EFFECT OF INTESTINAL HELMINTHIASIS ON NUTRITIONAL STATUS OF SCHOOLCHILDREN

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Abstract. This study was conducted in a rural agricultural area in Siniloan, Republic of the Philippines. The subjects were the school children. The nutritional status of 58 children infected with helminthiasis (*Ascaris lumbricoides* and *Trichuris trichiura*) was compared with that of 19 uninfected controls. Prevalence of *Ascaris* and *Trichuris* was 40.3% and 71.4% respectively, and 36.4% of infected children had both *Ascaris* and *Trichuris* infections. Statistically significant evidence of an adverse effect of helminthiasis on serum albumin levels was found, but no child had inadequate levels of other nutrients. Although helminthic infections increase the level of immunoglobulin E (IgE) in children endemically exposed to these parasites, there was no significant difference in the serum IgE among *Ascaris* or *Trichuris* infected groups in this study.

INTRODUCTION

Intestinal parasitic infection is a common afflication of much of the world's population and it has been and is an important problem in public health. Intestinal helminthiases are some of the major parasitic infections throughout the world. *Ascaris lumbricoides* and *Trichuris trichiura* infection are frequent in tropical and subtropical areas, where the prevalence of these infections is usually higher than that of hookworm infections. *Ascaris lumbricoides* and *Trichuris trichiura* are most prevalent in school-aged children who harbor both the highest prevalences and intensities of these infections (Bundy *et al*, 1988). The contribution of heavy *Ascaris* infection to malnutrition and vitamin deficiency has been studied and it was pointed out that the adverse effect of *Ascaris* infection on serum albumin levels and plasma vitamin C levels was found (Blumenthal and Shultz, 1976). Studies on hospitalized patients with Ascariasis have demonstrated a modest degree of malabsorption of fat, protein, and carbohydrate (Tripathy *et al*, 1971). The effect of *Ascaris lumbricoides* infection on intestinal absorption was studied in five children hospitalized in a metabolic ward, and it was reported that deworming resulted in a mean reduction of fecal fat from 9.9 to 2.3 % of dietary fat (Tripathy *et al*, 1972). In addition, the contribution of *Ascaris* to malnutrition has been assessed in field studies. The intervention study showed that the intensity of *Ascaris* infection was related to the degree of malnutrition, and a significant increment of height gain was found in the anthelminthic treatment group (Thein-Hlaing *et al*,1991). The study performed to assess intensity of helminth infections and nutritional status used anthropometric measurements; it showed that *Trichuris*-infected children with >1,000 eggs per gram feces (epg) had significantly lower nutritional status than lower epg or non-infected children (Hadju *et al*, 1995). It is well known that the production of large amounts of immunoglobulin E (IgE) is a characteristic feature of the immune response to helminthic infection in tropical populations. The problem that exists between helminthic infection, condition of hygiene and socioeconomic factors is widely acknowledged. There has been a growing concern that helminthic infections may detrimentally affect children's cognitive functions and contribute to their failing in school (Kvalsivig *et al,* 1991; Nokes *et al*, 1992; Simeon *et al*, 1995). The impact of geohelminth infections on children's educational achievement is now receiving attention. However, up to the present, studies were not measurement nutrients and it was suspected that their subjects

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were under generally poor nutrition status. The relationship between intestinal parasitic infection and nutritional status is an important problem. To date, not enough attention has been focused either on the intensity of infection or the proper design of such studies. This paper investigates the relationship between helminthiasis infection and nutritional status. It also assess the relation between an helminthiasis infection and serum IgE. The subjects are school children living in a rural agricultural area in Siniloan, Republic of the Philippines.

SUBJECTS AND METHODS

Subjects

The subjects were 142 school children (85 boys, 57 girls) with age ranging from 8 to 13 years old. They live in the municipality of Siniloan, Province of Laguna, Republic of the Philippines. No subjects had significant exposure to environmental pollutants. A questionnaire was used to obtain information regarding age, medical history and dietary habits. Sera were separated from blood samples and kept in the frozen state until used.

