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Use of Fecal Occult Blood Tests as Epidemiologic Indicators of Morbidity Associated with Intestinal Schistosomiasis during Preventive Chemotherapy in Young Children

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Abstract. There is a need for field-applicable markers to assess morbidity associated with intestinal schistosomiasis, especially in the context of preventive chemotherapy in young children. We investigated whether fecal occult blood (FOB) point-of-care tests could be used to assess intestinal pathology over a 12-month period in a cohort of 382 children (< 5 years of age). We found a strong association between egg-patent schistosomiasis and FOB at baseline (odds ratio [OR] = 3.1, $P < 0.0001$), 6 months (OR = 3.4, $P < 0.0001$), and 12 months (OR = 3.5, $P < 0.0001$), despite repeated chemotherapy. There were tendencies for prevalence of FOB to decrease in children who became egg negative and increase in those who became egg positive. Our results demonstrate overt disease in children less than five years of age. We therefore propose that FOB is useful for assessing dynamics of intestinal morbidity in young children at the community level and monitoring changes in morbidity after mass chemotherapy.

INTRODUCTION

Schistosomiasis is a neglected tropical disease caused by trematode parasites of the genus *Schistosoma*. These parasites infect some 207 million persons worldwide, particularly in sub-Saharan Africa.[1] Globally, it is estimated that schistosomiasis is responsible for 280,000 deaths per year and the loss of 1.53 million disability-adjusted life years.[2,3] However, this latter figure is likely to be an underestimate because schistosomiasis can cause a variety of subtle and hidden morbidities that are difficult to capture within this metric and hard to measure directly in communities to which schistosomiasis is endemic.

In sub-Saharan Africa, the intestinal form of schistosomiasis is largely attributable to *Schistosoma mansoni* and causes a spectrum of pathologic changes caused by detrimental reactions to eggs, produced by female worms, that have become trapped in host tissues.[2] For example, intestinal morbidity includes diarrhea, abdominal pain, intestinal granulomas and fibrosis, polyps and microulcerations, and colonic obstruction in extreme cases.[4–10] Perforation of the intestinal mucosa by *S. mansoni* eggs and colorectal polyps can cause the release of blood into the bowel, resulting in blood in feces and anemia if infections are heavy.[4,5]

Today, mass chemotherapy using the anthelminthic drug praziquantel (PZQ) acts as the cornerstone of all national schistosomiasis control programs. Up until now, the main aim of these programs has been control of morbidity associated with schistosomiasis, rather than infections *per se*.[11] As a consequence, school age children have been particularly targeted for mass chemotherapy because they were believed to have the highest infection intensities and to respond to PZQ with greater reductions in morbidity.[12] However, it is becoming clear that younger children less than six years of age can also be infected and treated safely with PZQ.[13,14] Despite the fact that the focus of national programs is on morbidity reduction, monitoring and evaluation of these programs is largely dependent on measuring infection intensities (egg output) as a proxy for morbidity. Thus, field-applicable morbidity markers are essential for defining the initial disease burden and monitoring the impact of preventive chemotherapy.

Fecal occult blood (FOB), which refers to cryptic blood in feces, has been used for a number of years as a marker of intestinal pathologic changes, particularly in association with colorectal cancer. A number of point-of-care tests have been developed,[15] and as such, they are simple to use and provide a result in minutes. Studies in Brazil, Zimbabwe, and the Philippines using guaiac-based methods for detection of FOB have demonstrated a positive correlation between FOB and intensity of *S. mansoni* or *S. japonicum* infection.[16–18] More recently, we reported a strong association between FOB (as assessed by an antibody-based test costing approximately $1.70 per person) and *S. mansoni* in baseline surveys of a cohort of young children (age = 0.5–6 years) and their mothers. We then proposed that FOB tests could be used for measuring morbidity associated with intestinal schistosomiasis but further study of longitudinal dynamics was needed.[19]

To extend this line of inquiry,[19] we have followed-up children in the cohort for one year, investigating the dynamics of FOB in the face of preventive chemotherapy. We report on a longitudinal analysis of the use of the FOB as an epidemiologic marker of *S. mansoni*-associated bowel morbidity in children 5 months–5 years of age.

