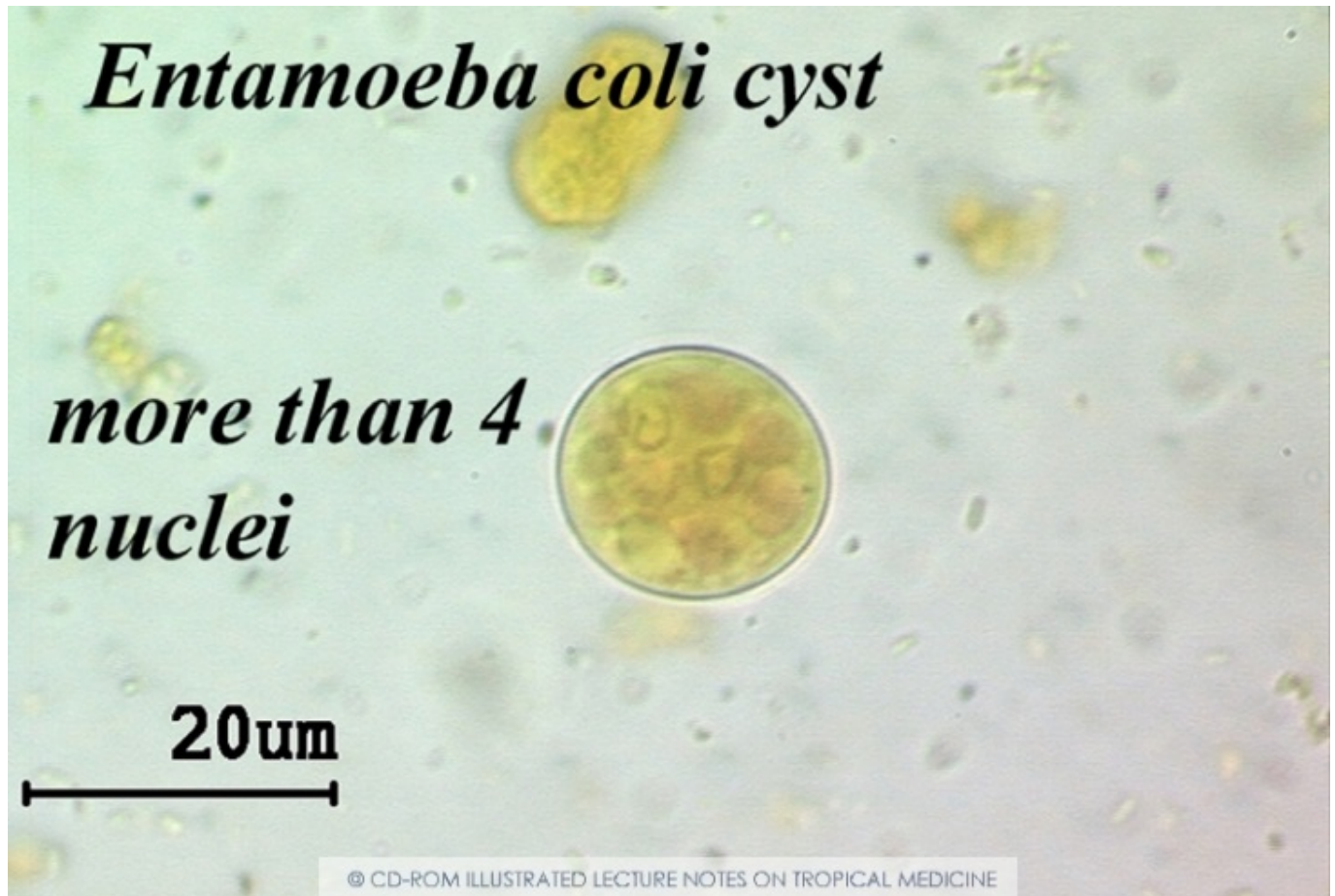
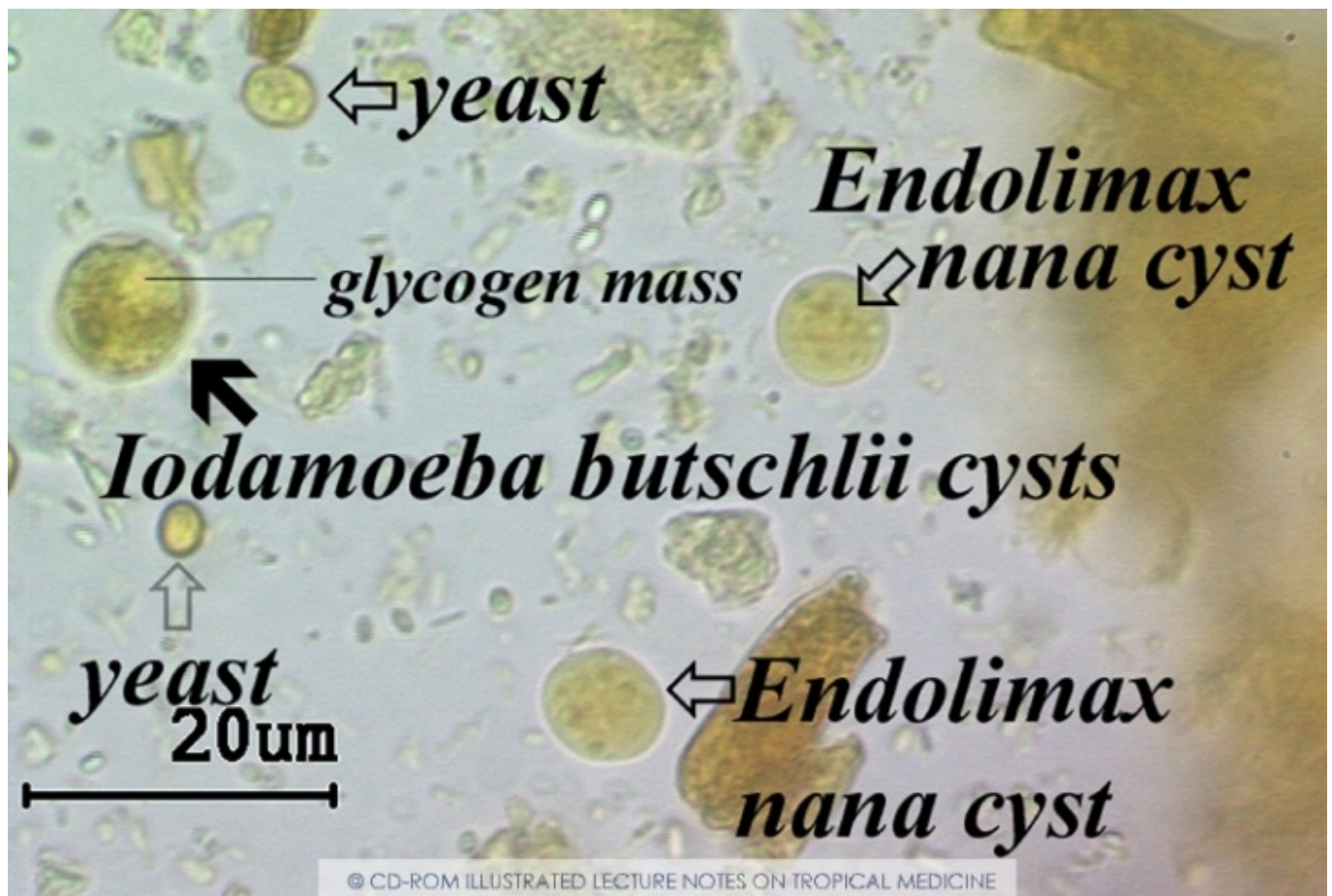


