I

Protozoa

1 Introduction

Protozoa are unicellular organisms in which the single cell is highly specialized to perform the functions of respiration, digestion, excretion and reproduction.

Their basic structure comprises a nucleus and cytoplasm. The nucleus is composed of a chromatinic rim or nuclear membrane enclosing a fine network of reticulum with a karyosome (inaccurately called the nucleolus), which is a condensed aggregate of chromatin. The location of the karyosome and the chromatin pattern is helpful in identifying different species of protozoa, e.g. the ciliated protozoan *Balantidium coli* has a micronucleus and macronucleus, whereas haemoflagellates have a kinetoplast or accessory nucleus.

The cytoplasm consists of an ectoplasm and endoplasm. The ectoplasm is the outer transparent layer that performs a protective as well as locomotory and sensory function. The endoplasm is the internal granular part of the cell and performs the nutritive, reproductive and excretory functions. A limiting membrane or plasma membrane binds the entire cell.

Protozoans exist in two main forms: the trophozoite, which is the active, invasive stage; and the cyst, which is the resistant stage that can survive unfavourable conditions.

Reproduction of protozoa may be asexual through binary or multiple fission, sexual through conjugation and syngamy or gametogony.

To survive, protozoa must be transmitted from one host to another. This is achieved by either transforming into the cyst stage or by changing their method of reproduction from asexual to sexual, e.g. in malaria, the sexual cycle or gametogony takes place in the mosquito and the asexual cycle or schizogony takes place in man. This process is known as alternation of generation. In leishmania and trypanosomes the haemoflagellate protozoa metamorphose into the amastigote (or leptomonad) forms and flagellate forms in both the insect and human host by means of asexual reproduction.

A classification of pathogenic protozoa is provided in Table 1 on p4.

Table 1 – Classification of pathogenic protozoa based on locomotive function

Class	Locomotion organ	Principal human pathogens
Mastigophora	flagella	*Leishmania*
		Trypanosoma
		Giardia★
		Trichomonas★
Rhizopoda	pseudopodia	*Entamoeba*
		Naegleria★ and *Acanthamoeba*
Ciliophora	cilia	*Balantidium*
Sporozoa	none	*Plasmodium*★
		Toxoplasma★
		Cryptosporidium★
		Isospora★
		Pneumocystis★

★Infections commonly encountered in Australia.

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Amoebiasis

PARASITOLOGY

Amoebiasis refers to infection by *Entamoeba histolytica* and is found most frequently in tropical and subtropical regions where the socio-economic status and sanitation are poor. Amoebiasis is indigenous to some developed countries like Australia. In New York, most reported cases of amoebiasis occur among the homosexual population, described with other intestinal parasitic and sexually transmitted diseases as the 'gay bowel syndrome' (Williams *et al.* 1971).

E. histolytica occurs as both trophozoite and cyst stages. The trophozoite is the invasive form; the cyst stage the infective form.

Man acquires infection by ingesting amoebic cyst-contaminated food or water (most outbreaks are water borne) (Fig. 2.1). Rarely, trophozoites can directly invade abraded genital organs ('gay bowel' transmission as in homosexuals). Four metacystic trophozoites are released into the small intestine from each cyst and migrate to the colon where they mature. These trophozoites invade the submucosa probably by releasing digestive enzymes such as protease, hyaluronidase and mucopolysaccharidase. The incubation period varies from 1 to 3 weeks.

Trophozoites measure 12–60 μm and have transparent ectoplasm with pseudopodia. The endoplasm is granular and may contain engulfed cells, particularly red blood cells, which indicate invasive activity (Fig. 2.2). The nucleus has a centrally located karyosome with fine peripheral chromatin (Fig. 2.3). Multiplication is by binary fission. Amoebic cysts measure 10–20 μm and contain two to four nuclei with the identical nuclear structure as trophozoites. In addition, chromatoidal bodies (cigar-shaped, darkly stained or refractile) are present.