METHODS

Study sites and participants. A longitudinal study, the Schistosomiasis in Mothers and Infants Project, was initiated in April 2009 to investigate infection dynamics of intestinal schistosomiasis and malaria in a closed cohort of young children and their mothers living in six lake shore communities in Uganda. Three of the study villages (Bugoigo, Walukuba, and Piida) are located in Buliisa District on Lake Albert, and three (Bugoto, Bukoba, and Lwanika) are located in Mayuge District on Lake Victoria.[19] The cohort was followed-up at 3, 6, and 12 months after baseline and offered treatment. A random sample of children in the cohort was tested for FOB at baseline. These same children were retested for FOB if they came to the 6 month and 12 month follow-ups. Only children for whom FOB results

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and *S. mansoni* egg counts were available for all three time points were included in the present analysis (n = 382). At baseline, the mean age of children in this study was 3.0 years (range = 5 months–5 years) and the male:female ratio was 1.03.

Questionnaires. Caregivers were interviewed in their local language and asked a series of questions on behalf of their children pertaining to health-seeking behavior, current signs and symptoms, history of previous infections and treatment, exposure to risk factors for *Schistosoma* infection, and knowledge of schistosomiasis and soil-transmitted helminths.

Rapid tests and enzyme-linked immunosorbent assays. Fecal occult blood was detected by using the Instalert OneStep Fecal Occult Blood Test Devices (Innovacon, Inc., San Diego, CA) according to the manufacturer's instructions. In brief, a specimen collection stick was stabbed into the fecal samples (first-day fecal samples) at three sites and the small amount of feces collected was homogenized in extraction buffer. Two drops of suspension was then applied to the test cassette. Results were read after five minutes and classified as negative (–), trace, weak positive (+), medium positive (++), and strong positive (+++). At each time point, a single urine sample from each mother and child was tested for schistosomiasis by using commercially available rapid tests, which detect schistosome circulating cathodic antigen (CCA; Rapid Medical Diagnostics, Pretoria, South Africa). Finger prick blood (50–100 μL) was taken from all study participants present at each time point and hemoglobin levels were measured as described.[20] Anemia was defined as a hemoglobin level < 110 g/L and categorized according to severity as reported.[20] The finger prick blood was allowed to clot and then centrifuged to obtain serum, which was tested on site for antibodies against schistosomiasis by using a soluble egg antigen enzyme-linked immunosorbent assay (SEA-ELISA) according to the manufacturer's instructions (Scimedx, Denville, NJ).

Parasitologic diagnosis. Microscopic diagnosis of *S. mansoni* and geohelminths (*Trichuris trichirua*, *Ascaris lumbricoides*, and hookworm) was conducted in the field on two fecal samples collected on consecutive days at baseline. Because of logistic and financial constraints, only one fecal sample was examined for follow-up surveys, as per World Health Organization survey guidelines.[21] Double Kato-Katz thick smears (2 × 41.7 mg) were prepared for each sample and inspected by microscopy at a magnification of 100×.[22] Results were expressed as mean egg count per gram (epg) of feces. The *S. mansoni* and geohelminth infection intensities were classified as recommended by the World Health Organization.[21] To control for potential confounding infections, thick and thin blood films were prepared for all study participants at each time point. The slides were stained and malaria parasites were counted as described.[23]

Treatment. At baseline all study participants were treated with PZQ as described.[19] At the 3 month and 6 month follow-ups, PZQ treatment decisions were based on a positive CCA test or Kato-Katz result. At 12 months, blanket treatment with PZQ was provided as an exit strategy for the cohort children living in Buliisa District. However, cohort children in Mayuge District were treated on the basis of results of CCA tests because one additional survey was planned in this district. At each time point, all study participants were offered albendazole treatment and persons who were malaria positive by rapid diagnostic test were offered treatment with artemether-lumefantrine (20 mg artemether/120 mg lumefantrine) on an outpatient basis.

