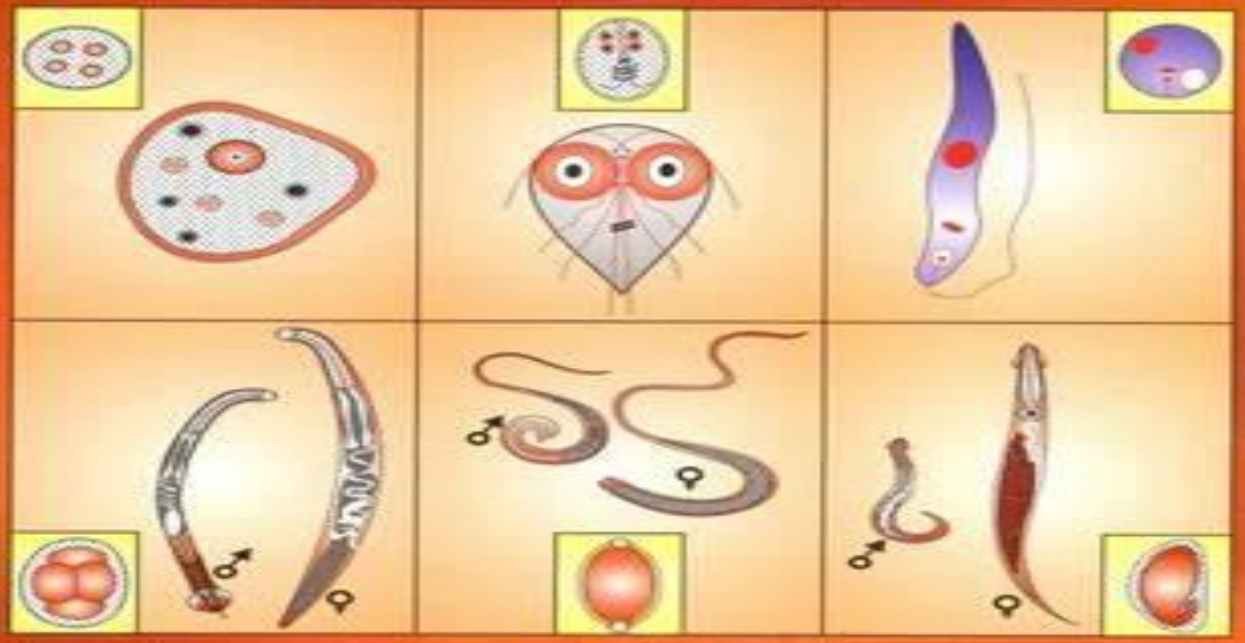
Tikrit University
College of Pharmacy

Microbiology II
-Medical Parasitology

MEDICAL PARASITOLOGY

Lec. 2: *Entamoeba histolytica*



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1. Subphylum Sarcodina (Amoebae)

Amoeboid organisms use pseudopodia for both locomotion and feeding. Only *Entamoeba histolytica* in this group is of medical importance.

ENTAMOEBA HISTOLYTICA

Entamoeba histolytica was first described by Losch in 1875 after being isolated in Russia from a patient with dysenteric stools. It derives its name from its ability to lyse virtually every tissue in the human body and in the bodies of experimental animals.

Geographical distribution

It is worldwide, with higher incidence of amoebiasis in tropical and subtropical regions that have poorsanitation and contaminated water. The average prevalence of infection in these areas is 10–15%, with as many as 50% of the population infected in some areas.

Many of the infected individuals are asymptomatic carriers who represent a reservoir for the spread of *E. histolytica* to others. Risk groups include male homosexuals, travellers and recent immigrants, and institutionalized populations. **On a global scale, it ranks second after malaria as a cause of death among people with parasitic infections produced by protozoa.**

Habitat

Trophozoites of *E. histolytica* reside in mucosa and submucosa of large intestine of man.

Morphology

The parasite exists in three morphological forms

1. Trophozoite
2. Precyst
3. Cyst

Trophozoite

It measures 10–60 μm (average 20–30 μm) in diameter.

