STATE-OF-THE-ART CLINICAL ARTICLE

Diagnosis and Management of Amebiasis

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Amebiasis in developed countries is most common in immigrants and travelers returning from the third world, and less common in men who have sex with men and immunosuppressed individuals. Infection is acquired by ingestion of food or water containing the cyst form of the parasite *Entamoeba histolytica*, which is the cause of amebic colitis and liver abscess. The trophozite invades the intestinal epithelium and causes disease by destroying host tissues. Amebic colitis commonly has a subacute onset with weight loss, and is characterized by diarrhea that usually contains occult or gross blood. Because *E. histolytica* is identical in appearance to the nonpathogenic parasite *Entamoeba dispar*, amebic colitis is best diagnosed by detection of *E. histolytica* in stool. Amebic liver abscess is 10 times more common in men than women. A history of alcohol abuse is common, and patients usually present with several weeks of fever and right upper quadrant pain. Most patients with liver abscess do not have concurrent dystentery. Standard treatment with metronidazole plus a luminal agent cures most patients with invasive amebiasis, and drug resistance has yet to be encountered.

E. dispar is the new species name for what had been called "noninvasive" or "nonpathogenic" *E. histolytica* [1–4]. (Earlier reports that *E. histolytica* and *E. dispar* could "convert" in culture [5] were an artifact of culture contamination [6].) *E.*

dispar, Entamoeba moshkovskii, Entamoeba coli, Entamoeba hartmanni, and *Endolimax nana* are nonpathogenic parasites of the human intestine. There has been no correlation established between infection with these organisms and gastrointestinal symptoms, and antiamebic treatment is not warranted. *Dientamoeba fragilis* and *Entamoeba polecki* have been occasionally implicated as a cause of diarrhea, and *Entamoeba gingivalis* is associated with periodontal disease [7].

E. histolytica is a pseudopod-forming nonflagellated protozoan parasite. The *E. histolytica* life cycle consists of an infective cyst and an invasive trophozoite form. The quadrinucleate cyst is resistant to gastric acidity and desiccation and can survive in a moist environment for several weeks. Only the trophozoite form invades human tissue. Some interesting differences between the biochemical pathways of *E. histolytica* and those of higher eukaryotes include its lack of glutathione, its use of pyrophosphate instead of ATP at several steps in glycolysis [8], a unique alcohol-aldehyde reductase [9], and its inability to synthesize purine nucleotides de novo. Molecular phylogenetic analysis of eukaryotic organisms based on sequence comparisons of small subunit rRNA has placed *Entamoeba* on the lowermost branches of the eukaryotic tree, closest to *Dictyostelium.* However, *E. histolytica* shares with higher branching eukaryotes mitochondrial genes that are contained within an apparently biochemically inert remnant organelle [10, 11]. Control of gene expression is fundamentally different from that of other eukaryotes, with a novel conserved sequence (GAAC element) in the RNA polymerase II promoter that specifies the rate and site of mRNA transcription [12]. The genome size of the parasite has recently been estimated to be slightly <20 megabases [13], and the sequencing of the entire genome is under way.

Pathogenesis

Infection occurs on ingestion of the cyst form of the parasite. Understanding of the mechanism of formation of the cyst has come from studies of the distantly related reptilian parasite *Entamoeba invadens.* Novel proteins and glycoconjugates are produced by *E. invadens* during encystation, and parasite galactose-containing molecules and receptors appear to be involved in regulating encystation [14]. The quadrinucleate cyst releases 8 trophozoites in the intestine, which colonize the in-

Table 1. Persons at increased risk for amebiasis.

Immigrants from developing countries
Travelers to tropics
Residents of institutions for mentally retarded
Men who have sex with men
Immunosuppressed persons

testine by adhering to colonic mucin glycoproteins via a galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin [15].

