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Intestinal amoebiasis, giardiasis and geohelminthiases: their association with other intestinal parasites and reported intestinal symptoms

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Abstract

In order to determine reported signs and symptoms that may predict an intestinal parasitic infection, 241 schoolchildren in western Côte d'Ivoire were interviewed with a simple questionnaire and their stool specimens were examined over several consecutive days. Special emphasis was placed on (i) assessing infections by *Entamoeba histolytica/E. dispar, Giardia duodenalis* and by intestinal worms, (ii) looking for associations between these parasites, and (iii) looking for associations between these parasites and commonly perceived intestinal signs and symptoms. Complete questionnaire results, intestinal helminth infections derived from 4 Kato–Katz thick smears, and intestinal protozoa infections assessed on a single day by a formalin–ether concentration procedure were obtained from 209 children (87%). A logistic regression modelling approach showed that an infection with *E. histolytica/E. dispar* was significantly associated with an *Entamoeba coli* infection. However, for *G. duodenalis*, hookworm and *Ascaris lumbricoides*, no association was found between any of these parasites and other intestinal parasites. In a multivariate analysis reported diarrhoea was the only symptom positively associated with an *E. histolytica/E. dispar* infection ($P = 0{\cdot}028$). Its diagnostic performance showed a low sensitivity (28%), a high specificity (85%) and moderate positive and negative predictive values (52% and 67%, respectively). Surprisingly, reported 'turning stomach' was less often reported by children infected with *G. duodenalis* (borderline significance, $P = 0{\cdot}057$). It is concluded that reported diarrhoea could be a symptom worth exploring further for the rapid identification of schoolchildren infected with *E. histolytica/E. dispar.*

Keywords: diarrhoea, *Ascaris lumbricoides, Entamoeba histolytica, Entamoeba dispar, Giardia duodenalis,* amoebiasis, giardiasis, geohelminthiasis, hookworm, intestinal parasites, disease perception, Côte d'Ivoire

Introduction

Amoebiasis, caused by *Entamoeba histolytica,* is believed to affect about 480 million people worldwide, and leads to about 40 000–110 000 deaths per year (WALSH, 1986; WHO, 1997). It has long been known that the majority of infected individuals are asymptomatic and that only about 10% develop disease (FARTHING *et al.*, 1996). The accumulation of recent biochemical, immunological and genetic data revealed that *E. histolytica* and *E. dispar* are 2 morphologically identical protozoan species (DIAMOND & CLARK, 1993). It is now generally accepted that only *E. histolytica* can cause invasive intestinal and extra-intestinal disease (WHO, 1997).

Giardiasis is a common protozoan infection of the intestinal tract and occurs worldwide. While there has been a long-lasting debate on the pathogenic significance of *Giardia duodenalis* (*Giardia lamblia*) there is now evidence that this parasite can cause both acute and persistent diarrhoea as well as vitamin and nutrient malabsorption, and that it may be responsible for growth and development retardment in children (ADAM, 1991; FARTHING, 1996).

Several recent studies investigated the aetiology of acute and chronic diarrhoea and demonstrated the pathogenic potential of *E. histolytica/E. dispar* (CHATTERJEE *et al.*, 1989; SHETTY *et al.*, 1990) and *G. lamblia* (SHETTY *et al.*, 1990; CHUNGE *et al.*, 1992). In a recent case–control study HENRY *et al.* (1995) found that an infection with *E. histolytica/E. dispar* was statistically associated with chronic diarrhoea. YOUNES *et al.* (1996) studied 60 patients with persistent or recurrent diarrhoea and identified *G. lamblia* as the most prevalent pathogen responsible for diarrhoea in 23·3% of these cases, followed by *E. histolytica* in 21·6% of the cases. The study by CHUNGE *et al.* (1992) also investigated the association between intestinal parasitic infections, unformed stool and reports of diarrhoea; they found significant correlations between the occurrence of both *E. histolytica* and *G. lamblia* and reported diarrhoea. The more severe forms of amoebiasis and giardiasis are rare and will not be considered here.

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Infections with other intestinal parasites may also cause intestinal signs and symptoms. This is especially the case with the geohelminths *Ascaris lumbricoides, Trichuris trichiura* and hookworms (*Necator americanus, Ancylostoma duodenale*) that can cause intestinal symptoms beside having other detrimental effects, such as vitamin malabsorption and contributing to anaemia (BUNDY, 1986; ADEDOYIN *et al.*, 1990; SHERCHAND *et al.*, 1996; TSHIKUKA *et al.*, 1996). On the other hand there is ample evidence that infections due to *Schistosoma mansoni* (classified here as an intestinal parasite because of the excretion of eggs in the faeces) can cause (bloody) diarrhoea, blood in stool, abdominal pain and colicky cramps (reviewed by GRYSEELS, 1992).

