

## FECAL OCCULT BLOOD TESTING OF *TRICHURIS*-INFECTED PRIMARY SCHOOL CHILDREN IN NORTHEASTERN PENINSULAR MALAYSIA

S. MAHENDRA RAJ

Department of Medicine, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia

**Abstract.** Stool specimens of 104 primary schoolchildren (mean  $\pm$  SD age =  $8.2 \pm 0.3$  years) were examined for helminth eggs and for occult blood to investigate the possibility that trichuriasis causes occult intestinal bleeding in the absence of the overt *Trichuris* dysentery syndrome. A commercially available guaiac test was used to detect fecal occult blood. Sixty-one children had *Trichuris* infection, 11 of whom had heavy infections ( $> 10,000$  eggs per gram of feces [epg]), and 53 had *Ascaris* infections. No hookworm infection was detected. Baseline screening yielded only one weakly positive occult blood test result in a child with a light (800 epg) *Trichuris* infection. Serial stool occult blood testing on the 11 subjects with heavy trichuriasis and 8 uninfected controls yielded a single weakly positive result in the control group. The results provide no evidence that trichuriasis predisposes to significant occult gastrointestinal bleeding in children in the absence of the dysenteric syndrome.

Intense infection with *Trichuris trichiura*, a nematode that inhabits the colon causes a chronic dysenteric illness in children.<sup>1</sup> However, this is a relatively uncommon event. Considering that the global prevalence of *Trichuris* infection is approximately 800 million,<sup>2</sup> there is a remarkable dearth of data on whether the infection predisposes to occult gastrointestinal bleeding in the absence of the overt *Trichuris* dysentery syndrome. There is evidence of an association between heavy *Trichuris* infection and anemia even when the dysenteric illness is not present.<sup>3,4</sup> Earlier studies that addressed the question of gastrointestinal bleeding and trichuriasis were conducted on small numbers of selected patients who may not be representative of the community.<sup>1–7</sup> A larger, more recent study was community-based but conducted on a population with a high prevalence of concomitant hookworm infection that could have obscured the role of trichuriasis in causing intestinal bleeding.<sup>8</sup> The aim of the present study was to determine if trichuriasis predisposed to occult colonic bleeding among early primary school children in northeastern peninsular Malaysia, a population with a relatively high prevalence of trichuriasis but a low prevalence of hookworm infection.

### MATERIALS AND METHODS

Stool specimens from 104 children attending a single school on the outskirts of the town of Kota Bharu in northeastern peninsular Malaysia were collected and examined for helminth eggs. All 117 children in the second grade were targeted for the study but stool specimens were obtained from only 104 subjects. Egg counts were determined using a modification of Stoll's method and expressed as eggs per gram of feces (epg).<sup>9</sup> Parasitologic examinations were generally performed on the day of stool collection. The stools were then stored at  $-20^{\circ}\text{C}$  for up to 48 hr before being tested for occult blood using a commercially available slide test kit. This test consisted of guaiac-impregnated paper upon which a thin smear of stool was made (Coloscreen<sup>®</sup>; Helena Laboratories, Beaumont, TX). A positive test result was indicated by a blue color change on adding two drops of the developer reagent on the slide. The guaiac test has been used in cancer screening programs and is based on the principle that guaiac undergoes an oxidative color change in the pres-

ence of hydrogen peroxide and the peroxidase activity of hemoglobin.<sup>10</sup>

