Amoebiasis: current status in Australia

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ABSTRACT

- *Entamoeba histolytica* is one of the most common parasitic infections worldwide, infecting about 50 million people and resulting in 40 000–100 000 deaths a year.
- In Australia, people at risk of infection include immigrants, travellers returning from countries of high endemicity, Indigenous people, and men who have sex with men.
- Clinical manifestations range from asymptomatic carriage to invasive disease. Amoebic colitis and amoebic liver abscess are the most common invasive manifestations observed in Australia.
- Diagnosis depends on a high index of suspicion and laboratory investigations. Molecular methods (using the polymerase chain reaction) are the most sensitive for identifying and differentiating *Entamoeba* species.
- Treatment should always include a luminal agent to eradicate colonisation, prevent spread and/or reduce the risk of invasive disease. Medical therapy can successfully cure invasive disease, including amoebic liver abscesses.

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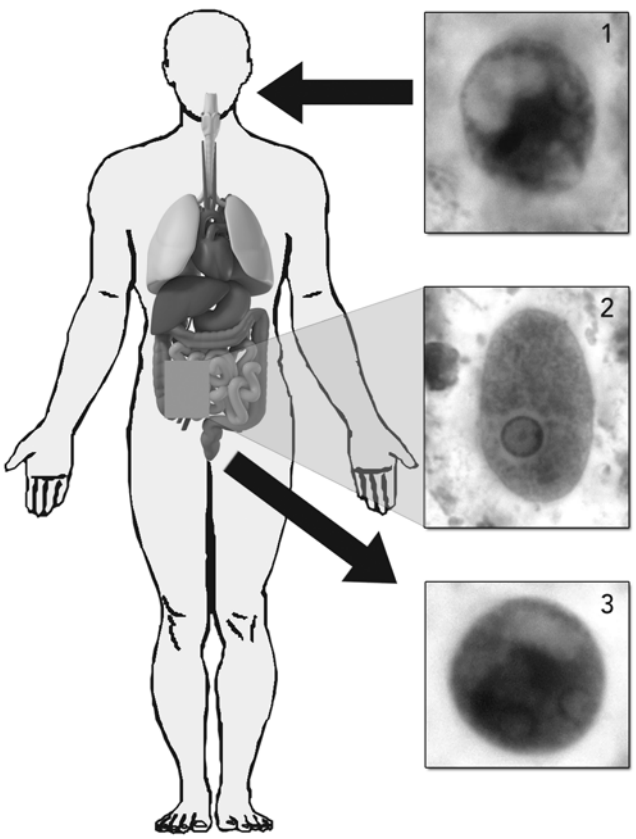
Amoebiasis, a disease caused by the intestinal protozoan parasite *Entamoeba histolytica*, is the third leading parasitic cause of death in humans after malaria and schistosomiasis. Globally, it is responsible for 40 000–100 000 deaths a year.[1,2] *E. histolytica* has a worldwide distribution and is endemic in Australia, with locally acquired disease occurring in northern Australia (predominantly in Indigenous people) and, recently, in Sydney, among men who have sex with men (MSM).[3-5] Other high-risk people in Australia include immigrants from countries of high endemicity (eg, India, South-East Asia) and travellers returning from such countries.[1,6]

The parasite

The genus *Entamoeba* comprises six species that colonise the human intestinal lumen. *E. histolytica* (Box 1) was initially thought to be a single species, but isoenzyme and molecular studies have led to the reclassification of *E. histolytica* into two morphologically identical species: the pathogenic *E. histolytica* and the non-pathogenic *E. dispar*.[1] *E. moshkovskii*, which is morphologically identical to *E. histolytica* and *E. dispar* but biochemically and genetically different, has been considered until recently to be primarily a free-living (non-pathogenic) amoeba. The early isolates of *E. moshkovskii* were free-living forms found in sewage, but human isolates have now been detected in North America, Italy, South Africa, Bangladesh, India, Iran and Australia.[6] The pathogenic role of *E. moshkovskii* is yet to be adequately defined, but recent studies suggest that infection with this species can cause diarrhoea and other intestinal disorders.[6] As *E. moshkovskii* is indistinguishable in its cyst and trophozoite forms from *E. histolytica* and *E. dispar*, it is not possible to differentiate the three species on the basis of traditional microscopic examination. Consequently, past studies on the prevalence of *E. histolytica* may be flawed if they did not consider the possible presence of *E. dispar* and *E. moshkovskii*.

