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Prelevance of parasitic etiology in patients with hemorrhagic rectocolitis. Implication of *Entamoeba histolytica* infection

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ABSTRACT

Entamoeba histolytica (Schaudinn, 1903), a protozoan with worldwide distribution, estimated to infect about 500 million people worldwide and registering higher rates in tropical countries, is also present in Romania. It has been difficult to distinguish, until recently, from the morphologically identical, but no pathological *Entamoeba dispar*. A number of 148 persons diagnosed with hemorrhagic rectocolitis were hospitalized at Fundeni Clinical Institute in the Gastroenterology Department between May 2007 and September 2008. *Giardia lamblia*, *Entamoeba coli*, *Blastocystis hominis*, *E. histolytica* protozoan species, and *Clostridium difficile* as unique etiologies, as well as the association of *Giardia lamblia* and *Clostridium difficile*, respectively *Entamoeba coli* and *Clostridium difficile* were detected in 139 patients. All patients were tested by ELISA (IgM detection) for the presence of *E. histolytica*, 3% of them being positive. Two of the positive patients belonged to high risk groups (male homosexuals and traveling in warmer areas endemic for *E. histolytica*), while one was infected locally. Taking into consideration the global warming registered also in Romania, especially during the winter time, it could be plausible that over-wintering mortality of *E. histolytica* cysts may decrease, hence leading to an increase in the future incidence of this infection in our country.

Keywords: *invasive amoebiasis, Entamoeba histolytica, Romania*

1. INTRODUCTION

It is estimated that *Entamoeba histolytica* (*Entamoeba dysenteriae*), a worldwide protozoan, causing intestinal invasion (amoebic dysentery or amoebic colitis), and extraintestinal infection (liver, lung and other organ abscesses) affects about 500 million people worldwide with 34 to 50 million symptomatic cases reported annually. *E. histolytica* registers higher infection rates in tropical and subtropical countries [1, 2, 3, 4, 5, 6, 7, 8, 9] and following malaria, the amoebiasis produced by *E. histolytica* is the second leading cause of death due to parasitic disease (it is estimated by the World Health Organization that about 70,000 people die annually worldwide, mostly from extra-intestinal amoebiasis, especially liver abscesses).

E. histolytica (*sensu lato* in present) has often been considered a protozoan with universal distribution, affecting about 10% of the world's population, with highest prevalence in warm weather climates. However, it has recently been shown that at least 90 % of these cases are due to *E. dispar*, a much commoner species. In most cases, the old published data on the epidemiology of the *E. histolytica* infection were based on methods that were unable to distinguish *E. histolytica* from the recently recognised, non-pathogenic parasite *E. dispar*; therefore, the true burden and distribution of amoebiasis worldwide remains unknown [10]. Because these two species cannot be clearly distinguished at the microscopic examination, a lot of cases are misdiagnosed. The *E. histolytica*

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infection is transmitted through contaminated food or water by oral ingestion of cysts, and sexual transmission among male homosexuals.

Brumpt's hypothesis (1925) of the existence of two morphologically identical amoeba species, long time not approved, was relatively recently accepted [11]. The gathering of biochemical, genetic and immunological data, *E. histolytica* was re-defined in 1993 to recognize the existence of two morphologically identical, but genetically distinct human parasites: *E. histolytica*, the aetiologic agent of invasive intestinal and extra intestinal amoebiasis, and *E. dispar*, a non-pathogenic intestinal parasite [7]. *E. histolytica senso stricto* species can be pathogenic, invasive, and very dangerous; while *E. dispar* is nonpathogenic, and commensal. The cases with clinical symptoms are due to *E. histolytica senso stricto* species and the asymptomatic cases are attributed to *E. dispar*, but frequently to *E. histolytica senso stricto* species as well, particularly in temperate zones. In luminal asymptomatic infections, commensal *E. histolytica* ingests bacteria and food particles by phagocytosis, but in some conditions, it has the ability to become pathogenic, by exhibiting histolytic and red blood cell engulfing activity. In contact with the intestinal walls, amoeba produce intestinal tissue invasion, and ingest destroyed tissue cells and red blood cells, causing ulcerative lesions. If *E. histolytica* reaches the bloodstream it can spread, most frequently to the liver, lung or brain where it causes metastatic lesions (amoebic abscesses). In hyper endemic zones, cutaneous and genital lesions may appear. The clinical symptoms are only observed in 10 % of *E. histolytica* infection cases, 90 % of them remaining asymptomatic, such persons, the so-called "healthy carriers" being important sources of infection, able to spread the parasite to others through poor hygiene.

