IMPACT OF INTESTINAL HELMINTH INFECTION ON ANEMIA AND IRON STATUS DURING PREGNANCY: A COMMUNITY BASED STUDY IN INDONESIA

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Abstract. A cohort study was carried out in Purworejo District, Central Java, Indonesia to investigate prevalence of anemia and low iron stores during pregnancy in relation to intestinal helminth infection. 442 pregnant women were followed until 5-10 weeks postpartum, during the period of April 1996 - August 1998. Information on intestinal helminths, hemoglobin and serum ferritin was collected each trimester. Highest prevalence of anemia in pregnancy (37.1%) was found in the second trimester, while the highest prevalence of low iron stores (49.5%) was found in the third trimester. Most pregnant women (69.7%) were infected with at least one species of pathogenic intestinal helminths. The most common helminth detected was *Trichuris trichiura* followed by *Necator americanus* (hookworm) and *Ascaris lumbricoides*. A significant negative association was found between hookworm infection and serum ferritin at the first trimester (p = 0.010). It is concluded that hookworm infection can interfere iron stores. Other causes of anemia among pregnant women should be considered. Anthelminthic therapy could be given to infected women before conception as public health strategy to improve iron status.

INTRODUCTION

Intestinal helminth infection affects millions of women of reproductive age in developing countries (Gillespie and Johnston, 1998). Intestinal helminths may cause anemia through reduced food intake, mal-absorption and endogenous nutrient loss. The main anemia-causing intestinal helminths are hookworms (*Ancylostoma duodenale*, *Necator americanus*), *Trichuris trichiura* and schistosomes, with hookworms being most common. Hookworms cause chronic intestinal blood loss by attaching to the mucosa of the upper small intestine and ingesting tissue and blood (Banwell and Schad,1978). In 1990, it was estimated that some 44 million women were both pregnant and infected with hookworm (Bundy *et al*, 1995). Infection during pregnancy could compromise maternal-perinatal health and nutritional status (Santiso, 1997; Villard *et al*, 1989; Weigel *et al*, 1996). The prevalence of intestinal helminth infection during pregnancy in Indonesia is little investigated, and so is its relationship with maternal and perinatal outcomes. Nutritional anemia is an important health problem in many developing countries, especially among pre-school children and pregnant women. Anemia is a major cause of maternal morbidity and mortality, and it also affects the outcome of pregnancy (Gillespie, 1998; Allen, 1997). The most prevalent cause of nutritional anemia during pregnancy is iron deficiency. In Indonesia, a nation-wide household survey of 1995 found nutritional anemia of 50.9% of pregnant women (Ministry of Health, Republic of Indonesia, 1995).

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The present study among pregnant women in Purworejo District, Central Java, Indonesia, had two main objectives: First, to investigate the prevalence of intestinal helminth infection, anemia and low iron stores; Second, to evaluate the relationship between intestinal helminth infection and iron status during pregnancy.

MATERIALS AND METHODS

The cohort study followed 442 pregnant women from early pregnancy until 5-7 weeks post delivery between April 1996 - August 1998. Those women were randomized from 2,173 women who had been recruited for a vitamin A and zinc supplementation trial during pregnancy between March 1996 - April 1998 in the surveillance area of the Community Health and Nutrition Research Laboratory, Purworejo District, Central Java, Indonesia (Hakimi *et al*, 1999). Informed verbal and written consent was obtained from all subjects. Women with severe helminth infection and women who suffered from anemia were to be treated during third trimester. About 56% of women received routine iron supplementation tablets given by the Primary Health Center in the first trimester. No malaria case was found among the women.

Information on maternal age, completed years of education, occupation, number of children and family size was collected in August-October 1996. Sanitation and hygiene variables included the sanitary facilities and type of flooring. These variables were categorized in close agreement with the Indonesian Demographic and Health Survey categorization scheme.