Killing of host cells by the extracellular amebic trophozoites requires contact via the parasite Gal/GalNAc lectin. Inhibition of the lectin with Gal/GalNAc prevents host-cell destruction, and cells that lack surface Gal/GalNAc are resistant to adherence and cytolysis [16, 17]. The Gal/GalNAc lectin is a multigene family of 260-kDa heterodimers consisting of heavy (170-kDa) and light (35/31-kDa) subunits linked by disulfide bonds [18]. By expression cloning, the carbohydrate recognition domain was identified within the lectin heavy subunit cysteine-rich region. Interestingly for a hepatic parasite, the carbohydrate recognition domain sequence was identical to the receptor-binding domain of hepatocyte growth factor, and it competed with hepatic growth factor for binding to the c-Met hepatic growth factor receptor [19]. Genetic proof that the lectin is required for virulence has come from the use of dominant negative mutants: inducible expression of the lectin cytoplasmic tail interfered with "inside-out" regulation of lectin activity and decreased liver abscess size by 90% [20].

Host cells are killed via the induction of an apoptotic cascade (figure 1). The parasite therefore does not so much kill the host as induce it to commit suicide [21]. Apoptotic killing occurs by a novel pathway that is not blocked by bcl-2 and does not require fas or the TNF-α receptor [21, 22]. Recently the amebae have been demonstrated to activate "effector" caspases immediately before destruction of the host cell. Inhibition of these human caspases blocks killing by the amebae [23]. Trophozoites also contain a pore-forming protein that is probably involved in the destruction of endocytosed bacteria. The purified protein is also capable of inducing necrotic death of eukaryotic cells [24]. Parasites that have invaded humans resist destruction by the complement arm of the innate immune system via lectin-mediated inhibition of assembly of the membrane attack complex [25]. Invasion is probably also promoted by the cytoskeleton-induced motility and by the secretion of proteases that degrade the extracellular matrix and antibody and that may also be involved in endocytosis [26–29].

Epidemiology

Amebic colitis and liver abscess are much more common in developing nations than in industrialized countries such as the United States. *E. histolytica* infection is probably second only to malaria as a protozoan cause of death. The best estimate is that 40–50 million cases of amebic colitis and liver abscess occur annually in the world, resulting in 40,000–110,000 deaths [1]. Most amebic infections occur in Central and South America, Africa, and Asia. The 1987–1988 Mexican national serosurvey, for example, demonstrated an 8.4% seropositivity for *E. histolytica*. In the year of the serosurvey there were an estimated 1 million cases of amebiasis and 1216 deaths due to *E. histolytica* infection in Mexico [30]. The prevalence of disease in the developing world is due to fecal-oral spread of infection via contaminated food and water. The vulnerability of the developed world to epidemics has recently been demonstrated by a large and apparently waterborne outbreak in Tbilisi in the former Soviet Republic of Georgia [31].

In the United States, immigrants from and travelers to developing countries are those most likely to develop amebiasis (table 1). A total of 2970 cases of amebiasis in the United States were reported to the Centers for Disease Control and Prevention in 1993; 33% of the patients were Hispanic immigrants and 17% immigrants from Asia or the Pacific Islands [32]. Travelers to the tropics are at a low but definite risk for acquiring amebic infection [33, 34]. One study of 2700 German citizens returning from tropical areas demonstrated a 0.3% incidence of *E. histolytica* infection [33]. Residents of institutions for the mentally retarded are also at increased risk for amebic colitis and liver abscess [35]. Men who have sex with men were in the past predominantly infected with the nonpathogenic ameba *E. dispar* [36], but recently invasive amebiasis has been seen in this group (with and without HIV infection) [37].

Amebic liver abscess is 7–12× more common in men than in women. In children, it is an unusual manifestation of amebiasis, and its distribution between the sexes is equal. The typical patient in the United States with amebic liver abscess is a Hispanic immigrant man aged 20–40 years. Infection with *E. histolytica* presents clinically within a year of immigration to the United States, although in unusual cases, it presents ≤12 years after immigration [37–42].

Clinical Findings

Asymptomatic colonization. Asymptomatic infection with *E. histolytica* is defined as the presence of *E. histolytica* in stool

Table 2. Clinical manifestations of *Entamoeba histolytica* and *Entamoeba dispar* infection.