The kit was validated *in vitro* by testing it on homogenized stool spiked with various concentrations of whole blood. The performance of the Coloscreen kit was tested initially on freshly prepared stool and later on stool stored for 72 hr, the first 24 hr of which was at room temperature and the remainder at  $-20^{\circ}\text{C}$ . The latter was done to simulate the anticipated storage conditions during the field study. As shown in Table 1, the *in vitro* sensitivity was high even at a hemoglobin concentration of 2 mg/g. Assuming a hemoglobin concentration of 11g/dL and a daily stool output of approximately 100 g, this concentration translates to a daily blood loss of approximately 2 ml/day. The upper limit of normal intestinal blood loss as determined by radioisotope studies have been reported to range from 1 to 3 ml/day.<sup>5,11,12</sup> The stool samples were moistened with drops of distilled water immediately prior to occult blood testing since rehydration has been shown to improve the sensitivity of the guaiac test.<sup>13</sup> The observer developing and reading the Coloscreen slide was blinded to the parasitologic result. Subjects with heavy *Trichuris* infections ( $> 10,000$  epg) and an equivalent number of randomly selected uninfected control subjects were each asked to furnish three additional samples of stool for repeat occult blood testing, which was again performed by an observer blinded to the parasitologic result.

The study was approved by the Research and Ethics Committee of the School of Medical Sciences, Universiti Sains Malaysia. Parental consent was obtained for all participating children.

### RESULTS

Of the 104 children, 53 were girls. The mean  $\pm$  SD age of the children was  $8.2 \pm 0.3$  years. Sixty-one were infected with *T. trichiura*, 53 with *Ascaris lumbricoides*, and none with hookworm. Thirty-two children had no evidence of helminthic infection. There was no difference in mean age and sex ratio between the *Trichuris*-positive and -negative groups. Among the *Trichuris*-positive children, the median intensity of infection was 4,000 epg with an interquartile range of 7,200 epg. Eleven children had heavy trichuriasis (median = 18,800 epg, range = 10,400–84,600 epg). Five

TABLE 1  
*In vitro* performance of the stool occult blood test kit

Hemoglobin concentration in spiked stool (mg/g)	Rate of positive test results	
	Freshly prepared stool	Stool stored for 72 hr
0	0/10	0/10
1	9/10*	3/10†
2	10/10	9/10*
5	10/10	10/10
10	10/10	10/10

\* The observed color change in the positive test results was unequivocal but generally weak.

† The observed color change in the positive test results was very faint.

of the 11 with heavy trichuriasis complied with the request to provide an additional three samples each while the rest provided nine additional samples. The stool recovery rate from children who served as controls for serial testing was lower, with 13 samples being obtained from eight subjects.

At baseline screening, all but one of the 104 stool samples tested were unequivocally negative for occult blood. The single positive test result was a weakly positive one observed in a child with a light (800 epg) *Trichuris* infection. Repeat fecal occult blood tests on serial samples from the children with heavy trichuriasis and controls also yielded negative results except for one weakly positive result on a single sample from an uninfected control subject.

#### DISCUSSION

There was no significant difference in the rate of stool occult blood positivity between *Trichuris*-positive and -negative children. Although the *in vitro* sensitivity of the Coloscreen test was clearly high, the *in vivo* sensitivity of detecting colonic bleeding may be lower due to factors such as the intermittent nature of bleeding or nonuniform mixing of blood in the stool. While even modest reductions in sensitivity are of critical diagnostic importance in individual patients being screened for lesions such as colon cancer, they are less crucial for the purpose of this study, which was to detect a difference in the propensity of colonic bleeding between groups. In the original design of the study, provisions were made to repeat occult blood testing after treatment based on the hypothesis that *Trichuris*-induced bleeding would be reversed by treatment. The baseline results clearly obviated the need for the interventional phase of the study. Furthermore, any reduction of *in vivo* sensitivity of the occult blood test due to the factors alluded to was at least partially compensated for by serial testing of stool in children with heavy *Trichuris* infection; the group most likely to have been occult blood-positive if the original hypothesis were true. The definition of heavy infection on the basis of egg counts is somewhat arbitrary, and the one used for this study (> 10,000 epg) was proposed by a World Health Organization expert committee.<sup>14</sup> It is acknowledged that some investigators consider 20,000 epg as a cut-off level between infections of moderate and heavy intensities.<sup>15</sup> The results of this study are compatible with those of the recent study by Stoltzfus and others, who found no correlation between *Trichuris* intensity and stool hemoglobin concentration after adjusting for hookworm intensity.<sup>8</sup>

The results of the present study show that occult colonic bleeding is not a high-risk event in *Trichuris*-infected children in the absence of the overt *Trichuris* dysentery syndrome. However, the possibility that it may be a low-risk event can only be excluded by testing even larger numbers of individuals.