We report a study that looked at the reliability and diagnostic performance of simple reported signs and symptoms for the identification of intestinal parasite infections. The marked relationship between reported blood in stool and intestinal schistosomiasis caused by *S. mansoni* is described elsewhere (UTZINGER *et al.*, 1998). In the present paper, emphasis is placed on *E. histolytica/E. dispar, G. duodenalis,* hookworm and *A. lumbricoides.* A multivariate analysis strategy using logistic regression was used to investigate the associations between the different intestinal parasites and to assess the most reliable signs and symptoms that may indicate intestinal protozoa and helminth infections.

Materials and Methods

Study area and population surveyed

The study was carried out near the town of Man in western Côte d'Ivoire between March and June 1997. All schoolchildren attending standard 4–6 from 3 neighbouring rural primary schools were enrolled. The objectives of the study were discussed with the village chiefs and the school directors and, after obtaining their consent, the sex and age of the children were recorded. The day before the first survey, children were issued with a small plastic container and they were asked to return the containers with a small portion of their morning stools. After stool collection, children were issued with a new container for stool collection the next day. This procedure was repeated over 5 consecutive days. Further details of the study area and the children surveyed are provided elsewhere (UTZINGER *et al.*, 1998).

Stool specimen analysis

The stool specimens were brought to the central laboratory in Man, the region's main town. First, they were analysed macroscopically by recording the consistency, placing special emphasis on liquid specimens. Second, a single 42-mg Kato–Katz thick smear was processed from each stool specimen according to KATZ *et al.* (1972). Within 30–150 min the slides were examined quantitatively under light microscopy by 1 of 4 experienced technicians. The total number of eggs of *S. mansoni*, hookworms, *A. lumbricoides* and *T. trichiura* was counted. This procedure with a varying clearing time resulted in some variability in sensitivity for hookworm detection since eggs are destroyed rapidly on the slide. As a quality control measure 10% of the slides were randomly selected and re-examined the following day for the presence of *S. mansoni, A. lumbricoides* and *T. trichiura* eggs.

On day 3, an additional 1–2-g portion of stool was collected and preserved in sodium acetate–acetic acid–formalin (SAF). The specimens were forwarded to a reference laboratory in Switzerland, processed according to MARTI & ESCHER (1990) and examined under light microscopy within 2 months. Helminth eggs that were counted included those of *S. mansoni*, hookworms, *A. lumbricoides* and *T. trichiura*. The presence of *E. histolytica/E. dispar* and *G. duodenalis* was assessed semi-quantitatively by distinguishing between 5 levels: 1+, 1–2 parasites per stool sample analysed; 3+, 1–2 parasites per microscope field; 5+, more than 10 parasites per microscope field. Categories 2+ and 4+ were in between. The presence of intestinal protozoa, such as *Entamoeba hartmanni, Entamoeba coli, Endolimax nana, Iodamoeba bütschlii, Chilomastix mesnili* and *Blastocystis hominis*, was also recorded.

Recent recommendations proposed that 'true' infection prevalence of *S. mansoni* should be expressed by the cumulative infection prevalence derived from readings of several repeated Kato–Katz thick smears from stool specimens collected over consecutive days (DE VLAS & GRYSEELS, 1992; DE VLAS *et al.*, 1992). In the present study, the cumulative results of 4 repeated Kato–Katz readings combined with the single SAF analysis were used as 'gold standard' for infection with *S. mansoni*, hookworm, *A. lumbricoides* and *T. trichiura*.

Reported signs and symptoms and treatment of infected children

In a first phase, 6 focus group discussions (FGDs) with groups of 8 schoolchildren were conducted according to DAWSON *et al.* (1993) to assess the perception of different intestinal signs and symptoms. The FGDs were held as mixed-gender groups and in French (the official school language in Côte d'Ivoire) with brief parts in Yacouba or Dioula (the main local languages). Following these FGDs a list of the 6 most common intestinal signs and symptoms was constituted: diarrhoea, blood in stool, worms in the faeces, 'running stomach' (*ventre qui coule*), 'turning stomach' (*ventre qui tourne*) and 'itching stomach' (*ventre qui gratte*).