Vitamin A and E determination

A 40 ml aliquot of ethanol containing IS (Tocopherol acetate, 100 mg/ml) was added to 0.2 ml of serum in a polypropylene microfuge tube. After vortex mixing, the specimen was extracted with 0.4 ml butanol-ethyl acetate (1:1 v/v) and further mixed for 1 minute. After vortex-mixing for another 1 minute, the sample was allowed to stand at -20ºC for 20 minutes before centrifugation at 15,000*g* for 2 minutes (Beckman Microfuge R centrifuge). The sample was allowed to stand at -20ºC until HPLC analysis. HPLC analysis was performed by isocratic elution. A Shimadzu LC-10AD was set at a flow-rate 0.8 ml/minute. The mobile phase, consisting of methanol-butanol-water (90:5:5, v/v). The analytical column used Capcel Pak 5C18 (Type AG120, 150 mm X 4.7 mm ID, 5 mm particle size, Shiseido). Vitamins A, E and an internal standard were detected by a UV-visible spectrometor (Shimadzu Model SPD-10A). Analytes were identified by retention time and quantitated by peak area with a Shimadzu Model CR-6A integrator (Kyoto, Japan).

Fatty acid determination

Serum samples (0.2 to 0.5 ml) were saponified with 1 ml of 1N-KOH solution in ethanol and 0.5 ml of 1 mg/ml pentadecanoic acid ethanol solution (internal standard) and heated at 75ºC for 1 hour. After saponification, 2 ml of 1N HCl was added and the fatty acids were extracted with n-hexane, dried under nitrogen stream, and redissolved in 0.5 ml of methanol-benzene (3:7). The fatty acid was esterified with 0.1 ml of 2M trimethylsilyl-diazomethane solution in n-hexane. After esterification, the fatty acid methyl esters were dried under nitrogen stream, and redissolved in n-hexane for GC analysis. GC analysis (GC-6A Shimadzu, Kyoto, Japan) was performed on a 30 m X 0.54 mm bonded fused silica DB-17 column (J and W Scientific) programed from 140 to 280ºC at 4 degrees/minute. Identification of fatty acid esters was made by comparison with authentic standards. Peak areas were calculated by Chromatopac C-R6A (Shimadzu, Kyoto, Japan).

Stool examination

All subjects were requested to submit fecal samples for examination. The compliance rate was 52.1% with 77 out of 142 submitted the samples. Using the Kato-Katz quantitative technique, the eggs were counted on all positive *Ascaris lumbricoides* and *Trichuris trichiura*. The intensity of infection was determined by the following criteria given by the WHO Expert Committee (WHO, 1987).

Statistical analysis

The data were analyzed by the Mann-Whitney test and Kruskal-Wallis, 1-Way ANOVA and Median test with the level of significance set at $p<0.05$.

RESULTS

Prevalence and intensity

The results of stool examination are shown in Table 1. The prevalence of *Ascaris* or *Trichuris* was 40.3% and 71.4% respectively. The percentages of children with light, moderate, or heavy infections were 64.5, 32.2, 3.2% respectively for *Ascaris* and 83.6, 14.5, 1.8% respectively for *Trichuris*. The infection rate was light in this area.

The prevalence and intensity of *Ascaris* or *Trichuris* infections by egg count category in two age-groups are shown in Table 2. The intensity of infection was determined by egg count category in two age-groups. The positive rate were 75.3% (58/77); 36.8% (28/77) in double infections with

Table 1
Prevalence and intensity of *Ascaris* and *Trichuris* infections.

	Ascaris lumbricoides		*Trichuris trichiura*	
Prevalence (%)	40.3 (31/77)		71.4 (55/77)	
Clasification:	Cases	Intensity (epg)	Cases	Intensity (epg)
Light	20	1,389 ± 982	46	292 ± 228
Moderate	10	12,484 ± 6,432	8	2,619 ± 1,865
Heavy	1	54,119	1	10,152

epg : egg per 1 gram of stool.
Ascaris : Light infection < 4,999 epg; Moderate infection 5,000-49,999 epg; Heavy infection > 50,000 epg.
Trichuris : Light infection < 999 epg; Moderate infection 1,000 9,999 epg; Heavy infection > l0,000 epg.
(WHO, 1987)

Table 2
Prevalence and intensity of *Ascaris* and *Trichuris* infcctions for age.