Acknowledgments: I thank Roslan Mustapha and Roziyani Hashim for assisting in the field work, as well as the teachers and pupils of Tawang School, Kelantan Malaysia for cooperation.

Financial support: The study was funded by the government of Malaysia.

Authors' address: S. Mahendra Raj, Department of Medicine, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, 16150, Malaysia.

#### REFERENCES

1. Bundy DAP, Cooper ES, 1989. *Trichuris* and trichuriasis in Humans. *Adv Parasitol* 28: 107-173.
2. Warren KS, Bundy DAP, Anderson RM, Davis AR, Henderson DA, Jamison JT, Prescott N, Senft A, 1993. Helminth infection. Jamison DT, Mosley VM, Measham AR, Bobadilla JL, eds. *Disease Control Priorities in Developing Countries*. New York: Oxford University Press, 131-160.
3. Robertson LJ, Crompton DWT, Sanjur D, Neisheim MC 1992. Hemoglobin concentrations and concomitant infections of hookworm and *Trichuris trichiura* in Panamanian primary schoolchildren. *Trans R Soc Trop Med Hyg* 86: 654-656.
4. Ramdath DD, Simeon DT, Wong MS, Grantham-McGregor SM, 1995. Iron status of schoolchildren with varying intensities of *Trichuris trichiura* infection. *Parasitology* 110: 347-351.
5. Mahmood A, 1966. Blood loss caused by helminthic infections. *Trans R Soc Trop Med Hyg* 60: 766-769.
6. Layrisse M, Aparcedo L, Martinez-Torres C, Roche M, 1967. Blood loss due to infection with *Trichuris trichiura*. *Am J Trop Med Hyg* 16: 613-619.
7. Lotero H, Tripathy K, Bolanos O, 1974. Gastrointestinal blood loss in *Trichuris* infection. *Am J Trop Med Hyg* 23: 1203-1204.
8. Stoltzfus RJ, Albonica M, Chwaya HM, Savioli L, Tielsch J, Schulze K, Yip R, 1996. Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. *Am J Trop Med Hyg* 55: 399-404.
9. Garcia LS, Bruckner DA, 1988. *Diagnostic Medical Parasitology*. New York: Elsevier Science Publishing Co., 395-396.
10. Bresalier RS, Kim YS, 1993. Malignant neoplasms of the large intestine. Steisenger NM, Fordtran JS, eds. *Gastrointestinal Disease: Pathophysiology/Diagnosis/Management*. Philadelphia: W. B. Saunders Company, 1449-1493.
11. Roche M, Perez-Gimenez ME, Layrisse M, Di Prisco E, 1957. Study of urinary and fecal excretion of radioactive chromium Cr<sup>51</sup> in man. Its use in the measurement of intestinal blood loss associated with hookworm infection. *J Clin Invest* 36: 1183-1192.
12. Herzog P, Holtermuller KH, Preiss J, Fischer J, Ewe K, Schreiber HJ, Berres M, 1982. Fecal blood loss in patients with colonic polyps: a comparison of measurements with <sup>51</sup>chromium labeled erythrocytes and with the Hemocult test. *Gastroenterology* 83: 957-962.
13. Macrae FA, St. John DJB, 1982. Relationship between patterns of bleeding and Hemocult sensitivity in patients with colorectal cancers or adenomas. *Gastroenterology* 82: 891-898.
14. WHO Expert Committee, 1987. Public Health Significance of Intestinal Parasitic Infections. *Prevention and Control of Intestinal Parasitic Infections*. *World Health Organ Tech Rep Ser* 749: 8-28.
15. Beaver PC, Jung RC, Cupp EW, 1984. Examination of specimens for parasites. *Clinical Parasitology*. Ninth edition. Philadelphia: Lea & Febiger: 733-758.