In a second phase, the head teachers interviewed all the children with a brief structured questionnaire based on the FGD findings. They asked the children individually whether they had experienced one of the above signs and symptoms during the last month. At the end of the interviews, all *S. mansoni*-infected children were treated with a single oral dose of praziquantel at the recommended standard dose of 40 mg/kg bodyweight (WHO, 1993). Infections with other helminths were treated 6 weeks later, using 2 oral doses of pyrantel (10 mg/kg bodyweight) given within 2 weeks.

Statistics

For data analysis, only those children who had (i) at least 4 samples examined by Kato–Katz, (ii) a SAF examination on day 3, and (iii) completed the questionnaire were considered. The parasitological and interview data were double entered and cross-checked using the EpiInfo software (version 6.04; Centers for Disease Control and Prevention, Atlanta, Georgia, USA).

Univariate statistics using the Kruskal–Wallis test were performed to assess the effect of infection status by *E. histolytica/E. dispar, G. duodenalis*, hookworm and *A. lumbricoides* with regard to age, sex and village. Logistic regression modelling techniques using the LOGISTIC software (DALLAL, 1988) were applied to assess the associations between (i) the different intestinal parasites and (ii) these parasites and the different intestinal signs and symptoms that were considered. For the 4 parasites of interest, baseline models were established, defining infected children as cases and incorporating the variables village, sex, age, all the other intestinal parasites and all reported signs and symptoms. A backward elimination technique was then applied to remove the non-significant associations. The adjusted odds ratio (including 95% confidence intervals), the likelihood ratio and the *P*-values were computed for the associations that remained significant.

Finally, the diagnostic performance of the most promising signs and symptoms was assessed by calculating the sensitivity, specificity and predictive values.

Results

Univariate statistics

At least 4 Kato–Katz thick-smear readings, complete results for the SAF examination on day 3 and responses to all questions were obtained from 209 children (87% of enrolled children). The median age was 12 years with a range between 8 and 16 years. There were statistically significantly more boys (135) than girls (74) (χ^2, 1 degree of freedom (d.f.) = 9·4, $P = 0{\cdot}002$), and this imbalance was more pronounced with increasing age.

The day-to-day variation and the cumulative results of the different helminth infections derived from the 4 repeated Kato–Katz readings have been presented in detail elsewhere (UTZINGER *et al.*, 1998). In brief, *S. mansoni* was the predominant helminth with a cumulative infection prevalence of 92.3%. It was followed by hookworms and *A. lumbricoides* with cumulative prevalences of 60·8% and 38·3%, respectively. Only 4 children were infected by *T. trichiura* (Table 1). The infection prevalences derived from the single SAF-conserved stool specimen were all lower than those resulting from the cumulative Kato–Katz readings. For *S. mansoni* and *A. lumbricoides* the SAF results considerably underestimated the cumulative infection prevalence; for hookworms, however, there was a good agreement. Combining the results of the single SAF examination with those of the 4 repeated Kato–Katz readings revealed the following: the additional SAF reading detected no additional *A. lumbricoides* infection but 1 additional *T. trichiura*, 3 additional *S. mansoni* and 21 additional hookworm infections (thus resulting in a cumulative hookworm infection prevalence of 70·8%; Table 1). These combined results were defined as 'gold standard' of helminth infections and were used in subsequent analysis.

The point prevalences of all intestinal protozoa infections are also presented in Table 1. The predominant species was *Entamoeba coli* with an infection prevalence of 66·5%. Other frequently observed protozoa were *B. hominis* and *E. histolytica/E. dispar* with point prevalences of 39·2% and 37·3%. The prevalence of *G. duodenalis* was 12·0%.

As the main focus of the present paper is on *E. histolytica/E. dispar, G. duodenalis*, hookworm and *A. lumbricoides* more detailed analysis was performed on these intestinal parasites.