Only one study has used adequate molecular techniques to determine the true prevalence of *E. histolytica*, *E. dispar and E. moshkovskii* in Australia. The study examined 5921 faecal samples submitted from patients with diarrhoea to a large metropolitan hospital in Sydney over a 4-year period. In 177 of the samples (3.0%), cysts and/or trophozoites microscopically resembling *E. histolytica/dispar/moshkovskii* were detected. Using molecular techniques, five patients were found to be infected with *E. histolytica*, 63 with *E. dispar* and 55 with *E. moshkovskii*. The study showed that, while *E. dispar* and *E. moshkovskii* are over 10 times more common, *E. histolytica* infections are nevertheless endemic in urban areas of Australia.[7]

1 Life cycle of *Entamoeba histolytica*



Infection occours by ingestion of mature cysts (1) from contaminated food, water or hands. Excystation occurs in the small intestine, releasing trophozoites (2), which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts (3). Both stages are passed in the faeces. The cysts are responsible for transmission, as they can survive for weeks in the external environment, whereas trophozoites are rapidly destroyed by the gastric environment or once they pass outside the body. (Original magnification x 1000; modified iron haematoxylin stain.) ◆

2 Possible complications of *Entamoeba histolytica* infection

Intestinal disease

Fulminant colitis

Toxic megacolon

Perianal disease with fistula formation

Extraintestinal disease

Liver abscess rupture:

- Pleuropulmonary disease (the most common complication, especially with right lobe abscesses)
- Intraperitoneal rupture
- Pericardial rupture (uncommon; usually associated with left lobe abscesses)

Other manifestations:

- Cerebral amoebiasis
- Genitourinary amoebiasis (rare; more common in women than men), eg, vaginal fistulae
- Primary cutaneous amoebiasis
- Amoeboma ◆

Groups at high risk of *E. histolytica* infection

High-risk groups include immigrants from countries of high endemicity, travellers to such countries, Indigenous Australians, and MSM.

Rates of asymptomatic carriage in immigrants to the United States are reported to be between 17% and 33%.[8] In contrast, immigrants to Australia have a documented carriage rate of about 2%.[9] In travellers returning to Australia, the carriage rate is unknown, and would vary greatly depending on the countries visited.[8] International data reflect similar asymptomatic carriage rates (0.3%–10%), but differences between the countries visited and/or precautions taken need to be considered.[6,10]

A number of cases of amoebiasis have been reported from northern Australia in Indigenous and non-Indigenous patients from both remote and urban regions.[3,11,12] While most cases predate the recognition of three separate species of *Entamoeba*, several recent cases have definitively identified *E. histolytica* as the cause of locally acquired invasive amoebiasis. The true prevalence of *E. histolytica* in northern Australian populations is unknown, as no studies using molecular techniques have been undertaken.

The prevalence of intestinal parasites in MSM is known to be higher than in heterosexual men. The possible explanation for this correlation is the practice of oral–anal sex.[13] As *E. histolytica* is transmitted via the faecal–oral route, MSM are at increased risk of being infected. In Taiwan and Japan, *E. histolytica* has emerged as an important parasitic infection among MSM.[14-17] This has also been documented in several Australian studies.[5,6] Clinicians should be aware of this association, as MSM are at risk of invasive disease.

Clinical presentation

Asymptomatic patients

In most *E. histolytica* infections, symptoms are absent or very mild and represent "non-invasive" disease.[1] Generally, asymptomatic patients never become symptomatic. They may excrete cysts for a short period of time, but the majority of these patients will clear the infection within 12 months. Patients with confirmed *E. histolytica* infection, even if they are asymptomatic, should be treated to eliminate the organism and prevent further transmission.

