

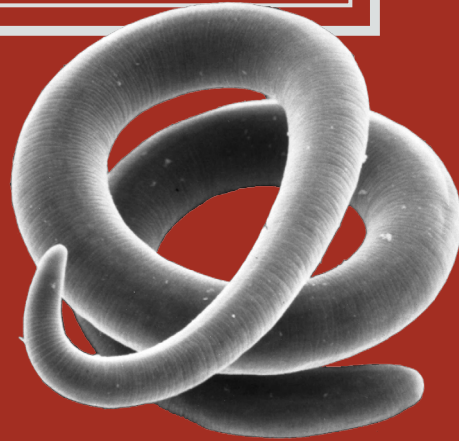
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Fifth Edition

Parasitic Diseases

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12. *Entamoeba histolytica* (Schaudinn 1903)

Introduction

Entamoeba histolytica is transmitted from person to person via the fecal-oral route, taking up residence in the wall of the large intestine. It is one of the leading causes of diarrheal disease throughout the world. Protracted infection can progress from watery diarrhea to dysentery (bloody diarrhea) that may prove fatal if left untreated. In addition, *E. histolytica* can spread to extra-intestinal sites causing serious disease wherever it locates. *E. histolytica* lives as a trophozoite in the tissues of the host and as a resistant cyst in the outside environment. Sanitation programs designed to limit exposure to food and water-borne diarrheal disease agents are effective in limiting infection with *E. histolytica*. Some animals (non-human primates and domestic dogs) can become infected with *E. histolytica*, but none serve as important reservoirs for human infection.

Entamoeba dispar is a morphologically identical, non-pathogenic amoeba, and is often misidentified as *E. histolytica* during microscopic examination of fecal samples.¹ Monoclonal antibodies are commercially available that identify only *E. histolytica*, distinguishing it from all other intestinal protozoans.² For reviews of the basic science and clinical information, see Reed and Ravdin³ and Martinez-Palomo.⁴ The full length of the genome of *Entamoeba histolytica* has been sequenced.⁵

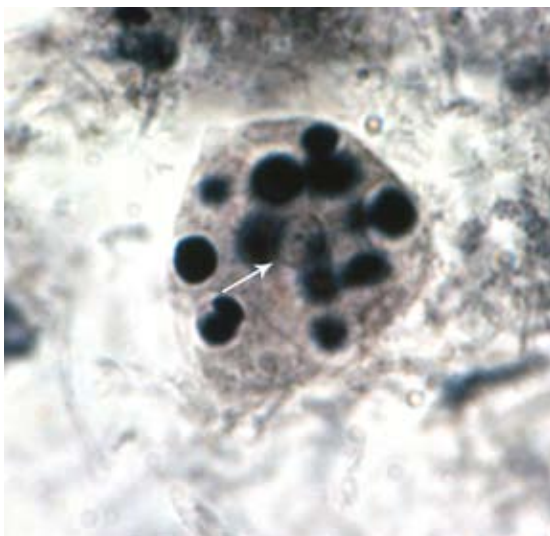


Figure 12.1. Trophozoite of *Entamoeba histolytica*. Note nucleus (arrow) and numerous ingested red cells. 35 μm .

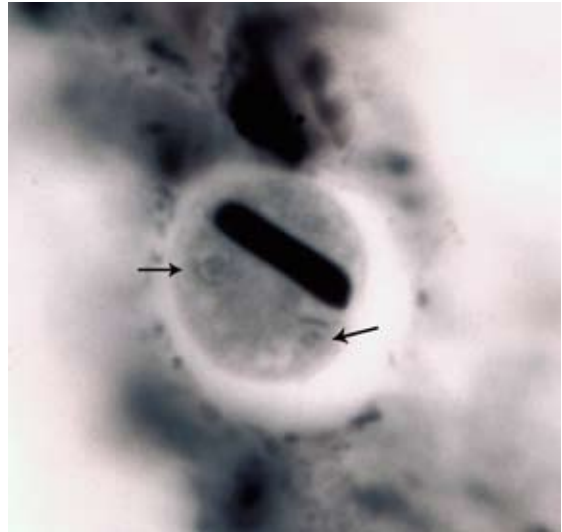


Figure 12.2. Cyst of *E. histolytica*. Two nuclei (arrows) and a smooth-ended chromatoidal bar can be seen. 15 μm .