In Europe (including Romania), the real number of cases of *E. histolytica* infection is unknown because the diagnosis has, until recently, been based on the microscopic detection of cysts or trophozoites. New researches, however, revealed that amongst these imported and indigenous infections with *E. histolytica sensu lato*, the *E. dispar* species occurred more frequently than *E. histolytica sensu stricto*. In the recent years, the amoebiasis has been diagnosed with a greater rate in Europe, as the number of European citizens (more susceptible to this disease) traveling to endemic areas has increased. Additionally, global warming, with its accompanying increase in mean temperatures, which allow the parasite's cysts to survive longer in new warmer and moister conditions (winter months included), correlated with the higher exchanges/larger movements of human populations, may, in coming years, increase the risk of amoebiasis induced by *E. histolytica*.

In Romania, many data gathered from experimental or clinical cases of invasive amoebiasis with intestinal, hepatic, pulmonary, cutaneous and urinary complications were reported [12] (the first autochthonous case of amoebic hepatic abscess with pulmonary complications) [13,14,15,16,17,18,19,20,21]. Grecu et al. also report a systemic amoebiasis case in 2006. Additionally, mention other authors [17,22,23] who have also diagnosed human amoebiasis.

The aim of the present study was to diagnose *E. histolytica* infections by differentiating them from those caused by other etiologic agents (protozoa, bacteria), which also cause ulcero-hemorrhagic recto-colitis or hepatic and pulmonary abscesses. This differentiation is of great importance in establishing not only a proper etiologic treatment, but also in determining the correct epidemiological approach and consecutive measures.

2. EXPERIMENTAL SECTION

148 patients were hospitalized at the Gastroenterology Department of Fundeni Clinical Institute with hemorrhagic rectocolitis between May 2007 and September 2008. They were tested by copro-parasitological examination (2 % eosin stain) to detect the disease-causing agent. Results of clinical and paraclinical investigations, such as digestive endoscopy, abdominal ultrasonography,

colonoscopy, or the biochemical, hematology, and bacteriology tests were correlated with patient history.

The clinical diagnosis of *E. histolytica* was based on data regarding travel to, or residence in endemic areas, on some gastrointestinal and general signs and symptoms and on colonoscopy, radiography (in intestinal amoebiasis), and radiography, ultrasonography, computer tomography (CT scan) (in liver and lung amoebiasis). Colonoscopy allowed for the recognition of classic ulcerative lesions, while the radiography was useful in establishing localized areas, cecal deformities, and abnormalities in hepatic and pulmonary abscesses. The ultrasounds and CT were used to detect liver abscesses.

The laboratory final diagnosis for *E. histolytica* was based both on the microscopic identification of the *Entamoeba* haematophagous trophozoites (in fresh liquid stools of patients with acute dysentery or in excisional biopsy pieces) and on ELISA tests, the latter being used to confirm the *Entamoeba* species. The microscopic examination served to distinguish the *Entamoeba* from other protozoan species, or macrophages, while the ELISA test alone was able to determine the *E. histolytica* species involved. Additional investigations were done: blood analyses, colon liver and lung tissue biopsies.

3. RESULTS SECTION

An unique etiology (*Giardia lamblia, Entamoeba coli, Blastocystis hominis, Entamoeba histolytica* protozoan species, and *Clostridium difficile* bacterial species) was identified in 73 % the 139 patients with ulcero-haemorrhagic recto-colitis, while in the rest of cases, two associated etiologic agents (*G. lamblia and C. difficile* and *E.coli* and *C. difficile*) were detected. The causative agent remained unidentified in 9 of the analyzed cases. Protozoan parasites were involved in 46 % of cases, while in 21 % of the cases, an association between *C. difficile* and a protozoan species was registered.

Table 1: Number of cases with protozoan and bacterial infections

No. crt.	**Aetiologic agents**	**Number of cases**
1	*G. lamblia*	34
2	*E. coli*	20
3	*B. hominis*	8
4	*E. histolytica*	5
5	*C. difficile*	40
6	*G. lamblia* and *C. difficile*	20
7	*E.coli* and *C. difficile*	10
8	No identified causal agent (saprophytic microbiota)	9
	Total	148

Four protozoan species (single or in association with a bacterium) were predominant (67 %) among the analyzed cases. Of these, *Giardia lamblia* was the most prevalent (37 %), single and in association with a bacterial strain. *C. difficile* also registered a high level of occurrence (48 %), both single or in association (Table 1, Figure 1). Five of the 139 analyzed patients were suspected of *E. histolytica* infection (Table 2).

