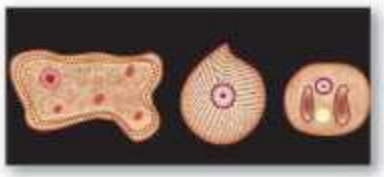


Amoebae 3

Amoebae are structurally simple protozoans which have no fixed shape. They are classified under **Phylum:** Sarcomastigophora, **Subphylum:** Sarcodina, **Super-class:** Rhizopoda and **Order:** Amoebida.

- The cytoplasm of amoeba is bounded by a membrane and can be differentiated into an **outer ectoplasm** and **inner endoplasm**.
- **Pseudopodia** are formed by the amoeba by thrusting out ectoplasm, followed by endoplasm. These are employed for locomotion and engulfment of food by phagocytosis.
- Reproduction occurs by fission and budding. Cyst is formed in unfavorable conditions and is usually the infective form for vertebrate host (e.g. *Entamoeba histolytica*).

Table 3.1: Classification of Amoebae

Intestinal amoebae	Free-living amoebae
Entamoeba histolytica *Entamoeba dispar* *Entamoeba coli* *Entamoeba polecki* *Entamoeba hartmanni* *Entamoeba gingivalis* *Endolimax nana* *Iodamoeba butschlii*	*Naegleria fowleri* *Acanthamoeba* spp. *Balamuthia mandrillaris*
Note: All intestinal amoebae are nonpathogenic, except *Entamoeba histolytica*	**Note:** All free-living amoebae are opportunistic pathogens

- Amoebae are classified as either free-living or intestinal amoebae (Table 3.1).
- A few of the free-living amoebae occasionally act as human pathogens producing meningoencephalitis and other infections, e.g. *Naegleria* and *Acanthamoeba*.
- The parasitic amoebae inhabit the alimentary canal.

Entamoeba Histolytica

History and Distribution

E. histolytica was discovered by Lösch in 1875, who demonstrated the parasite in the dysenteric feces of a patient in St. Petersburg in Russia.

- In 1890, William Osler reported the case of a young man with dysentery, who later died of liver abscess.
- Councilman and Lafleur in 1891 established the pathogenesis of intestinal and hepatic amoebiasis and introduced the terms '**amoebic dysentery**' and '**amoebic liver abscess**'.
- *E. histolytica* is worldwide in prevalence, being much more common in the tropics than elsewhere. It has been found wherever sanitation is poor, in all climatic zones from Alaska (61°N) to straits of Magellan (52°S).
- It has been reported that about 10% of world population and 50% of the inhabitants of developing countries may be infected with the parasite.
- The infection is not uncommon even in affluent countries, about 1% of Americans being reported to be infected.

- While the majority of infected humans (80–99%) are asymptomatic, invasive amoebiasis causes disabling illness in an estimated 50 million of people and causes 50,000 deaths annually, mostly in the tropical belt of Asia, Africa, and Latin America.
- It is the third leading parasitic cause of mortality, after malaria and schistosomiasis.

- Epidemiologically, India can be divided into 3 regions, depending on the prevalence of intestinal amoebiasis.
  - High prevalence states (>30%): Chandigarh, Tamil Nadu, and Maharashtra.
  - Moderate prevalence states (10–30%): Punjab, Rajasthan, Uttar Pradesh, Delhi, Bihar, Assam, West Bengal, Andhra Pradesh, Karnataka, and Kerala.
  - Low prevalence states (<10%): Haryana, Gujarat, Himachal Pradesh, Madhya Pradesh, Odisha, Sikkim, and Puducherry.