Historical Information

Losch, in 1875,⁶ described clinical features of infection with *E. histolytica* and reproduced some aspects of the disease in experimentally infected dogs. Quincke and Roos, in 1893,⁷ distinguished *E. histolytica* from *Entamoeba coli*, a non-pathogenic amoeba acquired by the oral-fecal route and often found in the stool of asymptomatic individuals. Schaudinn, in 1903,⁸ described the trophozoites and cysts of *E. histolytica*. He died at the age of 35 of overwhelming Amoebiasis, a tragic outcome of self-experimentation. Councilman and Lafleur, in 1891,⁹ described the main features of the intestinal pathogenesis caused by *E. histolytica*. Boeck, in 1925,¹⁰ was the first to culture *E. histolytica*, while Dobell, in 1928,¹¹ fully elucidated its life cycle.

Life Cycle

The trophozoite (Fig. 12.1) is a facultative anaerobe metabolizing glucose as its main source of energy.¹² The trophozoite measures 20–30 μm in diameter, and the cytoplasm contains a single nucleus with a centrally located nucleolus, often termed the karyosome. In addition, lysosomes, and a remnant mitochondrion called a “crypton,”¹³ or mitosome, are present.¹⁴ The latter organelle contains several mitochondrial genes encoding for proteins associated with heat-shock responses.¹⁵ Discovery of a calreticulin-like molecule of 51 kDa may indicate the presence of a rough endoplasmic reticulum and Golgi apparatus,¹⁶ although neither is visible on electron microscopy. The cyst

(Fig. 12.2) is smaller than the trophozoite (10–15 μm in diameter), and at full maturity contains four typically round *E. histolytica* nuclei. Each nucleus ultimately will give rise to an individual trophozoite. Immature cysts may contain a single, smooth-ended chromatoidal bar, a crystalline-like condensation of ribosomes, and any number of nuclei up to four.

Ingestion of a single cyst is all that is necessary to initiate infection, making this organism one of the most efficient pathogenic protozoa known to infect humans.¹⁷ Each cyst undergoes excystation in the small intestine. The details of the cellular and molecular events leading to excystation have yet to be discovered. It is known that the cyst must receive certain specific environmental cues from the host, including sequential exposure to an acidic and a basic pH environment, in order for the four trophozoites contained within to breach the cyst wall and enter the small intestine. The newly emerged trophozoites then divide, and the eight parasites are carried by peristalsis to the large intestine.

There the trophozoite penetrates the perimucosal space and attaches to epithelial cells using lectin-carbohydrate interactions. This event is cytotoxic. They engulf and kill only the living cells encountered there (Fig. 12.3). The trophozoite divides by binary fission¹¹, occupying increasingly larger areas of tissue as it does so. This activity eventually causes flask-shaped ulcers to develop (Fig. 12.4). Hematogenous or lymphatic spread is then possible, but this aspect does not play a role in the life cycle.

Some trophozoites, instead of dividing, encyst in the lumen of the ulcer. Little is known regarding the environmental cues or cellular and molecular events that lead to cyst formation. Apparently, Amoebic proteasome activity is necessary for the process, since treating cultures with lactacystin caused marked inhibition of cyst formation.¹⁸ Despite the fact that we have

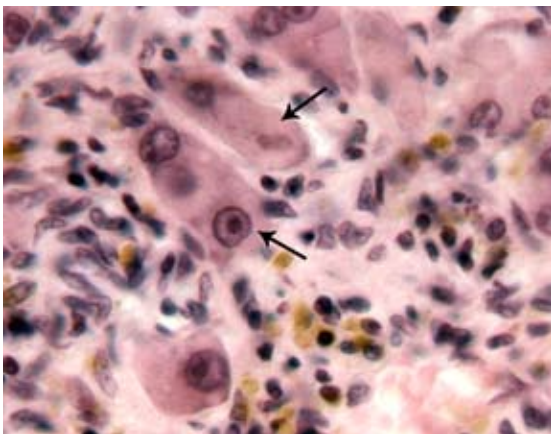


Figure 12.3. Trophozoites of *E. histolytica* in liver abscess (arrows). Note ingested host cells inside parasites.