Visual examination of the colon using colonoscopy remains a very useful diagnostic method to eliminate non-infectious causes of bloody diarrhoea, and to confirm uncertain cases of amoebic colitis. Imaging using ultrasound, computed tomography all have excellent sensitivity for the detection of liver abscesses arising from any cause; however, they cannot distinguish amoebic abscesses from pyogenic (bacterial pus-forming) abscesses (Figures 2, 3) or necrotic tumours [7].

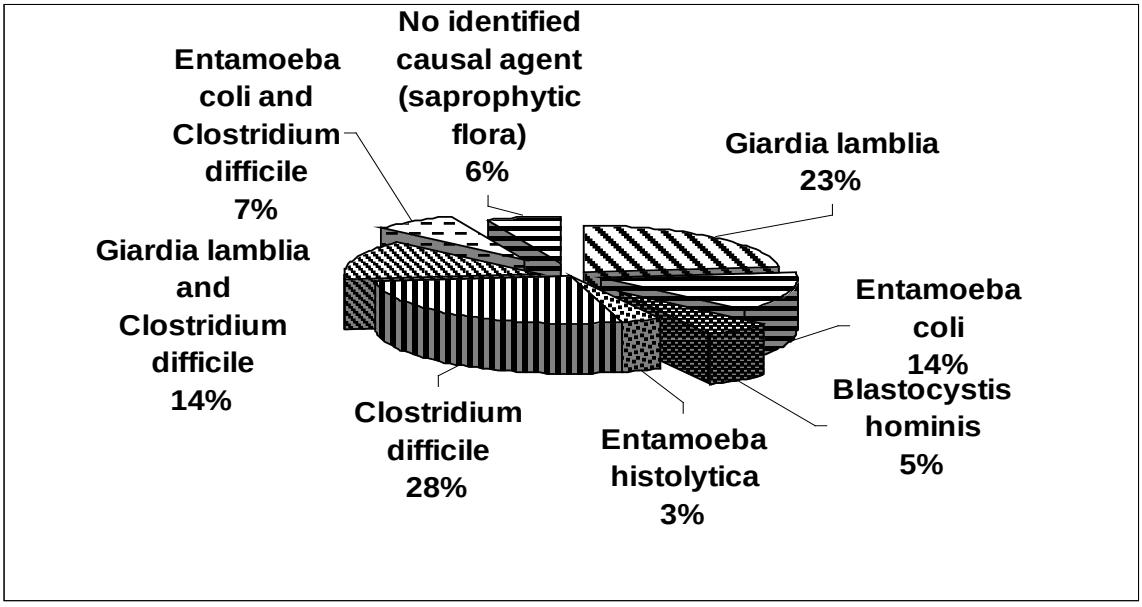

Figure 1: Frequency of ulcero-haemorrhagic rectocolitis cases with protozoan and bacterial etiology

Table 2: Epidemiologic and clinic diagnostic relevant data in five patients with amoebiasis

Case number, sex, age	**Possible source of infection**	**Pathology and clinical symptoms**	**Colonoscopy and radiography investigations, and colonic tissue biopsy results**
Case 1, male, 32 years old	Traveled as tourist to London, member of gay community	Acute recto colitis, severe dysentery with 10 daily stools containing blood, rectorrhagia (anal bleeding), weight loss.	**Radiography**: rectal ampulla, sigmoid and descending colon with classic ulcerative lesions. **Colonoscopy**: hyperemia, edema, numerous small ulcerative lesions. **Ultrasonography**: liver with inhomogeneous but normoechogenic structure, pancreas, spleen with normoechogenic structures.
Case 2, female, 64 years old	Resided several months in Florida, in the South of the United States of America	Acute rectocolitis, colic abdominal pain, severe dysentery with 10-15 daily stools, containing blood, mucus, pus.	**Radiography**: classic ulcerative lesions in sigmoid and descending colon. **Colonoscopy**: extensive ulcerative processes, strong edema mucosa. **Ultrasonography**: liver, pancreas, spleen with normoechogenic structure.
Case 3, male, 42 years old	Worked as builder plumber, in Malta	Severe dysentery with 12 daily stools containing blood, mucus, abdominal pain, fever, weakness, nausea.	**Colonoscopy**: ulcerative lesions, some of them being profound and edematous. **Colonic biopsy**: ulcerative lesions, hyperemia, frequent crater form ulcers. **Digestive endoscopy**: normal esophagus, stomach, duodenum. **Ultrasonography**: liver with hyperechodense structure, steatosis, normal pancreas. Lung without lesions.
Case 4, male, 28 years old	Worked in live-stock farm, in Spain	Severe dysentery with up to 30 daily stools containing blood, mucus, pus, rectorrhagia,	**Colonoscopy**: hemorrhage ulcerative lesions distributed over the whole mucosal colon, especially at rectum level, lesions which bleed to touch. **Thoracic radiography**: without pulmonary