Other types of non-pathogenic Entamoebae may be encountered in stools and need to be distinguished from *E. histolytica*. *E. hartmanni* is a smaller species measuring < 12 μm; *E. coli* is larger measuring 10–35 μm, containing two to eight large nuclei with splinter-shaped chromatoidal bodies and eccentrically located karyosomes. *Iodamoeba butschlii* has one nucleus with a large central karyosome and a prominent glycogen vacuole that stains brown with iodine. *Endolimax nana* is small (5–10 μm) with a prominent central karyosome, no peripheral chromatin and clear cytoplasm (Table 2.1).

Infection with *E. histolytica* can be intestinal or extra-intestinal.

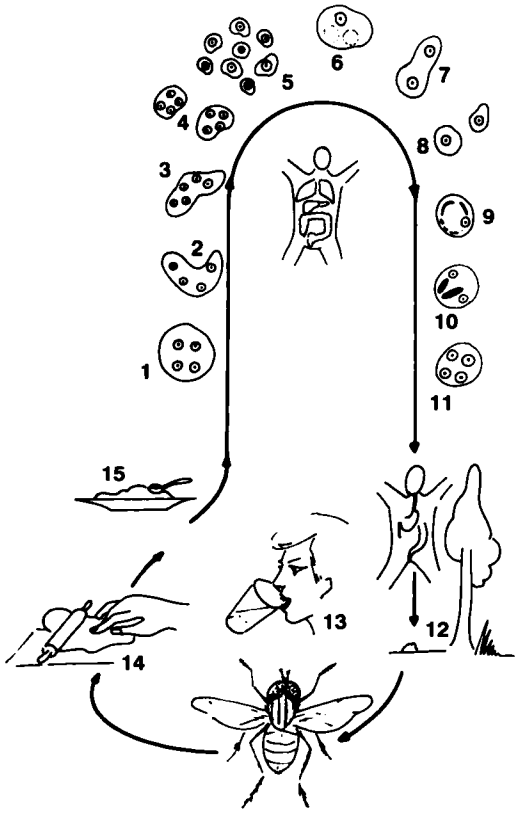


Figure 2.1 – Life cycle and transmission of *E. histolytica*. (1) The mature 4-nucleate cyst enters the alimentary canal; (1–5) the 4-nucleate amoeba that leaves the cyst and divides to form eight individual amoebae; (6–10) multiplication in the intestinal lumen; (9–11) cyst formation; (9) typical uninucleate stage with peripheral chromidial bodies; (10) binucleate cyst with chromidial bodies; (11) mature 4-nucleate cyst; (12–15) possible modes of infection: (13) drinking water contaminated with faeces; (14) by contaminated communal dishes; (15) flies transport cysts from faeces to food.

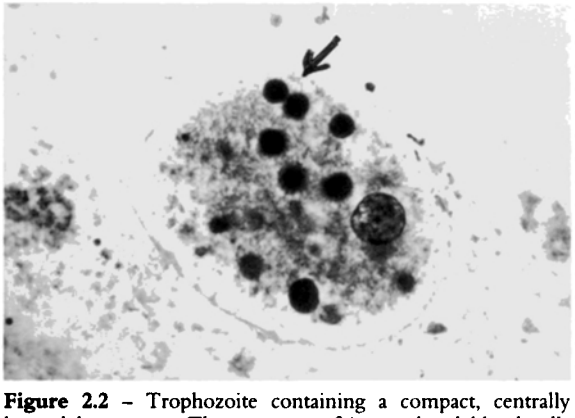


Figure 2.2 – Trophozoite containing a compact, centrally located karyosome. The presence of ingested red blood cells (arrow) is diagnostic of *E. histolytica* (trichrome ×1000).

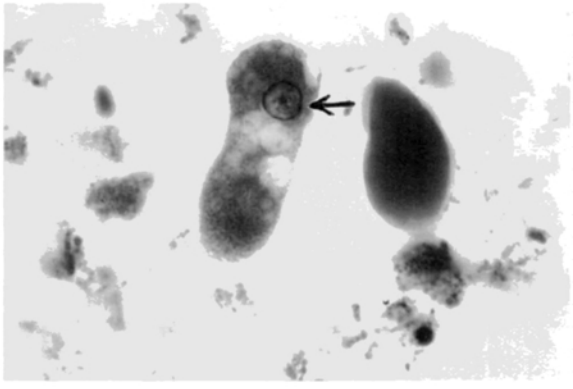


Figure 2.3 – Trophozoite showing the typical nuclear structure of *E. histolytica* with a centrally located, small, compact karyosome and finely granular chromatin distributed evenly over the inner surface of the nuclear membrane (arrow) (trichrome ×1000).