Statistical analysis. Data were entered by using EpiData™ (EpiData Association, Odense, Denmark) and converted to a spreadsheet by using Microsoft (Redmond, WA) Excel 2004 for Mac (version 11.5.6). Statistical analysis was carried out by using Stata version 9.2 (StatCorp LP, College Station, TX) and R version 2.8.1 (http://cran.r.project.org/bin/windows/base/). Age data were categorized (< 2, 2–4, and > 4 years of age). The geometric mean of Williams (GM_W) and arithmetic mean of positive samples (AM_{POS}) were calculated for *S. mansoni* infection intensities and 95% confidence intervals (CIs) for GM_W were determined by using the exact method.[24] To assess associations between *S. mansoni* infection and caregiver-reported signs/symptoms or FOB, logistic regression was carried out by using the symptom or FOB as a binary outcome variable (FOB negative and trace results were classified as negative and weak-strong positive results classified as positive) and *S. mansoni* infection as a categorical explanatory variable. Potential confounding variables (including lake, village, age, sex, presence of other infections, anemia, behavior, and previous treatment) were introduced into the logistic regression model in stepwise manner, and models were compared by using likelihood ratio tests. Because of small numbers of hookworm-infected persons at the 6 month and 12 month follow-ups, associations with FOB were investigated by using the Fisher exact test.[25]

Diagnostic performance. Sensitivity, specificity, positive predictive value, and negative predictive value of FOB were calculated by using *S. mansoni* infection status (as measured by the presence of eggs in feces) as a gold standard, and 95% CIs were estimated by using the binomial exact method.[26,27] Receiver operating characteristic analysis was performed using the same gold standard and plotting sensitivity against 100 – specificity to determine the area under the curve (AUC).[28] A number of new variables were generated consisting of FOB combined with one or more caregiver-reported signs/symptoms or with anemia. The diagnostic performance of these combined indicators was determined as described above.

Ethical approval and informed consent. The London School of Hygiene and Tropical Medicine (LSHTM 5538.09) and the National Council of Science and Technology, Kampala, Uganda, granted ethical approval for this study. Informed consent documented by signature or thumb print was obtained from each caregiver on behalf of her child or children.

RESULTS

The prevalence of egg-patent *S. mansoni* infection at baseline was 20.7% (GM_W = 37.7 epg, 95% CI = 26.6–53.6 epg, AM_{POS} = 130.2 epg, 95% CI = 76.1–184.2 epg, maximum value = 1,170 epg). At 6 months, the prevalence was 11.5% (GM_W = 69.6 epg, 95% CI = 47.3–102.3 epg, AM_{POS} = 172.4 epg, 95% CI = 49.9–294.9 epg, maximum value = 2,676 epg) and at 12 months it was 17.8% (GM_W = 50.3 epg, 95% CI = 34.6–73.2 epg, AM_{POS} = 193.8 epg, 95% CI = 94.9–292.7 epg, maximum value = 2,556 epg). In contrast, the prevalence of egg-patent hookworm infection at baseline was 15.7% (GM_W = 61.6 epg, 95% CI = 42.3–89.7 epg, AM_{POS} = 167.3 epg, 95% CI = 102.3–232.3 epg, maximum value = 1,080 epg). At 6 months, the prevalence was 2.1% (GM_W = 118.4 epg, 95% CI = 56.2–249.5 epg, AM_{POS} = 156.0 epg, 95% CI = 72.1–239.9 epg, maximum value = 288 epg) and at

TABLE 1

Prevalence levels of parasitic infections and morbidity indicators in 382 young children at baseline, 6 months, 12 months*