		abdominal pain, 38°C fever, dehydration, weight loss (15 Kg), cahecsis.	lesions. **Pan endoscopy**: normal esophagus, distorted duodenum. **Abdominal ultrasonography**: liver with echogenic structure, without abscesses, granular pancreas
Case 5, male, 54 years old	Resided in a Romanian village	Hospitalized with diagnosis of hepatic tumor (left lobe). Hepatomegaly, acute recto colitis, dysentery with (5-15) 12 daily stools, sometimes containing blood, thoracic anterior-inferior and abdominal pain.	**Colonoscopy**: strong edema mucosa, hemorrhage ulcerative lesions (which bleed to touch) extensively distributed, some of them being profound. **Biopsy in liver left lobe**: damaged hepatocytes, small fragments of hepatic parenchyma penetrated by amoeboidal cells, simulated hepatic abscesses. **Biopsy in left lung**: small fragments of necrotic tissue and amoeboidal cells.

Table 3: Laboratory findings in the five patients with *E. histolytica* infection

Case number, sex, age	**Coproparasitological exam results**	**Some data from the blood analysis**	**ELISA IgM test for *Entamoeba histolytica***
Case 1, male, 32 years old	Frequent *Entamoeba* trophozoites in the stools	Leukocytosis: 15,860/ µL; eosinophils: 8; Hemoglobin: 11.2g/dL; VSH: 26 mm/h; glycemia: 99 mg/dL; HIV: negative	Positive result: 1.441 cut off 0.500
Case 2, female, 64 years old	Frequent *Entamoeba* trophozoites in the stools	Leukocytosis: 11,400/ µL; eosinophils: 6; thrombocytes: 428,000; Hemoglobin: 10.3g/dL; glycemia: 73 mg/dL; HIV: negative	Positive result: 1.609 cut off 0.500
Case 3, male, 42 years old	Frequent *Entamoeba* trophozoites in the stools	Leukocytosis: 15,800/ µL; eosinophils: 5; Hemoglobin: 10.5 g/dL; thrombocytes: 416,000/u/L; glycemia: 94 mg/dl; HIV: negative	Positive result: 0.914 cut off 0.500
Case 4, male, 28 years old	Present *Entamoeba* trophozoites in the stools	Dynamics leukocytosis: 16,600-12,000-8,700-6,140/µL, thrombocytes: 627,000-588,000-448,000/uL; Hemoglobin: 8.6; 8.2; 9.8 g/dL (severe microcytic and hypochromic anemia); eosinophils: 10; VSH: 80-70-22 mm/1h; glycemia: 74mg/dL; HIV: negative	Positive result: 2.039 cut off 0.500
Case 5, male, 54 years old	Frequent *Entamoeba* trophozoites in the stools	Leukocytosis: 17,200/µL; eosinophils: 14; thrombocytes: 367,000; Hemoglobin: 9 g/dL; VSH: 104/134, glycemia: 105mg/dl; HIV: negative	Positive result: 2.441 cut-off 0.500

Microscopy has a poor sensitivity in distinguishing *E. histolytica* cysts and was used especially to check for the presence of other protozoan species or of *E. histolytica* haematophagous trophozoites. Because microscopy is unable to distinguish *E. histolytica* from *E. dispar*, it should no longer be relied upon to diagnose amoebiasis. To estimate the real proportions of *E. histolytica* and *E. dispar* in patients with amoebic infection, the PCR technologies or ELISA tests are needed [24,25]. Sensitive and specific molecular techniques that are able to distinguish these two organisms have been developed recently; they include (1) the detection of an *E. histolytica* antigen using an enzyme-linked immunosorbent assay (ELISA), (2) the use of the polymerase chain reaction (PCR) to amplify

