Cryptosporidiosis in patients with diarrhea and chronic liver diseases

Nasser Mousa¹, Ahmed Abdel-Razik¹, Hala El-Nahas², Atef El-Shazly², Mohammad Abdelaziz³, Marwa Nabih³, Magdy Hamed⁴, Mohammad Eissa⁴, Narmin Efffal⁵, Walled Eldars⁶

¹ Tropical Medicine Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
² Parasitology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
³ Clinical Oncology and Nuclear Medicine Department, Mansoura University, Mansoura, Egypt
⁴ Internal Medicine Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
⁵ Clinical Pathology Department, Mansoura University, Mansoura, Egypt
⁶ Medical Microbiology and Immunology Department, Mansoura University, Mansoura, Egypt

Abstract

Introduction: The aim of this study was to evaluate the epidemiology and clinical significance of Cryptosporidium in patients with diarrhea and chronic liver diseases.

Methodology: The study included 150 patients with chronic liver diseases and diarrhea, and 50 subjects with diarrhea as a control group. Stool samples were screened for the presence of Cryptosporidium by microscopic examination after modified Ziehl-Neelsen staining and detection of Cryptosporidium coproantigen by enzyme-linked immunosorbent assay (ELISA).

Results: The prevalence of Cryptosporidium infection in patients with chronic liver diseases was 30% (45/150) versus 14% (7/50) in controls. Cryptosporidium infection increased with the progression of chronic liver diseases from Child-Pugh class A to Child-Pugh class C (p< 0.001) and from model for end-stage liver disease (MELD) score ≤ 9 to MELD score > 9 (p< 0.031). Nine patients in Child-Pugh class C with diarrhea associated with Cryptosporidium infection developed hepatic encephalopathy, and only diarrhea was identified as a precipitating factor for hepatic encephalopathy.

Conclusions: Cryptosporidium is one of the important causes of diarrhea in patients with chronic liver diseases. The infection significantly increased with the progression of chronic liver diseases. In patients with advanced chronic liver diseases, Cryptosporidium infection may be a precipitating factor of hepatic encephalopathy.

Key words: Cryptosporidium; liver diseases; diarrhea, hepatic encephalopathy.


(Received 22 April 2014 – Accepted 19 August 2014)

Copyright © 2014 Mousa et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

The intestinal coccidian parasite Cryptosporidium has emerged as a significant human pathogen worldwide [1]. Globally, Cryptosporidium infection continues to be a significant health problem recognized to be an important cause of diarrhea in both immunocompromised and immunocompetent people [2,3]. It is an obligate intracellular extracytoplasmic protozoan parasite that is a major cause of diarrheal illness worldwide [4]. In developing countries, Cryptosporidium infection occurs mostly in children younger than five years of age and in AIDS patients [5].

C. hominis and C. parvum are responsible for the majority of human infections. C. hominis is found almost exclusively in humans, whereas C. parvum is found also in domestic livestock and wild animals. C. parvum, the most common cause of cryptosporidiosis in humans, can be divided into two major genotypes. These two genotypes, human and bovine, are also referred to as genotype 1 and genotype 2, respectively [6,7]. Although Cryptosporidium organisms are known to be zoonotic pathogens, human-to-human transmission seems to be the most common way of transmission, through the fecal-oral route, by the ingestion of Cryptosporidium oocysts through the consumption of food, drink, or recreational water contaminated with fecal matter [8,9].

Cell-mediated immunity appears to be the major component of the immune response to Cryptosporidium infection [10]. Patients with chronic liver failure, who have an impaired immune status, are often highly susceptible to infection [11]. Laboratory diagnosis of cryptosporidiosis is usually achieved by microscopic detection of Cryptosporidium oocysts in
stool specimens and staining techniques including acid-fast stains and immunofluorescence [12]. Little is known about the *Cryptosporidium* infection in chronic liver diseases and hepatocellular carcinoma, so this study aimed to elucidate the prevalence and clinical significance of *Cryptosporidium* infection in patients with diarrhea and chronic liver diseases.