Symptomatic patients

Invasive amoebiasis

Only a small proportion of people infected with *E. histolytica* will go on to develop clinical disease, the most frequent manifestations being amoebic colitis and amoebic liver abscess.[1] As amoebiasis is not a notifiable illness, the rate of invasive disease in Australia is unknown. Furthermore, as cases become more common (there were three cases at St Vincent's Hospital, Sydney, in 2006), their "newsworthiness" diminishes, with the result that further reports on such cases are less likely to be published.

Amoebic colitis

Patients with amoebic colitis present with a history of several weeks of abdominal pain and diarrhoea (usually characterised by blood, mucus and faecal leukocytosis). Fever occurs in less than 40% of patients. Toxic megacolon is a complication in about 0.5% of patients, and may be a consequence of inappropriate corticosteroid treatment.[2] Steroids play an important role in the management of inflammatory bowel disease. As amoebic colitis is

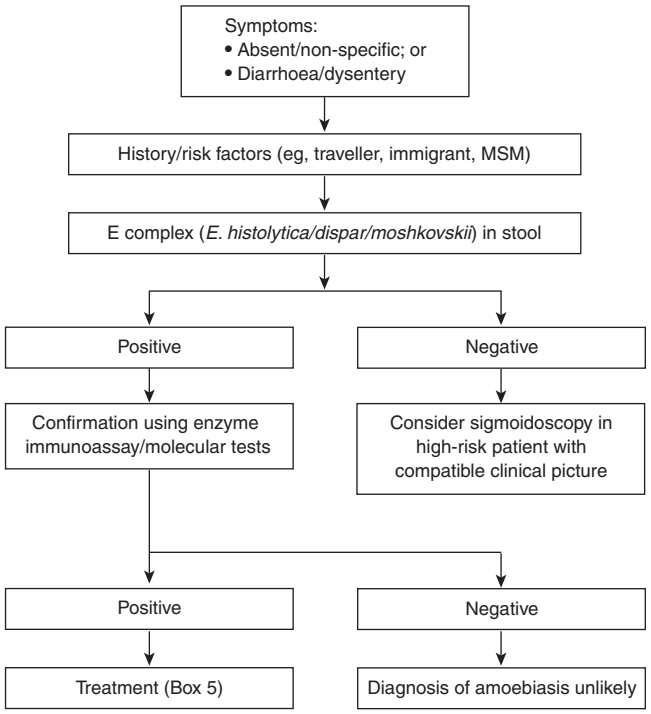
3 Diagnostic modalities

Diagnostic test	Presentation	Sensitivity	Specificity	Commercial kits available in Australia
Microscopy*				Permanent stain kits available from Oxoid Australia Pty Ltd (Adelaide, SA) and Fronine Laboratory Supplies Pty Ltd (Sydney, NSW)
Faeces	Colitis	30%–50%	10%–50%	
Liver aspirate	Liver abscess	< 5% (rarely seen)	100%	
Serology	Colitis	40%–60%	90%	Novagnost *Entamoeba histolytica* IgG kit (IHA) (Dade Behring, Deerfield, Ill, USA)
	Liver abscess	95%	98%	
Antigen detection	Colitis	80%–99%	86%–98%	Entamoeba CELISA Path kit (Cellabs, Sydney, NSW) and E. histolytica II kit (TechLab Inc, Blacksburg, Va, USA)
	Liver abscess	40%–100%	90%–100%	
Polymerase chain reaction	Colitis	80%–100%	85%–100%	Not commercially available
	Liver abscess	80%–100%	90%–100%	