Organism, factor, or response	Intensity of infection or morbidity	Baseline (95% CI)	6 months (95% CI)	12 months (95% CI)
Schistosoma mansoni KK	Any	20.7 (16.7–25.1)	11.5 (8.4–15.1)	17.8 (14.1–22.0)
	Light	15.2 (11.7–19.2)	7.1 (4.7–10.1)	12.8 (9.6–16.6)
	Medium	3.7 (2.0–6.1)	3.9 (2.2–6.4)	2.4 (1.1–4.4)
	Heavy	1.8 (0.7–3.7)	0.5 (0.06–1.9)	2.6 (1.3–4.8)
S. mansoni CCA	Any	17.3 (13.6–21.5)	21.3 (17.3–25.8)	16 (12.5–20.2)
S. mansoni ELISA	Any	48.4 (43.3–53.6)	51.6 (46.4–56.7)	35.6 (31.1–41)
Trichuris trichiura	Any	1.3 (0.4–3.0)	0.3 (0.007–1.4)	0.3 (0.007–1.4)
Hookworm	Any	15.7 (12.2–19.8)	2.1 (0.9–4.1)	7.3 (4.9–10.4)
	Light	15.7 (12.2–19.8)	2.1 (0.9–4.1)	7.1 (4.7–10.1)
	Medium/heavy	0	0	0.26 (0.007–1.4)
Malaria	Any	81.6 (77.2–85.4)†	71.1 (66.3–75.6)‡	70.2 (65.1–75.0)§
Anemia	Any	48.7 (43.6–53.8)	57.2 (52.1–62.2)‡	49.7 (44.6–54.9)
	Mild	25.1 (20.9–29.8)	21.0 (17.0–25.4)	21.5 (17.5–25.9)
	Moderate	22.8 (18.7–27.3)	20.5 (16.5–24.9)	26.7 (22.3–31.4)
	Severe	3.4 (1.8–5.7)	1.3 (0.4–3.0)	2.1 (0.9–4.1)
FOB	Any	20.2 (16.2–24.5)	24.6 (20.4–29.2)	14.9 (11.5–18.9)
	+	11.3 (8.3–14.9)	11.8 (8.7–15.4)	7.1 (4.7–10.1)
	++/+++	8.9 (6.2–12.2)	12.8 (9.6–16.6)	7.9 (5.4–11.0)
Abdominal pain	Any	31.8 (27.2–36.8)¶	32.3 (27.5–37.3)#	69.4 (64.4–74.0)**
Diarrhea	Any	36.6 (31.7–41.7)¶	28.2 (23.7–33.1)#	52.6 (52.6–47.3)**
Blood in feces	Any	11.9 (8.8–15.6)¶	6.2 (4.0–9.1)#	11.1 (8.1–14.8)**

*CI = confidence interval determined using the exact method; KK = Kato-Katz; CCA = circulating cathodic antigen; ELISA = enzyme-linked immunosorbent assay; FOB = fecal occult blood; + = weak positive; ++ = medium positive; +++ = strong positive.
†n = 374.
‡n = 381.
§n = 349.
¶n = 377.
#n = 372.
**n = 369.

12 months it was 7.1% (GM_W = 111.6 epg, 95% CI = 65.0–191.6 epg, AM_{POS} = 288.0 epg, 95% CI = 79.3–496.7 epg, maximum value = 2,760 epg). Only sporadic cases of infection with *A. lumbricoides* were detected at any time point, and the prevalence of *T. trichiura* infections was low (< 2% at baseline). The prevalence levels (and 95% CIs) of children infected with malaria at baseline, 6 months, and 12 months are summarized in Table 1.

Caregiver-reported signs and symptoms. At baseline, 31.8% of children were reported to have abdominal pain, 36.6% to have diarrhea and 11.9% to have visual blood in feces. The prevalence levels of these caregiver-reported signs and symptoms at 6 and 12 months are summarized in Table 1. Although after controlling for confounding factors there was evidence for an association between egg-patent *S. mansoni* infection and diarrhea at baseline (odds ratio [OR] = 2.9, 95% CI = 1.5–5.6, $P = 0.001$), this association was not maintained at 6 month and 12 month follow-ups (Table 2). For reported abdominal pain and visual blood in feces, there was no obvious positive association with *S. mansoni* infection at any time point, though there was evidence for an inverse relationship between *S. mansoni* infection status and abdominal pain at 12 months (Table 2).

Dynamics of *Schistosoma mansoni* infection and fecal occult blood. The dynamics of *S. mansoni* infection as measured by different diagnostic and morbidity markers is shown in Figure 1. The prevalence of egg-patent schistosomiasis decreased significantly between baseline and 6 months, but returned to baseline levels at the 12 month follow-up. Although there was no dramatic change in CCA (which infers presence of feeding worms) over the time course of the study, there was a substantial decrease in the prevalence of SEA-ELISA–positive persons between the 6 and 12 month follow-ups. In contrast, there was an increase in FOB prevalence between baseline and 6 months, followed by a significant decrease between 6 months and 12 months (Figure 1A and Table 1). Changes in FOB positivity over time are shown in Figure 1B, demonstrating that although only nine children were FOB positive for the whole year, 45 became positive over the 12 months of the study, 124 had been positive at some point but were negative after 12 months, and 201 were negative for the course of the study. Of the latter, 25 were egg positive for *S. mansoni*, most with light-intensity infections.