Infection with Various Protista

Non-*E. histolytica* intestinal amoebae



Entamoeba coli cyst in faeces. Cysts can obtain up to 8 nuclei. Copyright ITM



Iodamoeba butschlii in faeces. The glycogen mass will stain brown with an iodine stain.
Copyright ITM

At least 10 different amoeba species are found in the intestinal lumen or mouth. Some consider all amoebae apart from *E. histolytica* as non-pathogenic commensals, but more investigation is needed to clarify some issues especially regarding *Blastocystis hominis* and *Dientamoeba fragilis*. Pathogenicity is probably due to strain differences that are increasingly investigated. Genetic analysis indicates that *D. fragilis* is actually more closely related to *Trichomonas* than to amoebae.

1. *Entamoeba histolytica*
2. *Entamoeba dispar*
3. *Entamoeba moshkovskii*

4. *Entamoeba hartmanni*
5. *Entamoeba coli*
6. *Entamoeba polecki*
7. *Entamoeba chattoni*
8. *Entamoeba gingivalis*
9. *Endolimax nana*
10. *Iodamoeba butschlii*
11. *Blastocystis hominis*
12. *Dientamoeba fragilis*

E. dispar and ***E. moshkovskii*** are morphologically identical with *E. histolytica*. In order to distinguish between *E. histolytica* and *E. dispar* molecular tools such as PCR technology are used. Most antigen-detection tests cannot distinguish the two organisms, although one test (Wampole *E. histolytica* test) uses reagents that differentiate between *E. histolytica* and *E. dispar*. If trophozoites in stool contain RBCs, they are pathogenic *E. histolytica*, but if the trophozoites do not contain RBCs no species identification can be reached. Limited research has been carried out on *E. moshkovskii*. At present there are no good practical tests to distinguish this organism from the two other look-alikes. Its presence is suspected especially in people who have *E. histolytica* / *E. dispar*-like cysts in the stools, but who test negative for *E. histolytica*/*E. dispar* antigen. *E. moshkovskii* is highly resistant to the current amoebicidal drugs. The existence of these non-pathogenic look-alikes often results in clinical doubt and leads to overtreatment. Infections with non-pathogenic amoebae are much more frequent than infections with pathogenic *E. histolytica*.

E. hartmanni is a non-pathogenic intraluminal parasite which can only be distinguished from *E. histolytica* forms by its smaller dimensions.

Entamoeba coli is a non-pathogenic organism that is commonly mistaken for a pathogenic *E. histolytica*. Trophozoites move slowly and never contain red blood cells. *E. coli* cysts are larger (10-30 µm) and may contain up to eight nuclei.