Asymptomatic colonization (*E. histolytica* and *E. dispar*)
Intestinal amebiasis and its complications (*E. histolytica* only)
    Amebic colitis
    Ameboma
    Toxic megacolon
    Peritonitis
    Cutaneous amebiasis
Extraintestinal amebiasis (*E. histolytica* only)
    Amebic liver abscess
    Splenic abscess
    Brain abscess
    Empyema
    Pericarditis

in the absence of colitis or extraintestinal infection (table 2). Colonization with the morphologically identical parasite *E. dispar* is 3× more common in developing countries and at least 10× more common in developed nations [43–45]. *E. histolytica* colonization is frequently observed in high-risk settings. For example, in an urban refugee camp in Dhaka, Bangladesh, asymptomatic infection with *E. histolytica* was present in 5% and *E. dispar* in 13% of children aged 2–5 years. Colonization with *E. histolytica* carried a low but definite risk of development of invasive amebiasis: 2 of 17 children developed dysentery during a 1-year follow-up; the other children cleared the infection without specific treatment [46]. Since the stool ova and parasite microscopic examination cannot differentiate *E. histolytica* from *E. dispar* or *E. moshkovskii* [47], a diagnostic test specific for *E. histolytica* should be used (see Diagnosis, below). Only *E. histolytica* infection requires treatment [46, 48].

Dysentery or colitis. Patients with amebic colitis typically present with a several-week history of gradual onset of abdominal pain and tenderness, diarrhea, and bloody stools [49–52]

Table 3. Clinical findings for patients with amebic colitis.

Finding	Proportion of patients
History of immigration from or travel to area of endemicity	Most
Gradual onset, symptoms >1 week	Most
Diarrhea	94%–100%
Heme-positive stools	70%
Abdominal pain	12%–80%
Weight loss	44%
Fever >38°C	10%
Positive *Entamoeba histolytica*–specific stool antigen test	87%
Positive amebic serology	70%
Positive colonoscopic biopsies	80%

(table 3). In one series, patients with amebic colitis had an average duration of pre-hospital illness of 21 days, compared with 4 days for patients with shigellosis [50]. Because of the gradual onset, weight loss is a common finding. Surprisingly, fever is present in only the minority (8%–38%) of patients with amebic colitis. Colonic lesions can vary from mucosal thick-
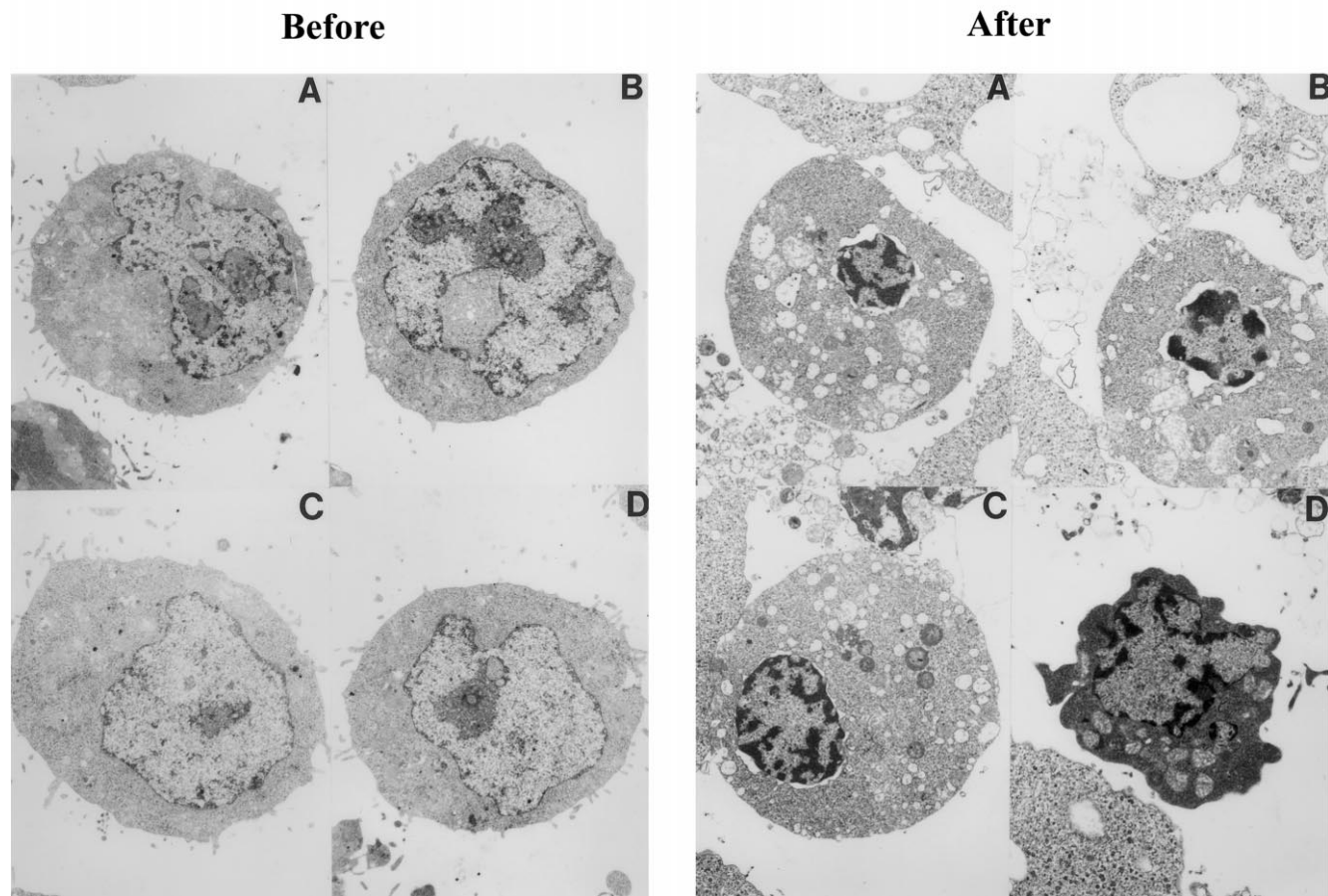

