referred to as **xenic cultures**. A good example of this type of culture is stool specimens cultured for *E. histolytica*. If the parasites are grown with a single known bacterium, the culture is referred to as **monoxenic**. An example of this type of culture is clinical specimen cultured with *Escherichia coli* as a means of recovering species of *Acanthamoeba* and *Naegleria*. If parasites are grown as pure culture without any bacterial associate, the culture is referred to as **axenic**. An example of this type of culture is the use of media for isolation of *Leishmania* spp. or *Trypanosoma cruzi*.

Animal inoculation

Animal inoculation is a sensitive means of detecting infection caused by blood and tissue parasites such as *Toxoplasma gondii*, *Trypanosoma brucei gambiense*, *T. b. rhodesiense*, *T. cruzi*, and *Leishmania* spp.

Xenodiagnosis

The technique of xenodiagnosis employs the use of laboratory-raised arthropod vectors to detect low levels of parasites in infected individuals. Classically this approach was used to diagnose Chagas' disease by allowing an uninfected reduviid bug to feed on an individual suspected of having the disease. Subsequently the bug was dissected and examined microscopically for the evidence of developmental stages of *T. cruzi*. This technique may be used in endemic areas.

Immunodiagnosis

Immunological tests are of two types:

1. Skin tests.
2. Serological tests.

Skin tests

These tests are performed by intradermal injection of parasitic antigens and are read as under:

1. ***Immediate hypersensitivity reaction:*** It reveals wheal and flare response within 30 minutes of injection. This reaction is seen in cases of hydatid disease, filariasis, schistosomiasis, ascariasis and strongyloidiasis.
2. ***Delayed hypersensitivity reaction:*** It reveals erythema and induration after 48 hours of injection. This reaction is seen in cases of leishmaniasis, trypanosomiasis, toxoplasmosis and amoebiasis.

Serological tests

These tests detect antibodies or antigens in the patient serum and other clinical specimens (Table 1.4).

Table 1.4. Important serological tests used for the diagnosis of parasitic infections

Test	Applications
Enzyme-linked immunosorbent assay and radioimmunoassay	Toxoplasmosis, toxocariasis, leishmaniasis, Chagas' disease, malaria and schistosomiasis
Indirect haemagglutination test	Amoebiasis, hydatid disease, filariasis, cysticercosis and strongyloidiasis
Indirect fluorescent antibody test	Amoebiasis, malaria, toxoplasmosis and schistosomiasis
Complement fixation test	Paragonimiasis, Chagas' disease and leishmaniasis
Agglutination tests	
• Direct agglutination	Visceral leishmaniasis
• Bentonite flocculation	Trichinellosis and hydatid disease

Molecular biological methods

These include DNA probes and polymerase chain reaction (PCR).

DNA probes

DNA probe is a radiolabelled or chromogenically labelled piece of single-stranded DNA complementary to a segment of parasitic genome and unique to a particular parasitic strain, species and genus. Specific probe is added to the clinical specimen. If the specimen contains the parasitic DNA, probe will hybridize with it which can be detected. DNA probes are available for the detection of the infection with *P. falciparum, W. bancrofti, T. b. gambiense, T. b. rhodesiense, T. cruzi* and *Onchocerca* spp.

Polymerase chain reaction (PCR)

PCR is a DNA amplification system that allows molecular biologist to produce microgram quantities of DNA from picogram amounts of starting material. It has been employed for the diagnosis of infections caused by *Giardia lamblia*, *Entamoeba histolytica*, *Plasmodium falciparum*, *Leishmania donovani*, *Toxoplasma gondii*, *Trichomonas vaginalis*, *Cryptosporidium parvum*, etc.

CLASSIFICATION OF PARASITES

Human parasites are classified within four eukaryotic kingdoms – Protozoa, Stramenopila, Fungi and Animalia (Tables 1.5 and 1.6). Kingdom is the highest taxonomic category. It is subdivided as follows:

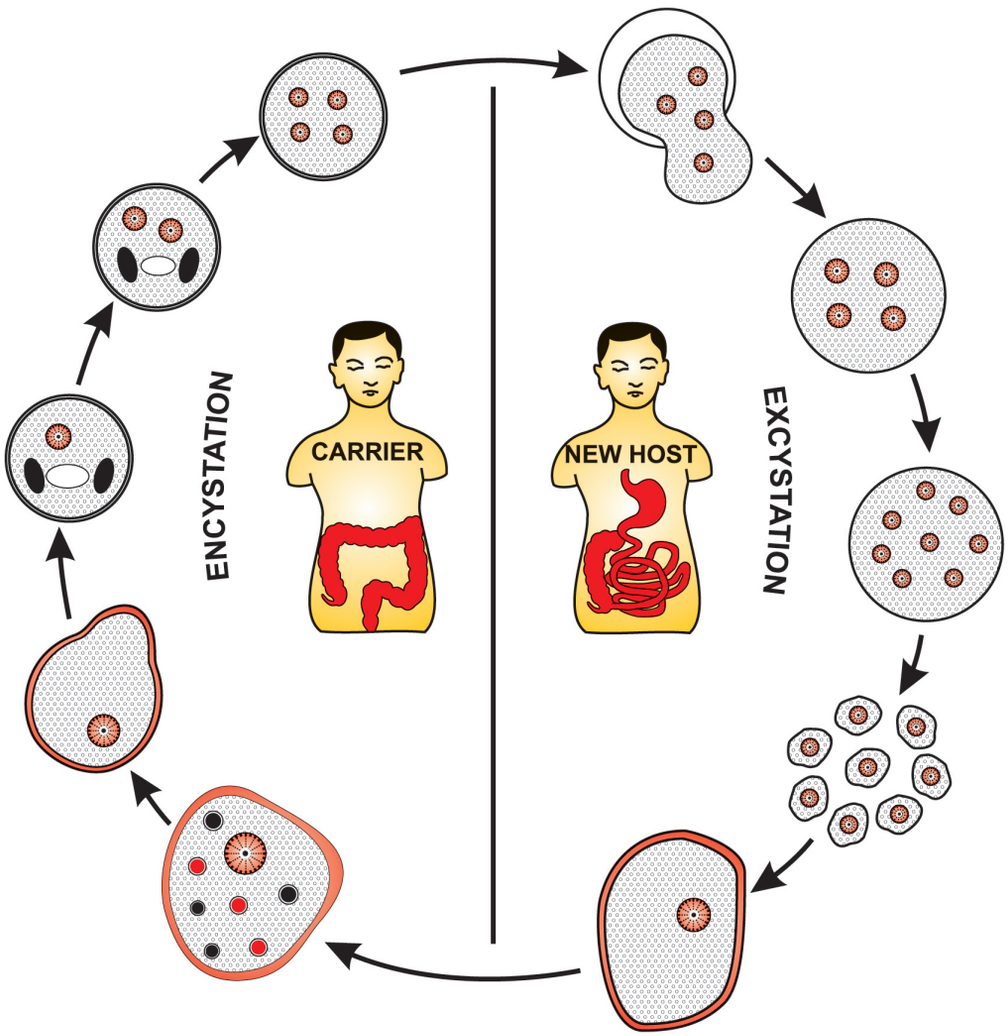


Fig. 3.2. Life cycle of *Entamoeba histolytica*.

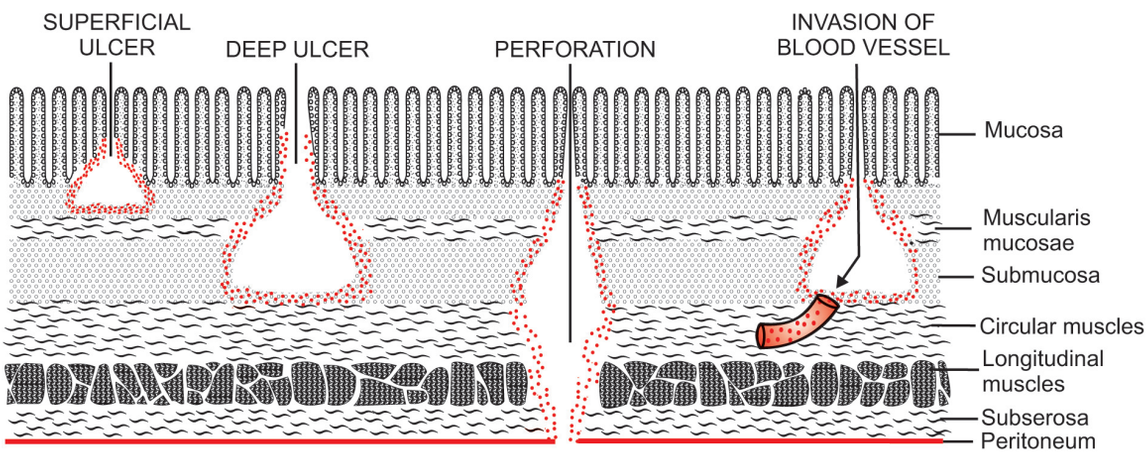


Fig. 3.3. Pathogenesis of intestinal amoebiasis.