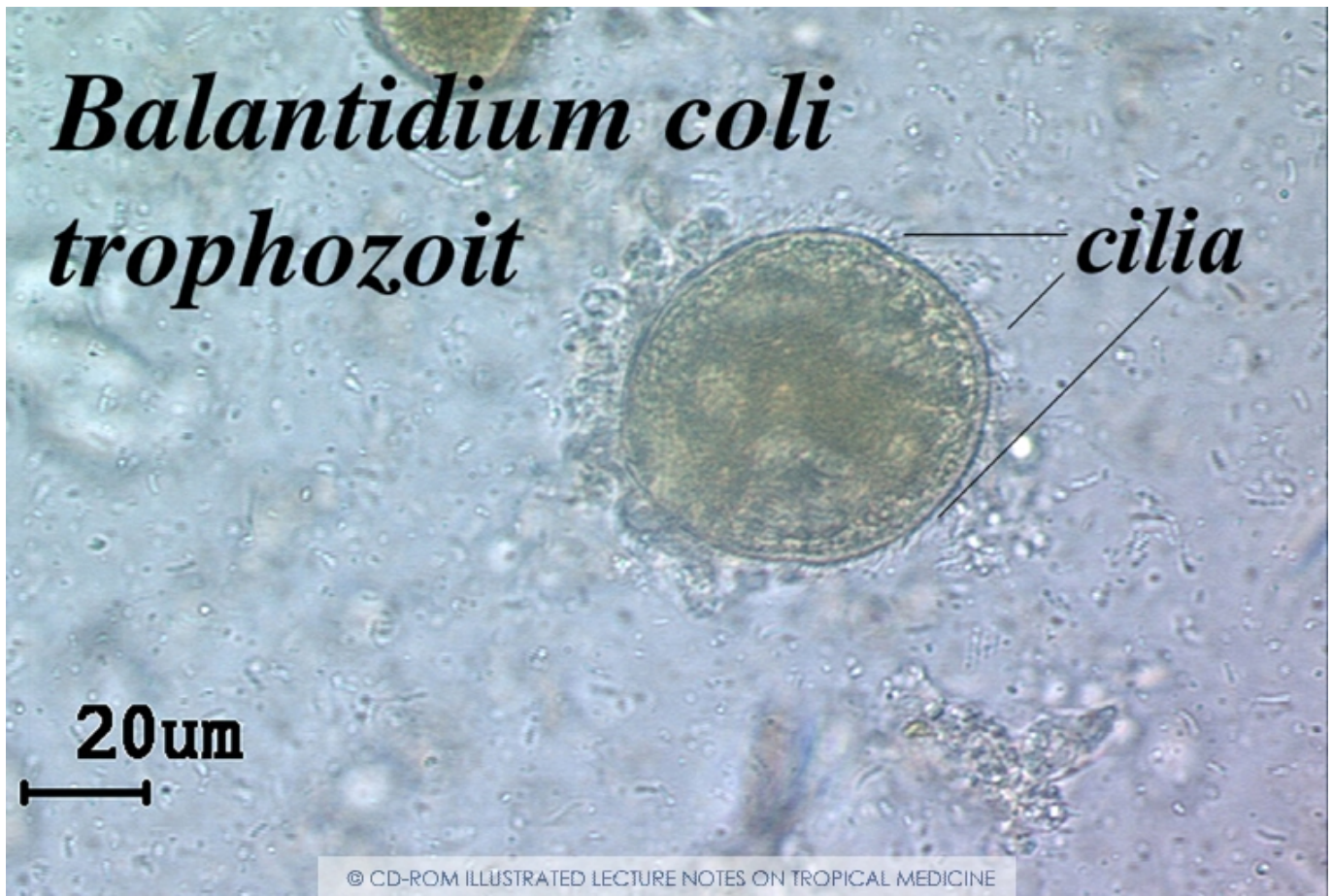
Endolimax nana is non-pathogenic. The trophozoites are small (up to 10 µm), move slowly with blunt hyaline pseudopods.

Iodamoeba butschlii also has small cysts, about 9 μm . These have only one nucleus and a glycogen mass which stains with iodine (Lugol), from which it gets its name.

Dientamoeba fragilis is an amoeboflagellate. The fact that it can develop flagella puts it in a different taxonomic group from the above-mentioned amoebae. It is more closely related to *Trichomonas* sp than to *Entamoeba histolytica*. It is a non-invasive intestinal parasite. Many infections are asymptomatic, but it has been associated with non-specific diarrhoea. It is very difficult to demonstrate with the microscope because the vegetative form is easily damaged (*fragilis* = breakable). No cyst stage is known and it is unclear if transmission via trophozoites can take place. One hypothesis as to how transmission of such a fragile microorganism is possible is that *Enterobius vermicularis* (pinworms) could function as vectors but solid evidence is lacking. If the faeces cannot be brought quickly to the laboratory (ideally < 10 min), they should be fixed in PVA (polyvinyl alcohol) or SAF (sodium acetate formalin), otherwise the parasite will most likely not be detected. There seems to be wide genetic variability between isolates, e.g. as demonstrated by differences in DNA melting temperature or variability of certain DNA markers. As more information will become available in the future; it is possible we will encounter a scenario like the one with *Entamoeba histolytica* (being pathogenic) and *Entamoeba dispar* (non-pathogenic): i.e. a heterogeneous species with genetic variants that have similar morphologies but different pathogenicities. It is clear that more study is needed. *Dientamoeba fragilis* infections can be treated with a 5-day course of metronidazole, but a single 2-gram dose (adult patient) of ornidazole it is easier and gives less side-effects. Paromomycin and iodoquinol can also be used and actually give higher cure rates.