Morphology

E. histolytica occurs in 3 forms (Fig. 3.1).
- Trophozoite
- Precyst
- Cyst.

Trophozoite

Trophozoite is the vegetative or growing stage of the parasite (Fig. 3.1A). It is the only form present in tissues.
- It is irregular in shape and varies in size from 12–60 µm; average being 20 µm.
- It is large and actively motile in freshly-passed dysenteric stool, while smaller in convalescents and carrriers.
- The parasite, as it occurs free in the lumen as a commensal is generally smaller in size, about 15–20 µm and has been called the **minuta form**.
- **Cytoplasm:** Outer ectoplasm is clear, transparent, and refractile. Inner endoplasm is finely granular, having a **ground glass appearance**. The endoplasm contains nucleus, food vacuoles, erythrocytes, occasionally leucocytes, and tissue debris.
- **Pseudopodia** are finger-like projections formed by sudden jerky movements of ectoplasm in one direction, followed by the streaming in of the whole endoplasm.
- Typical amoeboid motility is a **crawling** or **gliding** movement and not a free swimming one. The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. The cell has to be attached to some surface or particle for it to move. In culture tubes, the trophozoites may be seen crawling up the side of the glass tube.
- Pseudopodia formation and motility are inhibited at low temperatures.
- **Nucleus** is spherical 4–6 µm in size and contains **central karyosome**, surrounded by clear halo and anchored to the nuclear membrane by fine radiating fibrils called the **linin network**, giving a **cartwheel appearance**. The nucleus is not clearly seen in the living trophozoites, but can be clearly demonstrated in preparations stained with iron-hemotoxylin.
- The nuclear membrane is lined by a rim of chromatin distributed evenly as small granules.
- The trophozoites from acute dysenteric stools often contain phagocytosed erythrocytes. This feature is diagnostic as phagocytosed red cells are not found in any other commensal intestinal amoebae.
- The trophozoites divide by **binary fission** in every 8 hours.
- Trophozoites survive upto 5 hours at 37°C and are killed by drying, heat, and chemical sterilization. Therefore, the infection is not transmitted by trophozoites. Even if live trophozoites from freshy-passed stools are ingested, they are rapidly destroyed in stomach and cannot initiate infection.

Precystic Stage

Trophozoites undergo encystment in the intestinal lumen. Encystment does not occur in the tissues nor in feces outside the body.
- Before encystment, the trophozoite extrudes its food vacuoles and becomes round or oval, about 10–20

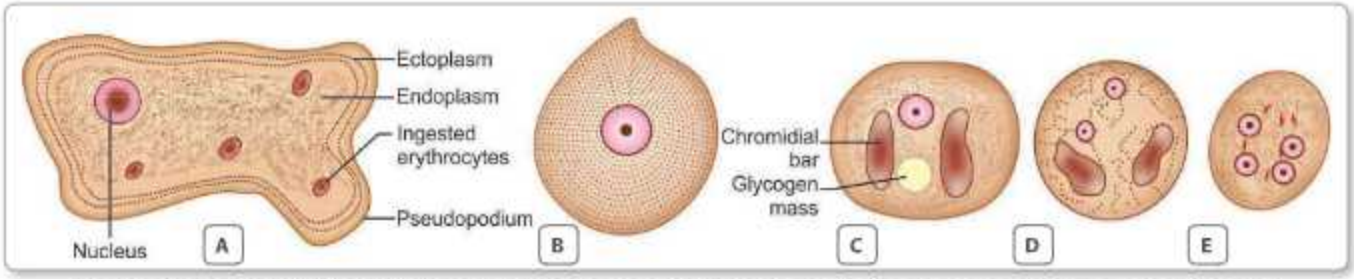


Fig. 3.1: *Entamoeba histolytica*. **A.** Trophozoite; **B.** Precystic stage; **C.** Uninucleate cyst; **D.** Binucleate cyst; **E.** Mature quadrinucleate cyst

μm in size. This is the precystic stage of the parasite (Fig. 3.1B).

- It contains a **large glycogen vacuole** and two **chromatid bars**.
- It then secretes a highly retractile cyst wall around it and becomes cyst.

Cystic Stage

The cyst is spherical in shape about 10–20 μm in size.

- The early cyst contains a single nucleus and two other structures—a mass of glycogen and 1–4 *chromatoid bodies or chromidial bars*, which are cigar-shaped refractile rods with rounded ends (Fig. 3.1C). The chromatoid bodies are so called because they stain with hematoxylin, like chromatin.
- As the cyst matures, the glycogen mass and chromidial bars disappear and the nucleus undergoes 2 successive mitotic divisions to form 2 (Fig. 3.1D) and then 4 nuclei. The mature cyst is, thus **quadrinucleate** (Fig. 3.1E).
- The cyst wall is a highly refractile membrane, which makes it highly resistant to gastric juice and unfavorable environmental conditions.
- The nuclei and chromidial bodies can be made out in unstained films, but they appear more prominently in stained preparations.
- With iron hemotoxylin stain, nuclear chromatin and chromatoid bodies appear deep blue or black, while the glycogen mass appears unstained.
- When stained with iodine, the glycogen mass appears golden brown, the nuclear chromatin and karysome bright yellow, and the chromatoid bodies appear as clear space, being unstained.