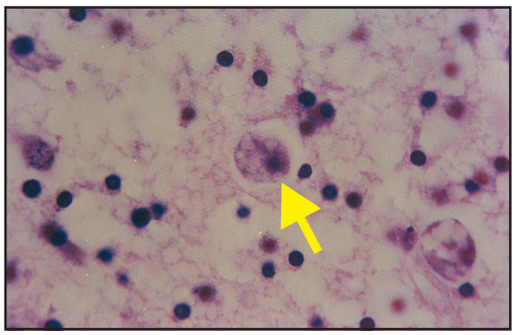


Fig. 3.19. *Acanthamoeba* in brain (haematoxylin and eosin stain, × 400).

with haematoxylin and eosin, Giemsa, Heidenhain's haematoxylin, Gomori's chromium haematoxylin, periodic acid-Schiff, Bauer chromic acid-Schiff, silver methenamine and indirect fluorescent antibody technique (IFAT). Rapid diagnosis of *Acanthamoeba* keratitis may be made by identifying amoebae or cysts in corneal scraping using procedures for Giemsa staining, calcofluor white staining and IFAT.

Culture

As in case of *N. fowleri, Acanthamoeba* may be cultured on non-nutrient agar spread with washed *E. coli* or *E. aerogenes* and incubated at 30°C instead of 37°C. *Acanthamoeba* does not have a flagellate stage but its trophozoites are identified by small spiky acanthopodia, and cysts are readily identified by their double-walled wrinkled appearance. Species identification may be made by IFAT.

Polymerase chain reaction (PCR)

PCR has also been applied for the diagnosis of *Acanthamoeba* infection.

Treatment

There is no satisfactory treatment for GAE. Total excision of the mass and treatment with ketoconazole, penicillin and chloramphenicol has been claimed to be useful. *Acanthamoeba* keratitis may be managed by use of combination of dibromopropamidine and propamidine isethionate ointment or drops, and neomycin drops. Topical miconazole and systemic ketoconazole; topical miconazole and neosporin with epithelial debridement; topical clotrimazole; oral itraconazole with topical miconazole and surgical debridement; and topical polyhexamethylene biguanide have also been claimed to be successful for the treatment of *Acanthamoeba* keratitis.

BALAMUTHIA MANDRILLARIS

B. mandrillaris is a newly described amoeba. Like *Acanthamoeba* spp. and unlike *N. fowleri,* it does not have a flagellate stage.

Morphology

Trophozoite

Trophozoites of *B. mandrillaris* (Fig. 3.20) are irregular or branching in shape. Their length ranges from 12–60 µm. They are sluggishly motile. In tissue culture, broad pseudopodia are usually seen; however, as the monolayer cells are destroyed, the trophozoites develop fingerlike pseudopodia.

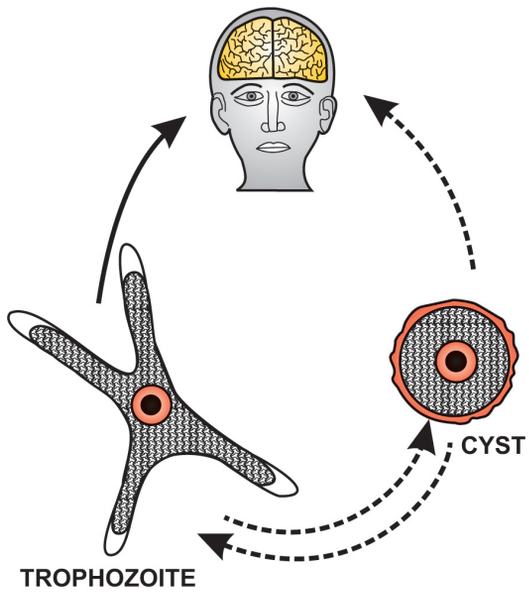


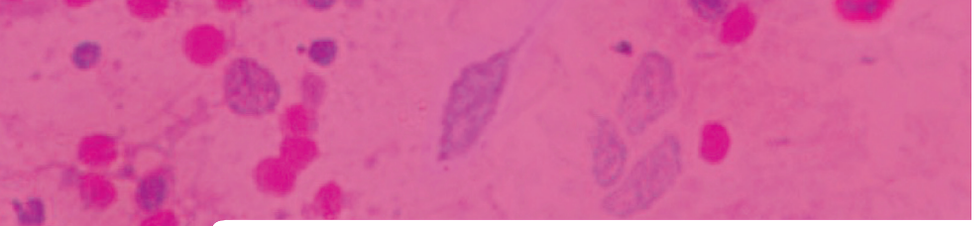
Fig. 3.20. Life cycle and pathogenicity of *B. mandrillaris.*

Cyst

It is spherical 6–30 µm in diameter with **two walls**. The inner wall is thin and spherical and the outer wall is thick and wrinkled much like the cysts of *Acanthamoeba*. Both trophozoites and cysts of *B. mandrillaris* are found in tissue.