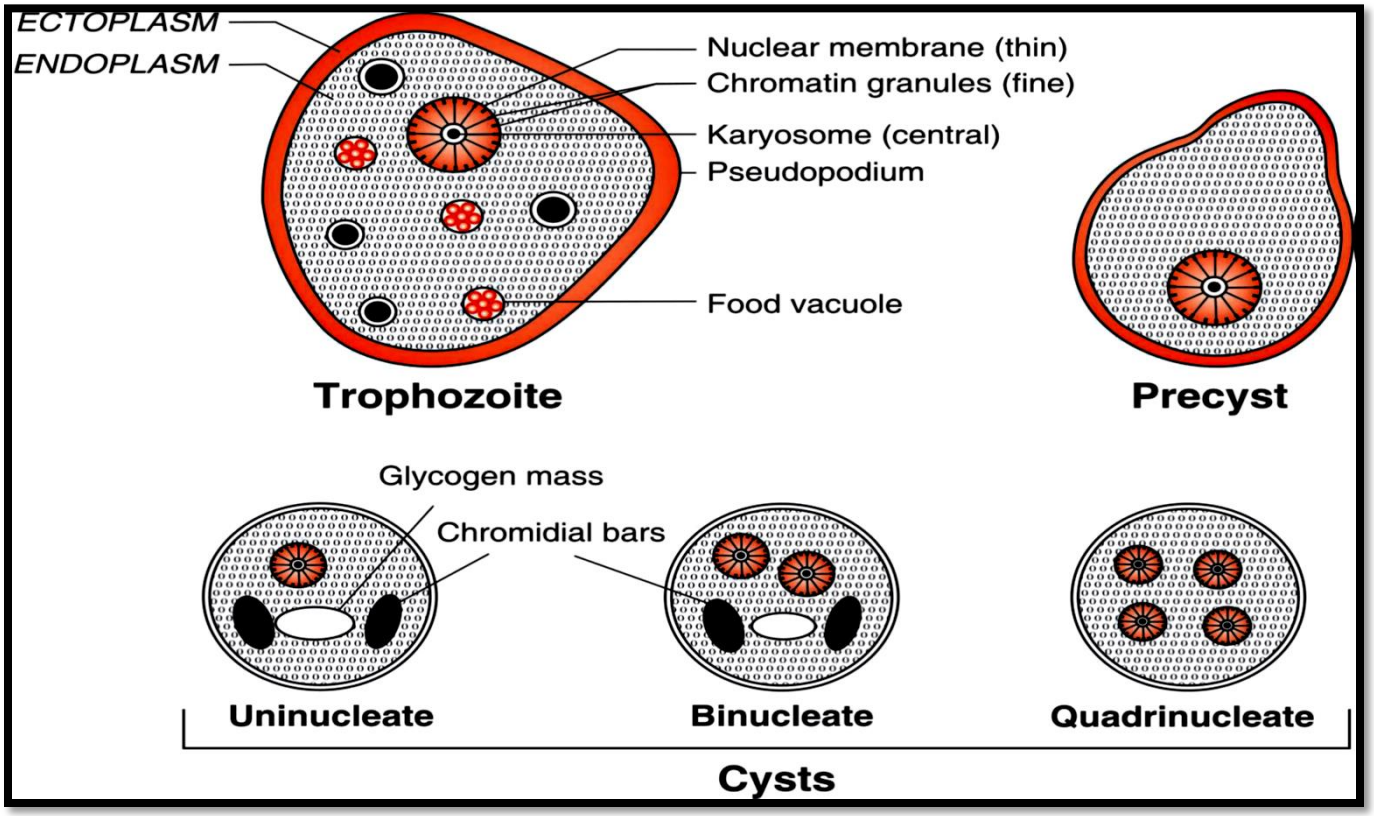
Cytoplasm

The cytoplasm of the trophozoite can be divided into **a clear outer ectoplasm and an inner finely granular endoplasm in which red blood cells, leucocytes and tissue debris are found within the food vacuoles.**

The presence of ingested erythrocytes is the characteristic feature of *E. histolytica* but not of *E. dispar*. Trophozoites are motile with active, unidirectional and purposeful motility. Movement results from long finger-like pseudopodial extensions of ectoplasm into which endoplasm flows. Trophozoite is the only form present in the tissues. It usually appears only in diarrhoeic faeces in active cases and survives only for a few hours.

Nucleus

It is spherical in shape varying in size from 4–6 μm in diameter. In stained preparations it shows **a central dot-like karyosome** which is surrounded by a clear halo. The nuclear membrane is delicate and is lined by a single layer of **fine chromatin granules**. The space between the karyosome and the nuclear membrane is traversed by linin network (achromatic fibrils) having spoke-like radial arrangement.



Precyst

It is smaller in size, varying from 10–20 μm in dia-meter. It is oval with a blunt pseudopodium projecting from the periphery. Food vacuoles disappear. There is no change in the nucleus which shows characteristics of the trophozoite.

Cyst

It is spherical, 10–15 μm in diameter. It is surrounded by a **thick chitinous wall** which makes it highly resistant to the gastric acid, adverse environmental conditions and the chlorine concentration found in potable water. It starts as a uninucleate body, but later the nucleus divides to form two and then four nuclei.

Uninucleate and binucleate cysts in addition also possess a **glycogen mass**, which stains brown with iodine, and 1–4 **chromidial or chromatoid bars**. These do not stain with iodine but appear as refractile oblong bars with rounded ends in normal saline preparations. With iron-haematoxylin stain they stain black in colour. **Cysts are present only in the lumen of the colon and in formed faeces.** Stools may contain cysts with 1–4 nuclei depending on their degree of maturation. ***E. histolytica*** **cysts are not produced in tissues.**

Life cycle

It passes its life cycle in **only one host** . Cysts are passed in faeces. Man acquires the infection by ingestion of **mature quadrinucleate cysts** in faecally contaminated food or water. Trophozoites can also be passed in diarrhoeal stools, but are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment.

In contrast, **cysts may remain viable in a humid environment and stay infective for several days**. Infection may also be acquired by anal-oral sexual practices among male homosexuals. Flies and cockroaches can also serve as vectors for the transmission of *E. histolytica* cysts.