Association between fecal occult blood and *S. mansoni* infection. Fecal occult blood was strongly associated with

TABLE 2

Association between questionnaire responses and *Schistosoma mansoni* infection in young children at baseline, 6 months, and 12 months*

	Baseline		6 months		12 months	
Questionnaire response	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Abdominal pain	1.5 (0.8–2.7)	0.17	0.7 (0.3–1.4)	0.32	0.4 (0.2–0.6)	< 0.0001
Diarrhea	2.9 (1.5–5.6)	< 0.001	0.6 (0.3–1.4)	0.26	0.7 (0.4–1.3)	0.24
Blood in feces	1.4 (0.6–3.1)	0.39	1.2 (0.3–4.1)	0.82	1.3 (0.6–2.9)	0.51

*OR = odds ratio; CI = confidence interval.

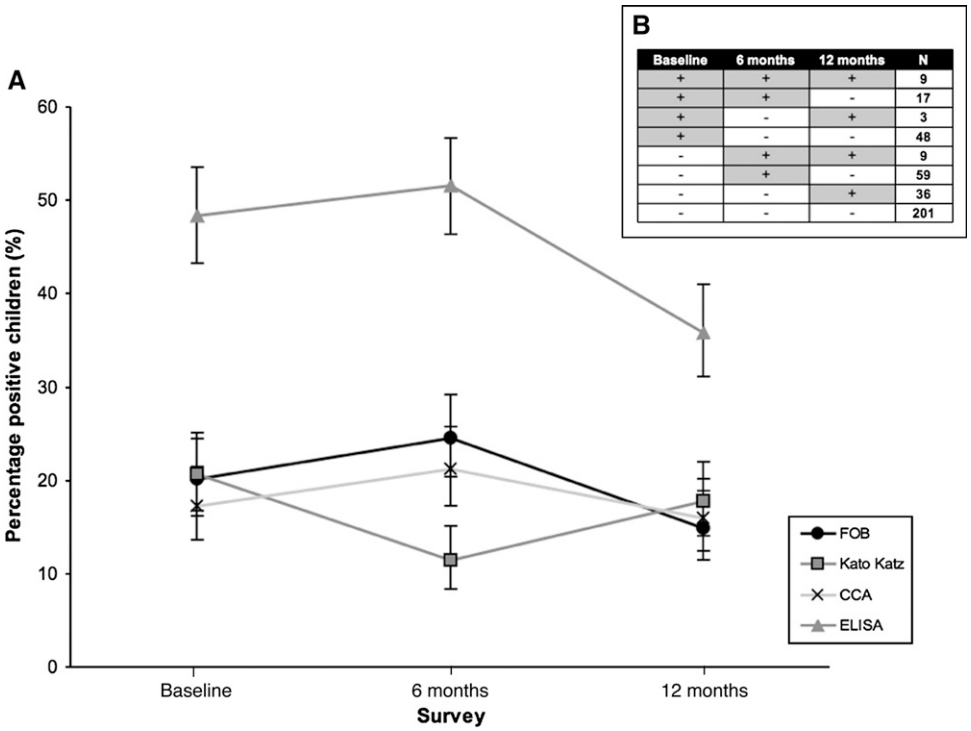

FIGURE 1. **A**, Prevalence levels of fecal occult blood (FOB) and *Schistosoma mansoni* infection as assessed by Kato-Katz, circulating cathodic antigen (CCA), and soluble egg antigen–enzyme-linked immunosorbent assay (ELISA) at baseline, 6 months, and 12 months. Error bars represent 95% confidence intervals. **B**, Dynamics of FOB over the course of the study. + = FOB positive; – = FOB negative; N = no. of children.

S.mansoni infection at baseline (OR = 3.1, 95% CI = 1.8–5.5, $P < 0.0001$), 6 months (OR = 3.4, 95% CI = 1.8–6.5, $P < 0.0001$), and 12 months (OR = 3.5, 95% CI = 1.8–6.9, $P < 0.0001$). At all three time points, medium and heavy *S. mansoni* infections were more strongly associated with FOB than light infections (Table 3). Controlling for previous treatment with PZQ did not have any effect on the association between FOB and intestinal schistosomiasis. In contrast, there was no evidence for an association between FOB and hookworm infection at baseline ($P = 0.49$), 6 months ($P = 0.23$), and 12 months ($P = 0.60$). Anemia was not associated with *S. mansoni* infection at baseline (OR = 0.8, 95% CI = 0.5–1.3, $P = 0.37$), 6 months (OR = 1.0, 95% CI = 0.5–1.9, $P = 0.96$), and 12 months (OR = 0.7, 95% CI = 0.4–1.3, $P = 0.27$), and there was also no association between FOB and anemia at any time point.