Stool specimens were collected each trimester. Helminth parasites specimen examination was performed using Kato-Katz thick smear methods (WHO, 1994a), and reviewed by an experienced parasitologist. A cardboard template with a hole of known capacity (30 mg) and a cellophane cover slip soaked in glycerine-malachite green solution were used. Quantities of *A. lumbricoides*, *T. trichiura* and *N. americanus* eggs were estimated from the numbers per gram stool about one hour after smear preparation. The results were classified as negative or positive if helminths were found to be either absent or present. Positive specimens were ranked by the level of infection as light, mild, and severe.

Iron status was assessed in a venous blood sample during each trimester. Serum hemoglobin was measured in a drop of whole blood at the women's house (HemoCue AB, Angelholm, Sweden). Remaining blood was brought to a field laboratory using a cool box and centrifuged at 1,000 rpm for 20 minutes at room temperature. Serum samples were transported to Yogyakarta in liquid nitrogen and stored at -70ºC until analyzed. Serum ferritin was assessed by an immunoradiometric assay (IMX ferritin, Abbott Laboratories, Illinois, USA).

In Purworejo, rice is the primary staple food, while cassava and sweet potato are consumed as snacks. Vegetables are eaten regularly, especially spinach and water spinach. Carrot is the most common source of carotene for the pregnant women. Bananas and papayas are available throughout the year, whereas mangoes, citrus fruit, water melons and other fruits are available seasonally. Fermented soya bean, called Tempe, is the most common vegetable protein consumed. Egg and fish are added to the diet if affordable. Notably, most pregnant women living near by the sea or river consume fish. Meat is a luxury item, which is not regularly consumed. Tea and plain water are common beverages among pregnant women.

The study was approved by the ethical review committee of the Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia.

Statistical analyses

Data were analyzed with the Statistical Package for Social Science (SPSS version 7.1, 1997). Due to skewed distribution ferritin values were transformed into logarithm scale. Anemia was defined as hemoglobin ≤ 11 g/dl (WHO, 1992) and low iron stores was defined as serum ferritin ≤ 12 μg/l (Cook and Skikne, 1989).

Association of ferritin and hemoglobin levels with intensity of helminth infection was studied using Pearson's correlation as well as linear and logistic regression analyses, where possible associated background factors including parity, infection, gestational age, number of iron tablets consumed and type of floor were controlled for. Finally, ferritin change over time was analyzed with a multivariate, repeated measures approach. Vitamin A and zinc supplementation had no effect on the relationship between intestinal helminth infection and iron status as evaluated by stratified analyses.

RESULTS

Among the 442 women recruited, only 420, 320 and 275 women completed blood and stool examinations at first, second and third trimester respectively (Table 1). During the study period, 30 women migrated, 19 women had abortion or premature delivery, and 3, 62 and 66 women refused to give stool samples at first, second and third trimesters, respectively. Non-compliance of stool sample submission was the foremost reason for incomplete measurements. No important differences were

Tabel 1
Socioeconomic background of pregnant women included in analyses, in Purworejo district, Central Java, Indonesia (in percentage unless indicated).

Variables	Had complete measurements at trimester I	Had complete measurements at trimester II	Had complete measurements at trimester III
No.	420	326	275
Age (years)	28.45±5.17	27.99±5.03[a]	27.83±4.80[a]
Gestational age (weeks)	11.26±2.21	23.30±1.24	33.33±1.43
Parity			
0-1	51.2	53.4	55.3
2-3	40.5	40.2	37.5
≥ 4	8.3	6.4	7.3
Education (years)			
0	2.6	2.1	2.2
1-6	56.0	55.8	53.5
≥ 7	41.4	42.0	44.4
Occupation			
Farmers	43.6	44.8	42.5
Housewives	34.3	35.3	33.8
Others	22.1	19.9	23.6
Living area			
Rural	91.9	92.3	93.5
Urban	8.1	7.7	6.5
Type of floor			
Earth	39.3	39.9	37.8
Cements/bricks	35.1	35.2	35.6
Tiles	25.6	24.8	26.7
Type of latrines			
Pond/river/yard	37.6	39.6	41.5
Without septic tank	11.2	10.7	9.6
With septic tank	51.2	49.7	48.9

[a]Significant difference between women with complete measurements (data shown) and women with incomplete measurements (data not shown) among the 442 women recruited. $p<0.05$, t-test.

noted at first trimester between women who had and women who did not have complete measurements. However, at second and third trimester, age was significantly higher among those who had incomplete measurements compared to those who had complete measurements.