INTESTINAL AMOEBIASIS

Presentation

Intestinal infection with *E. histolytica* results in different clinical patterns, varying from no symptoms to recurrent episodes, fulminant disease or milder forms. Fulminating intestinal amoebiasis usually affects elderly, debilitated patients and the immunosuppressed. Diarrhoea may be mild or severe, with or without blood or mucus. In severe cases with bloody diarrhoea, there may be associated systemic disturbances such as cramping, abdominal pain, fever, anorexia, tenesmus and flatulence. The faecal volume is smaller than in bacillary dysentery. Infrequently, the appendix may be involved, simulating acute appendicitis. Other differential diagnoses include ulcerative colitis, bacillary dysentery and Crohn's disease.

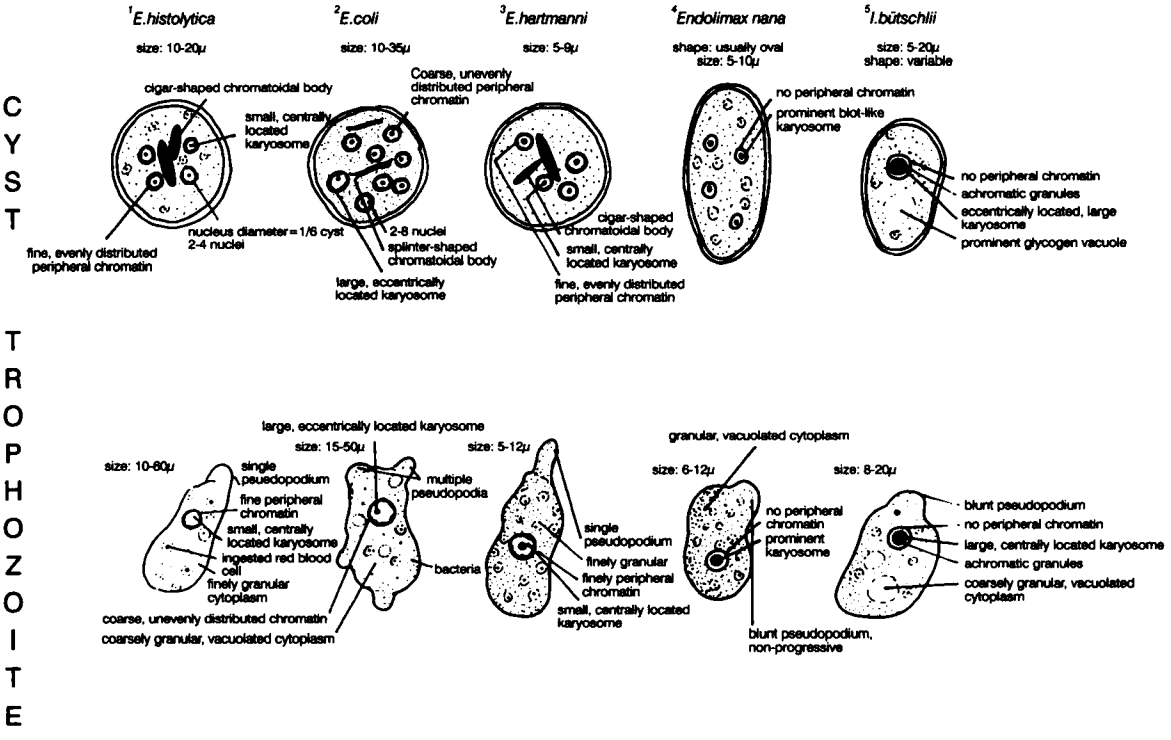
Complications

- Severe dehydration, hypovolaemia and electrolyte imbalance
- Acute haemorrhage and anaemia
- Toxic dilatation of the colon (toxic megacolon)
- Perforation with peritonitis
- Amoeboma (see below)