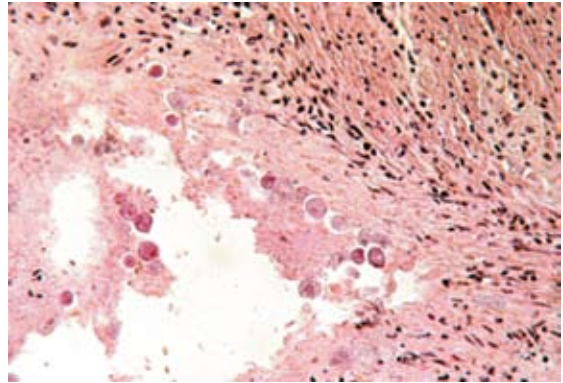


Figure 12.4. Low-magnification histologic section of Amoebic ulcer in small intestine. Organisms can be seen at living margin of ulcer.

known how to culture *E. histolytica* for over 70 years, encystment in vitro has never been achieved, although related species have been successfully induced to do so.¹⁹ During infection in the GI tract, cysts may be continuously produced and exit the host in feces. This stage can survive in warm, moist conditions for weeks without losing infectivity.

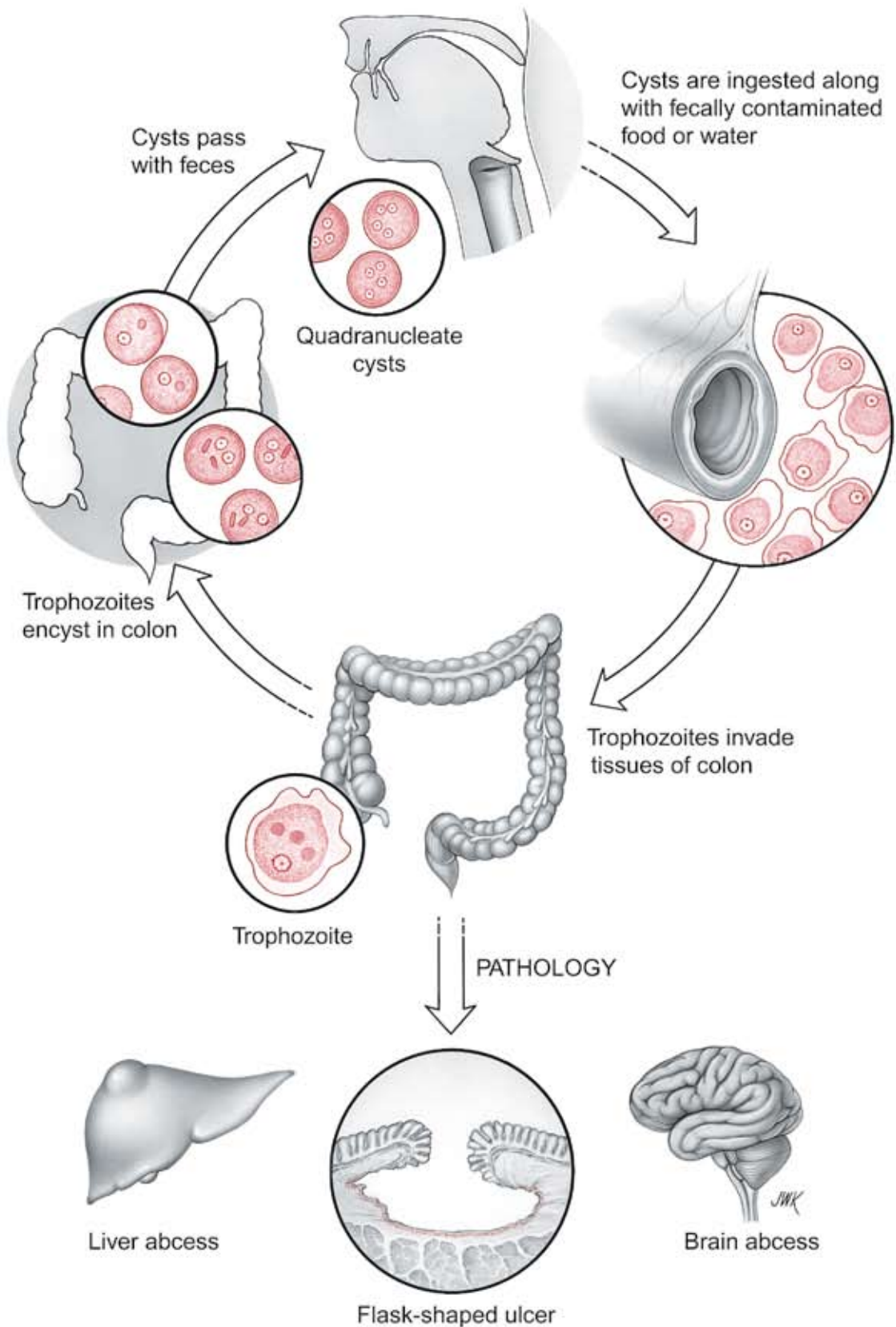
Cellular and Molecular Pathogenesis

Reviews dealing with the detailed aspects of its molecular biology and mechanisms of pathogenesis are available.^{4, 5, 20–23}

Amoebae must attach to host tissues as a necessary prerequisite for parasite-mediated cytotoxicity. Attachment is dependent upon interactions between epithelial cell membrane-bound N-acetyl-glucosamine and N-acetyl-galactosamine and at least two surface lectin proteins. The genes for both of the parasite lectins have been cloned and their cDNAs sequenced. One lectin is a 260 kDa protein,²⁴ while the other is 220 kDa. The heavy subunit of the 260 kDa lectin has a single transmembrane-spanning domain and a cytoplasmic domain related to β -2-integrins,^{25, 26} which may also participate in the attachment process. These surface lectins apparently also facilitate the parasite's evasion of the complement membrane attack complex,²⁷ although the mechanism(s) is not yet known. In vitro, *E. histolytica* can be inhibited from attaching to its target cells simply by adding free galactose to the medium (Fig. 12.5).²⁸ In this situation, cells and trophozoites co-exist. Attachment leads to cell death, which, at least in vitro, is calcium-dependent.²⁹ Several possible mechanisms for the actual killing of host cells have been proposed, all of which involve enzymes.³⁰

The trophozoite's surface membrane contains phospholipase A, neuraminidase, and a metallocollagenase.³¹ In addition, it secretes a minimum of four