	Total	Age group	
		≤ 10 years old	> 10 years old
Single infection:			
Cases	3	2	1
Ascaris lumbricoides (epg)	3,040 ± 2,127	3,024 ± 3,008	3,072
Cases	27	3	24
Trichuris trichiura (epg)	393 ± 481	281 ± 195	408 ± 507
Double infection:			
Cases	28	9	19
Ascaris lumbricoides (epg)	7,057 ± 11,358	10,958 ± 16,716	5,210 ± 7,622
Trichuris trichiura (epg)	1,211 ± 2,254	1,216 ± 1,683	1,209 ± 2,523
Prevalence of infection (%)	75.3 (58/77)	73.7 (14/19)	75.9 (44/58)
Single infection rate (%)	51.7 (30/58)	35.7 (5/14)	56.8 (25/44)
Double infection rate (%)	48.3 (28/58)	64.3 (9/14)	43.2 (19/44)

Ascaris and *Trichuris* and 39.0% (30/77) in single infections with *Ascaris* or *Trichuris*. The double infection rate for the age group under 10 age-years old and over 10 age-years old was 64.3% and 43.2% respectively in infected children. Almost half of the infected children (48%) had both *Ascaris* and *Trichuris* infections together. Arithmetic mean egg counts were 10,958 epg and 1,216 epg for *Ascaris* and *Trichuris,* respectively, reflecting intensity of infections in under 10 years old children. In over 10 years old children, the intensity of infections was 5,210 epg and 1,209 epg for *Ascaris* and *Trichuris* respectively. The under 10 years old children tended to have higher *Ascaris* intensity and double infection rate was higher than in the over 10 years old children. Among the under 10 years old children the arithmetic mean egg count and infection rate were 10,958 epg, and 64.3% (9/14), respectively. For over 10 years old children with double infection, these values were 5,210 epg and 43.2% (19/44), respectively.

Nutritional status

This study was analyzed using α-tocophenol acetate as an internal standard, and 6 fatty acids, namely mylistic acid, C14:0; palmitic acid, C16:0; stearic acid, C18:0; linoleic acid, C18:2; arachidonic acid, C20:4 and docosahexanoic acid, C22:6 and cholesterol (Chol) were analyzed in serum using pentadecanoic acid (C15:0) as internal standard. Except for the vegetable groups, the relation of dietary habit to the serum micronutrients and fatty acids content is shown Table 3. Rice was considered the most important staple dietary by all

Table 3
Concentration of serum micronutrients and fatty acids by dietary habits.

Micronutrients and fatty acids	Total (n=67)	Dietary habits: Fish and meat (n=33)	Meat and vegetable (n=21)	Fish and vegetable (n=13)	Median test p-value
Albumin (g/dl)	4.31 ± 0.25	4.30 ± 0.27	4.31 ± 0.24	4.35 ± 0.23	0.972
Total protein (g/dl)	7.63 ± 0.43	7.60 ± 0.45	7.64 ± 0.43	7.70 ± 0.40	0.809
Vitamin A (μg/dl)	79.6 ± 34.7	86.8 ± 36.1	82.0 ± 37.8	57.6 ± 9.6	0.021
Vitamin E (μg/dl)	1,200 ± 252	1,194 ± 223	1,264 ± 282	1,109 ± 263	0.343
Fatty acids (mg/dl)					
C14:0	9.0 ± 5.7	8.2 ± 5.0	9.5 ± 9.6	10.3 ± 5.5	0.071
C16:0	76.7 ± 20.1	78.9 ± 24.8	74.2 ± 14.6	75.2 ± 14.2	0.932
C18:0	76.8 ± 22.4	79.8 ± 14.6	75.7 ± 15.8	72.8 ± 14.6	0.932
C18:2n-6	76.7 ± 13.3	76.8 ± 13.1	78.5 ± 12.2	73.5 ± 15.7	0.685
C20:4n-6	18.1 ± 3.1	18.4 ± 3.4	18.2 ± 2.6	17.0 ± 2.9	0.328
C22:6n-3	6.0 ± 2.7	5.7 ± 3.2	6.5 ± 2.3	6.1 ± 1.9	0.826
Cholesterol (mg/dl)	89.1 ± 21.8	90.6 ± 26.1	86.4 ± 19.0	89.8 ± 13.2	0.567
Total lipid (mg/dl)	352.3 ± 64.1	357.8 ± 78.8	349.1 ± 47.6	344.6 ± 45.9	0.932

Table 4
Comparison of serum micronutrients and fatty acids by helminthic infection.