Pathogenicity

Like *Acanthamoeba*, *B. mandrillaris* causes GAE and single or multiple brain abscesses, primarily in immunocompromised individuals. The portal of entry and spread of *B. mandrillaris* infection is believed to be the same as for *Acanthamoeba* spp. Incubation period is not known. *B. mandrillaris* can be grown in tissue culture, preferably Vero cell.



BLOOD AND TISSUE FLAGELLATES

Blood and tissue flagellates possess a single nucleus, a single kinetoplast and a single flagellum. The kinetoplast consists of parabasal body and an adjacent dot-like blepharoplast. The blepharoplast and parabasal body are connected by one or more delicate fibrils. The flagellum arises from the blepharoplast. The portion of the flagellum extending from the blepharoplast to the surface of the body of the parasite is known as axoneme. Family Trypanosomatidae consists of six genera, of which *Leishmania* and *Trypanosoma* are pathogenic to man. Species of this family may exist in two or more forms.

LEISHMANIA

The genus *Leishmania* is widely distributed in nature. It has a number of species (Table 4.2) that are nearly identical morphologically. Differentiation is, therefore, based on a number of biochemical and epidemiological criteria – use of monoclonal probes to detect specific antigens, promastigote growth patterns *in vitro* in the presence of antisera, and vectors and reservoir hosts. The parasites of the Old World leishmaniasis (*L. donovani*, *L. infantum*, *L. tropica, L. major* and *L. aethiopica*) are transmitted to humans by the bite of female sandflies of the genus *Phlebotomus*; while those of the New World leishmaniasis (*L. peruviana, L. chagasi, L. mexicana* complex and *L.braziliensis* complex) are carried by sandflies of the genera *Lutzomyia* and *Psychodopygus*. The term 'New World' refers to the Americas and the 'Old World' is used for the rest of the world.

Leishmanias pass their life cycle in two hosts – invertebrate hosts and vertebrate hosts. Former are the sandflies and the latter are mammals in which the parasites reside within the phagolysosomal system of mononuclear phagocytic cells, typically macrophages. However, in the invertebrate hosts, the parasites are extracellular, development occurs exclusively in the gut and transmission is via the mouthparts during blood feeding.

Leishmaniasis is a collection of diseases, each with its own clinical manifestations and epidemiology. It is mainly a **zoonosis**, although in certain areas of the world there is primarily human-vector-human transmission. The World Health Organization estimates that 1.5 million cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis occur every year in 82 countries. Estimates indicate that there are approximately 350 million people at risk of acquiring leishmaniasis, with 12 million currently infected. In India, visceral leishmaniasis is a serious problem in Bihar, West Bengal, and eastern parts of Uttar Pradesh. Sporadic cases have been reported from Tamil Nadu, Pondicherry, Assam, Orissa and Gujarat.

Animal inoculation

The **hamster** is the laboratory animal of choice for the isolation of *Leishmania* spp. Young (2–4 months old) hamsters of either sex are inoculated intraperitoneally with aspirates or biopsy material obtained under sterile conditions from cutaneous ulcers, lymph nodes, spleen, liver, bone marrow, buffy coat cells or spinal fluid. It results in a generalized infection. Spleen impression smears should be examined for the presence of organisms. The infection develops slowly in hamsters. Several months may be required to produce a detectable infection. For this reason, culture procedures are usually selected as more rapid means of parasite recovery. Animals should be kept for 9–12 months before a negative report is given.

OLD WORLD LEISHMANIASIS

LEISHMANIA DONOVANI

Sir William Leishman in 1900 discovered this parasite in spleen smear of a soldier who had died of 'Dum Dum fever' or kala-azar contracted at Dum Dum, Calcutta now Kolkata. Leishman reported this finding from London in 1903, in which year Donovan also reported the same parasite in spleen smear of a patient from Madras now Chennai. The name *Leishmania donovani* was therefore given to this parasite.

Geographical distribution

L. donovani is endemic in Indian Subcontinent and East Africa.

Habitat

It is an **obligate intracellular parasite of reticulo-endothelial cells**, predominantly of liver, spleen, bone marrow and lymph nodes of man and other vertebrate hosts (dog and hamster) where it occurs in amastigote form.