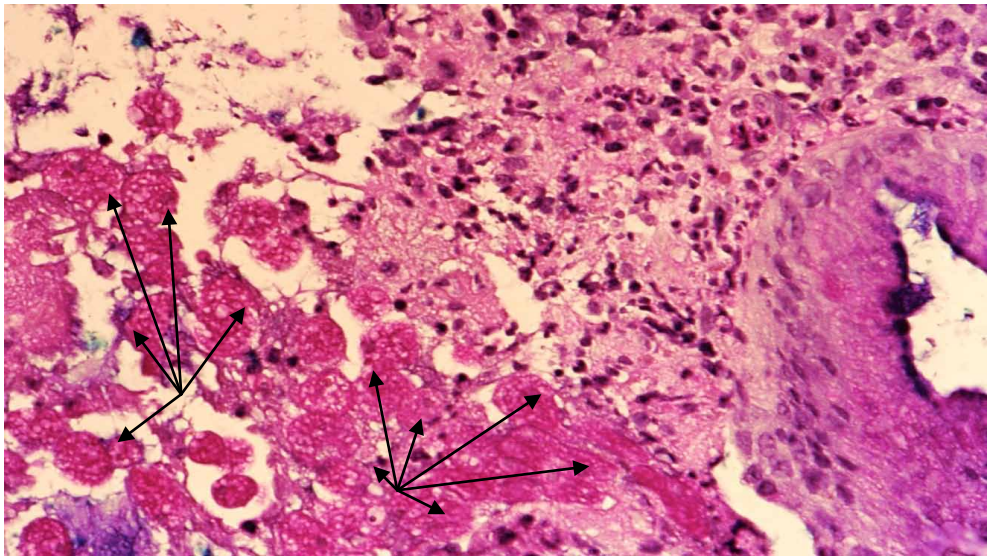

Figure 2: Lung tissue biopsy: *Entamoeba histolytica* trophozoits (X 20X, stained eosin smear).