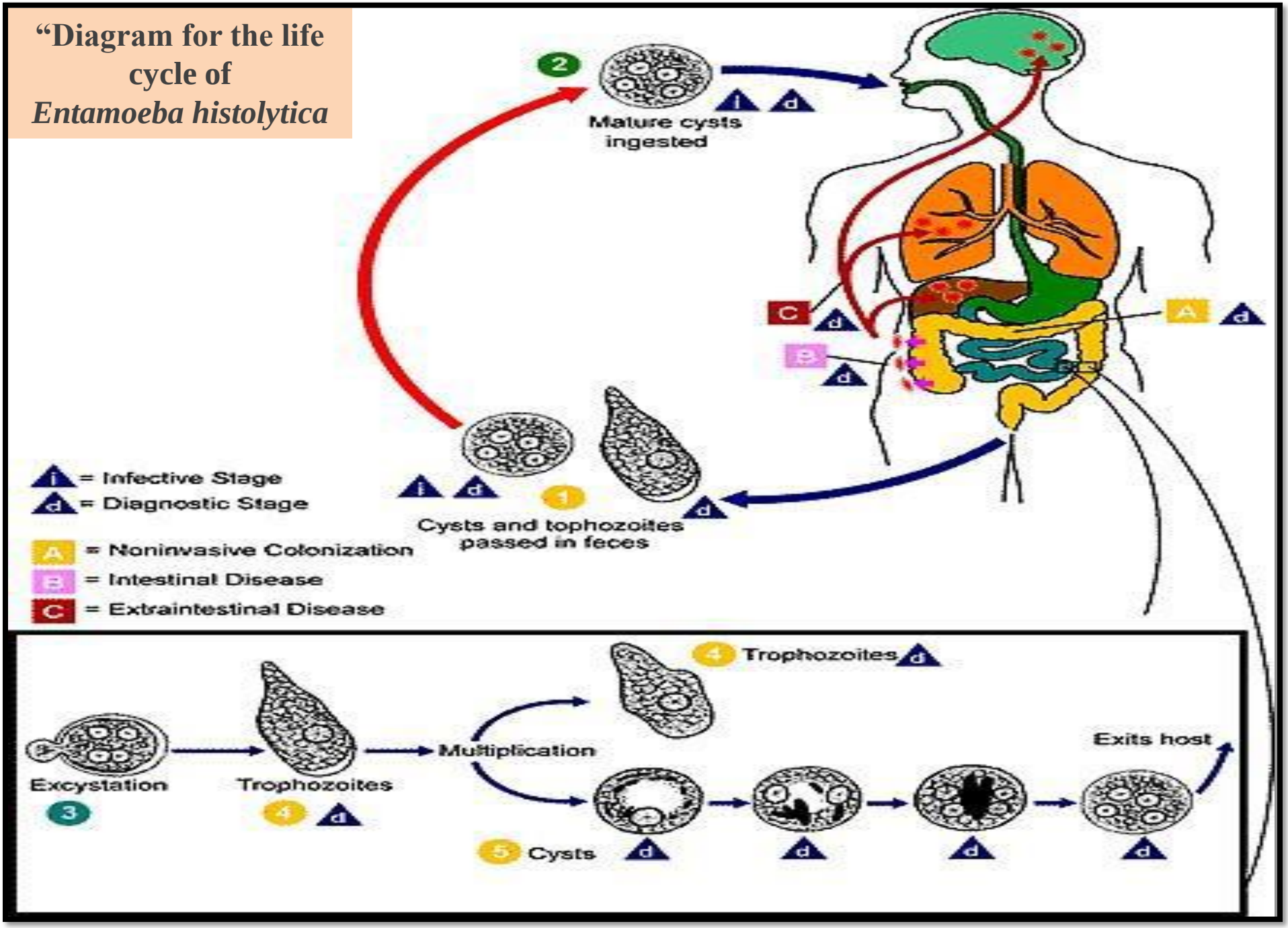
In the small intestine the cyst wall is lysed by trypsin and a single tetranucleate amoeba (**metacyst**) is liberated. Each nucleus divides by binary fission giving rise to eight nuclei. Almost immediately the cytoplasm becomes separated into as many parts as there are nuclei, thus from each mature cyst eight small amoebulae (**metacystic trophozoites**) are produced.