Table 1. Overall results of infections with intestinal parasites among 209 schoolchildren in Côte d'Ivoire screened on 4 consecutive days with Kato–Katz thick smears and on 1 day with an additional sodium acetate-acetic acid–formalin (SAF) concentration method

Intestinal parasite (method, examination)	No. of children positive	Prevalence (95% CI)
Helminths (Kato–Katz thick smears, 4 readings)		
Schistosoma mansoni	193	92·3 (87·9–95·6)
Hookworm	127	60·8 (53·8–67·4)
Ascaris lumbricoides	80	38·3 (31·7–45·2)
Trichuris trichiura	4	1·9 (0·5–4·8)
Helminths (SAF, 1 day)		
Schistosoma mansoni	114	54·5 (47·5–61·4)
Hookworm	111	53·1 (46·1–60·0)
Ascaris lumbricoides	13	6·2 (3·4–10·4)
Trichuris trichiura	2	1·0 (0·1–3·4)
Helminths (4 Kato–Katz thick smears & 1 SAF)		
Schistosoma mansoni	196	93·8 (89·6–96·6)
Hookworm	148	70·8 (64·1–76·9)
Ascaris lumbricoides	80	38·3 (31·7–45·2)
Trichuris trichiura	5	2·4 (0·0–2·6)
Protozoa (SAF, 1 day)		
Entamoeba coli	139	66·5 (59·7–72·9)
Blastocystis hominis	82	39·2 (32·6–46·2)
Entamoeba histolytica/E. dispar	78	37·3 (30·7–44·3)
Entamoeba hartmanni	60	28·7 (22·7–35·4)
Endolimax nana	52	24·9 (19·2–31·3)
Iodamoeba bütschlii	39	18·7 (13·6–24·6)
Chilomastix mesnili	28	13·4 (9·1–18·8)
Giardia duodenalis	25	12.0 (7·9–17·1)

Univariate statistics revealed that there was no significant association between an infection with *E. histolytica/E. dispar* and sex (Kruskal–Wallis H, 1 d.f. = 2·81, $P = 0{\cdot}094$), age (H, 8 d.f. = 8·24, $P = 0{\cdot}410$) and village (H, 2 d.f. = 0·78, $P = 0{\cdot}678$). Most *E. histolytica/E. dispar*-infected children showed a level of infection of 2+ (46%) and 3+ (24%).

Infections with *G. duodenalis* were also independent of sex (H, 1 d.f. = 0·91, $P = 0{\cdot}339$), age (H, 8 d.f. = 4·25, $P = 0{\cdot}834$) and village (H, 2 d.f. = 1·20, $P = 0{\cdot}549$). Fifty percent of the children who were infected with *G. duodenalis* had an infection level of 2+.

Hookworm infections were statistically significantly more often found in boys (H, 1 d.f. = 18·09, $P < 0{\cdot}001$) and older children were more likely to be infected with hookworms (H, 8 d.f. = 23·00, $P = 0{\cdot}003$). The association between a hookworm infection and village showed borderline significance (H, 2 d.f. = 5·81, $P = 0{\cdot}055$). Infections with *A. lumbricoides* were independent of sex (H, 1 d.f. = 0·48, $P = 0{\cdot}490$), age (H, 8 d.f. = 4·23, $P = 0{\cdot}836$) and village (H, 2 d.f. = 4·94, $P = 0{\cdot}085$).

Association with other intestinal parasites

Multiple intestinal parasite infections were very common in the study area, as shown in Figure 1. Interestingly, boys had a higher multiplicity of parasites than girls (Fig. 1a) while children aged 13–16 years had a higher multiplicity of parasites than younger children aged 8–12 years (Fig. 1b).

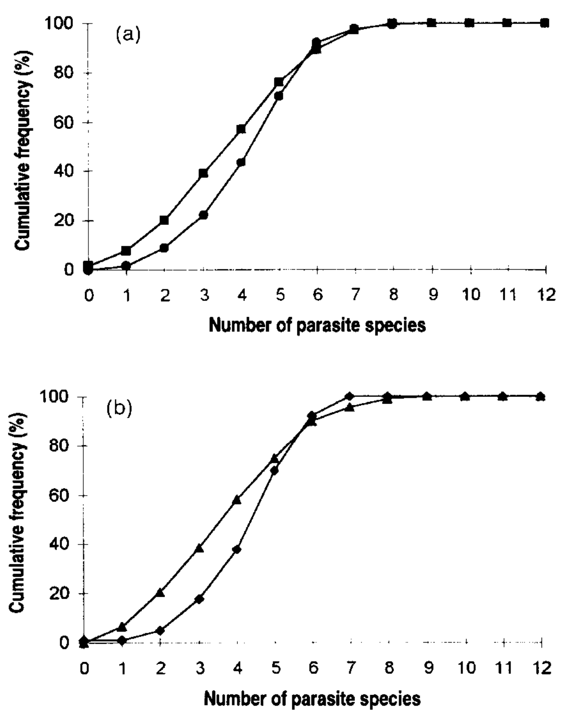

Fig. 1. Cumulative frequency (%) of multiparasitism among boys (●) and girls (■) (Fig. 1a) and stratified in 2 age-groups: 8–12 years (▲), 13–16 years (◆) (Fig. 1b).