Most of the pregnant women (69.7%) were infected with at least one species of pathogenic intestinal helminths. Overall, prevalence of *T. trichiura* infection was highest: 49.3%, 49.7% and 40.4% at first, second, and third trimester, respectively. This was followed by the prevalence of *N. americanus* and *A. lumbricoides* (Table 2). None of the women was diagnosed with a heavy helminth burden by estimation of egg concentration in the stool or by clinical criteria. Hence, none of the women was given anthelminthic treatment during pregnancy. Women infected with helminths were more likely to live in houses with soil floor, more often lacked sanitary facilities and were more likely to work as a farmer (data not shown).

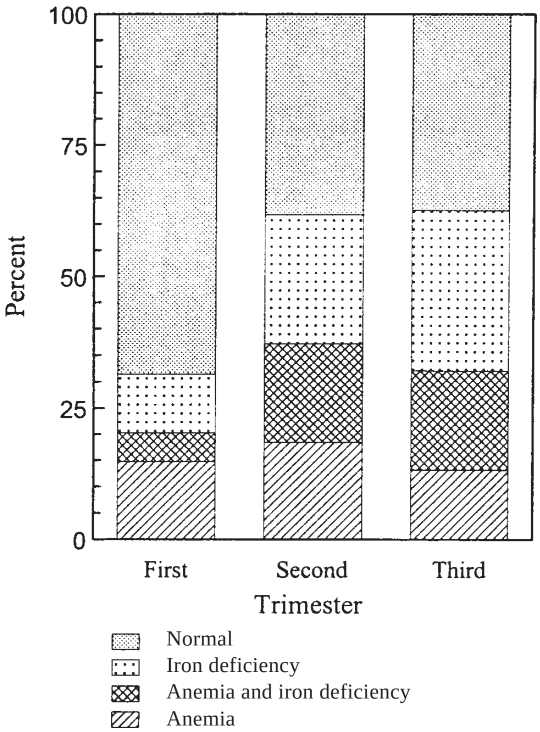

Fig 1–Prevalence of anemia and iron deficiency.

Table 2
Characteristics of pregnant women who had complete measurements of blood and stool by time of examination.

Characteristic	Trimester I	Trimester II	Trimester III
No. of women who had complete measurements	420	326	275
Necator americanus			
Infected (%)	23.8	23.3	19.3
Eggs/g feces[a]	344.0±629.8	329.8±740.3	287.0±484.7
Trichiuris trichiura			
Infected (%)	49.3	49.7	40.4
Eggs/g feces[a]	297.0±626.2	223.4±401.0	194.6±303.8
Ascaris lumbricoides			
Infected (%)	18.8	21.2	20.7
Eggs/g feces[a]	5,385.8±11,339.7	4,350.2±9,159.3	3,834.0±7,465.8
Haemoglobin (g/dl)[a]	12.00±1.39	11.37±1.30	11.59±1.49
Anemia[b] (%)	20.2	37.1	32.0
Severe anemia[c] (%)	0	0.3	0
Ferritin (μg/l)[a]	28.01±17.38	15.85±11.24	15.00±11.20
Low iron stores[d] (%)	16.7	43.3	49.5
Iron deficiency anemia[e] (%)	5.5	18.7	18.9
No of women consumed iron tablet (%)	55.7	92.0	97.8
No of iron tablet consumed[a]	10.5±9.2	35.2±23.2	61.2±34.9

[a]Mean±SD. [b]Hemoglobin < 11 g/dl. [c]Haemoglobin < 7 g/dl. [d]Ferritin < 12 μg/l. [e]Hemoglobin < 11 g/dl and Ferritin <12 μg/l

Table 3
Relationship between parasite load and haemoglobin and ferritin values for Purworejo women during first, second and third trimester.