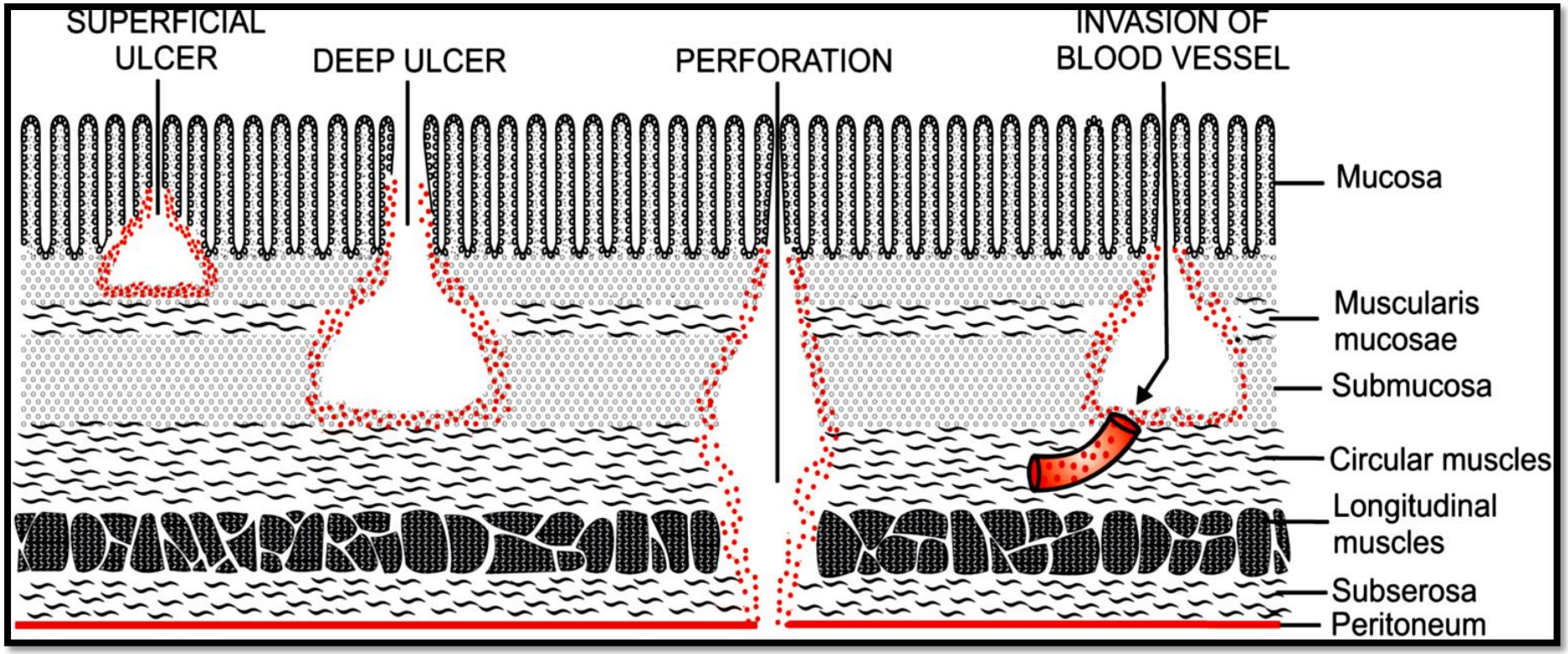
This process is known as ***excystation***. Metacystic trophozoites are carried in the faecal stream into the caecum. They invade the mucosa and ultimately lodgein the submucous tissue of large intestine. Here they grow and multiply by binary fission.

During growth, *E. histolytica* secretes a **proteolytic enzyme of the nature of histolysin** which brings about destruction and necrosis of tissue and produces flaskshaped ulcers . The amoebae are mostly present at the periphery of the lesion. At this stage, a large number of trophozoites are excreted along with blood and mucus in the stool leading to amoebic dysentery.

In a few cases, erosion of the large intestine may be so extensive that **trophozoites gain entrance into the radicles of portal vein** and are carried away to the liver where they multiply leading to amoebic hepatitis and amoebic liver abscess (Figs. 3.3–3.5).

"Diagram for the life cycle of *Entamoeba histolytica*





Pathogenesis of intestinal amoebiasis.

he host is toned down and patient has developed resistance, the lesions start healing and patient starts passing normal (formed) stools. The trophozoites, in the lumen of the large intestine, discharge undigested food particles and transform into precysts and then into mature quadrinucleate cysts. These are the infective forms of the parasite. This process is known as *encystation*. **Cyst formation occurs only within the intestinal tract**; once the stool has left the body, cyst formation does not occur.

Epidemiologically, India can be divided into three regions depending on the prevalence of intestinal amoebiasis. These include the regions of high prevalence (> 30%), moderate prevalence (10–30%), and regions of low prevalence (< 10%). Pregnancy, malnutrition, underlying metabolic diseases, and corticosteroid therapy predispose to more serious disease.