Micronutrients and fatty acids	Uninfected (n=15)	Single infection (n=26)	Double infection (n=26)	Median test p-value
Albumin (g/dl)	4.45 ± 0.23	4.33 ± 0.23	4.22 ± 0.25	0.168
Total protein (g/dl)	7.71 ± 0.48	7.65 ± 0.38	7.57 ± 0.45	0.949
Vitamin A (μg/dl)	71.1 ± 26.4	79.8 ± 30.2	84.3 ± 42.5	0.275
Vitamin E (μg/dl)	1,226 ± 207	1,131 ± 219	1,254 ± 297	0.358
Fatty acids (mg/dl)				
C14:0	7.8 ± 2.3	9.0 ± 4.3	9.7 ± 8.0	0.244
C16:0	73.9 ± 14.3	77.1 ± 12.8	78.0 ± 28.0	0.142
C18:0	77.3 ± 19.2	74.1 ± 12.6	79.2 ± 30.8	0.691
C18:2n-6	77.8 ± 9.9	75.2 ± 13.8	77.6 ± 14.8	0.975
C20:4n-6	18.0 ± 2.9	17.4 ± 2.8	18.8 ± 3.4	0.527
C22:6n-3	5.3 ± 2.6	5.8 ± 3.0	6.8 ± 2.5	0.275
Cholesterol (mg/dl)	91.9 ± 26.9	87.0 ± 16.8	89.7 ± 23.5	0.358
Total lipid (mg/dl)	352.1 ± 59.5	345.6 ± 46.1	359.6 ± 81.5	0.527

of the subjects, and it was generally consumed more than once a day. The subjects were divided: the majority (48.7%) who ate a combination of meat and fish followed by 30.3% meat and vegetables, 18.4% fish and vegetables and another 2.9% (2/69) vegetables only. No child in either group had low or deficient values in any biochemical determinations. There were no significant differences in the serum fatty acids, vitamin E, albumin and total protein among the 3 dietary habit groups. However, the serum vitamin A level of the fish and vegetable group was significantly lower than the other groups ($p < 0.05$). It was likewise shown that the serum levels of C18:2n-6 and C20:4n-6 of those who ate a combination of fish and vegetable tended to be lower compared to the other two groups. It was suspected that the eating habits were different between the age-groups under 10 years old and over 10 years old. Therefore, the serum micronutrients and fatty acid content

Table 5
Relation between infection intensity and serum total IgE levels.

	Total IgE levels (IU/ml)			
	No.	Median	Minimum	Maximum
Uninfected group	12	480	62	12,000
Ascaris infectcd group	3	612	0	54,119
Trichuris infectcd group	27	207	0	5,497
Double infected group	28	830	45	36,000

were compared in the two age-groups by using Mann-Whitney U-test: there was no significant difference in the serum micronutrients and fatty acids content between the two age-groups (data not shown).

Comparison of serum micronutrients and fatty acids according to helminthic infection is shown in Table 4. There was no significant difference in most of the serum micronutrients. However, the serum albumin level of the infected groups was lower than one of the uninfected groups. The serum albumin level tended to decrease with greater infection rate.

Infection and IgE

It is well known that the production of large amounts of immunoglobulin E (IgE) is a characteristic feature of the immnune response to helminthic infection. The relationship between helminthic infection and IgE level is shown in Table 5. The serum IgE of the infected group was higher except for the *Trichuris* infected group. It should seems that the serum IgE level increases with the intensity of helminthic infection. However, a correlation between serum IgE and helminthic infection intensity was not found.

DISCUSSION

This study aimed to show if there was a significant association between intestinal helminthiasis, nutritional status and IgE level. Since children are at highest risk of morbidity due to *Ascaris* and *Trichuris*, these findings suggest that gastrointestinal helminthiasis in children may be of particular significance to their health, especially the problem of nutritional status or growth reterdation (Lai Karen *et al*, 1995; Hadju *et al*, 1995). It has been reported that there is a relationship between helminthic infection and childhood nutritional status (Thein-Hlaing,1993). Although this study was performed including boys and girls, the significant difference between the group for infection was under the influence of gender (data not shown). Parraga *et al* (1996) examined the stools using the Kato-Katz method in all students 7-15 years of age at 17 public schools, and they reported that there were no significant difference between the infected and uninfected groups with respect to gender.