Entamoeba histolytica



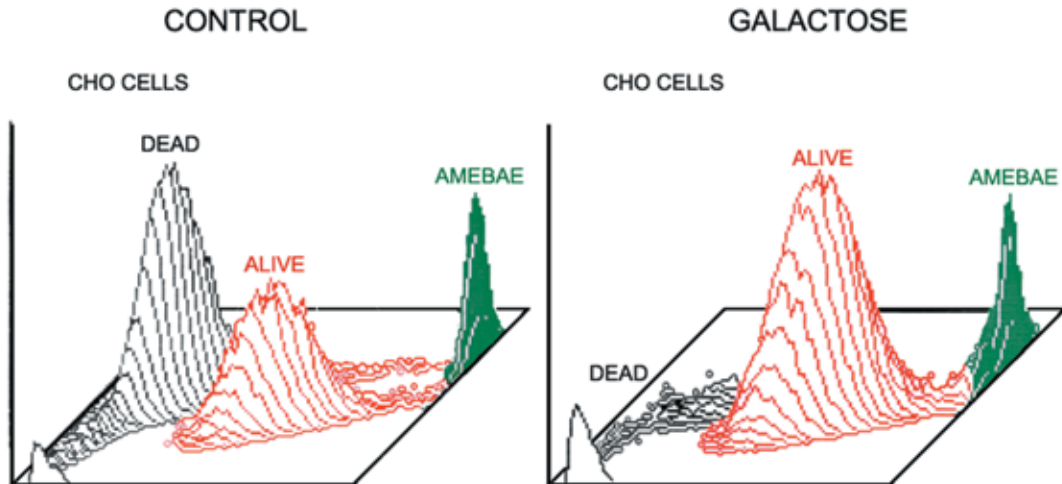


Figure 12.5. *in vitro* experiment showing that galactose-containing surface proteins are important for parasite cytotoxicity. Free galactose prevents attachment of amoebae to their target cells. CHO cells=Chinese hamster ovary cells. Redrawn after J. I. Ravdin.

cysteine proteases.³² These enzymes may also aid the parasite in moving through the extracellular matrix. Attachment elicits the secretion of a pore-forming peptide³³ that is biochemically related both in structure and function to saposins.³⁴ The pore-forming protein presumably plays a central role in lysing the host cell membrane. During attachment, the intracellular calcium levels of the target cell increase by 20 fold.³⁵ One intriguing finding is that the trophozoite may actually “lure in” new target cells, in this case lymphocytes, to the site of infection by upregulating the lymphotactic interleukin IL-8 in the surrounding colonic epithelial cells, while simultaneously inhibiting the upregulation of other cytokines known to play a role in inflammation.^{36, 37}

Protective immune mechanisms are short-lived and depend on the development of sIgA antibodies directed against parasite surface proteins involved in adherence to target cells.^{38–42}

In addition, cell-mediated killing of parasites can occur by induction of nitric oxide by the 220 kDa lectin, which up-regulates interferon- γ .^{43, 44} In experimental infections, polyclonal and monoclonal antibodies have been shown to be effective in protecting the host when they are directed against carbohydrate-binding lectins of the parasite,^{45, 46} emphasizing the central role these parasite proteins play in the pathogenesis of disease.

Clinical Disease

A comprehensive summary of all aspects of clinical disease is presented by Petri⁴⁷ and Wells and Arguedas.⁴⁸

Intestinal Amoebiasis

Many infected individuals are asymptomatic, and some go on to become carriers. Those who are symptomatic may experience a wide range of clinical manifestations. The most common consequence in symptomatic individuals is diarrhea, lasting more than a few days. Involvement of the entire bowel is usually associated with colicky pain, flatulence, alteration in the pattern of bowel movements, bloody stools, and eventually dysentery, indistinguishable from ulcerative colitis.

Generalized abdominal tenderness, with particular accentuation in the iliac fossae is frequently encountered on physical examination. Dysentery can either worsen, possibly resulting in a life-threatening situation, or resolve into a chronic state of ill health characterized by bouts of diarrhea, abdominal cramping, and abdominal discomfort. In the chronic condition, Amoeboma (large granuloma consisting of eosinophils, amoebae,

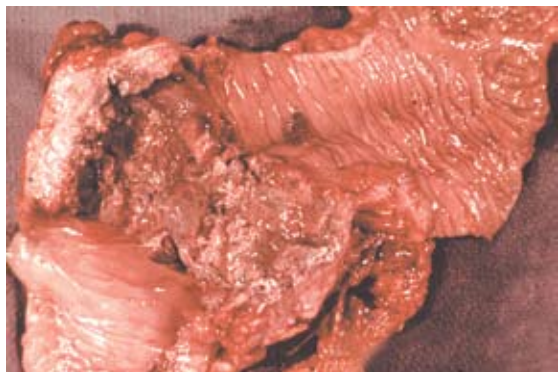


Figure 12.6. Portion of transverse colon showing extensive ulceration due to intestinal infection with *E. histolytica*.

and necrotic colonic tissue) are possible, presenting as palpable masses, and often misdiagnosed on barium enema as malignancies. If disease progresses, the colon may become atonic and may perforate at one or several points of ulceration (Fig. 12.6). If perforation occurs, symptoms and signs of peritonitis may develop. Acute colitis occurs more frequently in children.⁴⁷

The perforated, inflamed bowel may adhere to the abdominal wall, and the perforation may extend to the skin, causing cutaneous Amoebiasis, which can progress rapidly.⁴⁹ This situation may also occur in the perianal area as the result of invasion of the skin by the trophozoites emerging from the rectum.