Pathogenicity

E. histolytica causes intestinal and extraintestinal amoebiasis.

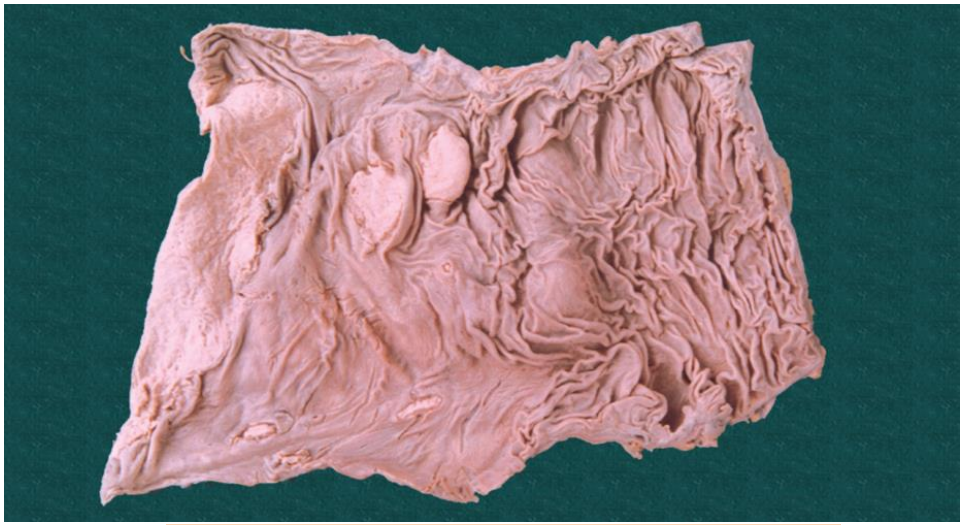
Intestinal amoebiasis

Intestinal amoebiasis indicates that organisms are confined to gastrointestinal tract. After an **incubation period of 1–4 weeks**, the amoebae invade the colonic mucosa, producing characteristic ulcerative lesions and a profuse bloody diarrhoea (**amoebic dysentery**).

The ulcers may be generalized involving the whole length of the large intestine or they may be localized in the ileo-caecal (caecum, ascending colon, ileo-caecal valve and appendix) or sigmoido-rectal (sigmoid colon and rectum) region. Ulcers are discrete with intervening normal mucosa. They vary in size from pin-head size to more than 2.5 cm in diameter.

They may be deep or superficial. Base of the deep ulcers is generally formed by muscular coat. However, superficial ulcers do not extend beyond muscularis mucosae. When destruction is not limited to the submucosa but extends deeper into the muscular layer, the following complications may arise:

- Local peritonitis
- Perforation and generalized peritonitis
- Pericaecal or pericolic abscess
- Sloughing and gangrene of large gut.



Amoebic ulcers large intestine.

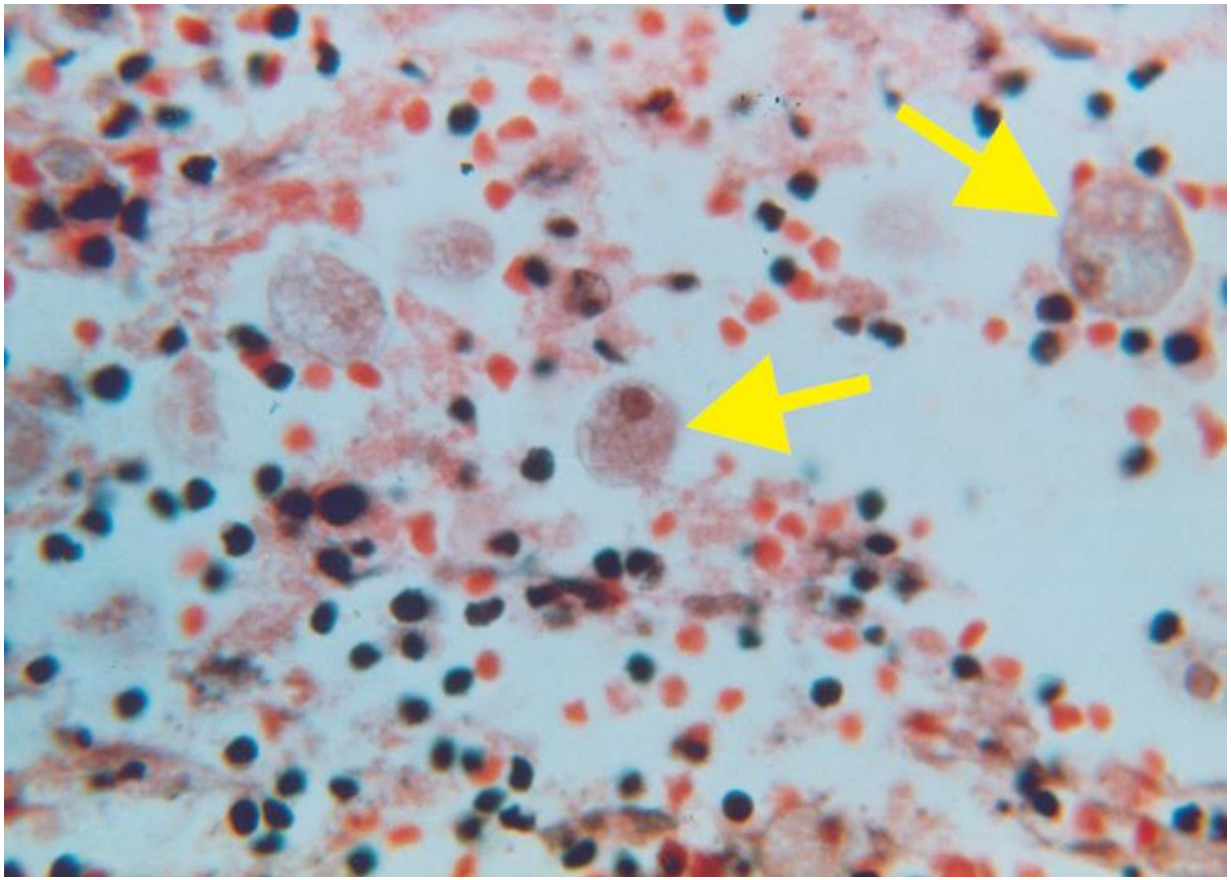
E. histolytica may also cause amoebic appendicitis and **amoebomas**. The latter are pseudotumoural lesions, whose formation is associated with necrosis, inflammation and oedema of the mucosa and submucosa of the colon.