First trimester

Parasite load	Haemoglobin (n=420)						Ln- Ferritin[a] (n=420)					
	Univariate analyses			Multivariate analyses[b]			Univariate analyses			Multivariate analyses[c]		
	β	SE	p	β	SE	p	β	SE	p	β	SE	p
N. americanus	-0.011	0.136	0.938	0.015	0.140	0.914	-0.173	0.067	0.010	-0.147	0.067	0.029
T. trichiura	0.072	0.080	0.368	0.071	0.082	0.317	0.014	0.040	0.724	0.084	0.040	0.832
A. lumbricoides	0.069	0.144	0.630	0.091	0.147	0.537	-0.034	0.071	0.633	0.032	0.071	0.996

Second trimester

Parasite load	Haemoglobin (n=326)						Ln- Ferritin[a] (n=326)					
	Univariate analyses			Multivariate analyses[b]			Univariate analyses			Multivariate analyses[c]		
	β	SE	p	β	SE	p	β	SE	p	β	SE	p
N. americanus	0.012	0.152	0.936	-0.01	0.157	0.949	-0.119	0.066	0.072	-0.109	0.067	0.105
T. trichiura	0.065	0.092	0.481	0.090	0.095	0.345	-0.033	0.040	0.407	-0.009	0.042	0.835
A. lumbricoides	0.048	0.153	0.754	0.015	0.158	0.926	-0.038	0.068	0.577	0.029	0.070	0.967

Third trimester

Parasite load	Haemoglobin (n=275)						Ln- Ferritin[a] (n=275)					
	Univariate analyses			Multivariate analyses[b]			Univariate analyses			Multivariate analyses[c]		
	β	SE	p	β	SE	p	β	SE	p	β	SE	p
N. americanus	-0.196	0.188	0.296	-0.213	0.193	0.271	-0.004	0.077	0.949	-0.007	0.078	0.929
T. trichiura	-0.025	0.110	0.824	-0.257	0.114	0.822	-0.049	0.045	0.274	-0.068	0.046	0.139
A. lumbricoides	-0.316	0.179	0.078	-0.328	0.192	0.088	-0.009	0.074	0.894	-0.005	0.078	0.936

[a]Logarithm scale of ferritin. [b]After controlling for parity, number of iron tablets consumed, gestational age and type of floor. [c]After controlling for parity, infection, number of iron tablets consumed, gestational age and type of floor.

Highest prevalence of anemia (37.1%) was found in the second trimester (Fig 1). Further, 43.3% of these women had low iron stores. Highest prevalence of low iron stores (49.5%) was found in the third trimester.

A significant negative relationship between hookworm infection and serum ferritin was found in the first trimester (p = 0.010) (Table 3). Even after controlling for parity, infection, number of iron tablets consumed, gestational age and type of floor, the relationship remained significant (p = 0.029). During second and third trimester this association was non-significant, probably due to smaller sample sizes.

No other significant associations were found in the correlation or linear regression analyses. Logistic regression revealed that during the first and second trimester, the risk of having low iron stores increased almost 5 times for women who had mild to severe hookworm infection, compared to women who had no hookworm infection (Table 4). This risk increase was significant in the first trimester.

No significant relationship was found between hookworm infection and serum ferritin status in the repeated measures analyses or between any other helminths and hemoglobin or ferritin status (data not shown).

Table 4
Relationship between parasite load and risk of anemia and low iron stores for Purworejo women during first, second and third trimester.