**Methodology**

**Patients**

This study was carried out on 150 patients (71 males and 79 females) with diarrhea and chronic liver disease between 3 September 2012 and 12 October 2013. Fifty subjects (32 males and 18 females) with diarrhea, free of any known liver diseases, matched for ethnicity, sex, and age, were recruited as a control group. The diagnosis of hepatitis C virus (HCV) was made by positive anti-HCV and reverse transcription polymerase chain reaction (PCR-RT), and the diagnosis of hepatitis B virus (HBV) was made by positive hepatitis B surface antigen (HBsAg) and PCR. Liver cirrhosis was diagnosed by history, clinical examination, abnormal liver function tests, abdominal ultrasound, and liver biopsy. Ascites was diagnosed by clinical examination, abdominal ultrasound, and abdominal computerized tomography (CT). Hepatocellular carcinoma (HCC) was diagnosed by abdominal ultrasound, triphasic CT, and elevated serum level of alpha fetoprotein.

All known causes of liver diseases were excluded based on analytical, clinical, and epidemiological data; control subjects were negative for both serum anti-HCV and HBsAg. Autoimmunity, metabolic, genetic disorders, nonalcoholic steatohepatitis (NASH), alcohol intake, clinical or biochemical history of acute hepatitis, and drug toxicity were excluded, and all cases were negative for anti-HIV antibodies. In brief, participants were recruited from three outpatient clinics and two centers in Mansoura University Hospital (Tropical Medicine Department, Clinical oncology and Nuclear medicine Department, and Internal Medicine Department). The study conformed to the medical ethics of the 1975 Declaration of Helsinki, and all participants provided written informed consent. Diarrhea was defined as three or more unformed stools in a 24-hour period. All hepatic patients using lactulose were excluded, to avoid the laxative effect. Also, all patients with immunosuppression (e.g., diabetes mellitus and HIV infection) were excluded.

Initially, the study populations were classified into four groups. Group A comprised 50 patients with liver cirrhosis without ascites, 46 patients with HCV-related infections, and 4 patients HBV-related infections. Group B comprised 50 patients with liver cirrhosis and ascites, 48 patients with HCV-related infections, and 2 patients with HBV-related infections. Group C comprised 50 patients with hepatocellular carcinoma (HCC), 44 patients with HCV-related infections, 2 patients with HBV-related infections, and 4 patients with combined HCV and HBV infections. None of the patients with HCC were receiving treatment (e.g., sorafenib and/or transarterial chemoembolization [TACE]). The diagnosis of HCC was made according to the Barcelona-2000 conference on the clinical management of hepatocellular carcinoma [13]. Group D comprised 50 individuals free of any liver diseases and complaining of diarrhea with matching age and sex as a control group.

All patients and controls included in the study were subjected to thorough clinical history, with the following points noted: age and sex, loss of weight, jaundice, vomiting, diarrhea and its characteristics, abdominal pain, right hypochondrial pain, dyspnea, cough and expectoration, discoloration of urine, history of drug intake of corticosteroids and cytotoxic drugs, and symptoms related to other body system affections. Full family and medical history of previous episodes of diarrhea, potential exposure to food poisoning, and general and abdominal examination included imaging using an ultrasound scan and triphasic CT were done.

**Assessment of the severity of the liver diseases**

Since patients with HCC can present with or without ascites, showing an overlap with groups A and B, the patients were further classified according to the modified Child-Pugh and model for end-stage liver disease (MELD) score to access the correlation between *Cryptosporidium* infection and the severity of liver diseases. The Child-Pugh score consists of five clinical features and is used to assess the prognosis of chronic liver diseases based on the degree of ascites, the plasma concentrations of bilirubin and albumin, the prothrombin time, and the degree of encephalopathy. The Child-Pugh score corresponds to the total points for each item. According to the sum of these points, patients can be categorized into Child-Pugh grades. A total score of 5 to 6 is considered grade A (well-compensated disease); 7 to 9 is grade B (significant functional compromise); and 10 to 15 is grade C (decompensated disease) [14]. The MELD score was calculated using the original formula without including the cause of liver disease [15]:
MELD score = 0.957 × loge (creatinine mg/dL) + 0.378 × loge (bilirubin mg/dL) + 1.120 × loge (international normalized ratio [INR]) + 6.43. The MELD score calculator from the UNOS website (www.unos.org) was used.