Figure 1. Apoptotic killing of host by *Entamoeba histolytica:* electron microscopic appearance of murine myeloid cells incubated in vitro with *E. histolytica* for 30 min at 37°C. Compacted chromatin, cytoplasmic condensation and convolution, membrane blebbing, and nuclear envelope swelling are illustrated. Magnification, ×9425. From [21] (used with permission).
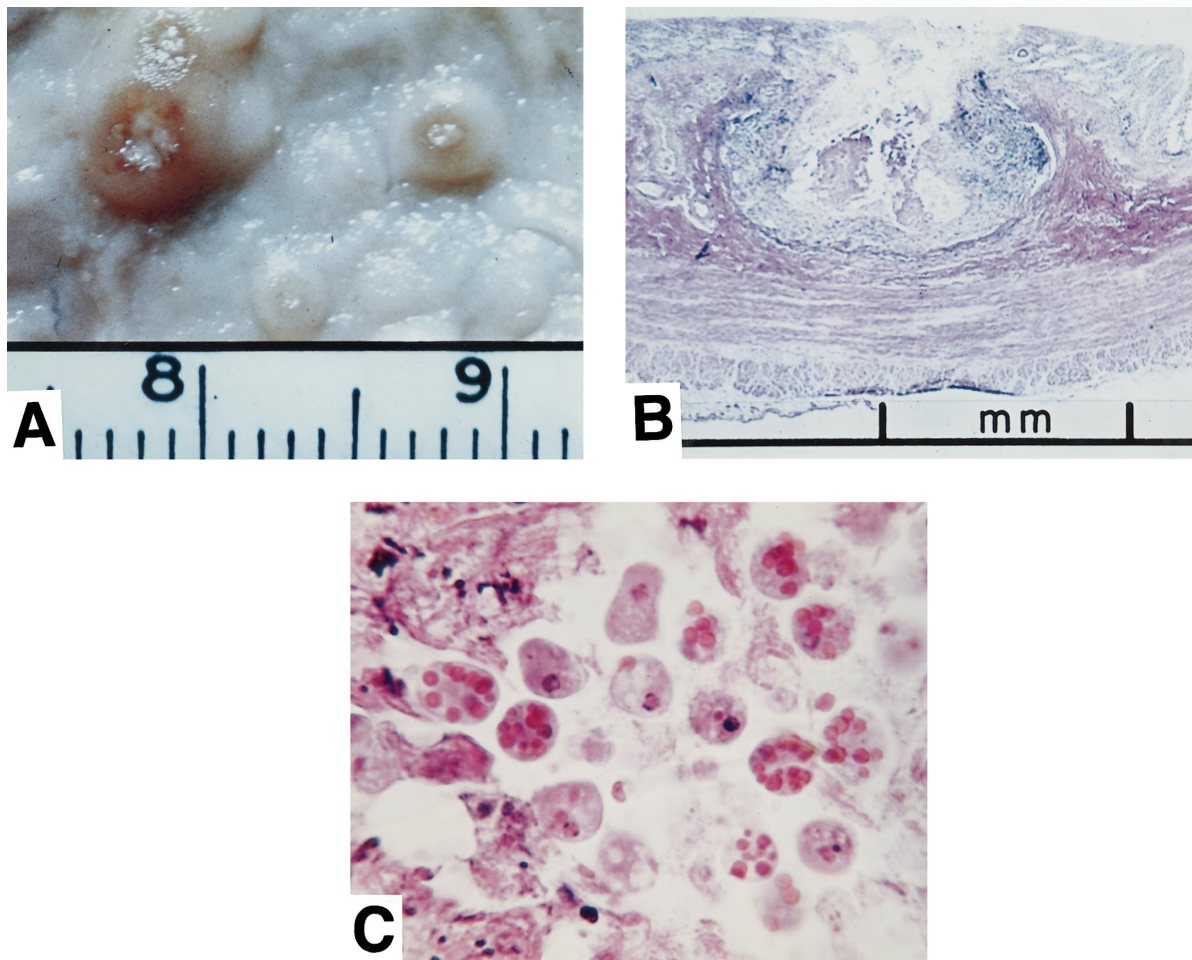
Figure 2. Gross and microscopic pathology of amebic colitis. *A,* Colonic ulcers of ~1 mm diameter. *B,* Cross-section of one of the ulcers, demonstrating characteristic flask shape. *C, Entamoeba histolytica* trophozoites in ulcer containing ingested red blood cells and surrounded by eosinophilic debris due to amebic destruction of submucosa. (From collection of the late Dr. Harrison Juniper.)