Extraintestinal Amoebiasis

Amoebae can erode the wall of the large intestine until the circulation of the submucosa is breached. In that case, parasites disseminate throughout the body. The most common extraintestinal site is the liver,^{48, 50} occasionally presenting as a medical emergency. Invasion of liver tissue may occur after symptomatic intestinal Amoebiasis, or in cases where the colonic infection is asymptomatic. Nearly half of all patients with Amoebic liver abscess do not have a history suggestive of Amoebic colitis.

Hepatic Amoebiasis is a slowly progressive, insidious disease that typically begins as a nonspecific febrile illness, with pain and tenderness in the right upper quadrant of the abdomen. There is frequently referred shoulder pain. Examination at that time may reveal only a slightly enlarged, tender liver, or it may reveal a mass. Most patients with hepatic Amoebiasis have involvement of the right hepatic lobe, but the left lobe of the liver can also be infected; the enlargement and tenderness can be central or even left-sided.

The lungs are the next most common extraintestinal sites of infection.^{51,52} The major pleuropulmonary findings include effusion, pleurisy, empyema, or lung abscess. Occasionally, an hepatobronchial fistula is formed, resulting in a productive cough, with large amounts of amoebae-containing necrotic material. Embolism is rare. Rupture into the pericardium is usually fatal.

Cerebral Amoebiasis rarely occurs. The onset is usually abrupt and is associated with a high mortality rate unless diagnosed early on in the infection.⁵³

There is growing evidence suggesting that infection with HIV places those individuals at greater risk for developing extraintestinal Amoebiasis with more serious pathological consequences than those without HIV infection.⁶⁸



Figure 12.7. Trophozoite of *E. histolytica* in stool of patient suffering from Amoebic dysentery. Note Charcot-Leyden crystal “pointing” to nucleus. Also note numerous red cells in parasite cytoplasm. 30 μ m.

Diagnosis

Definitive diagnosis depends upon two approaches: detection of antigens in stool² or PCR on stool or tissue samples.⁵⁴ Either of these two modalities will most likely replace microscopy, based on their sensitivity, specificity, rapidity, ease of execution, and cost. An ELISA-based test is now in common use that is both rapid and specific for distinguishing these two organisms.² Unfortunately, only fresh or frozen fecal samples can be examined by these two methods.⁶⁸

Microscopy is still the only diagnostic modality in many facilities. If red blood cells are seen in the cytoplasm of a suspected trophozoite (Fig. 12.7), then a positive diagnosis of *Entamoeba histolytica* can be made. Without this telltale marker, misdiagnoses in favor of *Entamoeba dispar* will still be commonplace.

The presence of Charcot-Leyden crystals (Fig. 12.8) in stool are frequently seen when patients are suffering from disease caused by *E. histolytica*, but they are also seen with heavy infection caused by *Trichuris trichiura*, and therefore are not pathognomonic for Amoebiasis. PCR can also be useful for diagnosis of liver disease when used on aspirates derived from the abscess.⁵⁵

Since infection with *E. histolytica* invariably leads to long-lasting antibody production, antibody-based tests⁵⁶ are sometimes difficult to interpret, especially when done during chronic infection. The IHA and IFA tests are used together to rule in the possibility of extraintestinal disease, but are not definitive proof of infec-

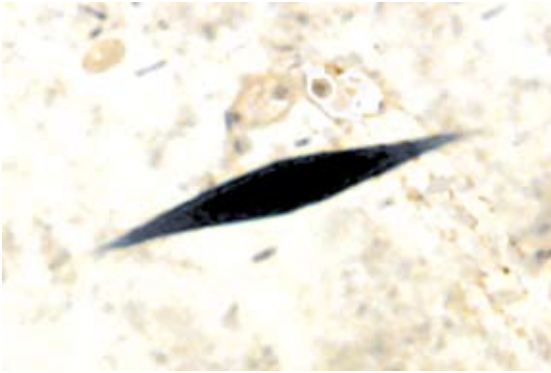


Figure 12.8. Charcot-Leyden crystal in stool of patient suffering from Amoebic dysentery. These crystals can also be found in patients infected with *Trichuris trichiuria* and *Strongyloides stercoralis*.

