RBC, Hb, Hct, MCH, MCHC and Alb) between hookworm infected and helminth-free groups before deworming (M0). *p*-values were obtained by Mann -Whitney *U* test.

Table 3
High degree significance of RBC, Hb, Hct and albumin levels in heavily and moderately infected children, at the beginning of the study ($M_0$).

| Parameters | Hw-free + low infection | | Moderate + heavy infection | | *p*-value |
|---|---|---|---|---|---|
| | Median | Range | Median | Range | |
| RBC | 4.8 | 3.92-8.60 | 4.61 | 3.76-6.79 | 0.018 |
| Hb | 12.9 | 9.2-18.9 | 12.3 | 5.7-17.6 | 0.002 |
| Hct | 39.4 | 30.3-54.8 | 37.6 | 23.6-52.1 | 0.001 |
| Alb | 4.5 | 4.0-4.9 | 4.4 | 3.5-4.9 | 0.004 |

Hw, hookworm
RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; Alb, albumin




F, free of hookworms; L, light hookworm infection; M, moderate hookworm infection; H, heavy hookworm infection

Fig 3–Laboratory results among children with various intensities of infection.

controls; boys predominated in the worm-positive group.

**Blood analysis**

The blood tests show among all subjects prior to treatment, the prevalence of hypochromic microcytic anemia was 12/239 (5.0%), of which 9/182 (4.9%; all from Nakhon Si Thammarat) were in the hookworm-infected group and 3/57 (5.3%; all from Nan) were in the helminth-free group.

Before deworming the RBC, Hb, Hct, MCH, and MCHC levels in the hookworm-infected group were significantly lower than the helminth-free group by Mann-Whitney *U* test ($p < 0.05$) (Fig 2). The Hb, Hct and MCHC levels paralleled intensity of infection; *p*-values comparing the differences between the heavily-infected group with the helminth-free group for Hb, Hct and MCHC levels were 0.002, 0.003 and 0.001; the *p*-values comparing the differences between the moderately-infected group with the helminth-free group were 0.017, 0.008 and 0.045, respectively (Fig 3). For Hb, Hct and MCHC significant differences were seen between the heavily and lightly infected groups.

When the hematological data in the heavily and moderately infected groups were combined and compared with the combined data of the lightly infected and helminth-free groups, significant differences were observed in RBC, Hb, Hct and Alb values with *p*-values of 0.018, 0.002, 0.001 and 0.004, respectively (Table 3). When the Hb and Hct data for the heavily and moderately infected groups were combined and compared with the lightly infected group and the helminth-free group individually (Table 4), significant differences were observed except for the Hb level in the lightly-infected group ($p = 0.087$). Combining the data, the cut-off values of Hb of <12.3 g/dl and Hct of <37.6% could be used as criteria for anemia due to hookworm infection in this cohort. Since the Hct levels in the lightly-infected group (37.6%) were significantly lower ($p = 0.027$) than the helminth-free group (38.7%), the Hct level is the most

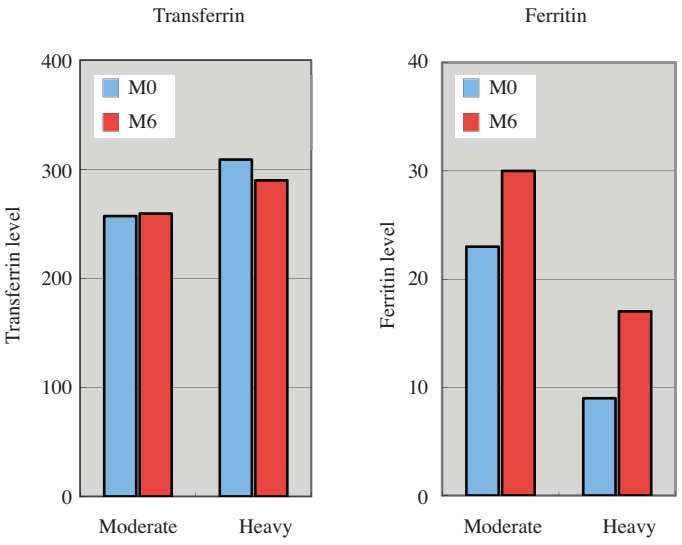



Fig 4–Ferritin and transferrin levels among cases at M0 and M6.

sensitive parameter for anemia tested to suggest hookworm infection.