Multivariate statistics of relations between the intestinal parasites

The logistic regression analysis for investigating the relationship between an infection with *E. histolytica/E. dispar* and other intestinal parasites is presented in Table 2. It revealed that only infections with *Entamoeba coli* were associated with an *E. histolytica/E. dispar* infection (adjusted odds ratio (OR) = 2·44, 95% confidence interval (CI) 1·28–4·67, $P = 0{\cdot}005$). An infection with *G. duodenalis* showed no significant association with any other intestinal parasite.

Further logistic regression analysis revealed that there was no significant association between a hookworm infection and any other intestinal parasite. The significant associations that were found in the univariate analysis between a hookworm infection and both sex and age also remained in the logistic regression (Table 2). However, the borderline significance between a hookworm infection and village did not appear in the logistic regression analysis (Table 2). An infection with

Table 2. Logistic regression analysis for *Entamoeba histolytica/E. dispar*, *Giardia duodenalis*, hookworm and *Ascaris lumbricoides*, being defined as 'case' to assess significant associations with other parasites and reported signs and symptoms

Parasite	Association	Adjusted odds ratio (95% CI)	Likelihood ratio statistics (1 d.f.)	*P*-value
E. histolytica/E. dispar	*Entamoeba coli*	2·44 (1·28–4·67)	7·78	0·005
	'Diarrhoea'	2·19 (1·09–4·40)	4·81	0·028
Giardia duodenalis	'Turning stomach'	0·42 (0·17–1·06)	3·63	0·057
Hookworm	Sex	3·13 (1·64–6·25)	11·98	<0·001
	Age	1·56 (1·23–1·97)	14·66	<0·001
Ascaris lumbricoides	Village	1·58 (1·05–2·37)	4·93	0·026

A. lumbricoides showed no significant association with any other intestinal parasite. However, the logistic regression analysis revealed a significant association between an *A. lumbricoides* infection and village (adjusted OR = 1·58, 95% CI 1·05–2·37, $P = 0·026$; Table 2).

Stool consistency

The macroscopical stool inspection on day 3 recorded a total of 21 liquid/unformed specimens (10%). The cumulative number of children with at least 1 liquid stool specimen over 4 consecutive days was 52 (25%). There was no significant association between observed liquid stool specimens and an infection with *E. histolytica/E. dispar* and/or *G. duodenalis*. Furthermore, no significant association was found between reported diarrhoea over the previous month and observed liquid stool specimens.

Reported signs and symptoms

Only one significant association between intestinal parasite infections and reported signs and symptoms was found in our analysis. Children infected with *E. histolytica/E. dispar* reported significantly more often having had diarrhoea during the previous month when compared with those not infected (adjusted OR = 2·19, 95% CI 1·09–4·40, $P = 0·028$; Table 2). The diagnostic performance of reported diarrhoea as a predictor for an infection with *E. histolytica/E. dispar* is characterized by a low sensitivity: 28·2% (95% CI 18·9–39·7%), a high specificity: 84·7% (95% CI 77·2–90·2%), a moderate positive predictive value: 52·4% (95% CI 36·6–67·7%) and a moderate negative predictive value: 66·5% (95% CI 58·7–73·5%).

It was interesting to note that children who were infected with *G. duodenalis* reported less often having had a 'turning stomach' during the previous month (adjusted OR = 0·42, 95% CI 0·17–1·06, $P = 0·057$; Table 2). This finding is difficult to interpret and might be due to chance.

Discussion

The high endemicity of intestinal amoebiasis and giardiasis was confirmed in the present study with schoolchildren from western Côte d'Ivoire, who showed point prevalences derived from a single SAF examination of 37·3% and 12·0%, respectively. It is likely that the true infection prevalences of these 2 intestinal protozoa are even higher, as single stool examinations fail to identify all infections, especially the light ones. In a previous study, examinations of multiple stool samples and the use of a mathematical model revealed that the probability of correctly identifying an infection with *E. histolytica/E. dispar* and *G. duodenalis* was 61% and 75%, respectively, for a single sample (MARTI & KOELLA, 1993).