ening only to flask-shaped ulcerations to necrosis of the intestinal wall (figure 2). In at least 70% of cases, the stool is positive for blood (gross or microscopic). Infection with *Shigella dysenteriae* and *Shigella flexneri* was more common in children in Dhaka with *E. histolytica* or *E. dispar* infection, potentially complicating the management of amebiasis [43].

The differential diagnosis of an illness with diarrhea containing gross or occult blood should include infectious etiologies (including amebiasis and infections with *Shigella, Salmonella, Campylobacter,* and enteroinvasive and enterohemorrhagic *Escherichia coli*) and noninfectious causes (including inflammatory bowel disease, ischemic colitis, arteriovenous malformation, and diverticulitis). It can be difficult to make the diagnosis of amebic colitis, since the presentation of the illness may be insidious or chronic, bleeding may occur without diarrhea, and fever is an unusual finding. Once the diagnosis has been entertained, conclusive diagnosis can also be problematic, because a single stool examination for parasites is insensitive, histo-pathologic confirmation of infection on biopsy specimens may be difficult, and serological tests for antibodies to amebae are not always positive. The best initial diagnostic approach at this time is detection of *E. histolytica* antigen in stool (figure 3).

Unusual manifestations of amebic colitis include acute necrotizing colitis, ameboma (granulation tissue in colonic lumen mimicking colonic cancer in appearance), cutaneous amebiasis, and rectovaginal fistulas. Acute fulminant or necrotizing colitis occurs in ~0.5% of cases, usually requires surgical intervention, and has a mortality of >40%. Abdominal pain and distension and rebound tenderness are present in most patients with fulminant colitis, and indications for surgery include perforation and persistence of abdominal distension and tenderness while undergoing antiamebic therapy. Partial or total colectomy with exteriorization of the ends is recommended over primary anastomosis, since anastomoses may fail because of the friable condition of the bowel wall [51, 52].

Liver abscess.   Roughly 90% of patients with amebic liver

Amebiasis

Table 4. Clinical findings for patients with amebic liver abscess.