**Laboratory investigations and serological tests**

Fasting (12 hours) serum and plasma samples were obtained to test serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), α-fetoprotein (Liaison AFP kit, DiaSorin, Vercelli, Italy). HBV, HCV, and HIV were screened using an enzyme-linked immunosorbent assay (ELISA) technique (INNOTEST HCV Ab IV; Fujirebio Europe N.V, Ghent, Belgium) 100% sensitivity and 100% specificity, respectively (National Serologic Reference Laboratory, Australia; http://www.nrl.gov.au). The laboratory investigations were done to exclude other diseases such as diabetes mellitus and other malignant diseases such as leukemia by using standard clinical chemistry analyzers. Stools were examined by direct smear for parasitic diseases.

**Collection of fecal samples**

Three stool samples from each patient in the study were collected to increase sensitivity. Each individual in the study was supplied with two containers, one for direct examination by concentration method [16] and for staining with modified Ziehl-Neelsen stain [17]. The second container was preserved for detection of *Cryptosporidium* coproantigen by ELISA. Fecal samples were collected in dry, clean, labeled plastic containers with tight-fitting covers containing no preservatives; stool contamination was avoided. Macroscopic examination was first conducted focusing on several aspects: consistency, presence of blood and mucus, and macroscopic parasitic elements. Microscopic examination was done using a direct wet smear technique to test for the presence of oocysts of parasitic protozoa [18].

The *Cryptosporidium* antigen was detected in the stool by ELISA. The method was performed according to the instructions supplied with the commercial ELISA kit (Ridascreen Cryptosporidium, C1201, R-Biopharm AG, Germany).

**Molecular diagnosis of the species**

DNA extraction was performed using the QIAamp DNAstool kit (QIAGEN, Hilden, Germany) following the manufacturer’s instructions. Species identification was carried out by nested amplification of the *Cryptosporidium* oocyst wallprotein (COWP) gene followed by RsaI restriction enzymedigestion [19]. The digestion pattern of the amplicon fragment (543–554 bp) for *Cryptosporidium parvum* is 34, 106 and 413 bp fragments, and for *Cryptosporidium hominis* is 34, 106, 130 and 284 bp fragments.

**Statistical analysis**

A computer program, SPSS for Windows version 11.5.0, was used for data analysis. The descriptive data were given in mean (± SD). The Chi-square test, Student’s t-test, and Fischer’s bicaudal exact test were used for the analytical assessment. The differences were considered to be statistically significant when the p value obtained was less than 0.05.