Amoeboma

There may be no preceding history of amoebiasis. The presentation is that of chronic diarrhoea, anorexia, anaemia, abdominal pain and loss of weight. Up to 5% of patients with intestinal amoebiasis may present with

Table 2.1 – Comparison of cysts/trophozoites of amoebae in stools



a localized granulomatous reaction in the bowels, which is usually solitary but may be multiple. The tumour-like masses occur in the caecum and rectum and have been mistaken for carcinoma and abdominoperineal resections have been performed. Biopsy and histological examination is necessary for proper distinction.

Pathology

Intestinal amoebiasis produces mucosal ulcerations which are most frequently seen in the caecum, although the ascending colon, sigmoid colon, appendix and, less frequently, the ileum can be involved. Early lesions consist of minute erosions with congestion and oedema of the surrounding mucosa. When fully developed, 'flask-shaped' ulcers with overhanging edges are present (Fig. 2.4) and the intervening mucosa, apart from congestion and oedema, is normal. Sometimes, mucosal inflammation can be generalized and mimics bacillary dysentery. When multiple ulcers are present, the appearance can resemble pseudomembranous colitis.

Microscopically, superficial erosions of the mucosa are filled with inflamed granulation tissue. Trophozoites are rarely seen in these lesions and serological tests are needed to confirm the diagnosis. Fully developed ulcerated lesions show necrosis with absent or scanty inflammatory reaction with occasional eosinophils. The trophozoites, present at the junction of the necrotic debris and viable tissue, are characteristically surrounded by a clear halo with a tiny central karyosome, delicate nuclear membrane and a 'bubbly' or vacuolated cytoplasm, which sometimes contains ingested red cells (Fig. 2.5). Inflammatory reaction, when present in the adjacent mucosa, consists of plasma cells, lymphocytes, eosinophils and only a few neutrophils. The organisms require distinction from macrophages. The periodic acid-Schiff (PAS) stain is useful to highlight amoebae but macrophages will also be stained so that nuclear details must be studied for correct identification.

Investigations

1. *Stool examination* – diagnosis is based on the finding of motile trophozoites in freshly examined stool or mucosal ulcer smears. Three separate stool samples should be examined. The stools contain pus

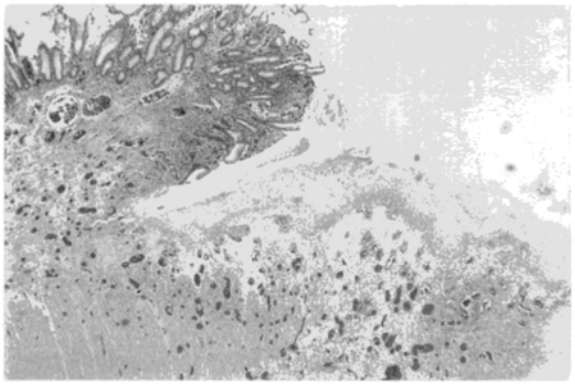


Figure 2.4 – Flask-shaped ulcer caused by *E. histolytica* (H&E × 100).

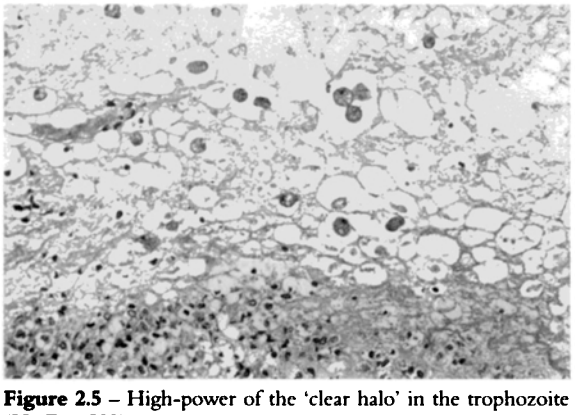


Figure 2.5 – High-power of the 'clear halo' in the trophozoite (H&E × 200).

cells, but in much smaller quantities than in bacillary dysentery. The demonstration of amoebic cysts does not necessarily signify acute infection. The ocular micrometer, a glass disc with linear scale etched on it, is recommended by the WHO for the examination and identification of parasites (González-Ruiz and Bendall 1995). It is useful for the differentiation of the cysts of *E. histolytica* from *E. hartmanni*. Size is the most obvious difference. *E. hartmanni* cysts are 4–8 μm diameter and those of *E. histolytica* are 7.5–15 μm and accurate measurement allows proper distinction.