First trimester

Parasite load	Anemia[a]					Low iron stores[b]				
		Univariate analyses		Multivariate analyses[c]			Univariate analyses		Multivariate analyses*	
	No.	OR	95% CI	OR	95% CI	No.	OR	95% CI	OR	95% CI
N. americanus										
Negative	320	1.000		1.000		320	1.000		1.000	
Low	89	1.219	0.692 - 2.148	1.248	0.683 - 2.280	89	1.500	0.822 - 2.736	1.197	0.631 - 2.269
Mild-Severe	11	0.948	0.200 - 1.494	1.045	0.216 - 5.054	11	4.931	1.448 - 16.79	4.530	1.292 - 15.89
T. trichiura										
Negative	213	1.000		1.000		213	1.000		1.000	
Low	99	0.509	0.263 - 0.982	0.505	0.253 - 1.008	99	0.941	0.496 - 1.785	0.887	0.451 - 1.746
Mild-Severe	108	0.941	0.534 - 1.645	0.987	0.558 - 1.747	108	0.989	0.534 - 1.832	0.977	0.516 - 1.853
A. lumbricoides										
Negative	341	1.000		1.000		341	1.000		1.000	
Low	68	0.986	0.500 - 1.869	1.113	0.575 - 2.156	68	1.946	1.070 - 3.584	1.764	0.901 - 3.381
Mild-Severe	11	0.404	0.051 - 3.191	0.370	0.045 - 3.016	11	0.573	0.072 - 4.572	0.338	0.041 - 2.805

Second trimester

Parasite load	Anemia[a]					Low iron stores[b]				
		Univariate analyses		Multivariate analyses[c]			Univariate analyses		Multivariate analyses*	
	No.	OR	95% CI	OR	95% CI	No.	OR	95% CI	OR	95% CI
N. americanus										
Negative	249	1.000		1.000		249	1.000		1.000	
Low	70	1.267	0.740 - 2.169	1.266	0.082 - 2.169	70	1.183	0.696 - 2.011	1.091	0.623 - 1.910
Mild-Severe	7	2.447	0.536 - 11.17	2.549	0.541 - 12.01	7	8.649	1.027 - 72.88	6.079	0.778 - 57.81
T. trichiura										
Negative	163	1.000		1.000		163	1.000		1.000	
Low	95	1.373	0.824 - 2.288	1.401	0.822 - 2.387	95	0.877	0.526 - 1.463	0.769	0.448 - 1.319
Mild-Severe	68	0.877	0.486 - 1.582	0.774	0.413 - 1.450	68	1.193	0.681 - 2.091	0.949	0.524 - 1.719
A. lumbricoides										
Negative	257	1.000		1.000		257	1.000		1.000	
Low	62	1.167	0.890 - 2.705	1.385	0.772 - 2.483	62	1.214	0.697 - 2.117	1.017	0.566 - 1.828
Mild-Severe	7	0.260	0.032 - 2.145	0.240	0.029 - 2.008	7	0.829	0.194 - 3.542	0.672	0.153 - 2.950

Third trimester

Parasite load	Anemia[a]					Low iron stores[b]				
		Univariate analyses		Multivariate analyses[c]			Univariate analyses		Multivariate analyses*	
	No.	OR	95% CI	OR	95% CI	No.	OR	95% CI	OR	95% CI
N. americanus										
Negative	221	1.000		1.000		221	1.000		1.000	
Low	46	1.164	0.597 - 2.272	1.265	0.636 - 2.516	46	1.055	0.559 - 1.988	1.153	0.602 - 2.208
Mild-Severe	7	2.911	0.635 - 13.35	2.969	0.633 - 13.94	7	1.406	0.308 - 6.426	1.468	0.318 - 6.782
T. trichiura										
Negative	163	1.000		1.000		163	1.000		1.000	
Low	57	1.122	0.596 - 2.212	1.220	0.639 - 2.328	57	0.933	0.511 - 1.703	0.953	0.516 - 1.760
Mild-Severe	54	1.037	0.541 - 1.989	0.989	0.503 - 1.945	54	1.075	0.584 - 1.978	1.181	0.632 - 2.207
A. lumbricoides										
Negative	217	1.000		1.000		217	1.000		1.000	
Low	49	2.167	1.163 - 4.036	2.414	1.225 - 4.360	49	0.687	0.371 - 1.274	0.799	0.417 - 1.533
Mild-Severe	8	1.462	0.339 - 6.301	1.282	0.273 - 6.021	8	1.637	0.382 - 7.016	1.779	0.397 - 7.971

[a]Haemoglobin < 11 g/dl. [b]Ferritin < 12 μg/l. [c]After controlling for parity, number of iron tablets consumed, gestational age and type of floor. After controlling for parity, infection, number of iron tablets consumed, gestational age and type of floor.