Life Cycle

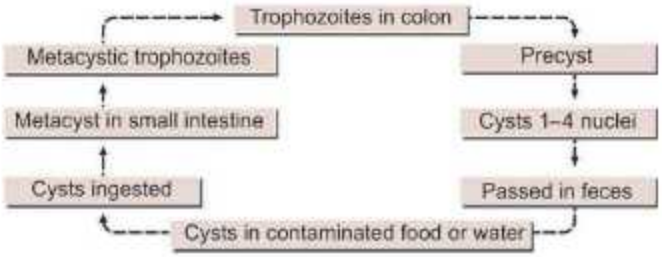
E. histolytica passes its life cycle only in 1 host-man (Flowchart 3.1 and Fig. 3.2).

Infective form: Mature quadrinucleate cyst passed in feces of convalscents and carriers. The cysts can remain viable under moist conditions for about 10 days.

Mode of transmission: Man acquires infection by swallowing food and water contaminated with cysts.

- As the cyst wall is resistant to action of gastric juice, the cysts pass through the stomach undamaged and enter the small intestine.
- **Excystation**: When the cyst reaches caecum or lower part of the ileum, due to the alkaline medium, the cyst wall is damaged by trypsin, leading to excystation.
- The cytoplasm gets detached from the cyst wall and amoeboid movements appear causing a tear in the cyst wall, through which **quadrinucleate amoeba** is liberated. This stage is called the **metacyst** (Fig. 3.2).
- **Metacystic trophozoites:** The nuclei in the metacyst immediately undergo division to form **8 nuclei**, each of which gets surrounded by its own cytoplasm to become **8 small amoebulae** or **metacystic trophozoites**.
- If exystation takes place in the small intestine, the metacystic trophozoites do not colonize there, but are carried to the caecum.
- The optimal habitat for the metacystic trophozoite is the submucosal tissue of caecum and colon, where they lodge in the glandular crypts and grow by binary fission (Fig. 3.2).
- Some develop into precystic forms and cysts, which are passed in feces to repeat the cycle.
- The entire life cycle is, thus completed in one host.

In most of the cases, *E. histolytica* remains as a commensal in the large intestine without causing any ill effects. Such persons become carriers or asymptomatic cyst passers and are responsible for maintenance and spread of infection in the community. Sometimes, the infection may be activated and clinical disease ensues. Such latency and reactivation are the characteristics of amoebiasis.



Flowchart 3.1: Life cycle of *Entamoeba histolytica* (Schematic)

Pathogenesis and Clinical Features

- *E. histolytica* causes intestinal and extraintestinal amoebiasis.
- **Incubation period** is highly variable. On an average, it ranges from 4 days to 4 months.
- Amoebiasis can present in different forms and degree of severity, depending on the organ affected and the extent of damage caused.

Intestinal Amoebiasis

The lumen-dwelling amoebae do not cause any illnesss. They cause disease only when they invade the intestinal tissues. This happens only in about 10% of cases of infection, the remaining 90% being asymptomatic.

- Not all strains of *E. histolytica* are pathogenic or invasive. Differentiation between pathogenic and non-pathogenic strains can be made by susceptibility to complement-mediated lysis and phagocytic activity or