2. *Serology* – antibodies to *E. histolytica* may be detected in the serum by a variety of immunological methods, e.g. isoenzyme electrophoresis of *E. histolytica* can distinguish the zymodeme pattern of pathogenic or invasive amoebae from non-pathogenic, non-invasive amoebae (Garcia and Bruckner 1993).
3. *Histologic examination* – sigmoidoscopy and biopsy for histologic examination provides definitive diagnosis.

Treatment

Patients with intestinal amoebiasis require rehydration, blood transfusion for haemorrhage, and correction of electrolyte imbalance. Surgery is indicated for peritonitis developing from perforation, and proper resuscitation with fluids, nasogastric intubation, and a combination of antibiotics such as metronidazole, gentamicin and ampicillin are required. In uncomplicated cases, the drugs used should control tissue invasion and eliminate amoebae in the intestinal lumen

Tissue amoebicidals

- Metronidazole 500–750 mg/day for 7–10 days for adults. Children > 12 years adult dose, 7–12 years one-half adult dose, 3–7 years one-third adult dose and infants one-quarter adult dose
- Tinidazole in a single dose 50 mg/kg, which gives almost a 100% cure rate, while others have reported lower cure rates. Adult dose is 2 g
- Paromomycin 30 mg/kg/day for 5 days (Sullam *et al.* 1986)

Tinidazole and metronidazole are best avoided in the first trimester of pregnancy. Tissue amoebicides should be used together with lumen amoebicides for complete eradication.

Lumeinal amoebicidals

- Diloxanide furoate – dose 20 mg/kg/day in divided doses for 10 days, has a very high cure rate. It is generally not indicated for children < 2 years
- Tetracycline 15 mg/kg/day for 10 days. It is generally not indicated for children < 9 years

Successful treatment of intestinal amoebiasis is achieved when three consecutive stool samples, examined 14 days after completion, are negative for *E. histolytica*.

EXTRA-INTESTINAL AMOEBIASIS

Extra-intestinal amoebiasis may occur as amoebic hepatitis, abscesses in the liver, brain or pericardium, cutaneous amoebiasis or pulmonary amoebiasis.

Amoebic hepatitis

Amoebic hepatitis is generally preceded by intestinal disease although there may be no definite preceding history. Unlike a definite abscess, there is no localized lesion. The liver enlargement may be accompanied by fever. Serological tests are usually positive while liver function tests are normal. Amoebic hepatitis occurs in endemic areas and should be suspected particularly in patients with a history of amoebic colitis.

Amoebic liver abscess

This is the commonest extra-intestinal lesion. Amoebic liver abscesses have an insidious onset. A history of preceding intestinal amoebiasis is generally obtainable. There is a long latent period between intestinal infection and liver abscess. The commonest site of the abscess is the right lobe. Presenting symptoms are fever, pain in the right hypochondrium, sometimes referred to the right shoulder tip. Pain may be aggravated by side to side movement and may be pleuritic. The liver is enlarged and tender, with intercostal tenderness on the right side. Abscesses on the left side may present as tender epigastric masses. Clinically, it is not possible to distinguish between an amoebic and non-amoebic liver abscess.

Patients with pleuritic chest pain, enlarged liver, fever and abdominal pain, and a space-occupying lesion on CT and ultrasonography have pyogenic liver abscess, malignancy, congenital hepatic cyst and hydatid disease as differential diagnoses. Differentiation from a primary pyogenic abscess may be difficult as it is often difficult to demonstrate amoebae in the pus. Aspiration, which is not usually done for amoebic liver abscesses, reveals sterile pus. Hepatocellular carcinoma is often associated with chronic liver disease or portal hypertension. Serum α-fetoprotein is usually raised in hepatocellular carcinoma. Hydatid disease may present as a solitary liver abscess. The clinical history and serology for hydatid disease and amoebiasis may distinguish the two conditions.