The specificity of FOB for predicting egg-patent *S. mansoni* infection was 84.2% (95% CI = 79.6–88.1%) at baseline, 78.4% (95% CI = 73.6–82.7%) at 6 months, and 88.5% (95% CI = 84.5–91.8%) at 12 months. In contrast, the sensitivity was 36.7% (95% CI = 26.1–48.3%) at baseline, 47.7% (95% CI = 32.5–63.3%) at 6 months, and 30.9% (95% CI = 20.2–43.3%) at 12 months. These findings are reflected in the results of receiver operating characteristic analysis, which showed AUC values of 0.60 (95% CI = 0.55–0.66) at baseline, 0.63 (95% CI = 0.55–0.71) at six months, and 0.60 (95% CI = 0.54–0.66) at 12 months (Table 4). Attempts to enhance diagnostic scores by combining FOB with one or more caregiver-reported symptoms or anemia resulted in improvements in specificity but a substantial decrease in sensitivity, and a reduction in AUC score in comparison with FOB alone (Supplemental Table 1).

To determine whether FOB prevalence levels changed with alterations in *S. mansoni* egg patency over time, FOB prevalence levels were stratified on the basis of *S. mansoni* infection status at baseline, 6 months, and 12 months (Table 5). Although the number of persons in most categories was small, there was an overall tendency for FOB prevalence to decrease as children became egg negative and increase as children became egg positive. In children who were egg positive at baseline and egg negative at 6 and 12 months the reduction in FOB prevalence between baseline and 6 months was marginal, but was more substantial 6 months later. In contrast, there was a tendency for FOB prevalence to increase before egg patency in children who

TABLE 3
Association between FOB and *Schistosoma mansoni* infection in young children at baseline, 6 months, and 12 months*

	Baseline		6 months		12 months	
S. mansoni infection status	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Negative	1.0	–	1.0	–	1.0	–
Positive	3.1 (1.8–5.5)	< 0.0001	3.4 (1.8–6.5)	< 0.0001	3.5 (1.8–6.9)	< 0.0001
Light infection	1.5 (0.8–3.1)	0.22	2.1 (0.9–4.8)	0.08	2.5 (1.1–5.3)	0.02
Medium or heavy infection	17.0 (6.0–48.6)	< 0.0001	7.3 (2.6–20.6)	< 0.0001	8.3 (3.1–22.0)	< 0.0001

*OR = odds ratio; CI = confidence interval.

TABLE 4

Diagnostic performance of FOB using egg-patent *Schistosoma mansoni* as a gold standard*

FOB vs. KK and microscopy	Baseline	6 months	12 months
Sensitivity (%)	36.7 (26.1–48.3)	47.7 (32.5–63.3)	30.9 (20.2–43.3)
Specificity (%)	84.2 (79.6–88.1)	78.4 (73.6–82.7)	88.5 (84.5–91.8)
PPV (%)	37.7 (26.9–49.4)	22.3 (14.4–32.1)	36.8 (24.4–50.7)
NPV (%)	83.6 (79.0–87.6)	92.0 (88.3–94.9)	85.5 (81.2–89.2)
AUC	0.60 (0.55–0.66)	0.63 (0.55–0.71)	0.60 (0.54–0.66)

*FOB = fecal occult blood; CI = confidence interval; KK = Kato-Katz; PPV = positive predictive value; NPV = negative predictive value; AUC = area under the curve (from receiver operating characteristic analysis).

were egg negative at baseline and 6 months but egg positive at 12 months.

DISCUSSION

There is a clear need for field applicable morbidity markers, particularly in the context of young children and detection of early stage morbidity.[29] In this study, we investigated whether FOB could be used as a marker to assess bowel morbidity associated with intestinal schistosomiasis over time and after PZQ treatment. We have followed-up a cohort of 382 young children (5 months–5 years of age) in Uganda for one year and have demonstrated a convincing association between FOB and *S. mansoni* infection at baseline, 6 months, and 12 months set within a context of repeated PZQ and albendazole treatment. In addition, we have found that there was a tendency for FOB prevalence to increase from baseline to 12 months in children who become infected with *S. mansoni* and to decrease in children who become negative for egg-patent schistosomiasis. In contrast, there was no consistent association between caregiver-reported signs and symptoms (abdominal pain, diarrhea, and blood in feces) or anemia (measured by capillary hemoglobin concentration) and *S. mansoni* infection over the duration of the study.