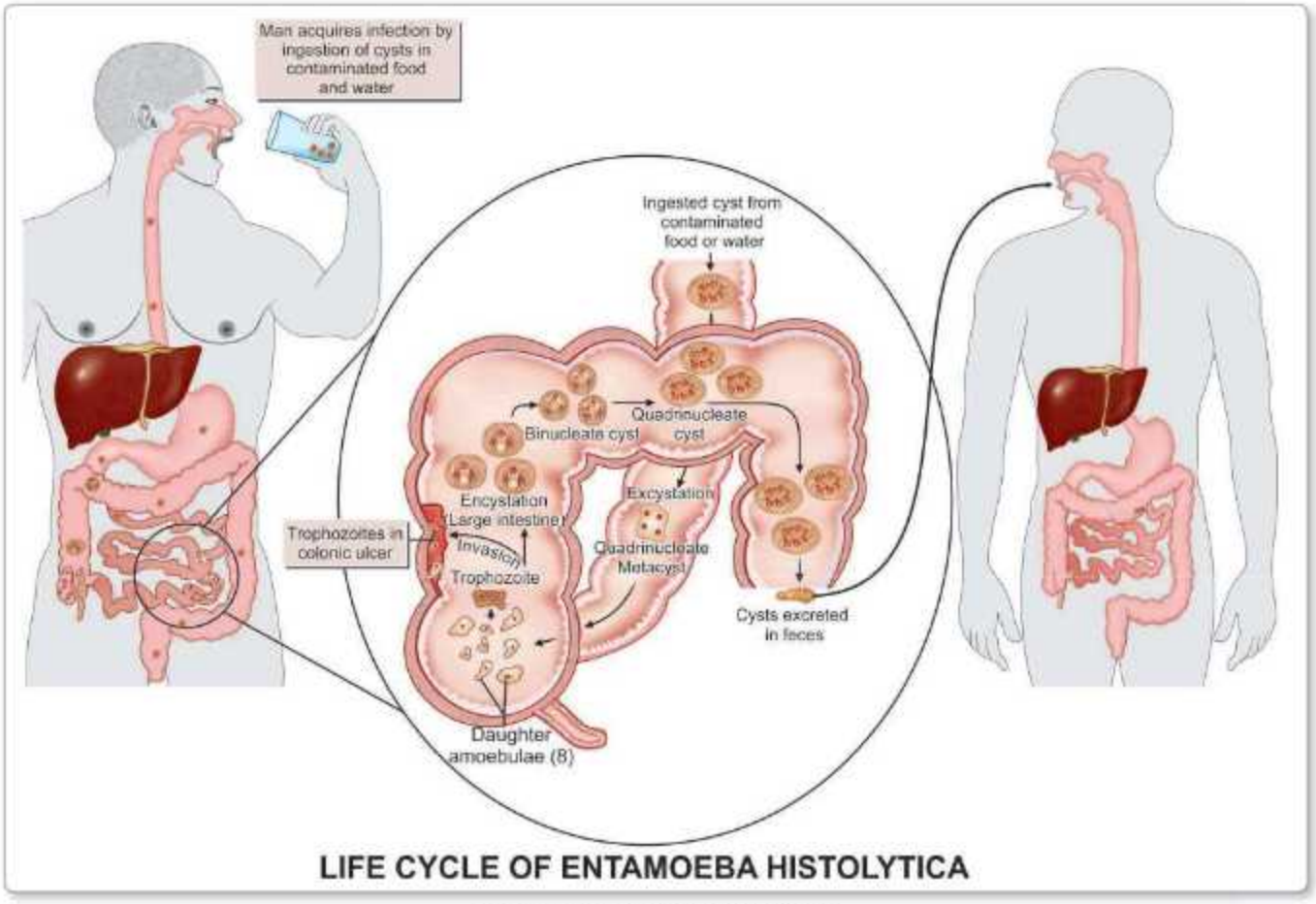


Fig. 3.2: Life cycle of *Entamoeba histolytica*

by the use of genetic markers or monoclonal antibodies and zymodeme analysis. (see box on page number 18)

- The metacystic trophozoites penetrate the columnar epithelial cells in the **crypts of Liberkühn** in the colon.
- Penetration of the amoeba is facilitated by the motility of the trophozoites and the tissue lytic enzyme, **histolysin**, which damages the mucosal epithelium. Amoebic **lectin** another virulence factor mediates adherence.
- Mucosal penetration by the amoeba produces discrete ulcers with pinhead center and raised edges. Sometimes, the invasion remains superficial and heals spontaneously. More often, the amoeba penetrates to submucosal layer and multiplies rapidly, causing lytic necrosis and thus forming an abscess. The abscess breaks down to form an ulcer.
- **Amoebic ulcer** is the typical lesion seen in intestinal amoebiasis (Fig. 3.3). The ulcers are **multiple** and are confined to the colon, being most numerous in the **caecum** and next in the **sigmoido-rectal region**. The intervening mucous membrane between the ulcers remains healthy.

Fig. 3.3: Intestinal amoebiasis: Specimen showing amoebic ulcer in colon

Factors affecting virulence of *Entamoeba histolytica*

- Amoebic cystine proteinase, which inactivates complement factor C3 is an important virulence factor.
- Amoebic lectin and ionophore protein are other virulence factors.
- Host factors such as stress, malnutrition, alcoholism, corticosteroid therapy, and immunodeficiency influence the course of infection.
- Glycoproteins in colonic mucus blocks the attachment of trophozoites to epithelial cells, therefore alteration in the nature and quality of colonic mucus may influence virulence.
- Virulence may also be conditioned by the bacterial flora in the colon.