tion. Intestinal Amoebiasis must always be considered in any patient with protracted diarrhea and in all patients with dysentery. The diagnosis must also be considered in patients presenting with intraluminal colonic masses, because of the development of Amoebomas that resemble carcinoma of the colon. In extraintestinal Amoebiasis, identification of the lesion by the various modalities and the presence of a travel history compatible with Amoebiasis, in parallel with identification of amoebae in the colon, points to the diagnosis.

The erythrocyte sedimentation rate is typically increased, and the total PMN count may be low during active infection. Radiography of the abdomen may show enlargement of the liver and a fixed, raised diaphragm. In cases of perforation of the diaphragm, there may be evidence of consolidation of the left lower lobe of the lung or its lower segment, and a pleural effusion. A radionuclide or a CT scan often reveals the abscess; it may also show additional abscesses, which are rare. On ultrasonography, an Amoebic liver abscess usually appears as a round hypodense area that is contiguous to the liver capsule, usually without significant wall echoes.⁴⁷ Direct extension to the right pleural space and the lung is the most common form of intrathoracic Amoebiasis, but hematogenous spread may cause metastatic Amoebiasis in other portions of the lung and the pleura, as well as in other organs, notably the brain. Amoebic pericarditis can occur in the same manner.⁵⁷

Treatment

Metronidazole is the drug of choice⁵⁸ for the intestinal and extraintestinal infection. It can be given in equivalent doses orally or intravenously. Apparently, there are no naturally occurring metronidazole-resistant strains

of *E. histolytica*,⁵⁹ but they can be easily induced under laboratory conditions.⁶⁰ It is probably only a matter of time before they appear in human populations. Liver abscesses resolve slowly, despite treatment with the recommended high doses of metronidazole. This drug also has a few limitations and some adverse side effects. Use of alcohol is prohibited during treatment, as it induces a side effect similar to that caused by disulfiram therapy. Furthermore, it does not affect the cyst stage. Therefore, a cysticidal agent is also indicated. In fact, the latter alone may be adequate for treating asymptomatic cyst passers and those with non-dysenteric Amoebic colitis. Diloxanide furoate and iodoquinol (diiodohydroxyquin)⁶¹ both are effective at killing cysts. Iodoquinol is a relatively nontoxic drug.

There are few reports of patients surviving Amoebic abscess of the brain,⁵³ since, unfortunately, they are typically diagnosed too late. In the case of infection involving the pleural cavity, quick aspiration of an expanding pericardial effusion, combined with aggressive anti-Amoebic therapy, has saved lives of most of those suffering from this rare manifestation of the infection.⁶²

An Amoebic liver abscess⁴⁸ should be aspirated in the following circumstances: (1) if there is no clinical improvement within 48-72 hours despite appropriate medical therapy; (2) for abscesses greater than 10 cm in diameter; (3) when there is marked elevation of the diaphragm; (4) for abscesses in the left lobe; and (5) when there is negative serology, which might raise suspicion of a pyogenic abscess.^{63, 64}

Prevention and Control

Good public health practice, starting with ensuring the safety of drinking water supplies, and in some cases, watershed management, are the best long-term approaches to controlling most waterborne diarrheal disease agents. Thorough screening of food handlers by periodic stool examinations can identify carriers whose occupations would place the general public at risk. Recurrent outbreaks of Amoebiasis in mental institutions can be prevented by strictly adhering to appropriate sanitary practices, coupled with routine stool examinations of the patients. All infected individuals should receive treatment.

Vaccines against both the intestinal and extraintestinal infection may soon become available, based on recent encouraging progress made in the laboratory.⁶⁵⁻⁶⁸ Its primary use would be to vaccinate children living in hyper-endemic zones.

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