**Results**

**Clinical and epidemiological characteristics of Cryptosporidium infection in study populations**

There was no statistically significant difference between both groups in terms of age and sex. Statistical analysis of the epidemiological data revealed that tap water was the only source of drinking water in the chronic liver diseases and control groups, with no significant difference. Also, no significant differences were found between both groups in terms of direct contact with pets (p = 0.72) and direct contact with sheep/cows (p = 0.73). Vomiting was more significant in the chronic liver diseases group than in the control group (p < 0.05), while there was no difference in terms of fever (p = 0.48). The number of bowel movements per day varied from three to ten. The duration of diarrhea ranged from three to six days, and all patients with *Cryptosporidium* infection showed no intractable diarrhea in both groups (Table 1). Nine patients with diarrhea associated with *Cryptosporidium* infection developed hepatic encephalopathy; all were class C Child-Pugh score. Four patients with liver cirrhosis with ascites developed hepatic encephalopathy (grade 1-2), five patients with HCC developed hepatic encephalopathy (grade 1-2), while no patients with liver cirrhosis without ascites developed hepatic encephalopathy. Only diarrhea was identified as a risk factor for the development of hepatic encephalopathy in patients after the exclusion of other precipitating factors (e.g., gastrointestinal bleed, hyponatremia, and hypokalemia). Nitazoxanide is the only broad-spectrum antiparasitic drug that has been approved in the United States for treatment of cryptosporidiosis [20].
Table 1. Clinical and epidemiologic characteristics of *Cryptosporidium* infection in patients with chronic liver diseases and controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Chronic liver diseases (n = 150)</th>
<th>Controls (n = 50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)(mean ± SD)</td>
<td>56.5 ± 7.4</td>
<td>56.5 ± 5.7</td>
<td>0.49</td>
</tr>
<tr>
<td>Sex (male : female)</td>
<td>71 : 79</td>
<td>32 : 18</td>
<td>0.50</td>
</tr>
<tr>
<td>Clinical features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Associated fever</td>
<td>19 (12.6%)</td>
<td>5 (10%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Associated vomiting</td>
<td>16 (10.56%)</td>
<td>3 (6.0%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Water source (tap water)</td>
<td>150 (100%)</td>
<td>50 (100%)</td>
<td>NS</td>
</tr>
<tr>
<td>Direct contact with pets</td>
<td>54 (36%)</td>
<td>16 (32%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Direct contact with sheep/cows</td>
<td>68 (45%)</td>
<td>15 (36%)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Table 2. *Cryptosporidium* infection in patient groups and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>Positive cases</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Liver cirrhosis without ascites (n = 50)</td>
<td>11 22</td>
<td>A vs B = 0.19</td>
</tr>
<tr>
<td>B: Liver cirrhosis with ascites(n = 50)</td>
<td>18 36</td>
<td>A vs E = 0.43</td>
</tr>
<tr>
<td>C: Hepatocellular carcinoma (n = 50)</td>
<td>16 32</td>
<td>B vs C = 0.8</td>
</tr>
<tr>
<td>D: Total chronic liver diseases (n = 150)</td>
<td>45 30</td>
<td>B vs E = 0.02</td>
</tr>
<tr>
<td>E: Controls (n = 50)</td>
<td>7 14</td>
<td>D vs E = 0.04</td>
</tr>
</tbody>
</table>

Table 3. *Cryptosporidium* infection

<table>
<thead>
<tr>
<th>Child-Pugh class</th>
<th>Number of +ve cases</th>
<th>% of +ve cases</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 29)</td>
<td>4</td>
<td>10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>B (n = 38)</td>
<td>6</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>C (n = 72)</td>
<td>35</td>
<td>48.6</td>
<td></td>
</tr>
<tr>
<td>MELD Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MELD ≤ 9 (n = 72)</td>
<td>16</td>
<td>22.22</td>
<td>0.031</td>
</tr>
<tr>
<td>MELD &gt; 9 (n = 78)</td>
<td>30</td>
<td>38.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Comparison between results of examination of acid-fast stained smears and antigen detection of *Cryptosporidium*

<table>
<thead>
<tr>
<th>AFS detection in 200 subjects</th>
<th>Total</th>
<th>Ag detection in 200 subjects</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>–ve</td>
<td>+ve</td>
<td>–ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Number</td>
<td>148</td>
<td>52</td>
<td>200</td>
</tr>
<tr>
<td>%</td>
<td>74%</td>
<td>26%</td>
<td>100%</td>
</tr>
<tr>
<td>AFS –ve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFS +ve</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AFS: acid-fast stained; Ag: antigen

Table 5. Biochemical characteristics in the study populations

<table>
<thead>
<tr>
<th>Positive cases of <em>Cryptosporidium</em></th>
<th>Negative cases of <em>Cryptosporidium</em></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (iu/L)</td>
<td>41 ± 37.5</td>
<td>49 ± 53.4</td>
</tr>
<tr>
<td>AST (iu/L)</td>
<td>38 ± 26.98</td>
<td>51 ± 22.96</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.1 ± 0.5</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>1.90 ± 0.97</td>
<td>1.30 ± 0.92</td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>8.64 ± 3.18</td>
<td>4.56 ± .81</td>
</tr>
<tr>
<td>INR</td>
<td>1.7 ± 0.37</td>
<td>1.4 ± 0.33</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.4 ± 0.5</td>
<td>0.9 ± 0.9</td>
</tr>
</tbody>
</table>