Based on electrophoretic mobility of 6 isoenzymes (acetylglucosaminidase, aldolase, hexokinase, NAD-diaphorase, peptidase and phosphoglucomutase), *E. histolytica* strains can be classified into at least 22 zymodemes. Of these only 9 are invasive and the rest are noninvasive commensals. The zymodemes show a geographical distribution. Even in endemic areas, nonpathogenic zyodemes are far more common than pathogenic ones, which account only about 10% of the total population.

It has been proposed that pathogenic and nonpathogenic strains though morphologically identical may represent 2 distinct species—the pathogenic strains being *E. histolytica*, and the nonpathogenic strains reclassified as *E. dispar*. Trophozoites of *E. dispar* contain bacteria, but no RBCs.

- Ulcers appear initially on the mucosa as raised nodules with pouting edges. They later break down discharging brownish necrotic material containing large numbers of trophozoites.
- The typical amoebic ulcer is **flask-shaped** in cross section, with mouth and neck being narrow and base large and rounded.
- Multiple ulcers may coalesce to form large necrotic lesions with ragged and undermined edges and are covered with brownish slough.
- **The ulcers generally do not extend deeper than submucosal layer**, but amoebae spread laterally in the submucosa causing extensive undermining and patchy mucosal loss. Amoebae are seen at the periphery of the lesions and extending into the surrounding healthy tissues. Occassionally, the ulcers may involve the muscular and serous coats of the colon, causing perforation and peritonitis. Blood vessel erosion may cause hemorrhage.
- The superficial lesions generally heal without scarring, but the deep ulcers form scars which may lead to strictures, partial obstruction, and thickening of the gut wall.
- Occassionally, a granulomatous pseudotumoral growth may develop on the intestinal wall from a chronic ulcer. This amoebic granuloma or **amoeboma** may be mistaken for are malignant tumor.

Lesions in chronic intestinal amoebiasis

- Small superficial ulcers involving only the mucosa.
- Round or oval-shaped with ragged and undermined margin and flask-shaped in cross-section.
- Marked scarring of intestinal wall with thining, dilatation, and sacculation.
- Extensive adhesions with the neighboring viscera.
- Formation of tumor-like masses of granulation tissue (amoeboma).

Clinical Features of Intestinal Amoebiasis

The clinical picture covers a wide spectrum from noninvasive carrier state to fulminant colitis.

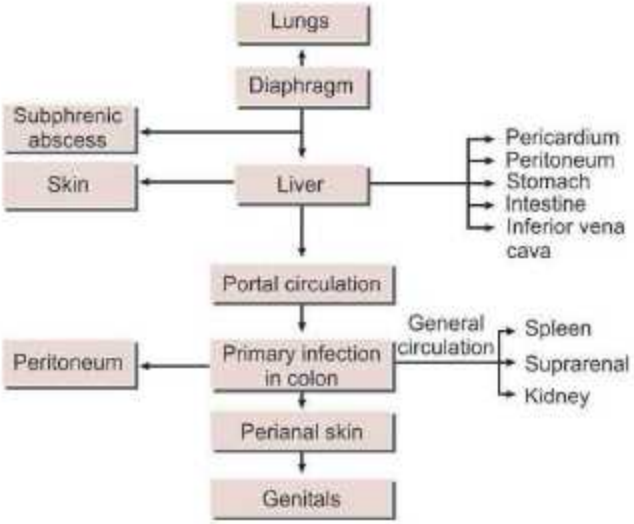
- The incubation period is highly variable from 1–4 months.
- The clinical course is characterized by prolonged latency, relapses and intermissions.
- The typical manifestation of intestinal amoebiasis is amoebic dysentery. This may resemble bacillary dysentery, but can be differentiated on clinical and laboratory grounds. Compared to bacillary dysentery, it is usually insidious in onset and the abdominal tenderness is less and localized (Table 3.2).
- The stools are large, foul-smelling, and brownish black, often with bloodstreaked mucus intermingled with feces. The RBCs in stools are clumped and reddish-brown in color. Cellular exudate is scanty. Charcot-Leyden crystals are often present. *E.histolytica* trophozoites can be seen containing ingested erythrocytes.
- The patient is usually afebrile and nontoxic.
- In fulminant colitis, there is confluent ulceration and necrosis of colon. The patient is febrile and toxic.
- Intestinal amoebiasis does not always result in dysentery. Quite often, there may be only diarrhea or vague abdominal symptoms popularly called '**uncomfortable belly**' or '**growling abdomen**.'
- Chronic involvement of the caecum causes a condition simulating appendicitis.