The use of questionnaires enquiring about symptoms, such as blood in feces and diarrhea, to determine which communities are at risk for disease has been investigated extensively, and it is has been shown that self-reported blood in feces is the symptom most strongly associated with *Schistosoma* infection.[30] In the present study, we found that only diarrhea, as reported by the caregiver, was associated with *S. mansoni* infection at baseline (i.e., before PZQ treatment). This finding contrasts with those of earlier studies in which blood in feces and diarrhea were found to correlate with intestinal schistosomiasis.[19] The difference is likely because of a smaller sample size in the present study; thus, a weak association might not be detected. Interestingly, there was no consistent association between any signs and symptoms and *S. mansoni* infection over the course of this longitudinal study and treatment. At 12 months, there was an inverse relationship between abdominal pain and *S. mansoni* infection. The latter observation may be caused by other infections, which cause abdominal pain, but which are either less prevalent or cause less abdominal pain in children who are positive for *S. mansoni*. Overall, these results suggest that self-reporting or caregiver-reporting of signs and symptoms are not a robust method for monitoring morbidity after PZQ treatment.

TABLE 5

Prevalence of FOB at baseline, 6 months, and 12 months stratified by *Schistosoma mansoni* infection dynamics*

Egg-patent *S. mansoni*			n/N (%)		
Baseline	6 months	12 months	Baseline	6 month	12 month
–	–	+	3/29 (10.3)	12/29 (41.4)	8/29 (27.6)
–	+	+	1/2 (50.0)	2/2 (100)	0/2 (0)
–	+	–	0/8 (0)	0/8 (0)	2/8 (25.0)
+	–	–	11/29 (37.9)	9/29 (31.0)	4/29 (13.8)
+	+	–	4/13 (30.8)	9/13 (69.2)	3/13 (23.1)
+	–	+	2/16 (12.5)	4/16 (25.0)	4/16 (25.0)
+	+	+	12/21 (57.1)	10/21 (47.6)	9/21 (42.9)
–	–	–	44/264 (16.7)	48/264 (18.2)	27/264 (10.2)

*FOB = fecal occult blood.

Although we and others have demonstrated an association between FOB and prevalence or intensity of *S. mansoni* or *S. japonicum* infection,[16–19] to our knowledge, this is the first longitudinal analysis to demonstrate a continued association between FOB and egg-patent *S. mansoni* infection after PZQ treatment. We found that FOB tests were highly specific for *S. mansoni* infection at all time points, but not sensitive, indicating that a number of persons with intestinal schistosomiasis did not show detectable levels of FOB, likely persons with low infection intensities (Table 3). Thus, FOB tests would not be appropriate for diagnosis of intestinal schistosomiasis at an individual or population level, but do have potential for monitoring changes in intestinal morbidity associated with schistosomiasis at a community level. Consistent with the specificity of FOB for *S. mansoni* infection, there was no association between FOB and hookworm infection. However, it must be remembered that prevalence level and intensity of this helminth infection were low in the study cohort. In areas where hookworm prevalence level and intensity are higher, an association with FOB may be observed and the specificity of FOB for *S. mansoni* infection may be lower.

The potential confounding effects of malaria infections were controlled for in the analysis and other infections including *A. lumbricoides*, *T. trichiura*, *Strongyloides stercoralis*,[31] and *Entameba histolytica* (Betson M, unpublished data) were at low levels in this cohort. However, we cannot rule out the presence of additional confounding infectious agents, for which we did not control. These agents could include bacterial pathogens such as *Shigella* or enterohemorrhagic *Escherichia coli* and protists such as *Giardia*. There are few reports on the population prevalence of such pathogens and their associations with blood in feces in Uganda. In a cross-sectional survey of patients who came to health facilities with bloody diarrhea in Mbarara District, Uganda, *Shigella* was isolated from 35% of fecal samples.[32] In eastern Kenya, prevalence levels of *Shigella*, *Campylobacter*, and *Salmonella* in patients with bloody diarrhea were 44%, 7%, and 3%, respectively.[33] Among children with diarrhea in southwestern Uganda, 8%

were infected with Shiga toxin–producing *E. coli* (STEC).[34] In contrast, no Shiga toxin–producing *E. coli* was found among infants with diarrhea in Kampala.[35] Low prevalence levels of *Giardia lambia* and *E. histolytica* were found among school children in Kampala,[36] whereas in western Uganda, 41% of persons were infected with *Giardia,* but no association was found between *Giardia* infection and gastrointestinal symptoms.[37]