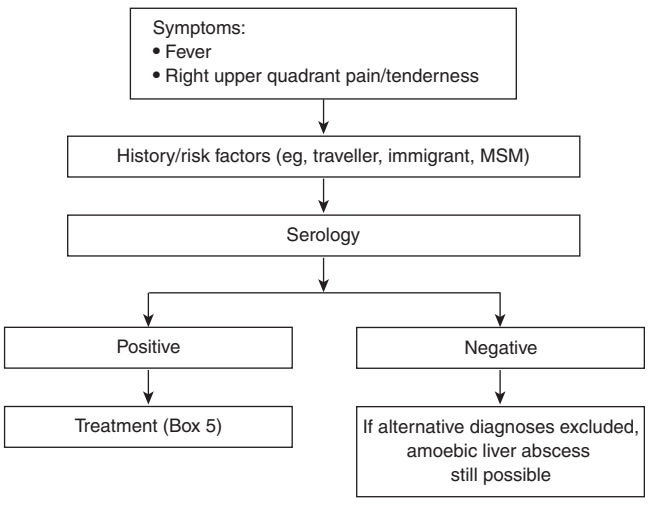
* Sensitivities are based on use of permanent stains. Sensitivity of microscopy increases when colonoscopy specimens are used. ◆

4 Diagnosis of amoebiasis

A: Intestinal amoebiasis



B: Amoebic liver abscess



MSM = men who have sex with men. ◆

relatively uncommon in Australia, and as inflammatory bowel disease is part of the differential diagnosis of amoebic colitis, clinicians may overlook the less common diagnosis and treat patients inappropriately with corticosteroids. Progression to fulminant or necrotising colitis occurs in 0.5% of patients and is associated with a high mortality rate (40%) secondary to perforation, peritonitis or massive bleeding. Uncommon manifestations include amoebomas, rectovaginal fistulas and cutaneous involvement (especially after inappropriate steroid use) (Box 2).

Amoebic liver abscess is the most common extraintestinal manifestation of amoebiasis seen in Australia and has been reported in travellers and immigrants. Patients usually present within 5 months of contracting the disease. However, prolonged latency may occur.[10,11] Clinical symptoms include fever (in 87%–100% of patients), malaise, and right upper quadrant pain with no concomitant colitis (in 60%–70% of patients). Biochemical parameters are usually abnormal but non-specific. Imaging studies (ultrasound, computed tomography and magnetic resonance imaging) have excellent sensitivity for detection of liver abscesses but are unable to distinguish amoebic liver abscesses from pyogenic abscesses or necrotic tumours. *E. histolytica* cysts and trophozoites are rarely found in the stools of patients with liver abscess, the majority of patients having no intestinal symptoms or history of dysentery. Therefore, the diagnosis depends on a high index of suspicion (eg, consistent travel history) and appropriate laboratory investigations. Complications associated with abscess rupture are dependent on which body cavity they rupture into. Such complications are rare, with the last documented case in Australia being in 1977.[11] Mortality rates are low (< 1%) with appropriate treatment.

Diagnostic techniques

The diagnosis of amoebiasis is confirmed either by detecting *E. histolytica* parasites in the faeces or by detecting an antibody response to the parasite in the serum (Box 3, Box 4).

Detection of *E. histolytica* in the faeces

Microscopy relies on identifying *E. histolytica* cysts and trophozoites. It is performed on fixed faecal smears stained with a permanent stain (iron haematoxylin or trichrome). Infection with *E. histolytica* cannot be diagnosed on the basis of morphological criteria alone. Historically, the presence of haematophagous trophozoites (trophozoites containing ingested red blood cells) was regarded as suggestive of *E. histolytica* infection. However, this finding is rarely seen and has been found to occur also in non-pathogenic species.

Unfortunately, many laboratories do not routinely perform permanent stains as they are time-consuming and laborious and require specific expertise. Multiple samples are required, as the organism is shed intermittently and the sensitivity of microscopy is poor.[18] As *E. histolytica* is morphologically identical to the non-pathogenic species *E. dispar* and *E. moshkovskii*, microscopy can not distinguish between the three species, and further testing is required for speciation.[19-21]

Colonoscopy and flexible sigmoidoscopy are useful in patients with acute colitis when *E. histolytica* infection is suspected on clinical grounds but not detected in stool samples. Examinations of scrapings and biopsies for trophozoites have a higher sensitivity than examinations of faecal specimens.

Culture techniques have been used to detect *E. histolytica* for close to 100 years. However, culture methods are time-consuming, laborious and often unrewarding, with a sensitivity of only about 50%. Further testing is required for speciation. Thus, culture methods are restricted to specialised parasitology research laboratories.

