DETERMINATION OF FECAL OCCULT BLOOD IN PRIMARY SCHOOLCHILDREN INFECTED WITH *TRICHURIS TRICHIURA*

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Abstract. A correlation of *Trichuris trichiura* infection and fecal occult blood detection was conducted in 146 primary schoolchildren in Narathiwat Province, Thailand. The Kato-Katz thick smear method was used for determining egg counts and stated as eggs per gram of feces (epg). The number of *T. trichiura* eggs was categorized as class I (1-499 epg), class II (500-4,999 epg), and class III (> 5,000 epg), according to the relation between infection intensity and reduced hemoglobin concentration. Each fecal sample was processed to detect occult blood using a guaiac-based test (Hema-Screen™, USA) and an immunochromatographic-based test (HEXAGON OBTI test™, Germany). There were 50 schoolchildren without parasitic infection in the control group. Of 96 cases with *T. trichiura* infection, 85 and 11 children were classified in the class I and class II groups, respectively, but no subjects were in the class III group. Positive occult blood detection results in the control, class I, and class II groups using the guaiac and the immunochemical tests were 0, 3.5, and 9.1% (p=0.19), and 0, 2.4, and 36.4%, (p<0.0001) respectively. This study suggests that *T. trichiura* infection with an intensity of 500 epg or greater may be associated with intestinal bleeding.

INTRODUCTION

Trichuris trichiura is a major infecting intestinal nematode in the world population. The total global number of people with *T. trichiura* infection had been estimated to be 1,049 million, including 114 million preschool-aged children and 233 million school-aged children (Stephenson, 2000). In southern Thailand, schoolchildren are also susceptible to trichuriasis, due to a suitable climate for parasite development and unsanitary conditions (Muennoo *et al*, 2000; Tomono *et al*, 2003; Loymek *et al*, 2004). The prevalence rate of *T. trichiura* infection reported for school-aged children has varied from 28 to 70% (Muennoo and Rojekittikhun, 2003). The burden of infection is a significant source of human ill-health and an important public health problem, especially for children in developing countries (Gillespie, 2001). The mechanism of damage to the colonic mucosa is by the worm threading into the epithelium of the colon producing an inflammatory response (Garcia, 2001). The clinical spectrum of trichuriasis varies from asymptomatic infection to *Trichuris* dysentery syndrome, which is characterized by chronic mucous-bloody diarrhea. Rectal bleeding and prolapse are also associated with trichuriasis. Blood loss occurs from both the feeding activities of the parasites and extensive damage to the colonic mucosa (Crompton, 1998; Gillespie, 2001).

Data regarding an association between heavy *T. trichiura* infection and lower blood hemoglobin concentrations (Robertson *et al*, 1992) and intestinal blood loss (Layrisse *et al*, 1967) had been reported. There is scant information regarding fecal occult blood detection in light of *T. trichiura* infection. The available data have been derived from epidemiological studies of occult bleeding in schoolchildren co-infected with hookworm and *T. trichiura*, but simultaneous

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infection may impede the role of trichuriasis in inducing intestinal blood loss (Stoltzfus *et al*, 1996). A previous investigation showed that there was no correlation between light *T. trichiura* infection and stool occult blood tests using the guaiac test (Raj, 1999). However, these results could have been hampered by the relatively low sensitivity and specificity of the conventional test. Immunochemical tests for fecal occult blood detection have been demonstrated to be more sensitive and specific than the traditional technique (Allison *et al*, 1996; Rozen *et al*, 2000). Determination of stool occult blood was therefore conducted in children infected with *T. trichiura* using both guaiac-based and immunochemical-based tests.

MATERIALS AND METHODS

In February 2003, a cross-sectional study of schoolchildren attending four primary schools in Mueang and Takbai districts, Narathiwat Province, Thailand, was conducted to evaluate the correlation between *T. trichiura* infection and fecal occult blood detection. Parental informed consent was obtained for all participants. Stool specimens of 356 children were collected without dietary or drug restrictions. Fecal examinations for parasites were performed. The occult blood tests were processed and interpreted by experienced technicians who were blinded to the parasitologic results.

The Kato-Katz thick smear method was used to determine egg counts, stated as eggs per gram of feces (epg) (Katz *et al*, 1972). The number of *T. trichiura* eggs were classified as class I (1-499 epg), class II (500-4,999 epg), and class III (> 5,000 epg), according to the relation between infection intensity and reduced hemoglobin concentration as described by Robertson *et al* (1992). Each fecal sample was processed to detect occult blood using a guaiac-based test (Hema-Screen™, Stanbio, Texas, USA) and an immunochromatographic-based test (HEXAGON OBTI test™, Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany). The diagnostic procedures were conducted per manufacturers' directions. According to the production instructions, Hema-Screen™ can detect 10 mg of hemoglobin per gram of feces. A positive reaction is indicated by the appearance of a blue-green color between 30 seconds and two minutes. The HEXAGON OBTI test™ can reveal hemoglobin as low as 50 µg per gram of dry stool. A positive result is determined by the appearance of two lines on the test at 5 minutes.

The data were analysed by the chi-square test using Epi Info Version 6 and Microsoft Excel. The level of significance was set at 0.05.

Table 1
Background characteristics of the control and study populations.