Extraintestinal Amoebiasis

The various extraintestinal lesions in amoebiasis have been summarized in Flowchart 3.2 and depicted in Fig. 3.5.

Hepatic Amoebiasis

Hepatic involvement is the most common extraintestinal complication of amoebiasis. Although trophozoites reach



Flowchart 3.2: Sites affected in amoebiasis

the liver in most cases of amoebic dysentery, only in a small proportion do they manage to lodge and multiply there. In the tropics, about 2–10% of the individuals infected with *E. histolytica* suffer from hepatic complications.

- The history of amoebic dysentery is absent in more than 50% of cases.
- Several patients with amoebic colitis develop an enlarged tender liver without detectable impairment of liver function or fever. This acute hepatic involvement (**amoebic hepatitis**) may be due to repeated invasion by amoebae from an active colonic infection or to toxic substances from the colon reaching the liver. It is probable that liver damage may not be caused directly by the amoebae, but by lysosomal enzymes and cytokines from the inflammatory cells surrounding the trophozoites.

Complications and sequelae of intestinal amoebiasis

Fulminant amoebic colitis
- Toxic megacolon
- Perianal ulceration
- Amoeboma

Extraintestinal amoebiasis
- Amoebic hepatitis
- Amoebic liver abscess
- Amoebic appendicitis and peritonitis
- Pulmonary amoebiasis
- Cerebral amoebiasis
- Splenic abscess
- Cutaneous amoebiasis
- Genitourinary amoebiasis

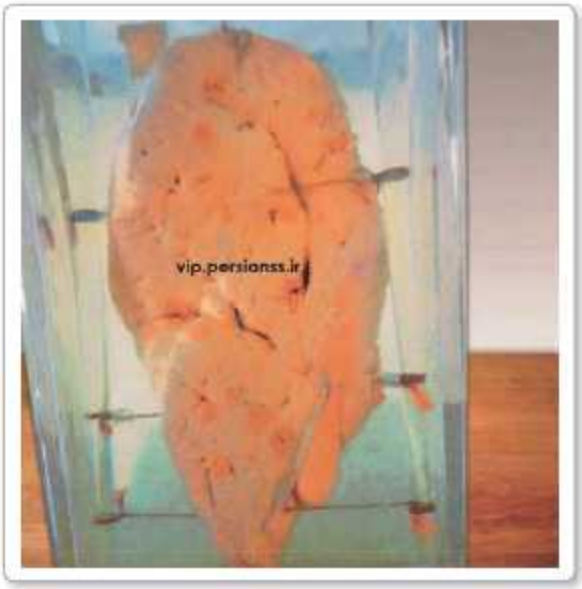


Fig. 3.4: Specimen showing amoebic liver abscess

- In about 5–10% of persons with intestinal amoebiasis, **liver abscesses** may ensue (Fig. 3.4). The center of the abscess contains **thick chocolate brown pus (anchovy sauce pus),** which is liquefied necrotic liver tissue. It is bacteriologically sterile and free of amoeba. At the periphery, there is almost normal liver tissue, which contains invading amoeba.
- Liver abscess may be multiple or more often solitary, usually located in the upper right lobe of the liver. Jaundice develops only when lesions are multiple or when they press on the biliary tract.
- Untreated abscesses tend to rupture into the adjacent tissues through the diaphragm into the lung or pleural cavity, pericardium, peritoneal cavity, stomach, intestine, or inferior vena cava or externally through abdominal wall and skin.
- The incidence of liver abscess is less common in women and rare in children under 10 years of age.

Pulmonary Amoebiasis

Very rarely, primary amoebiasis of the lung may occur by direct hematogenous spread from the colon bypassing the liver, but it most often follows extension of hepatic abscess through the diaphragm and therefore, the lower part of the right lung is the usual area affected (Fig. 3.5).

- Hepatobronchial fistula usually results with expectoration of **chocolate brown sputum**. Amoebic empyema develops less often.
- The patient presents with severe pleuritic chest pain, dyspnea, and non-productive cough.