Antigen detection methods use monoclonal antibodies directed against various proteins of *E. histolytica*. Some of these assays determine the presence of the *E. histolytica/dispar/moshkovskii* group, while others are specific for *E. histolytica* only. The two stool

5 Treatment of amoebiasis

Asymptomatic carriage (treat with *luminal* amoebicide ONLY)

- Oral paromomycin* 500 mg three times daily for 7 days

Invasive disease (treat with tissue amoebicide and luminal amoebicide)

- Oral metronidazole 750–800 mg three times daily for 6–10 days

  OR
- Oral tinidazole 2 g once daily for 2–3 days (up to 10 days) and oral paromomycin* 500 mg three times daily for 7 days

Liver abscess (treat with tissue amoebicide and luminal amoebicide)

- Oral or intravenous metronidazole 750–800 mg three times daily for 14 days

  OR
- Oral tinidazole 2 g once daily for 5 days and oral paromomycin* 500 mg three times daily for 7 days

* Paromomycin is now the luminal agent of choice (Special Access Scheme approval is required). Alternative luminal agents are diloxanide furoate (however, production was ceased in 2003 and the drug is unavailable in Australia) and iodoquinol (availability in Australia is limited). ◆

antigen detection kits commercially available in Australia — namely, the Entamoeba CELISA Path kit (Cellabs, Sydney, NSW) and the E. histolytica II kit (TechLab Inc, Blacksburg, Va, USA) — are able to distinguish *E. histolytica* from *E. dispar* and *E. moshkovskii*. For the more commonly used kit, sensitivities range from 80% to 99% and specificities from 86% to 98%.[18,22-24] However, in a recent Australian study, the TechLab *E. histolytica* enzyme-linked immunosorbent assay (ELISA) test was found to cross-react with *E. dispar* and *E. moshkovskii*, thus limiting the utility of the test.[6] The main advantage of these tests is that they are rapid (same day) and their interpretation is less subjective than microscopy.

Molecular methods using the polymerase chain reaction amplify *E. histolytica* genes from extracted faecal DNA. Sensitivity and specificity are high (80%–100% and 100%, respectively).[22,25,26] The advantage of molecular detection is that it is extremely sensitive (able to detect < 1 parasite) and reliably able to differentiate non-pathogenic *Entamoeba* species from *E. histolytica*. Drawbacks of this method are the high level of expertise required and the cost. The availability of the test is limited, but at our institution it has proved extremely valuable.

Antibody tests (serology) are specific but have varying sensitivity, depending on the presence or absence of invasive disease and the type of invasive disease. The sensitivity of serology is about 95% for amoebic liver abscess and 84% for invasive intestinal disease.[2] Antibody testing to diagnose carriage of *E. histolytica* is unhelpful, as the sensitivity is only 8%.[2] A positive antibody test confirms the suspicion of invasive amoebic disease provided the patient has not had a disease episode in the recent past, as antibody titres can remain high for years after successful therapy.

Treatment

Asymptomatic carriers of *E. histolytica* should be treated with a luminal agent to minimise the spread of disease and the risk of developing invasive disease.[1]

In patients with invasive disease, metronidazole should be used in conjunction with a luminal agent to eradicate the organism (Box 5).

The role of surgery is generally limited to patients with complications of invasive disease. Surgical drainage is generally unnecessary in amoebic liver abscess, as cure can be achieved with medical therapy alone.[27,28] The role of radiologically guided percutaneous therapeutic aspiration in uncomplicated amoebic liver abscess is controversial,[29] but it has been shown to be of some clinical benefit in patients with large abscesses. Aspiration should be considered in patients with an uncertain diagnosis, lack of response to medical therapy (persistent fever > 4 days), and large abscesses at risk of rupturing (especially left lobe abscesses, as these may rupture into the pericardial space).

Conclusion

Clinicians should be aware of *E. histolytica* infection, as it is present in Australia as both a local and imported disease. High-risk patients include immigrants, Indigenous people and MSM. Without the appropriate clinical suspicion and laboratory investigations, the diagnosis may be missed, with possible harmful consequences.

Competing interests

None identified.

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