DISCUSSION

Intestinal helminth infection was common among pregnant women in Purworejo District, Central Java. Seventy percent of the women had at least one pathogenic species in their stool samples. *T. trichiura* was the most common worm, followed by hookworm and *A. lumbricoides*. However, the intensity of infections was low. Only a few other studies of intestinal helminth infection during pregnancy to compare was exist. Among pregnant women in Nepal, the prevalence of helminth infection was 78.8%, 56.2% and 7.9% for hookworm, *A. lumbricoides* and *T. trichiura*, respectively (Navitsky *et al*, 1998). In Uganda, Kiwanuka and colleagues (1999) found hookworm to be the most common helminth (50% of infected cases). In contrast, in Guatemala Villard *et al* (1989) found *A. lumbricoides* to be the most prevalent helminth among pregnant women who came to the antenatal clinic in Guatemala City.

Among our Indonesian women, anemia was most prevalent in the second trimester (37.1%), while low iron store was most prevalent in the third trimester (49.5%). The highest prevalence of iron deficiency anemia also was found in the third trimester. However, these levels are lower than national figure for anemia in pregnant women, 50.9% (Ministry of Health, Republic of Indonesia, 1995). Decline in hemoglobin and serum ferritin levels during the progress of pregnancy is partly due to hemodilution combined with rapid mobilization of maternal iron stores. During pregnancy the requirement of hemotopoietic nutrients is increased to meet the need of the growing fetus (Calvo and Sosa, 1991). Increased daily requirement for iron especially in the last trimester cannot be met by diet alone but will also partly be met by maternal reserves. Frequent pregnancies as well as poor consumption of heme iron and promoters of non-heme iron absorption together with high consumption of inhibitors of non heme iron absorption lower these reserves. However, anemia in tropical countries has multiple contributory causes. Iron deficiency has been shown to be a major cause, but malaria, thalassemia and megaloblastic anemia can also contribute (Crane *et al*, 1972). Finally, the role of hookworm and *T. trichiura* in causing iron blood loss and reduced absorption is well documented with hookworm having the most significant effect on anemia (Roche and Layrisse, 1966; Layrisse *et al*, 1987, Pritchard *et al*, 1991; Crompton and Whitehead, 1993). *A. lumbricoides* has a small but significant inverse correlation with hemoglobin (Curtale *et al*, 1993; Stolzfus *et al*, 1997).

The role of hookworm as a cause of anemia is consistent with the findings of the present study. There was a negative relationship between hookworm burden and plasma ferritin level, particularly in first trimester of pregnancy. Among Nepali pregnant women, 32% of moderate to severe anemia and 29% of iron deficiency anemia was attributable to hookworm infection (Dreyfuss *et al*, 1996). Surprisingly, among our Indonesian women there was no effect of worm burden on hemoglobin levels. In contrast, Shield *et al* (1981) found an inverse correlation between hookworm egg counts and both hemoglobin and plasma ferritin levels. However, our findings are similar with those of studies among pregnant women in Uganda (Kiwanuka *et al*, 1999) and among adult men and women population in Indonesia (Bakta *et al*, 1993) and in Papua New Guinea (Pritchard *et al*, 1991). Those studies found associations between hookworm and serum ferritin levels, but not between hookworm and hemoglobin. The absence of correlation between hookworm burden and hemoglobin in the present study may be explained by the lower intensities of infection, with no severe infection. It has been reported that an effect of hookworm burden on degree of anemia is only seen when intensity is in excess of 2000 eggs/g (Layrisse and Roche, 1964). Among our women, mean egg count was 287 - 344 eggs/g.