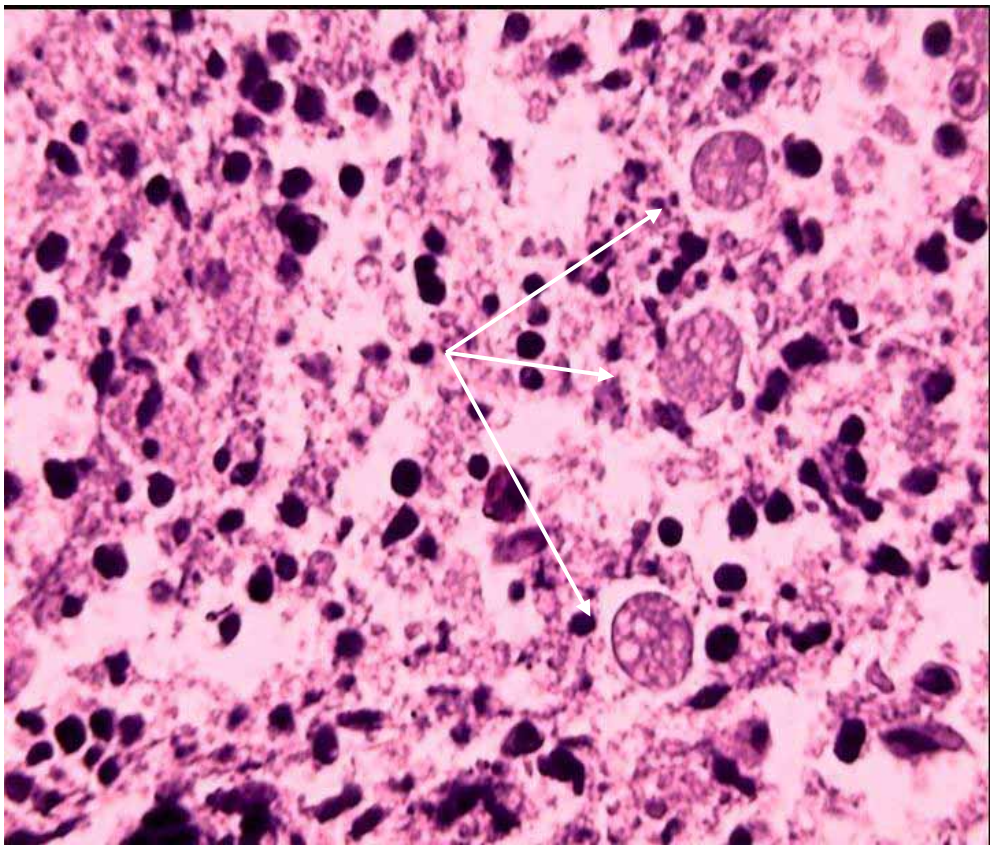

Figure 3: Lung tissue biopsy: *Entamoeba histolytica* trophozoits (X 40X, stained eosin smear).

amoebic DNA, and (3) the culture of stool samples followed by isoenzyme analysis. Of these three test methods, only antigen detection using ELISA can be performed rapidly and easily, reason for which it became the method of choice for clinical use in the developing world, where the morbidity and mortality caused by *E. histolytica* is the greatest [7]. PCR and antigen detection had comparable sensitivities when performed directly on fresh stool specimens, but only the TechLab *E. histolytica* antigen detection test was both rapid and technically simple.

In the five cases suspected of *E.histolytica* infection, a history of travel or short time residence in some endemic area, characteristic intestinal and general signs and symptoms (signs of inflammation,

severe dysentery with stools containing blood, mucus and shreds of necrotic mucosa, accompanied by acute abdominal pain, fever, weakness, dehydration, weight loss), and some blood analysis results (leukocytosis between 11,400 and 16,600 µL), guided the diagnosis towards amoebiasis (Table 2). A differentiation from other intestinal amoeba and, in one case, from other hepatic diseases (viral or bacterial hepatitis, hydatid cyst, malignancy, gallbladder infections) was made. Infection with this specific genus of amoeba was based on colonoscopy and radiography and, in one case, even on colonic tissue biopsy, and especially on copro parasitological exams, as well as on frequently detected *Entamoeba* trophozoites. The amoeba was located in the large intestine and in the cecal region (both in intestinal lumen and wall).

The presence of the invasive *E. histolytica* pathogen was established by *ELISA IgM test for E. histolytica*, which was positive in all five of the cases (Table 3). Ultrasonography and X-ray examination revealed that an extra intestinal amoebiasis was absent in four of the patients (without pulmonary and liver abscesses). A male patient (54 years old), residing in a rural locality near the town of Oradea had both an intestinal and secondary hepatic amoebiasis. The left liver lobe had two abnormal zones: one (27 mm diameter), surrounded by a hypoecogene zone and another (28 mm diameter), imprecisely delimited. The right liver lobe had inhomogeneous echo structure, with a transonic, hypoecogene and necrotic area inside. She complained of pain in the epigastria that radiated to the inferior abdomen. The possible source of infection was analyzed: in four patients an allochtonous source, correlated to temporary residence, travel or community gay member in endemic zone for amoebiasis (Malta, Spain, Florida) [5], and in one case an autochtonous source was suspected (male, 54 year old, not traveler, not gay, from Romanian western rural area, near Oradea village).

4. CONCLUSIONS

The amoebiasis diagnosis is not easy to establish, due to the lack of a specific diagnostic method, and the best results can be obtained by corroboration of the clinical and laboratory data.

Results of clinical and laboratory investigations obtained from 148 patients hospitalized in the Gastroenterology Department, Fundeni Clinical Institute, Bucharest, with a preliminary diagnosis of hemorrhagic recto colitis, established four protozoan (*Giardia lamblia*, *Entamoeba coli*, *Blastocystis hominis*, *Entamoeba histolytica*) and one bacterial (*Clostridium difficile*) species as etiological agents in 139 of the analyzed patients. Of these, 30 registered a polyparasitism caused by two protozoan species (*Giardia lamblia* and *Entamoeba coli*) and the *C. difficile* bacterium.

More specific investigations were sought for five of the patients: signs and symptoms analysis, colonoscopy, radiography, ultrasonography, computer tomography, colonic, hepatic and pulmonary tissue biopsy, coproparasitological exams, blood analysis, ELISA IgM test for *E. histolytica*. An intestinal amoebiasis was confirmed, and a hepatic infection was discovered in one of the five patients.

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