For ***Blastocystis hominis***, see below (separate chapter).

Balantidium coli



Balantidium coli trophozoite. Notice the numerous cilia. Copyright ITM

Balantidium coli is a large protozoon. The trophozoite measures 30-200 μm x 40-60 μm . The whole surface of the trophozoite is dotted with countless cilia. These are very characteristic and because of this, it is classified as a ciliate (compare with *Paramecium*). *Balantidium coli* is the only ciliate pathogenic to humans. Transmission occurs from pigs to humans and from human to human in poor hygiene situations, also via water or food contaminated with cysts including poorly cooked pork sausages. As with amoebiasis the infection may be intraluminal and latent or invasive in the intestinal wall and symptomatic. In the invasive forms ulcerations of the intestinal wall are found which are quite similar to those of amoebiasis, with the same complications and the same clinical forms. Liver abscesses caused by *B. coli* have been observed but are extremely rare. Diagnosis is parasitological by direct stool microscopy or enrichment techniques. In a fresh preparation, *B. coli* can be very quickly recognised due to its swift manner of propulsion. Under the microscope, the creature is

difficult to keep in the field of vision due to its relatively high speed. Treatment is not always simple. Tetracyclines (10 days) have been used as well as imidazoles in high doses.

Flagellum

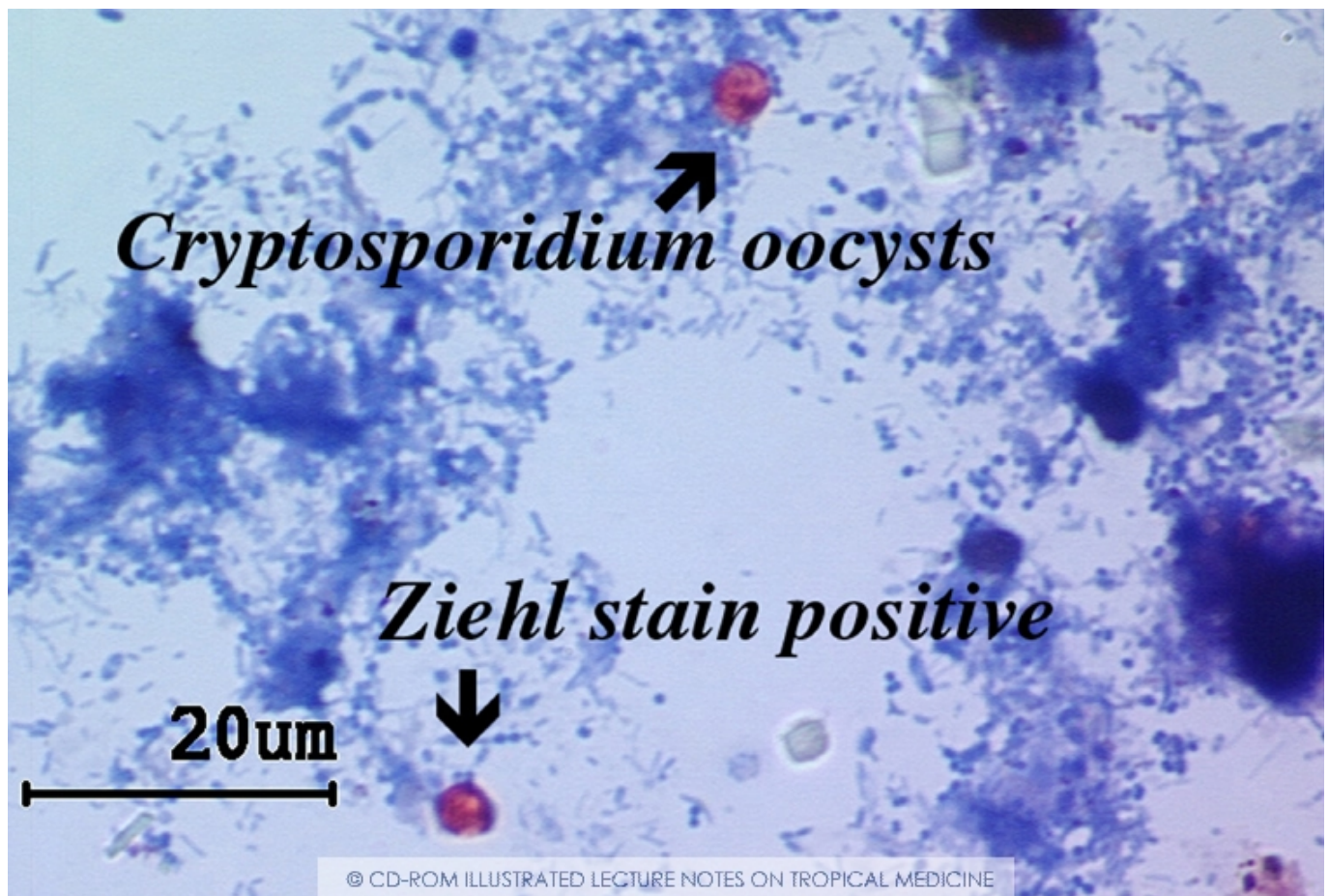
One important organelle to move in an aqueous environment is the flagellum. A certain group of micro-organisms (flagellates) take their name from the fact that they possess flagella. The term flagellum (L. flagellum = whip) is used, however for two totally different organelles. Some micro-organisms are dotted with myriads of these organelles which work in a coordinated way and which are then called “cilia”.