As mentioned earlier, *T. trichiura* infection is also known to produce an intestinal blood loss (Roche and Layrisse, 1966). *T. trichiura* was the most prevalent helminth among the Indonesian pregnant women. However, again the worm burden was light and may not have contributed to iron deficiency anemia. This is similar to findings from Benin (Hercberg *et al*, 1986). Here, *A. lumbricoides* also was common, but it did not contribute significantly to a lower ferritin and hemoglobin level. Likewise, among children in Zanzibar *Trichiuris* and *Ascaris* were common but they did not contribute significantly to intestinal blood loss (Stoltzfus *et al*, 1996).

Any public health intervention aimed at reducing anemia prevalence, to be effective, should include the control of soil transmitted helminths. Helminth infection thrives and persists in communities in need of better housing, clean water, appropriate sanitation, greater access to health care and education as well as increased personal earnings (Crompton, 1999). Therefore, in a setting where intestinal helminth infection is common, anthelminthic mass treatment should be performed with careful attention to sanitation and health education. Anthelminthic treatment, especially combined with supplemental iron, will help prevent iron deficiency anemia and its consequences during pregnancy (Atukorala *et al*, 1994). Anthelminthic treatment should be carried out among pregnant women after the first trimester, when there is no risk of teratogenicity (WHO, 1994b). Anthelminthic programs among adolescent and young adult women, before their reproductive career, should be considered very important for improving their iron status.

Our study showed that helminth infection, anemia and low iron stores were common among pregnant women in Central Java, Indonesia, although no women had severe helminth infections. It is concluded that hookworm infection has a negative effect on iron stores, which indicated by serum ferritin. The study demonstrates the need to search for other important causes of anemia than low iron stores and intestinal helminths, especially among pregnant women. Effects of dietary intake, levels of vitamin B12 and folate also should be investigated.

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REFERENCES

Allen LH. Pregnancy and iron deficiency: Unresolved issues. *Nutr Rev* 1997; 55: 91-101.

Atukorala TMS, de Silva LDR, Dechering WHJC, Dassenaike TSC, Perera RS. Evaluation of effectiveness of iron-folate supplementation and anthelminthic therapy against anemia in the plantation sector of Sri Lanka. *Am J Clin Nutr* 1994; 60: 286-92.

Bakta IM, Widjana DP, Sutisna P. Hookworm infection and iron stores: a survey in a rural community in Bali, Indonesia. *Southeast Asian J Trop Med Public Health* 1993; 24: 501-4.

Banwell JG, Schad GA. Hookworm. *Clin Gastroenterol* 1978; 7: 129-55.

Bundy DAP, Chan MS, Savioli L. Hookworm infection in pregnancy. *Trans R Soc Trop Med Hyg* 1995; 89: 521-2.

Calvo EB, Sosa EM. Iron status in non-pregnant women of child-bearing age living in Greater Buenos Aires. *Eur J Clin Nutr* 1991; 45: 215-20.

Cook JD, Skikne BS. Iron deficiency definition and diagnosis. *J Intern Med* 1989; 226: 349-55.

Crane GG, Haornabrok RW, Kelly A. Anemia in the coast and highlands of New Guinea. *Hum Biol Oceania* 1972; 1: 234-41.

Crompton DWT. How much human helminthiasis is there in the world? *J Parasitol* 1999; 85: 397-403.

Crompton DWT, Whitehead R. Hookworm infections and human iron metabolism. *Parasitology* 1993; 107: S137-45.

Curtale F, Tilden R, Muhilal, Vaidya Y, Pokhrel RP, Guerra R. Intestinal helminths and risk of anemia among Nepalese Children. *Panminerva Med* 1993; 35: 159-66.