Intestinal helminthiasis

The prevalence and intensity of *Ascaris* and *Trichuris* in these schoolchildren can be compared with other survey of children of almost same age. A study in a slum area of Ujung Pandang municipality, South Sulawesi, Indonesia, found that the prevalences of *Ascaris* and *Trichuris* infection were 98% and 92% respectively, which is very high compared with this study (Hadju *et al*, 1995).

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Nutritional status

The levels of vitamin A and vitamin E in this study ranged from 26 μg/dl to 185 μg/dl; for vitamin E 593 μg/dl to 2,008 μg/dl. These levels were almost the same as those reported for healthy Swedish children between the ages of 8 and 13 years (Drott *et al*, 1993). The ranges of vitamin A and vitamin E were 21.1-150.5 μg/dl and 280.0-1,320.0 μg/dl respectively. The serum vitamin A level was not significantly different between the infected group and the uninfected group. A relationship between serum vitamin E and helminth infection was not found. No child in the uninfected or the infected group had “low” or “deficient” serum albumin levels. Tripathy *et al* (1971) had reported that the serum albumin level in heavily *Ascaris* infected children (mean 199,000 egg/g feces, range 56,000-449,000), had a mean value of 3.0

g/dl (range 2.55-4.10). Our results were 4.33+/-0.23 g/dl in a single infection and 4.22+/-0.25 g/dl in a double infection, respectively. The intensity of *Ascaris* infection had a mean of 7,075 egg/g feces in this study. The apparent difference in results of the two studies is considered to be in infection intensity. Our subjects had light or moderate intensity, and thus the intestinal absorption of protein was not affected by *Ascaris.*

One of the most abundant polyunsturated acids produced by plants, linoleic acid (n-6 series fatty acid) cannot be made by animals yet this fatty acid is necessary to maintain animals in a healthy condition. We compared the serum fatty acid level of the helminth-infected group to the uninfected group in the same area, but the mean age of subjects was higher. The results showed that the amounts of palmitic acid (C16:0) and stearic acid (C18:0) in the infected group were markedly reduced compared with the uninfected group (Yamamoto *et al*, 1997). In this study, essential fatty acid, C18:2n-6 level was compared in uninfected and single or double infected groups. There was no significant difference between the infected group and the uninfected group. This result suggests that the absorption of fatty acids was not affected by *Ascaris* and *Trichuris* infection. The study aimed to assess the influence of intestinal helminth infection on intestinal permeability in early primary schoolchildren. The result suggests that the influence of intestinal helminths is negligible in relation to the cumulative effects of other factors in children from the same socioeconomic background (Raj *et al*, 1996).

Helminthic infection and immunogloblin E (IgE)

Helminthic infection can stimulate serum IgE in children endemically exposed to these parasites. In this study, there was no significant difference in the serum IgE among *Ascaris* and *Trichuris* infected groups. On the contrary, it has been reported that the total serum IgE level increases from relatively low values in the low *Ascaris* infected group through to high concentrations in the high *Ascaris* infected group, and the high *Ascaris* infected group divided relatively low socioeconomic level (LSEL) (Lynch *et al*, 1987). LSEL focuses attention on various problems that exist between helminth infection, conditions of hygiene and socioeconomic factors. In addition, it had been reported that there is perfect correlation between IgE levels in the slum children and the degree of poverty (Lynch *et al*, 1992). LSEL, especially poverty, would affect the nutritional status of children. In the children infected with *Ascaris*, it was reported that the total IgE concentration was significantly higher in malnourished children than in their well-nourished counterparts and there was significant inverse correlation between nutritional status and IgE level (Hagel *et al*, 1995), however, their results suggest that the intestinal helminths do not act alone in producing elevated IgE levels. The nutritional status of subjects was relatively good in this study. Our results agreed with the above evidence, that the intestinal helminths do not act alone in producing IgE in these intensity levels of *Ascaris* and *Trichuris*.

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