ALT: alanine aminotransferase; AST: aspartate aminotransferase; AFP: alpha-fetoprotein

1587
In this study, in addition to fluid and electrolyte replacement, nitazoxanide 500 mg twice daily for three days was used for treatment of all positive cases of cryptosporidiosis. All patients recovered from hepatic encephalopathy. In this study, other parasitic causes of diarrhea in patients with chronic liver diseases were found, such as Entamoeba histolytica in 3.9% (6/150), Giardia lamblia in 5.3% (8/150), Strongyloides stercoralis in 1.33% (2/150), and mixed infection by Cryptosporidium and Giardia lamblia in 6.6% (10/150). In the control group, Entamoeba histolytica was found in 6% (3/50), Giardia lamblia in 4% (2/50), Strongyloides stercoralis in 2% (1/50), and mixed infection by Cryptosporidium and Giardia lamblia in 6% (3/50). However, all parasitic causes of diarrhea other than Cryptosporidium were excluded, as the focus was on only Cryptosporidium in this study.

Cryptosporidium infection in patients groups and controls

The prevalence of Cryptosporidium infection in patients with liver cirrhosis without ascites was 22% (11/50), in patients with liver cirrhosis with ascites 36% (18/50), while the prevalence in patients with hepatocellular carcinoma was 32% (16/50) and in controls with diarrhea was 14% (7/50). The prevalence of Cryptosporidium infection was significant in liver cirrhosis with ascites versus the control group (p = 0.02). The results showed that the prevalence of positive Cryptosporidium infection in patients with chronic liver diseases was significantly higher than that in controls (p = 0.04) (Table 2). The prevalence of Cryptosporidium infection by species was 65.0% for C. parvum and 35% for C. hominis, including five mixed infections.

Cryptosporidium infection according to the severity of the liver diseases

When the patients with chronic liver diseases were classified based on their Child-Pugh score, the prevalence of Cryptosporidium infection increased progression of chronic liver diseases as demonstrated by a significant increase of the rate of infection from Child-Pugh class A to Child-Pugh class C (p < 0.001) (Table 3A) and according to MELD score, from a MELD score ≤ 9 to a MELD score > 9 (p < 0.031) (Table 3B).

Comparison between the results of examination of acid-fast stained smears and antigen detection for Cryptosporidium

Using Cryptosporidium coproantigen detection, Cryptosporidium was detected in 53 (26.5%) of the 200 samples included in this study. Using an acid-fast stain, Cryptosporidium was detected in 52 (26%) of the 200 samples included in this study. Comparison between the results of antigen detection and examination of acid-fast stained smears for Cryptosporidium revealed that one sample was positive by antigen detection and negative by microscopic examination of acid-fast smears (Table 4).

Biochemical characteristics of the study populations

No significant differences were found in serum liver enzymes between patients with and without Cryptosporidium infection. Patients with Cryptosporidium infection showed a significant decrease in serum albumin and a significant increase in serum bilirubin, INR, creatinine, and alpha-fetoprotein versus patients without Cryptosporidium (p < 0.05, p < 0.001, p < 0.05, and p < 0.001, respectively) (Table 5).