Characteristic or finding	Proportion of patients
Sex, men/women	10/1
History of immigration from or travel to area of endemicity	Most
History of alcohol abuse	Many
Fever	85%–90%
Right upper quadrant pain	84%–90%
Hepatomegaly	30%–50%
Weight loss	33%–50%
Symptom duration of >4 weeks	20%–50%
Diarrhea	20%–33%
Cough	10%–30%
Positive amebic serology	70%–95%
WBC count >12,000/μL	80%
Elevated level of alkaline phosphatase	70%
Elevated levels of bilirubin and serum aspartate and alanine aminotransferases	20%

abscess are young adult males, although among children and infants the male/female ratio is equal. Liver abscess may present acutely, with fever and right upper abdominal tenderness and pain, or subacutely, with prominent weight loss and less frequent fever and abdominal pain [37–42] (table 4). Most often, patients with liver abscess will present without concurrent colitis, although they sometimes have a history of dysentery within the last year. A history of alcohol abuse is common. A more chronic presentation of 2–12 weeks of weight loss, fever, and abdominal pain has been reported in a subset of patients with single abscesses. In a recent series of patients from San Francisco, one-third were HIV-infected and did not have a history of residence or travel to an area of endemicity [37].

Findings from physical and laboratory examination include right upper quadrant pain, fever of 38.5°C–39.5°C, leukocytosis, and elevated levels of serum transaminases and alkaline phosphatase. An elevated right hemidiaphragm is a common finding on chest radiograph. Early evaluation of the hepatobiliary system with ultrasound, CT, or MRI is essential to demonstrate the abscess in the liver.

The differential diagnosis of the lesion in the liver should include pyogenic abscess (less probable if the gallbladder and ducts appear normal), hepatoma, and echinococcal cyst (usually an incidental finding unrelated to the acute fever and abdominal pain) (table 5). Eighty percent of the time, amebic abscess is single and is in the right lobe of the liver (figure 4). However, the most common location for a pyogenic abscess is also in the right lobe, so location is not helpful in distinguishing the etiology of an abscess. Patients with pyogenic abscesses are more likely to be aged >50 years, to have preexisting illness, to present with jaundice, pruritus, sepsis, or shock, and to have a palpable mass.

Aspiration of the abscess is occasionally required to diagnose amebiasis. Amebae are visualized in the pus in only the minority of cases. If the abscess is pyogenic, however, the responsible bacteria should be identified by gram staining and/or culture.

Antibodies to *E. histolytica* are present in the serum of 86%–97% of patients on acute presentation with amebic liver abscess and therefore are very useful diagnostically. Because a significant proportion of the population in developing countries is seropositive, antibody tests are less specific in residents of or immigrants from the developing world (see Diagnosis, below).

Complications of amebic liver abscess include abscess rupture and secondary bacterial infection of the abscess. Impending abscess rupture was associated in 1 study with dyspnea, elevated right hemidiaphragm and pleural effusion, jaundice, anemia, and diabetes mellitus. Patients with these associated risk factors and who do not respond within 72 hours to antiamebic therapy may benefit from percutaneous drainage of the abscess. Aspiration of the abscess is otherwise not required as part of the treatment and can be complicated by secondary bacterial infection. Intrathoracic and intraperitoneal rupture of an amebic liver abscesses can be adequately treated with antiamebic therapy without surgery if secondary bacterial infection is absent.

Unusual extraintestinal manifestations of amebiasis include direct extension of the liver abscess to pleura or pericardium, cutaneous amebiasis, and brain abscess [53].

Laboratory Diagnosis of Amebiasis

The World Health Organization has recommended that intestinal infection be diagnosed with an *E. histolytica*–specific test [1]. The classic stool ova and parasite examination is therefore obsolete. The TechLab (Blacksburg, VA) *E. histolytica* stool antigen detection test is the only one available that is
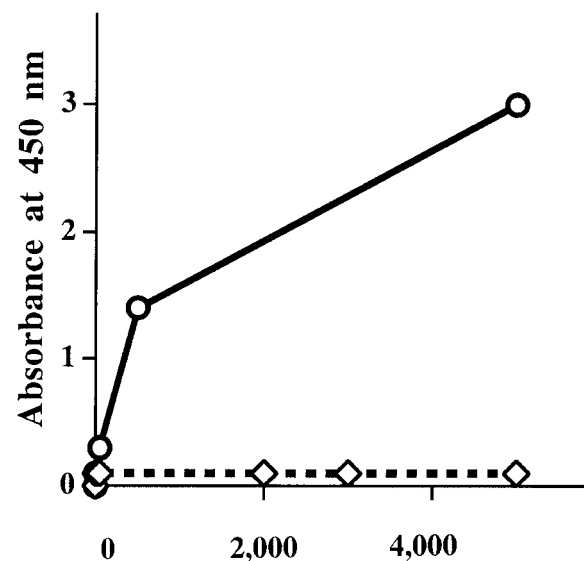

Figure 3. Antigen detection test for *Entamoeba histolytica*. Antigen test uses monoclonal antibodies specific for Gal/GalNAc lectin of *E. histolytica,* allowing rapid identification of *E. histolytica* (○) in stool. Closely related but nonpathogenic parasite *Entamoeba dispar* (◇) is not recognized by monoclonal antibodies.