**Effects of deworming on hematological data**

As early as 2 months (M2) after deworming, Hb, Hct, MCV, total protein and albumin levels, in the treated subjects improved markedly and were comparable with the helminth-free group (Table 5). The RBC level in the hookworm-infected group ($4.61x10^6$ dl) at M2 was slightly higher than the helminth-free group (Table 5), but was in the normal range ($4.2-6.3x10^6$/dl). At M12 all lab values were not significantly different (Table 5).

Ferritin and transferrin levels were tested in 22 heavily and 41 moderately infected cases at M0 and M6 (Fig 4). Because of the wide range of normal values for these parameters, nearly all the cases had values within the normal range. Before deworming (M0) the mean ferritin level for the heavy infection group was 8.0 g/dl (4.0-30.0), which was significantly ($p=0.004$) lower than the mean ferritin level in the moderately infected group of 20 g/dl (1.0-90.0). The low ferritin level remained in heavily-infected children, even at M6; however by M12 no significant difference remained between the moderately infected and heavily infected groups. The normal ferritin range in children is 20-25 g/dl (Wiratsethasin, 2003). No significant differences in transferrin levels were seen between heavily and moderately infected cases at M0 and M6 (Fig 4).

**Evaluation of hematological improvement**

Improvement in hematological levels among cases was seen at M2 and M6 with higher increases in Hb, Hct and RBC levels seen in cases than controls (Table 6). By one year (M12) all cases were free from worm infestation and their blood profiles had improved; 16 subjects (9.1%) had continuous (M0<M2<M6<M12) improvement. The hematological values in the helminth-free group remained unchanged.

## DISCUSSION

Prior to treatment (M0), 5.0% (12/239) of subjects had hypochromic microcytic anemia. Anemia was evident in both hookworm-infected (4.9%) and helminth-free (5.3%) groups. All the anemia cases in the helminth-free group were from Nan Province, where rice, fish and vegetables are the main sources of food and beef and pork are rarely served to children, and inadequate iron rich food intake is uncommon (Wiratsethasin, 2003).

Table 4
Median values (range) for hemoglobin (Hb) and hematocrit (Hct) in hookworm-infected and helminth-free groups at $M_0$.

| | H + M | L + F | L | F |
|---|---|---|---|---|
| Hb (mg/dl) | 12.3 (5.7-17.6) | 12.9 (9.2-18.9) | 12.4 (9.6-14.7) | 13.5 (10.5-18.9) |
| | | H+M *vs* L+F : $p = 0.002$ | | |
| | | H+M *vs* L : $p = 0.087$[a] | | |
| | | H+M *vs* F : $p = 0.000$ | | |
| Hct (%) | 37.6 (23.6-52.1) | 39.4 (30.3-54.8) | 38.7 (32.9-44.4) | 40.4 (31.7-54.8) |
| | | H+M *vs* L+F : $p = 0.001$ | | |
| | | H+M *vs* L : $p = 0.027$ | | |
| | | H+M *vs* F : $p = 0.000$ | | |

H, heavy infection ; M, moderate infection; L, light infection; F, helminth-free group
[a]Not significant; Hb, hemoglobin; Hct, hematocrit

Table 5
Comparison of median values (range) of indicators measured after deworming ($M_2$ and $M_{12}$) between hookworm-infected and helminth-free children (Mann-Whitney *U* test).