Complications occur as a result of extension of the liver abscess. The abscess may rupture into the pleural or peritoneal or pericardial cavity, colon or portal vein and hepatic vein with resultant thrombosis.

Investigations

1. *Blood film examination* – white cell count is raised with predominance of polymorphonuclear leukocytes
2. *Stool examination* - generally produces negative results
3. *Chest X-ray* – shows elevation of the right hemidiaphragm (Fig 2.6). CT scan or ultrasound will reveal one or more space-occupying lesions of the abdomen
4. *Serology* – extra-intestinal amoebiasis generally shows a positive serology with antibodies being present in > 90% of patients with invasive amoebiasis. The serological test was traditionally the amoebic indirect haemagglutination test (IHA) which is positive in 82–98% of patients with symptomatic amoebic colitis but ELISA assays are now producing comparable results

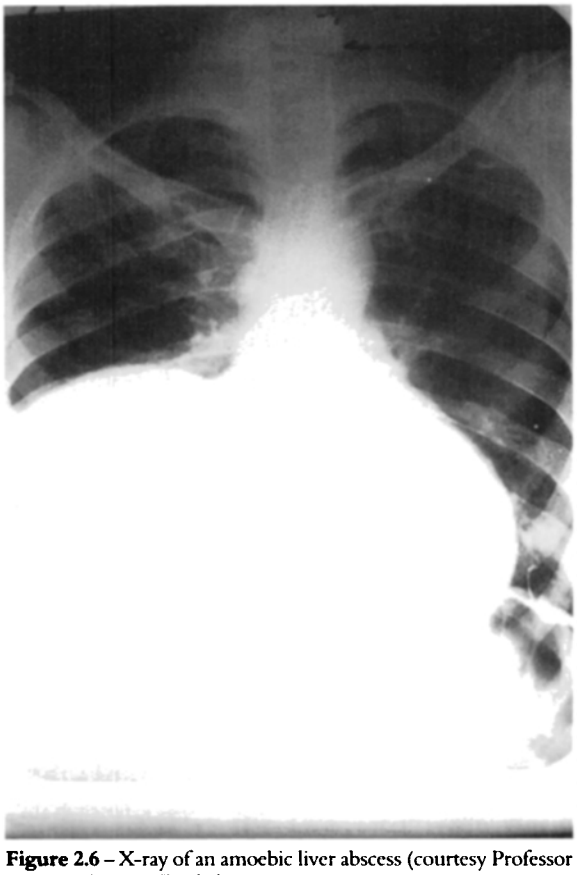


Figure 2.6 – X-ray of an amoebic liver abscess (courtesy Professor C. Kirre, Harare, Zimbabwe).

Pulmonary amoebiasis

Usually secondary to hepatic amoebiasis. It commonly occurs in the right lower lobe of the lung as a result of rupture of a right lobe of the liver abscess. Rupture into the pleural cavity will produce an empyema.

Haematogenous or lymphatic spread can also cause pulmonary amoebiasis.

Cutaneous amoebiasis

Cutaneous amoebiasis is secondary to extension of rectal infection to the perineum or as a result of diagnostic or therapeutic aspiration of liver abscess or empyema, or rupture of an amoebic abscess through the anterior abdominal wall.

Cerebral amoebic abscess

Cerebral amoebic abscesses are very rare and usually solitary. Clinical manifestations are those of a space-occupying lesion or meningitis. Prognosis is poor unless diagnosed and treated early. A CT scan of the brain will demonstrate a space-occupying lesion.