Characteristics	Control group	Study group
Number	50	96
Gender, male : female	1:1.6	1:1.1
Age range (years)	7-12	7-12
Mean age ± SD (years)	9.2 ± 0.3	8.8 ± 0.4

RESULTS

A total of 146 schoolchildren within the age group of 7 to 12 years were enrolled in the study. Fifty subjects without parasitic infection served as a control population. Of the 96 children infected with *T. trichiura*, 85 and 11 cases were categorized into class I and class II respectively, but no subjects were found to be in class III. The baseline characteristics of the control and study groups are shown in Table 1.

There were no positive fecal occult blood results in the control group. In cases with *T. trichiura* infection, the positivity rates for occult blood determination using the Hema-Screen™, and HEXAGON OBTI™ tests were 4.2% and 6.3%, respectively. Positive results on occult blood detection for group II were more frequent than in group I, as shown in Table 2. There were no statistically different results in blood detection between the class I and class II groups ($p=0.19$) using the guaiac-based test, but there was a significant difference ($p<0.0001$) using the immunochemical test.

DISCUSSION

In *T. trichiura* infection, the severity of mechanical damage to the epithelium of the colon is apparently related to the worm burden. Adult

Table 2
Comparison of occult blood detection correlated with *Trichuris trichiura* intensity.

Test	Positive occult blood detection			p-value
	Control (0 epg) n = 50	Class I (1-499 epg) n = 85	Class II (500-5,000 epg) n = 11	
Hema-Screen™	0 (0%)	3 (3.5%)	1 (9.1%)	0.19
HEXAGON OBTI™	0 (0%)	2 (2.4%)	4 (36.4%)	<0.0001

Table 3
Estimation of blood loss and hemoglobin loss per gram of feces correlated with *Trichuris trichiura* intensity.

	Class I	Class II
Number eggs/g feces (epg)	1-499	500-5,000
Number of worms/g feces	1-7	7-70
Blood loss (ml)/g feces/day	0.005-0.035	0.035-0.35
Hemoglobin loss (mg)/g feces/day	0.55-3.85	3.85-38.5

worms are embedded in the colonic mucosa with their piercing stylets and feed on host blood (Crompton, 1998). The amount of fecal blood loss from a whipworm is about 5 µl per worm per day which is 6-10 times and 30-50 times less than with *Necator americanus* and *Ancylostoma duodenale*, respectively (Layrisse *et al*, 1967). The association between trichuriasis with decreased blood hemoglobin concentrations and iron deficiency anemia was evidenced in infection intensities greater than 5,000 epg and 10,000 epg, respectively (Robertson *et al*, 1992). Intestinal bleeding related to *T. trichiura* infection has been established in heavily infected children (Layrisse *et al*, 1967). On the other hand, light *T. trichiura* infection has not been associated with iron deficiency anemia (Greenberg and Cline, 1979), or with fecal occult blood detection using the guaiac test (Raj, 1999). In addition, the latter report also found no significant occult gastrointestinal bleeding in children with heavy trichuriasis (10,400-84,600 epg) in the absence of dysenteric syndrome. Our findings are in concordance with a study by Raj (1999) in which conventional testing provided no conspicuous evidence for occult intestinal bleeding in children with non-severe *Trichuris* infection. However, immunochromatographic testing demonstrated a higher occult blood positive rate with higher worm burdens (500-4,999 epg) than with lower worm burdens (1-499 epg).

Fecal occult blood tests are generally used to detect gastrointestinal bleeding. The most commonly relied upon test being the guaiac-based test. This conventional method is based on the peroxidase-like activity of hemoglobin in catalyzing the oxidation by peroxide of a chromogen, however, it is nonspecific for human blood. Consumption of some oral medications and certain foods can also cause false positive results. Immunochemical stool occult blood tests have been established to offer a better sensitivity and specificity than guaiac-based tests (Allison *et al*, 1996; Rozen *et al*, 2000). They specifically identify human hemoglobin from the lower gastrointestinal tract. Additionally, dietary restrictions are not needed (Ko *et al*, 2003). Since the whipworms inhabit the colon, the immunochemical-based tests were expected to be suitable for our design to determine stool occult blood with *T. trichiura* infection.

Regarding radioactive studies to determine intestinal bleeding, the amount of fecal blood loss per whipworm is about 5 µl per worm per day. The egg output was evaluated at approximately 70 epg per worm per day (Layrisse *et al*, 1967). Thus, estimated daily blood loss is about 35 µl and 350 µl per day in the class I and class II groups, respectively (Table 3). In a previous study, a hemoglobin concentration of 11 g/dl and a stool output of about 100 g/day were assumed (Raj, 1999). A daily hemoglobin loss per gram of feces was evaluated to be 0.55-3.85 mg in class I group and of 3.85-38.5 mg in class II group.

According to the manual instructions, the guaiac test is not sensitive enough to detect occult blood in class I group. In addition, the immunochemical test should be sensitive to detect blood loss in both groups. In our study, the immunochromatographic-based test demonstrated a significantly higher rate of positive results of occult blood determination in the class II group (36.4%) than in the class I group (2.4%). Whereas, the conventional method showed no statistical difference in the positivity rates for blood detection between the two groups. It is probable that there is no association between the total number of worms recovered after anthelminthic treatment and the number of eggs per gram of feces (Layrisse *et al*, 1967). Non-uniformed distribution of blood loss in feces may also affect the results. Consecutive fecal sample collections are recommended for occult blood detection. This was limited in this study due to the cross-sectional design.

The present study demonstrates that *T. trichiura* infection with an intensity of 500 epg or greater may be correlated with intestinal bleeding. However, larger numbers of children should be further investigated to confirm this outcome.

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