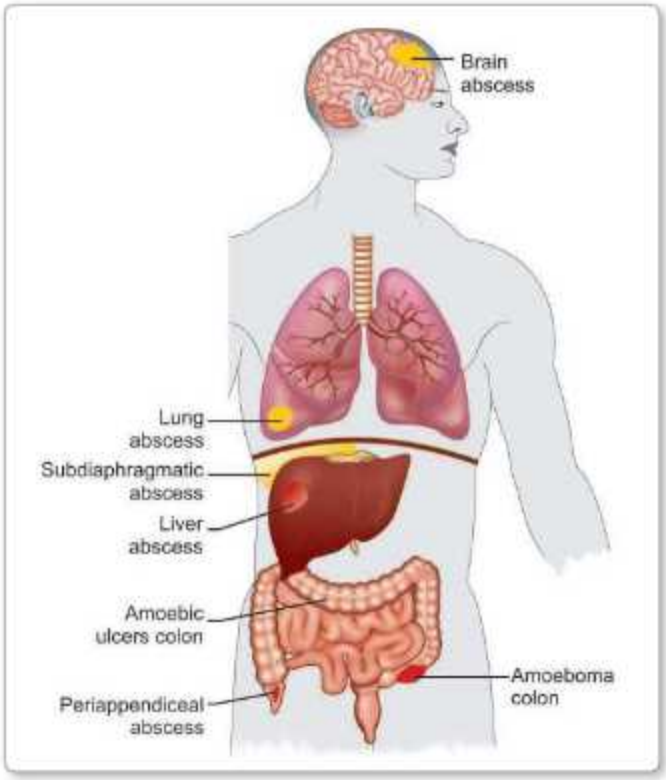


Fig. 3.5: Lesions of Amoebiasis

Metastatic Amoebiasis

Involvement of distant organs is by hematogenous spread and through lymphatics. Abscesses in kidney, brain, spleen, and adrenals have been noticed. Spread to brain leads to severe destruction of brain tissue and is fatal.

Cutaneous Amoebiasis

It occurs by direct extension around anus, colostomy site, or discharging sinuses from amoebic abscesses. Extensive gangrenous destruction of the skin occurs. The lesion may be mistaken for condyloma or epithelioma.

Genitourinary Amoebiasis

The prepuce and glans are affected in penile amoebiasis which is acquired through anal intercourse. Similar lesions in females may occur on vulva, vagina, or cervix by spread from perineum. The destructive ulcerative lesions resemble carcinoma.

Laboratory Diagnosis

Diagnosis of Intestinal Amoebiasis

Stool examination

Intestinal amoebiasis has to be differentiated from bacillary dysentery (Table 3.2). The stool should be collected into a wide mouth container and examined without delay. It should be inspected macroscopically as well as microscopically, (Flowchart 3.3A).

Table 3.2: Differential Features of Amoebic and Bacillary Dysentery

Features	Amoebic dysentery	Bacillary dysentery
Clinical		
Onset	Slow	Acute
Fever	Absent	Present
Toxicity	Absent	Present
Abdominal tenderness	Localised	Generalised
Tenesmus	Absent	Present
Stool		
Frequency	6–8 per day	Over 10 per day
Odor	Offensive	Nil
Color	Dark red	Bright red
Nature	Feces mixed with blood and mucus	Blood and mucus with little or no feces
Consistency	Not adherent	Adherent to container
Reaction	Acid	Alkaline
Microscopy		
Cellular exudates	Scanty	Abundant
Red blood cells	Clumped yellowish brown	Discrete or in rouleaux, bright red
Macrophages	Few	Several, some with ingested red blood cells
Eosinophils	Present	Absent
Charcot-Leyden crystals	Present	Absent
Motile bacteria	Present	Absent
Amoeba	Motile trophozoites with ingested red blood cells	Absent

- **Macroscopic Appearance:** The stool is foul-smelling, copious, semi-liquid, brownish black in color, and intermingled with blood and mucus. It does not adhere to the container.
- **Microscopic Apprearance: Saline preparation**
  - The cellular exudate is scanty and consists of only the nuclear masses (pyknotic bodies) of a few pus cells, epithelial cells, and macrophages.
  - The RBCs are in clumps and yellow or brown red in color.
  - Charcot-Leyden crystals are often present. These are diamond-shaped, clear and refractile crystals.