To date, no studies on these pathogens and associations with blood in feces or FOB have been carried out in the areas where our study was conducted. Such bacterial or protist infections may account for fecal occult blood at baseline in 48 children who were negative for *S. mansoni* infection by microscopy. However, 22 (46%) of these children were positive by SEA-ELISA, and 3 (7%) of 45 were positive for CCA, highlighting the diagnostic insensitivity of the Kato-Katz test and suggesting that these children may have eggs trapped in their intestinal mucosa even if they are not detectable in their feces.

Although we were not able to demonstrate a significant reduction in the prevalence of FOB over a 12-month period, this finding was likely caused by the fact that there was also no significant reduction in overall prevalence and intensity of egg-patent *S. mansoni* infection in this cohort over the duration of the study. Intriguingly, however, there was a reduction in the number of SEA-ELISA–positive persons between 6 and 12 months, suggesting a reduction in the accumulation rate of eggs or a dampening of the immune response, perhaps immunotolerance in the younger child.

When FOB results were stratified by *S. mansoni* infection status at baseline, 6 months, and 12 months, we did observe a reduction in FOB prevalence in children who became negative for egg-patent *S. mansoni* (and the reverse in those children who became egg patent). Although the number of persons in each category was small, there was some indication that there may be a lag between becoming egg negative and a reduction in FOB. For example, in children who were *S. mansoni* positive at baseline but then negative at 6 months and 12 months, there was a small reduction in FOB prevalence at 6 months but by 12 months FOB prevalence was at background levels. This finding suggests that it may take up to 12 months for the gut to heal properly, perhaps because of polyps in the intestinal mucosa that take time to resolve or ongoing inflammatory responses to trapped and dying eggs. Interestingly, in children who were egg negative at baseline and 6 months but egg positive at 12 months, there was also some suggestion that they became FOB positive before they were egg patent. At six months, eight (67%) of these FOB-positive children were positive by SEA-ELISA, suggesting that *S. mansoni* eggs were already present in their gut lining. Thus, FOB may be able to detect early stages of bowel pathologic changes even before infections are detectable by microscopy. This finding is consistent with previous work demonstrating that first *S. mansoni* infections in young children are detected earlier by SEA-ELISA than by microscopy.[38]

Of particular note is that our cohort consisted of young children, who likely present an early stage of pathologic changes associated with intestinal schistosomiasis and in whom the bowel is continuing to develop and mature. It is important to note that despite their young age, 21% of children were egg patent at the start of the study and 20% demonstrated bowel morbidity as detected by the FOB test. These data add to the mounting evidence that young children are at risk of *S. mansoni* infection and demonstrate overt schistosomiasis-associated morbidity.[13,14] Fecal occult blood may prove a highly appropriate intestinal morbidity marker for persons such as young children who have acquired their infections recently because they may show greater intestinal bleeding than long-standing infections, perhaps a consequence of the fact that there is a reduction in the immune response to schistosomiasis over time and also variation in the numbers of eggs produced by adult worms against a background of preventive chemotherapy.[39] Interestingly, previous treatment did not have an effect on the association between FOB and *S. mansoni* infection, suggesting that over the time course of this study, treatment with PZQ did not reduce intestinal morbidity on subsequent re-infection.

In conclusion, we have shown in a cohort of young children in Uganda that FOB continues to be strongly associated with the prevalence and intensity of *S. mansoni* infection over a period of one year and after repeated PZQ and albendazole treatment, unlike self-reported symptoms and anemia. Thus, we propose that this rapid test could be useful for control programs to evaluate the extent and dynamics of intestinal morbidity in young children at a community level and to monitor changes in morbidity after mass chemotherapy.

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Note: Supplemental table appears at www.ajtmh.org.

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