Discussion

Infection by Cryptosporidium has been reported across the globe and identified in patients from 3 days to 95 years of age [21]. Shrestha et al. (1993) found that oocysts of Cryptosporidium were detected in the stools of 20% of patients with liver disease [22]. In Egypt, Cryptosporidium parvum prevalence was found to range from 0%–47%. The risk factors for this protozoa, including population, ecology, and environmental findings, suggest that zoonotic transmission and water transmission modalities are the principal routes of infection [23]. Recently Badawy et al. (2012) reported a 31.1% prevalence of Cryptosporidiumparvum among Egyptian military recruits [24]. Previous studies demonstrated that severe liver injury and liver failure are closely associated with reduced cellular immunity [25]. Reduced cellular immune function could contribute to an increased susceptibility to infection in subjects with chronic liver diseases, including infection with Cryptosporidium. In this study, we found that 32% of patients with hepatocellular carcinoma and diarrhea harbored Cryptosporidium, compared to 22% in patients with liver cirrhosis without ascites and 36% in patients with liver cirrhosis with ascites.
In AIDS patients, Cryptosporidium infection can promote disease progression and worsen the prognosis of the disease [26]. Unlike the negative effect Cryptosporidium infection has on AIDS patients, our results demonstrated that Cryptosporidium infection does not appear to be problematic in patients with chronic liver diseases, as five patients (10%) with HCC and four patients (8%) with liver cirrhosis with ascites developed hepatic encephalopathy (grade 1-2), and all patients recovered from hepatic encephalopathy. This result in agreement with that of Hunter and Nichols (2002), who found that in cancer patients (other than those with hematological malignancies), cryptosporidiosis did not appear to be as problematic as it was in hematological malignancies [27]. In this study, Cryptosporidium infection increased with progression of chronic liver diseases from Child-Pugh class A to Child-Pugh class C. This result may be explained by the impaired cellular immune response in patients with chronic liver disease [25]. Several studies investigated the epidemiology and prognosis of Cryptosporidium infection in patients with malignant disease. Tanyukel et al. (1995) reported that Cryptosporidium oocysts were detected in 18 (17.0%) of 106 patients with diarrhea and various cancers [28]. On the other hand, a low prevalence of Cryptosporidium was found in an Indian study that involved in 560 patients with cancer and diarrhea; oocysts were found in only seven patients (1.3%). Of these seven patients, five had hematological cancers [29]. In our study, a high prevalence of Cryptosporidium oocysts was found in our malignant patients with hepatocellular carcinoma (32%). The high prevalence found in our study compared to the prevalence found in other studies could be explained by differences in subject selection, as we selected only malignant patients with hepatocellular carcinoma rather than those with hematological malignancy. In addition, the high prevalence of Cryptosporidium infection in our patients suggests that these protozoa are widespread in our environment. This high prevalence of infections with these opportunistic parasites among patients with cancer may result from the patients’ reduced immunity [30].

C. hominis and C. parvum are the most common species in humans [31]. Our study showed a predominance C. parvum over C. hominis. This result was in agreement with the results of another study in Egypt, where the predominant species were C. parvum over C. hominis [32], but contradictory to the results of another study in Egypt that showed an elevated prevalence of C. hominis over C. parvum [33]. Regarding the method of detection of Cryptosporidium infection, in the present work, both Ziehl-Neelsen stain and coproantigen detection methods were used. The number of positive cases was 53 (26.5%) by the antigen detection test and 52 (26%) by the Ziehl-Neelsen stain method. In accordance with these results, Bialek et al. (2002) found that the sensitivity of antigen enzyme immunoassay (EIA) and direct fluorescent antibody (DFA) were similar (94% and 95%, respectively) [34].

Conclusions

Cryptosporidium is an important cause of diarrhea in patients with chronic liver diseases. The infection significantly increases with progression of chronic liver diseases. In patients with advanced chronic liver diseases, Cryptosporidium infection may be a precipitating factor for hepatic encephalopathy. The clinical significance of this parasitic infection in patients with diarrhea and chronic liver diseases requires further study.

Acknowledgements

We thank the hospital staff for their assistance with the collection of stool samples.

References


**Corresponding author**

Nasser Mousa MD  
Tropical Medicine, Mansoura University  
Postal code: 35516, Mansoura, Egypt  
Phone: + 0201227029213  
Email: mousa_medic@yahoo.com

**Conflict of interests:** No conflict of interests is declared.