Cryptosporidiosis

C. parvum is the most common parasite in this group in human infections, but *C. meleagridis*, *C. canis*, *C. muris* and *C. felis* are also found in immune-compromised persons with acute diarrhoea.

Biological classification

Cryptosporidia are coccidia and belong to the Apicomplexa phylum. Coccidia form an order of unicellular eukaryotic micro-organisms, which includes the following human pathogens: *Toxoplasma gondii*, *Sarcocystis* sp., *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli*. Microsporidia do not belong to the Coccidia and form a totally different taxonomic group. DNA analysis of *Cryptosporidium* suggests that there could be more than twenty different species.



Cryptosporidium parvum, Ziehl stain. Copyright ITM

Transmission to humans occurs from calves, dogs and cats. Transmission via drinking water or via insufficiently chlorinated water in swimming pools happens frequently. This species is resistant to standard chlorination. The parasite was first observed in humans in cases of persistent diarrhoea in patients with immunosuppression and since 1981 in cases of AIDS. Since 1983 the infection has frequently been recognized as a cause of benign and brief diarrhoea, both in children and adults, and it is one common aetiologies of travellers' diarrhoea.

Life cycle details

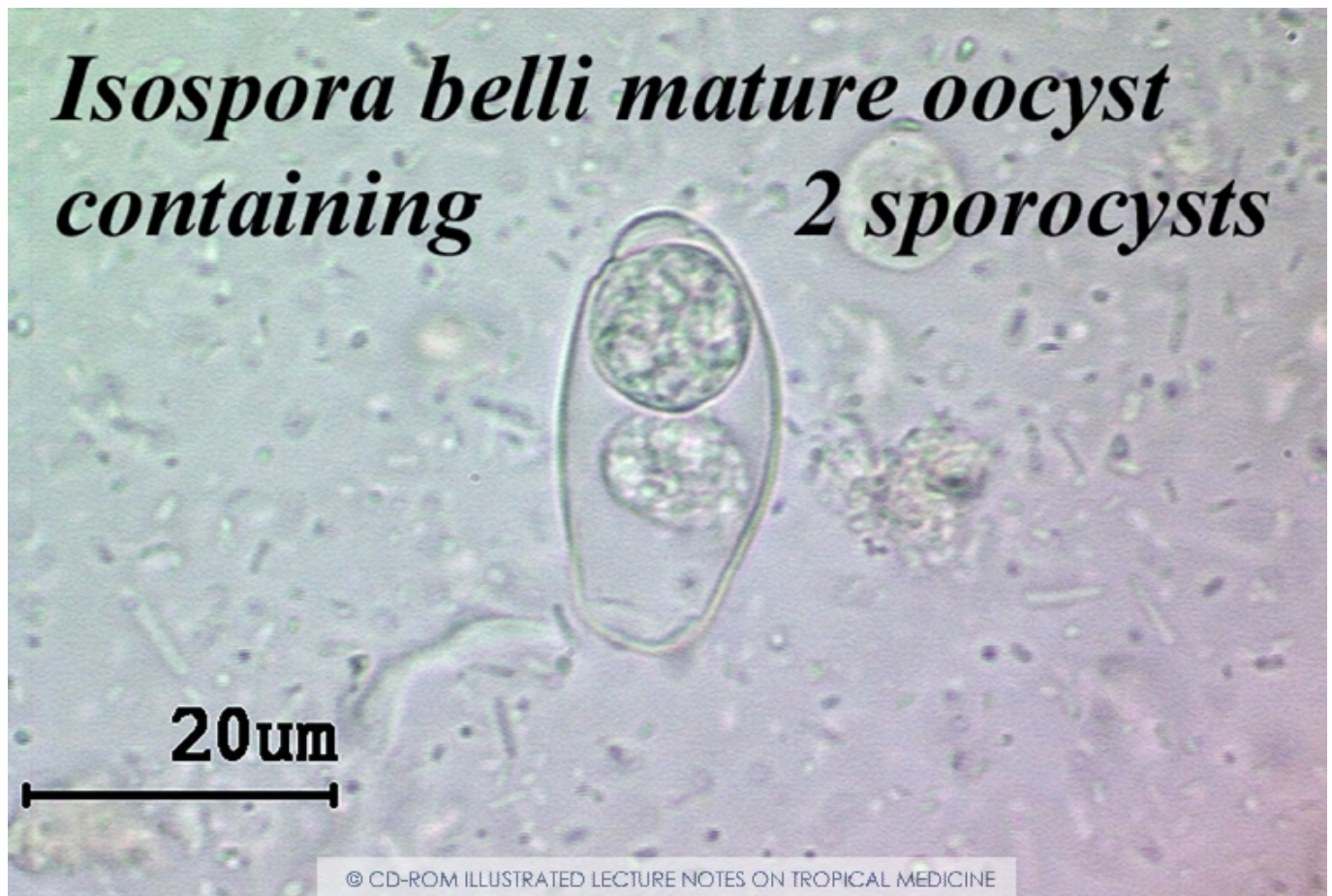
The complete cycle of the parasite, sporogony and schizogony, takes place in the same