Hookworm was the most prevalent geohelminth. The cumulative infection prevalence after 4 repeated Kato–Katz readings and a single SAF examination was 70·8%. The second most frequently observed geohelminth was *A. lumbricoides*, with a cumulative infection prevalence of 38·3%. The day-to-day variation in the egg output of hookworms and *A. lumbricoides* was considerable, hence a single Kato–Katz reading would have resulted in a substantial underestimation of the 'true' infection prevalences. Recommendations that the infection prevalence of *S. mansoni* should be assessed by repeated Kato–Katz readings (DE VLAS & GRYSEELS, 1992; DE VLAS *et al.*, 1992) seem therefore to be also relevant for geohelminths. Care is needed in assessing the infection prevalence of hookworms for which Kato–Katz thick smears are not ideal because the eggs tend to get overcleared rapidly by the glycerol in the stain. It is likely that the rather long and varying clearing time in our study (30–150 min) has led to some variation in the sensitivity of hookworm detection. This is the likely explanation for the fact that the single SAF examination was able to identify an additional 10% hookworm infections, while this was not the case for the other geohelminths.

Diagnosis of protozoa was made by light microscopy which has the disadvantage that it fails to distinguish between the cysts of *E. histolytica* and *E. dispar* (DIAMOND & CLARK, 1993) and such infections must therefore be reported as *E. histolytica/E. dispar*. New and more sophisticated techniques—such as the polymerase chain reaction technique, isoenzyme analysis and antigen detection—are necessary for specific identification (GONZÀLEZ-RUIZ & WRIGHT, 1998). Unfortunately, such diagnostic techniques are not yet available for routine use in developing countries and appropriate methods are urgently needed (WHO, 1997). Recent work by RIVERA *et al.* (1998) in the Philippines suggests that a PCR approach might be suitable for this purpose.

This limitation represents a serious issue in our study since *E. histolytica* is thought to be responsible for nearly all the reported morbidity (CLARK, 1998) and since *E. dispar* is the more frequent infection with for example a ratio of 10:1 in South Africa (GATHIRAM & JACKSON, 1985). Consequently our assessment of the diagnostic performance of reported signs and symptoms is based on a substantial mis-classification on the infection side and this might explain the low sensitivity of reported diarrhoea in our findings.

Despite this limitation, reported diarrhoea was found to be significantly associated with *E. histolytica/E. dispar* infections. This confirms previous reports by other epidemiological studies that *E. histolytica/E. dispar* was associated with acute or chronic episodes of diarrhoea (CHATTERJEE *et al.*, 1989; SHETTY *et al.*, 1990; YOUNES, 1996). A further case–control study revealed that an infection with *E. histolytica/E. dispar* is statistically significantly more often reported in patients with diarrhoea than in controls (HENRY *et al.*, 1995).

Our diagnostic work was preceded by a careful qualitative analysis aimed at identifying the most commonly perceived intestinal signs and symptoms in the study children. Although we might have failed to identify some perceived signs or symptoms, especially the ones that do not have equivalents in the biomedical understanding of disease, it is likely that the list that we produced was a good basis for this work. As a result, it is rather unlikely that we missed a sign or symptom that might be equally good as diarrhoea for predicting infections with *E. histolytica/E. dispar*.

These observations raise some hope that perceived/reported diarrhoea may be of use in the rapid identification for *E. histolytica/E. dispar* infections, at least for the population studied. A potential confounding effect can be expected in areas with high levels of *S. mansoni* infections, since infected individuals report diarrhoea more often compared with non-infected individuals (GRYSEELS, 1992). In our own work in the same area we found an odds ratio of 1·7 (95% CI 0·8–3·6) for this association (UTZINGER *et al.*, 1998).

In summary, reported diarrhoea was the only useful symptom for the identification of *E. histolytica/E. dispar* infections in our population surveyed, while no useful sign or symptom was found for the other intestinal parasites. The specificity of this symptom was relatively high (85%) but its sensitivity was low (28%) which led to only moderate predictive values. The low sensitivity was probably a consequence of our inability to distinguish between pathogenic *E. histolytica* and non-pathogenic *E. dispar* infections. It is therefore recommended that the relationship between reported diarrhoea and specific *E. histolytica* infections should be further investigated with adequate diagnostic techniques in several places. Such investigations may conclude that reported diarrhoea is a reliable symptom for the rapid and low-cost identification of communities at a high risk of the pathogenic *E. histolytica* infections.

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