| | $M_2$ | | *p*-value | $M_{12}$ | | *p*-value |
|---|---|---|---|---|---|---|
| | Hw | Helm-free | | Hw | Helm-free | |
| Hb | 12.1 (5.1-19.2) | 12.0 (9.2-15.7) | 0.811 | 12.4 (9.7-17.9) | 12.3 (10.0-16.8) | 0.632 |
| Hct | 36.9 (12.7-58.7) | 36.1 (30.4-45.8) | 0.245 | 38.8 (31.3-54.8) | 38.4 (30.8-51.2) | 0.363 |
| MCV | 81.2 (52.0-93.3) | 83.0 (59.0-93.3) | 0.051 | 81.7 (55.7-94.6) | 80.3 (56.0-92.5) | 0.245 |
| MCH | 26.7 (13.7-35.2) | 27.8 (19.7-29.9) | 0.002 | 26.3 (17.1-30.2) | 26.1 (18.1-29.9) | 0.479 |
| MCHC | 32.5 (24.5-40.2) | 33.3 (29.2-35.1) | 0.005 | 32.0 (28.6-34.4) | 32.3 (29.4-33.8) | 0.138 |
| RBC | 4.61 (1.5-6.8) | 4.58 (3.6-5.8) | 0.040 | 4.7 (4.0-6.5) | 4.8 (4.1-6.5) | 0.825 |
| Protein | 7.8 (6.6-9.5) | 7.7 (6.7-9.8) | 0.253 | 7.7 (6.3-9.8) | 7.6 (6.5-8.4) | 0.051 |
| Albumin | 4.8 (3.2-5.6) | 4.8 (3.9-6.1) | 0.833 | 4.6 (3.6-5.7) | 4.6 (4.0-5.3) | 0.646 |

Hw, hookworm; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cells

Table 6
Percentages of subjects who showed continuous laboratory value improvement throughout the study ($M_0$<$M_2$<$M_6$<$M_{12}$).

| Group | *n* | No. of subjects with improved blood value(s) (%) | | | |
|---|---|---|---|---|---|
| | | Total | Hb | Hct | RBC |
| Hookworm-infected | 175 | 16 (9.1) | 6 (3.4) | 12 (6.9) | 6 (3.4) |
| Helminth-free | 53 | 0 | 0 | 0 | 0 |

Hb, hemoglobin; Hct, hematocrit; RBC, red blood cells

In this study, hookworm-infected schoolchildren had lower RBC, Hb, Hct, MCH, MCHC and albumin levels than the helminth-free group (Fig 2). Of those hematological tests, the Hct level seemed to be more sensitive than the other blood values. In our study, a Hb < 12.3 g/dl or a Hct < 38.7% could be used as cut-off values for hookworm infection, even though the results were within the normal ranges (Hb = 12-18 g/dl and Hct = 37-54%). We also measured ferritin and transferrin levels among heavily infected patients. Low ferritin values in heavily infected cases remained until M6 after treatment, after which they increased to the normal range by the end of the study (M12). The ferritin test was specific, but expensive and is not available as a routine laboratory test in the community. However, assessment of iron level is useful. An intervention with iron supplementation to reduce iron depletion and prevent iron deficiency anemia after deworming assists the iron level to return to normal (Phuc *et al*, 2009; Casey *et al*, 2010).

The anemic status of the hookworm-positive group significantly improved by 2 months after deworming and the hematological values of all participants gradually reached normal levels by 12 months (Table 5). Even in individuals who improved slowly (9.1% of the hookworm-infected children), continuous improvement (M0<M2<M6<M12) in Hb, Hct and/or RBC values were observed; this corresponds with a previous report by Beasley *et al* (1999). Such a drastic improvement in anemic status clearly indicates the anemic status of cases in the study was mainly due to hookworm infection, but not due to iron deficiency caused by poor nutrition. Anthelminthic treatment achieved hematological improvement.

This study shows mild anemia in schoolchildren may not be evident on physical exam. Hookworm control still relies on the frequent use of antihelminthic drugs, either through deworming programs targeting school-aged children or integrated control programs (Hotez, 2008). There are currently many kinds of effective anthelminthic drugs. Albendazole, a broad-spectrum anthelminthic gives 97.2% and 88.9% cure rates and 97.2% and 95.0% egg-reduction rates in ascariasis and hookworm infections, respectively (Ow-yang and Hanjeet, 1986). Mebendazole gives a 71.4% cure rate with light *Trichuris*-infection (Anantaphruti *et al*, 1993). However, the high rates of hookworm reinfection and possible emergence of drug resistance will ultimately require the development of new control

tools. Helminth infection control and the prevention of anemia in developing tropical countries are of considerable public health importance for the prevention of under-nutrition. Active and continuous deworming and health promotion programs remain essential for schoolchildren to control hookworm infection in rural areas of Thailand.

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