Table 5. Comparison of findings for patients with pyogenic vs. amebic liver abscess.

	Pyogenic abscess	Amebic abscess
Male/female ratio	1/1	10/1
Age, years	>50	20–40
Aspirate or blood cultures	Positive	Negative
Biliary disease	Yes	No
Diabetes mellitus	Yes	No
Immigrant from or traveler to developing world	No	Yes

specific for the pathogenic ameba *E. histolytica* [4, 43, 54] (figure 3). All other antigen detection tests currently available cross-react with *E. dispar* [54], which is a problem because *E. dispar* infection is 3–10× more common than *E. histolytica* infection. Published experience with the TechLab *E. histolytica* stool antigen detection test has shown a sensitivity of 87% and a specificity of >90% compared with culture. Stool ova and parasite examination, in addition to being nonspecific, misses one-half to two-thirds of all *E. histolytica* colonic infections detected by culture [4, 43, 46]. A second-generation *E. histolytica* stool antigen test with improved sensitivity will be available shortly [55]. At this time culture and PCR detection of the parasite are research tools and not practical or approved for clinical diagnostic use [4, 54].

An important adjunct to antigen detection is the detection of serum antibodies to amebae. Especially in the case of amebic liver abscess, in which most patients do not have detectable parasites in stool, the presence of antibodies to amebae can be very useful in diagnosis. Tests for antibodies to amebae are ~90% sensitive for amebic liver abscess and 70% sensitive for amebic colitis. A major problem with current serological tests is that the patient continues to test positive for years after an episode of amebiasis. As a result, currently available serological tests show that a substantial number (10%–35%) of residents of developing countries have antibodies to amebae. Since the vast majority of patients with invasive amebiasis in developed countries are immigrants from developing nations, serological tests may not be as specific as one would hope.

Colonoscopy may be helpful in the diagnosis of amebic colitis if antigen detection tests are negative. Colonoscopy is preferable to sigmoidoscopy for the diagnosis of amebic colitis because disease may be localized to the cecum or ascending colon. Cathartics or enemas should not be used to prepare the patient because they will interfere with the identification of the parasite. Wet preparations of material aspirated or scraped from the base of ulcers should be examined for motile trophozoites. The appearance of amebic colitis may resemble that of inflammatory bowel disease, with granular, friable, and diffusely ulcerated mucosa. Large ulcers with sharply defined borders and pseudomembranes may also be present. The detection rate of trophozoites on histopathologic examination of colonic biopsy specimens from patients with amebic colitis varies in different reports from all to only some of the patients. Biopsy specimens

should be taken from the edge of the ulcers. Periodic acid–Schiff stains the parasites a magenta color, increasing the ease of detection in biopsies [56]. *E. histolytica* has been shown to invade carcinomas, which causes diagnostic confusion.

Ultrasound, CT, and MRI studies of the liver are equally sensitive at detecting amebic abscesses and equally incapable of specifically differentiating an amebic from a pyogenic abscess. Characteristically, an amebic liver abscess will appear on ultrasound as a homogeneous hypoechoic round or oval lesion. On contrast CT scan, amebic abscesses usually appear as rounded, well-defined, low-attenuation lesions, the wall commonly enhancing with contrast. The appearance of the abscess cavity on CT is variable: some appear homogeneous, and others show septations or observable levels of fluid or debris. Follow-up imaging studies are not indicated: 6 months after successful treatment, ultrasonography showed that only one-third to two-thirds of amebic liver abscesses had disappeared [57, 58].