host. People become infected by swallowing thick-walled, resistant oocysts. Once in the intestine the parasites excyst and release sporozoites. They penetrate epithelial cells via the apical membrane. After maturation of the sporozoite there is asexual reproduction via schizogony with the formation of merozoites. These may either penetrate a new epithelial cell to repeat the cycle (type 1 merozoites) or undergo further intracellular changes (type 2 merozoites) to the sexual form of the parasite. The macrogamont is the female form, the microgamont the male form. The microgamont releases microgametes. After fertilisation and the formation of zygotes, thin-walled oocysts are produced, which after meiosis release sporozoites in their turn which amplifies the infection (auto-infection). Thick-walled oocysts are released into the lumen of the intestine, and are directly infectious via the faeco-oral route. *C. parvum* induces apoptosis in epithelial cells.

The parasites may be found throughout the entire digestive tract and even in the mucosa of the respiratory tract but are usually limited to the duodenum and jejunum. The incubation period is 4 to 12 days (usually 7-10 days) and is followed by moderately severe diarrhoea without fever usually and with little abdominal pain. Asymptomatic infections may occur. If there is no underlying immunosuppression, spontaneous recovery occurs within a few weeks. It is estimated that 4 to 10% of all cases of diarrhoea in children in tropical environments can be attributed to *Cryptosporidium*. In patients who have a deficiency in cellular immunity (such as in HIV infection), the diarrhoea is more pronounced can be chronic for several months and recurrent. Fulminant infection with cholera-like diarrhoea may occur in patients with fewer than 50 CD4 T-cells/mm³. Sometimes the protista enter the biliary tract, resulting in sclerosing cholangitis, strictures and papillary stenosis. Diagnosis is difficult and requires invasive procedures such as retrograde cholangiography (ERCP [endoscopic retrograde cholangiography]). A biliary tract reservoir may contribute to the chronic course of infection. Diagnosis is based on looking for the parasite in the faeces on smears stained with modified Ziehl-Neelsen or Kinyoun staining. The small dimensions of the parasites and their similarity to yeast cells were responsible for the fact that infection in humans was only recognised in 1976. The parasites can easily be recognised on intestinal biopsy material obtained by endoscopy. There are other diagnostic techniques, such as immunofluorescence, antigen-capture ELISA and PCR, but these are not available in most tropical settings. Treatment is mainly symptomatic and can be quite difficult in AIDS patients. The best practical method in these patients is via HAART (highly active antiretroviral therapy). Paromomycin (Humatin®, Gabbrolal®) is a non-absorbed aminoglycoside and is of limited use. At present the drug of

choice is nitazoxanide (Alinia®, Cryptaz®, 500 mg tablets or syrup) but this drug is unaffordable. Cryptosporidium cysts are very resistant to chlorination (much more so than Giardia cysts, although even those have a certain resistance to standard concentrations in drinking water).

Cystoisosporosis



Isospora belli mature oocyst containing two sporocysts. Copyright ITM

Cystoisospora belli is a coccidian parasite of the duodenum and proximal small intestine (jejunum) in humans. It is cosmopolitan but more frequent in a tropical environment. The previous name was *Isospora belli*. No reservoir hosts other than man are known. The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist. The sexual and asexual cycles occur in the same host. The parasites are located