Amoebomas are generally single, but occasionally multiple masses usually found in the vertical segments of the large intestine – the caecum, the sigmoido-rectal region of the colon, the ascending colon, and the hepatic and splenic angles of the colon. The condition is usually acute with dysentery, abdominal pain and a palpable mass in the corresponding area of the abdomen.

Extraintestinal amoebiasis

About 5% individuals with intestinal amoebiasis, 1–3 months after the disappearance of the dysenteric attack, develop hepatic amoebiasis. Trophozoites of *E. histolytica* are carried as emboli by the radicles of the portal vein from the base of the amoebic ulcer in the large intestine . The capillary system of the liver acts as an excellent filter and holds these parasites. They multiply in the liver and lead to cytolytic action.

The amoebae cause obstruction of the portal venules resulting in anaemic necrosis of hepatic cells. The destruction starts here and continues in concentric layers. Necrosis is followed by cytolysis. Small miliary abscesses coalesce to form big liver abscess .



Trophozoites of *Entamoeba histolytica* in liver aspirate showing cytolysis of liver cells (haematoxylin and eosin stain, × 400).

Amoebic liver abscess

It varies greatly in size. It has been reported in patients of all ages, but predominates in adults between 20–60 years. It has a marked preference for the right lobe of the liver and it is at least three times more frequent in males than in females. The wall of the abscess cavity is ragged with shreds of connective tissue running across the abscess cavity. **A section through the margin of the liver abscess can be differentiated into three zones:**

1. A necrotic Centre filled with thick pus with no amoebae.
2. An intermediate zone consisting of degenerated livercells, a few red blood cells, leucocytes and occasional trophozoites of *E. histolytica*.
3. An outer zone of nearly normal hepatic tissue just being invaded by amoebae.

Sign and symptoms

The centre of an amoebic liver abscess contains a **viscous red-brown (anchovy sauce appearance)** or grey-yellow fluid consisting of cytolysed liver cells, red blood cells and leucocytes. It is referred to as ‘pus’ but contains very few pus cells.

Since the amoebae actively multiply in the walls of the abscess, the last few drops of pus obtained from the lesion are most likely to yield recognizable trophozoites of the parasite. The signs and symptoms of amoebic liver abscess vary but, in general, the onset is abrupt with pain in the right hypochondrium radiating towards the right shoulder and scapular area.

The pain usually increases with deep breathing and coughing. Fever between 38° and 40°C is found in all patients with amoebic liver abscess.

On palpation, the liver is soft and smooth in contrast to the rough hard irregular character of the liver in patients with cirrhosis and hepatocellular carcinoma. Jaundice is present in 8% of the patients. When jaundice is severe, multiple abscesses should be suspected.