Pathology

Amoebic abscess occurs most frequently in the right lobe of the liver and results from trophozoites from the bowel entering the portal system via the portal veins. In the very early stages, a number of small necrotic foci form in the centres of the liver lobules and these coalesce into one or more big abscesses. As the abscess develops, the liver enlarges and contains grey, ill-defined globular patches up to 25 mm in diameter. These liquefy and coalesce, forming the characteristic ragged abscess cavities filled with viscid, chocolate brown pus (anchovy sauce) which contains necrotic liver tissue and blood. Trophozoites can often be found in this pus as well as in large numbers in the abscess wall but cysts are never found.

Characteristically, the abscess displays three zones: a centre containing yellow or grey opaque material, a mid-zone of stroma and a shaggy outer fibrinous wall invaded by trophozoites. The adjacent liver is oedematous and infiltrated with chronic inflammatory cells. Very rarely, abscesses may calcify and require differentiation from a hydatid cyst.

The pathology of amoebic abscesses in other sites such as lung and brain are similar in appearance. In the brain, the left hemisphere is more frequently involved and abscesses are usually multiple. Gliosis and non-specific inflammation is seen around the abscess. Cutaneous lesions can be ulcerative or condylomatous.

Abscesses in other sites are very similar in appearance. In the brain, the left hemisphere is more frequently involved and abscesses are usually multiple. Gliosis and non-specific inflammation is seen around the area of liquefactive necrosis. In the skin, the lesions can be ulcerative or condylomatous.

Treatment

As a rule, amoebic abscesses in various sites are treated by resection and collections of pus in various spaces such as the pleura are treated by drainage. Specific chemotherapy with amoebicidal drugs should be given in all instances. Pericardial abscesses are associated with very high mortality rate. These are similarly treated with surgical drainage and amoebicidal drugs and the patients should be treated in an intensive care unit.

The drug treatment for extra-intestinal amoebiasis is the same regardless of the site.

- Metronidazole 40 mg/kg/day in three divided doses for 10 days is effective
- Tinidazole 35 mg/kg in divided doses given 12 h apart for 5 days has a high cure rate

In the past, dehydroemetine 1 mg/kg/day maximum 65 mg/day for 10 days was the drug of choice. However, it is more toxic and requires cardiac monitoring.

Tissue amoebicides should be combined with luminal amoebicides for hepatic amoebiasis (see above). Surgical intervention in hepatic amoebiasis is rarely necessary today except for large abscesses and impending rupture. Successful treatment of hepatic amoebiasis is the disappearance of symptoms and signs and reduction in size of the abscess on CT scan within 10 days.

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3

Non-intestinal Amoebiasis

Non-intestinal amoebiasis is caused by a group of free-living soil amoebae. The two species that cause disease in humans are Acanthamoeba and Naegleria. Acanthamoeba was thought to be the causative agent in the first reported case of primary amoebic meningo-encephalitis in 1965, seen in an Australian patient (Fowler and Carter 1965), but this case was later found to be caused by *Naegleria fowleri* (Bull *et al.* 1968). The distribution of primary amoebic meningo-encephalitis is universal and includes Australia, Belgium, Brazil, Czechoslovakia, the UK, India, New Zealand, Nigeria and the USA (Lowande *et al.* 1980).

NAEGLERIA FOWLERI

Parasitology

Primary amoebic meningo-encephalitis is a very rare infection of the meninges caused by *Naegleria fowleri*. Between 1955 and 1972, 13 probable and confirmed cases of primary amoebic meningo-encephalitis occurred in the Spencer Gulf region of South Australia (Dorsch *et al.* 1983). It was subsequently reported in Queensland, New South Wales, and Western Australia and until 1983, all cases had been fatal (Dorsch *et al.* 1983). Ten pathogenic strains were isolated from the domestic water supply in the northern part of South Australia and *N. fowleri* was isolated from the surface soil in the same area. Its prevalence is universal and up to 1978, > 80 cases had been reported worldwide, of which 38 were from the USA.

Humans acquire the disease by swimming in fresh water lakes and poorly chlorinated outdoor or heated indoor pools, which are contaminated by the parasite. Transmission has been shown to be by water carried through pipelines for several hundred kilometres as some infected patients had never been immersed in water and the only source of infection was pipeline water supply. It was thought chlorine levels had dropped by the time it reached the supply destination