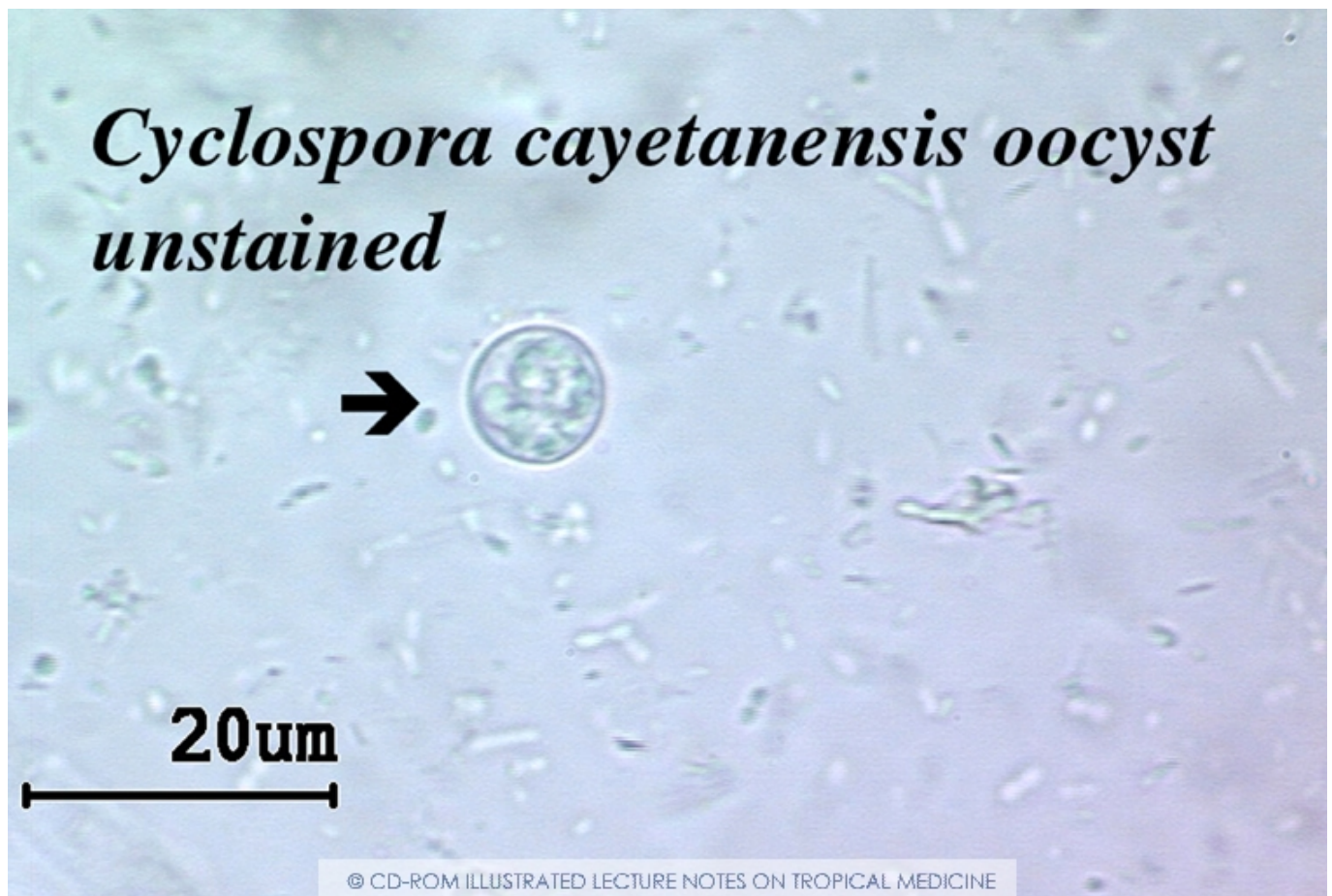
intracytoplasmic, unlike *Cryptosporidium*. There is a prepatent period of about 9-10 days. Infection may be latent or lead to diarrhoea for one to two weeks occasionally with mild fever, headache, malaise and abdominal pain. The stools tend to be soft, watery or foamy, with an offensive smell, suggesting malabsorption. In immunosuppressed people the infection can become chronic. In such cases oocyst shedding can continue for years. Diagnosis is difficult and is based on stool examination, and biopsy of the duodeno-jejunal mucosa, in which the parasites are not very numerous. Charcot-Leydig crystals (derived from eosinophils) are occasionally found in stools samples of isosporiasis cases.

The infection can be severe and prolonged in case of immunosuppression (in particular in AIDS). It is like cryptosporidiosis a frequent cause of prolonged/recurrent cholangitis in AIDS patients through invasion of the biliary tract.

The condition can be treated with cotrimoxazole (e.g. Bactrim forte® 4 x 1 tablets/day for 10 days). If there is diminished sensitivity or resistance, either pyrimethamine (Daraprim®) 25 mg/day x 20 weeks or the combination ornidazole (e.g. 2 gram on day 1, 15, 30) with albendazole (400 mg BD x 30 days) is used. Ciprofloxacin is also moderately effective (70% cure rate).

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Cyclospora cayetanensis



Cyclospora cayetanensis in faeces, unstained. The parasite is about double the size of *Cryptosporidium parvum*. Copyright ITM

Cyclospora cayetanensis is a protozoon which belongs to the Coccidia. The name is derived from the morphology (the sporocysts are spherical) and from a Peruvian university (most of the epidemiological and taxonomic work has been carried out at the Universidad Peruana Cayetano Heredia, Lima, Peru). Distribution is probably cosmopolitan, but the species is only common in regions with poor hygiene. Protista can be detected in surface water with special techniques. No reservoir is known to date.

After swallowing mature (i.e. sporulated) oocysts, there is excystation after contact with bile salts. The released sporozoites penetrate the jejunal enterocytes. Infected persons eliminate non-sporulated oocysts in their faeces. Until they sporulate, which takes days or weeks these

parasites cannot infect a new host. This delay makes direct human to human transmission unlikely.

The protista are present in the duodenum and jejunum and cause persistent watery diarrhoea, often accompanied by significant abdominal discomfort, nausea, tiredness and anorexia and sometimes with mild fever. The symptoms may last several weeks. In particular, non-immune persons such as travellers or small children, will be symptomatic. Cotrimoxazole is used in treatment. This protozoon also causes persistent diarrhoea in HIV-positive persons. If patients cannot tolerate cotrimoxazole, the rather less effective ciprofloxacin may be used.

Sarcocystosis

General

Sarcocystis species are parasites of mammals, birds and reptiles. Human sarcocystosis (syn. sarcosporidiosis) is rarely diagnosed. For some species humans are the definitive host i.e. the host in which sexual reproduction (gametogony followed by sporogony) is completed. In this case there is intestinal sarcocystosis. Humans may also act as accidental dead-end intermediate hosts – where asexual reproduction (schizogony) takes place – for several other species and in these cases there is muscular sarcocystosis.

Intestinal sarcocystosis

Intestinal Sarcocystis sp.