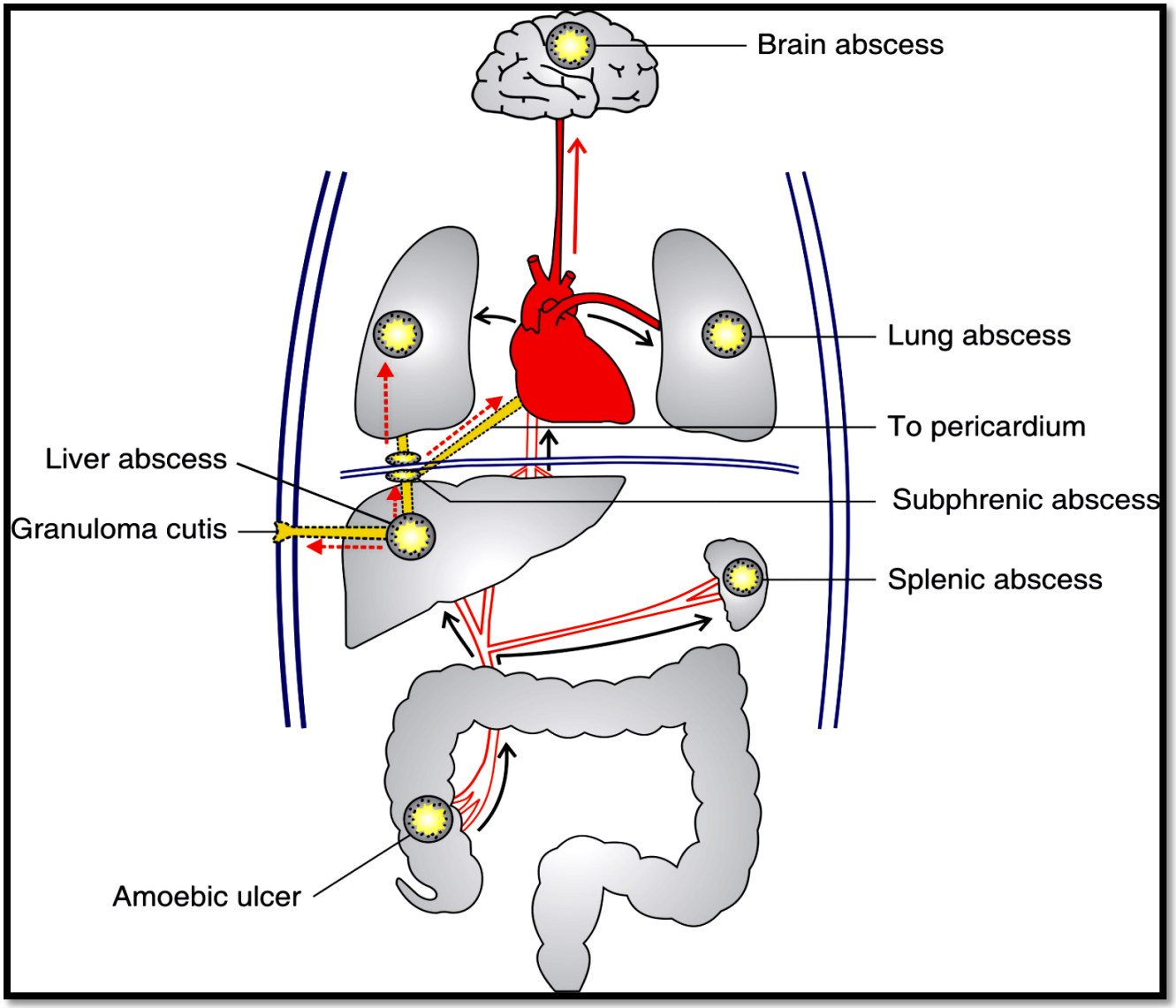
Complications of amoebic liver abscess

With the continued lysis of liver tissue, the abscess may grow in various directions coming in contact with neighbouring organs through which its contents may be discharged :

• **A right-sided liver abscess** may rupture externally. In such cases amoebae may cause infection of the skin leading to *granuloma cutis*. It may rupture into the lungs and the pus containing trophozoites of *E.histolytica* may be expectorated. It may also rupture into right pleural cavity leading to *empyema thoracis*, below the diaphragm causing *subphrenic abscess* and into the peritoneal cavity producing *generalized peritonitis*.

• **A left-sided liver abscess** may rupture externally through the anterior abdominal wall leading to *granuloma cutis*, into the stomach leading to *haematemesis*, and into pleural cavity and pericardial cavity leading to *empyema thoracis* and *pericarditis* respectively.

• **A liver abscess situated on the inferior surface** may rupture into bowel and peritoneal cavity, and the one situated on the posterior surface may rupture into inferior vena cava which is invariably fatal. From the liver, *E. histolytica* may enter into general circulation involving other organs of the body like lungs, brain, spleen, skin, etc. Both faecal and sigmoidoscopic examinations for the parasite are negative in approximately half of the patients in extraintestinal disease.



Diagrammatic representation of routes of origin and locations of extraintestinal amoebiasis. Solid arrows indicate haematogenous spread and interrupted arrows indicate direct spread of amoebae.

Immune response

Both humoral and cellular immune responses are generated following infection with *E. histolytica*. Humoral immune response in patients with invasive intestinal amoebiasis is initiated by a short and transient local secretory response, followed by an increase in systemic antibodies. IgA anti-*E. histolytica* antibodies have also been found in human milk, colostrum and saliva. Circulating antibodies to *E. histolytica* can be detected as early as one week after the onset of symptoms and persist for more than three years after an invasive amoebic episode. All classes of immunoglobulins are involved, but there is predominance of IgG. Humoral antibodies do not appear to be protective against *E. histolytica*.

Cell-mediated immune response probably has a role in limiting invasive amoebiasis and the rarity of recurrence of amoebic liver abscess. **The existence of an effective cell-mediated immunity is supported by:**

• the results of a few studies of passive transfer of immunity with cells; and

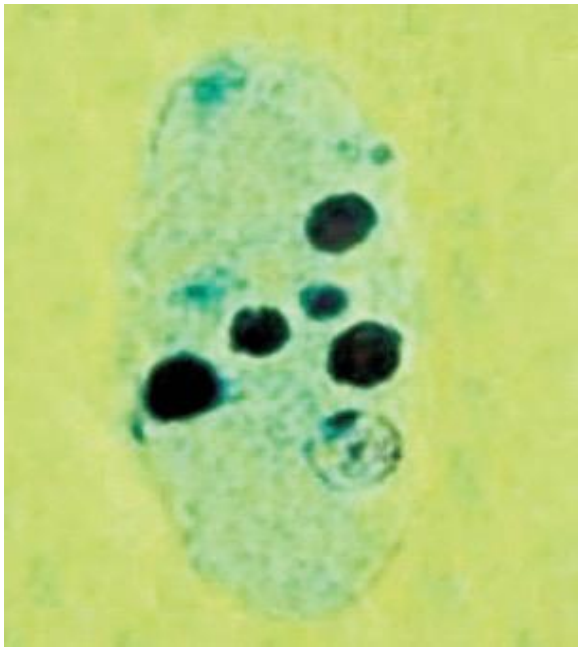
• the cytolytic effect of activated lymphocytes and macrophages against *E. histolytica.* Cell-mediated immune response can be detected by delayed hypersensitivity to antigens of *E. histolytica*, macrophage migration inhibition and blast transformation.