Dreyfuss ML, Shresta JB, Khatry SK, *et al*. Relationship between iron status and helminth infection among pregnant women in Nepal [Abstract]. *Faseb J* 1996; 10: A730.

Gillespie S. Major Issues in the control of iron deficiency. Ottawa: Micronutrient Initiative, 1998.

Gillespie S, Johnston JL. Expert consultation on anemia determinants interventions. Ottawa: Micronutrient Initiative, 1998.

Hakimi M, Dibley MJ, Surjono A, Nurdiati D, Ninuk TH, Ismadi SD. Impact of vitamin A and zinc supplements on maternal postpartum infections, in Rural Central Java [Abstract]. Report of XIX International Vitamin A Consultative Group Meeting - Vitamin A and other micronutrients: Biologic Interactions and integrated interventions. Durban, South Africa. 8-11 March, 1999.

Hercberg S, Cahuliac M, Galan P, *et al*. Relationship between anemia, iron and folacin deficiency, haemoglobineopathies and parasitic infection. *Hum Nutr Clin Nutr* 1986; 40C: 371-9.

Kiwanuka GN, Isharaza WH, Mahmoud S. Iron status of pregnant women at first antenatal booking in Mbarara University Teaching Hospital. *Trop Doc* 1999; 29: 228-30.

Layrisse M, Aparcado L, Martinez-Torres C, Roche M. Blood loss due to infection with Trichiuris trichiura. *Am J Trop Med Hyg* 1987; 16: 613-9.

Layrisse M, Roche M. The relationship between anemia and hookworm infection. *Am J Hyg* 1964; 79: 279-301.

Ministry of Health, Republic of Indonesia. Survei kesehatan rumah tangga (SKRT) 1995, survei morbiditas maternal (house hold survey, survey of maternal morbidity). Jakarta: National Institute for Health Research and Development, Ministry of Health Republic of Indonesia, 1995.

Navitsky RC, Deyfuss ML, Shrestha J, Khatry SK, Stoltzfus RJ, Albonico M. Ancylostoma duodenale is responsible for hookworm infections among pregnant women in the rural plains of Nepal. *J Parasitol* 1998; 84: 647-51.

Pritchard DI, Quinnell RJ, Moustafa M, *et al*. Hookworm (*Necator americanus*) infection and storage iron depletion. *Trans R Soc Trop Med Hyg* 1991; 85: 235-8.

Roche M, Layrisse M. Nature and causes of hookworm anemia. *Am J Trop Med Hyg* 1966; 15: 1032-102.

Santiso R. Effects of chronic parasitosis on women's health. *Int J Obstet Gynaecol* 1997; 58: 129-36.

Shield JM, Vaterlaws AL, Kimber RJ, *et al*. The relationship between hookworm infection, anemia and iron status in a Papua New Guinea highland population and the response to treatment with iron and mebendazole. *PNG Med J* 1981; 24: 19-34.

Stoltzfus RJ, Albonico M, Chwaya HM, *et al*. Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. *Am J Trop Hyg* 1996; 55: 399-404.

Stoltzfus RJ, Dreyfuss ML, Chwaya HM, Albonico M. Hookworm control as a strategy to prevent iron deficiency. *Nutr Rev* 1997; 55: 223-32.

Villard J, Klebanoff M, Kestler S. The effect on fetal growth of protozoan and helminthic infection during pregnancy. *Obstet Gynecol* 1989; 74: 915-20.

Weigel M, Calle A, Armijos RX, Vega IP, Bayas BV, Montenegro CE. The effect of chronic intestinal parasitic infection on maternal and perinatal outcome. *Int J Gynecol Obstet* 1996; 52: 9-17.

World Health Organization. Bench aids for the diagnosis of intestinal parasites. Geneva: World Health Organization, 1994a.

World Health Organization. Report of the informal consultation on hookworm infection and anemia in girls and women. Geneva: World Health Organization, December, 1994b.

World Health Organization. The prevalence of anemia in women: A tabulation of available information, 2nd ed. Geneva: World Health Organization. 1992.