Treatment

Colonization with *E. histolytica* should be treated with a luminal agent alone; *E. dispar* infection does not require treatment (table 6) [59]. Oral drugs that are effective against luminal infection include diloxanide furoate (available only through the Centers for Disease Control and Prevention; causes frequent gastrointestinal disturbances and rare diplopia), paromomycin (rarely causes ototoxicity and nephrotoxicity but frequently causes gastrointestinal disturbances), and iodoquinol (rarely causes optic neuritis and atrophy with prolonged use). The recommended duration of treatment with paromomycin is 7 days, with diloxanide furoate is 10 days, and with iodoquinol is 20 days [60]. In a case in which luminal agents cannot be used, it seems a reasonable (if unproven) approach to treat luminal infection with metronidazole and test for cure with the stool antigen detection test.

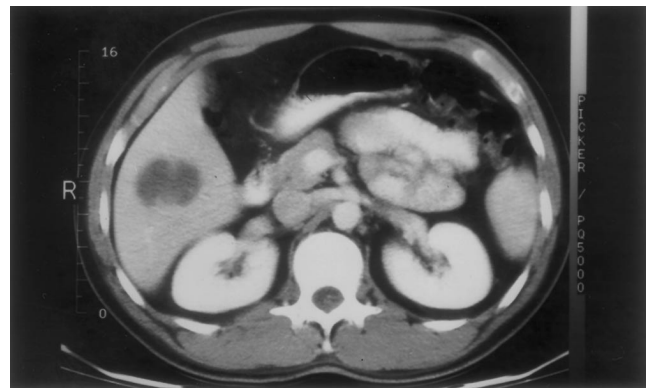Invasive amebiasis (e.g., colitis, liver abscess) should be

Figure 4. Imaging of hepatic amebic abscess by CT. Patient was young Hispanic man from Charlottesville, Virginia, who had last lived in Mexico a year before.

Table 6. Treatment of amebiasis.

Colonization	
Entamoeba dispar	No treatment needed
Entamoeba histolytica	Luminal agent (paromomycin, diloxanide furoate, or iodoquinol)
Invasive disease	Metronidazole followed with luminal agent; poor response: aspirate or add chloroquine

treated with metronidazole for 10 days. Although metronidazole has some unpleasant side effects, such as headache, nausea, metallic taste, and a disulfiram-like reaction to alcohol, reaction is rarely severe. Uncommon neurological side effects, such as vertigo or encephalitis, or neutropenia may necessitate discontinuation of treatment. Therapy with metronidazole should be followed with a luminal agent, since patients are otherwise at risk of relapsing from residual infection in the intestine [60]. The majority of patients with amebic liver abscess defervesce after 3–4 days of treatment with metronidazole. Chloroquine and/or percutaneous drainage of the liver abscess are options in addition to metronidazole treatment for the rare patient who does not respond to metronidazole alone [61, 62].

Prevention

Prevention of amebiasis at present requires interruption of the fecal-oral spread of the infectious cyst stage of the parasite. Because cysts are resistant to low doses of chlorine or iodine, in developing countries water must be boiled before it is safe to drink, and raw vegetables must be washed with soap and then soaked in vinegar for 15 min before they can be eaten. Since amebiasis often spreads through a household, it is prudent to screen family members of an index case for intestinal *E. histolytica* infection.

On the horizon is the development of a vaccine to prevent disease in residents of and travelers to the developing world. Both the amebic adherence lectin and serine-rich antigen have proven effective in the prevention of liver abscess in animal models of the disease [62–67]. The lectin is a particularly attractive candidate antigen because it is required to initiate contact-dependent cytolysis, mediates evasion of the complement membrane attack complex, and is antigenically conserved among geographically distinct isolates of *E. histolytica*.

Current obstacles to vaccine development include an incomplete understanding of mechanisms of immunity in humans and in animal models of the disease. Protection against liver abscess can be partially transferred with antibodies in rodent models [19, 64]; however, it is likely that protection will also require cellular immune responses against the ameba. For example, macrophages and neutrophils that were activated with IFN-γ and TNF-α could kill *E. histolytica* trophozoites in vitro, whereas in the absence of the cytokines these cells were themselves killed by the amebae [68, 69]. Another obstacle to be overcome is the lack of well-studied intestinal models of infection, which has blocked testing of any of the current prototype vaccines for protection from intestinal infection. Progress in this field continues, however, to move at a rapid pace. It is exciting to consider that since humans are the only significant reservoir of infection, a vaccine that blocked colonization could lead to elimination of amebiasis.

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