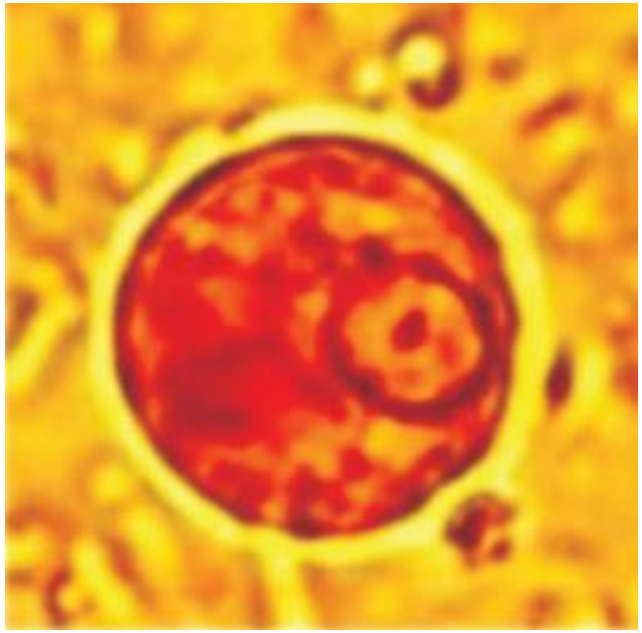
Laboratory diagnosis

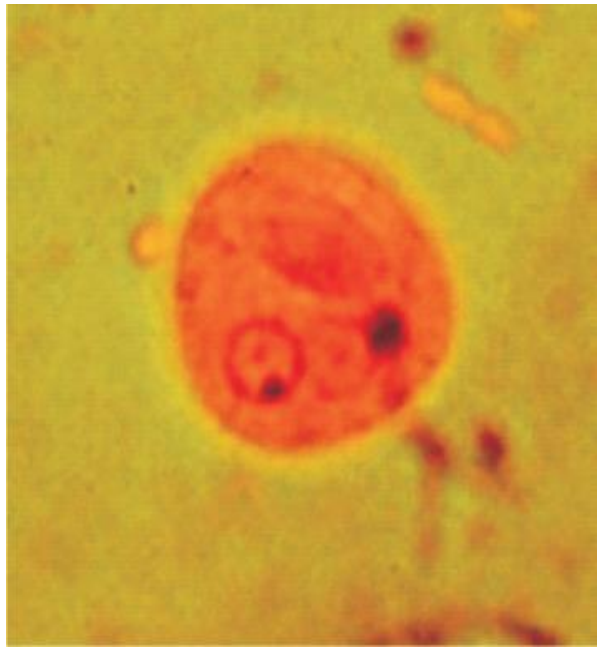
Intestinal amoebiasis

Entamoeba histolytica must be differentiated from other intestinal nonpathogenic amebae. The nonpathogenic *Entamoeba dispar* is morphologically identical to *E. histolytica*, and differentiation must be based on isoenzymatic or immunologic analysis. Molecular methods are also useful in distinguishing between *E. histolytica* and *E. dispar*.

Microscopic identification of feces samples is the common method for diagnosing *E. histolytica* to search for cysts and trophozoites in the stool This can be accomplished using wet mount and permanently stained preparations such as iodine or trichrome or by flotation or sedimentation method for stool samples.







A B C

Trichrome stain of trophozoite (A), and wet mount in iodine of uninucleate (B), and binucleate (C) cysts of Entamoeba histolytica in stool, (× 400).

The typical stool in amebic dysentery consists of exudates, mucous, blood and maybe little fecal material; however, we are mainly looking for the cyst stage. In liquid stool, trophozoite may also be found, but only the cyst stage is present in the solid stool.

The blood examination shows moderate leukocytosis. In the serological tests, in the later stages of invasive amoebiasis antibodies appear. Tests including ELISA, IHA and IFA.

In the histological examination, the trophozoites can be identified in the aspirates or biopsy samples obtained during colonoscopy or surgery. The molecular methods include the DNA probe and PCR.

Treatment

Very effective drug for the therapy of amoebiasis is metronidazole or its analogs tinidazole and ornidazole. Because metronidazole is so well absorbed in the gut, levels may not be therapeutic in the colonic lumen, and the drug is less effective against cysts. Hence patients with amoebic colitis also should receive a luminal agent to eradicate any *E. histolytica* trophozoites residing within the gut lumen. The nonabsorbed aminoglycoside *paromomycin* and the 8-hydroxyquinoline compound *iodoquinol* are effective luminal agents.