Intestinal Sarcocystis Copyright ITM



Sarcocystis, pseudocyst in muscle. Copyright ITM

Sarcocystis bovi hominis and *Sarcocystis sui hominis* are parasites of humans. Infection occurs due to eating raw or insufficiently cooked meat from cattle or pigs containing tissue cysts (intestinal infection cannot be triggered by the ingestion of sporocysts). The sexual cycle takes place within the cytoplasm in the cells of the human intestinal mucosa. The sporocysts which are released with the faeces are infectious for the intermediate host. These infections are cosmopolitan and generally asymptomatic. They can nevertheless trigger enteritis with peripheral hypereosinophilia. The diagnosis is based on faecal examination. Sometimes the parasites will be detected in surgical resected intestinal specimens. Gastro-intestinal disease is often self-limiting and does not need treatment. There is no known effective treatment.

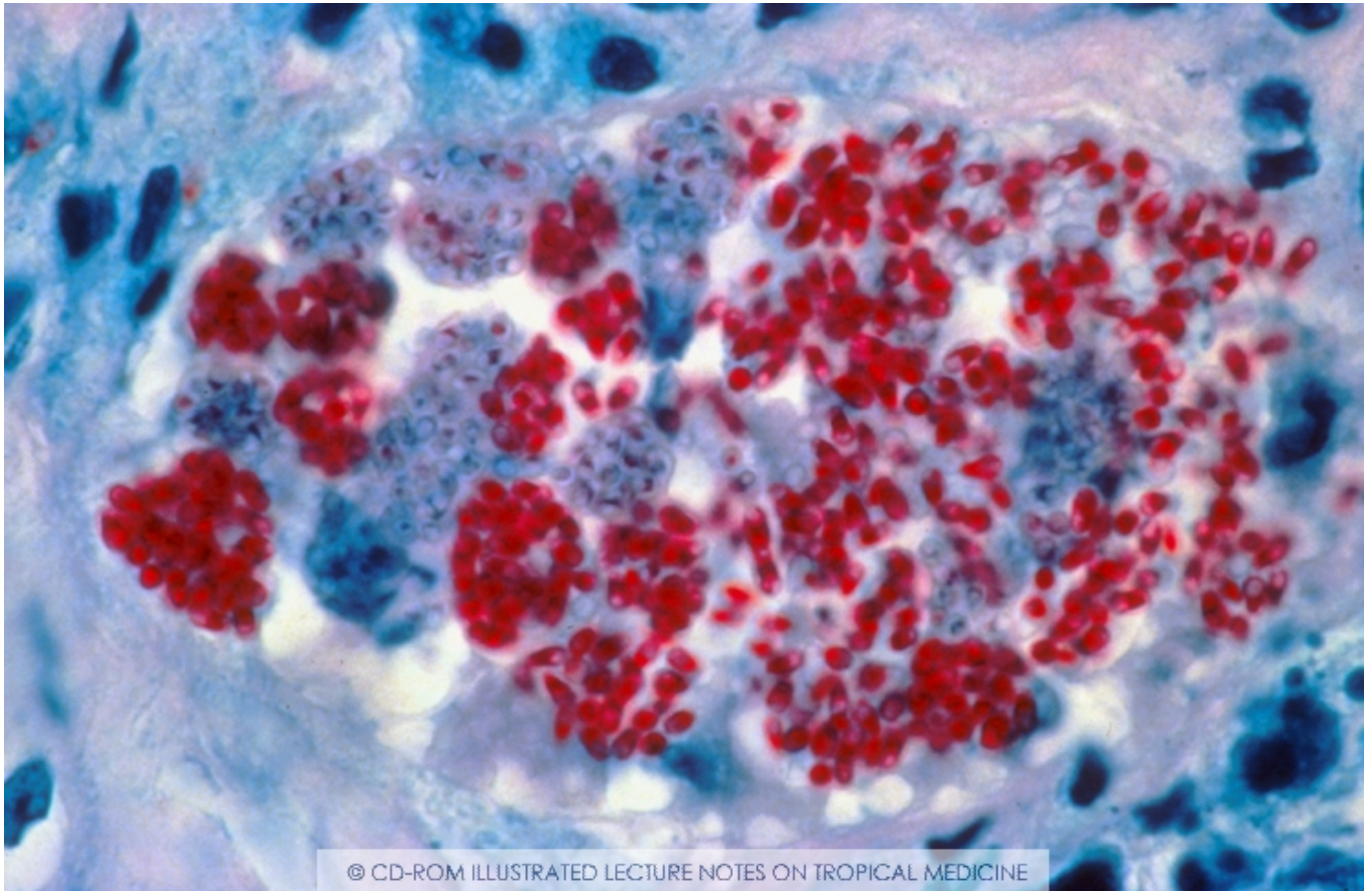
Muscular sarcocystosis

Muscle infection is caused after swallowing sporocysts (faeces of an infected predator). Each sporocyst releases 4 sporozoites. These penetrate the intestinal wall. Reproduction begins in the vascular endothelium. After dissemination of merozoites there is invasion of skeletal and cardiac muscle tissue and possibly the central nervous system (in animals). The merozoites develop first to metrozoites and then to cystozoites. These tissue cysts remain dormant until the host is eaten by a predator after which the intestinal cycle begins. The tissue cysts gave the genus its name (Gr. sarx = flesh).

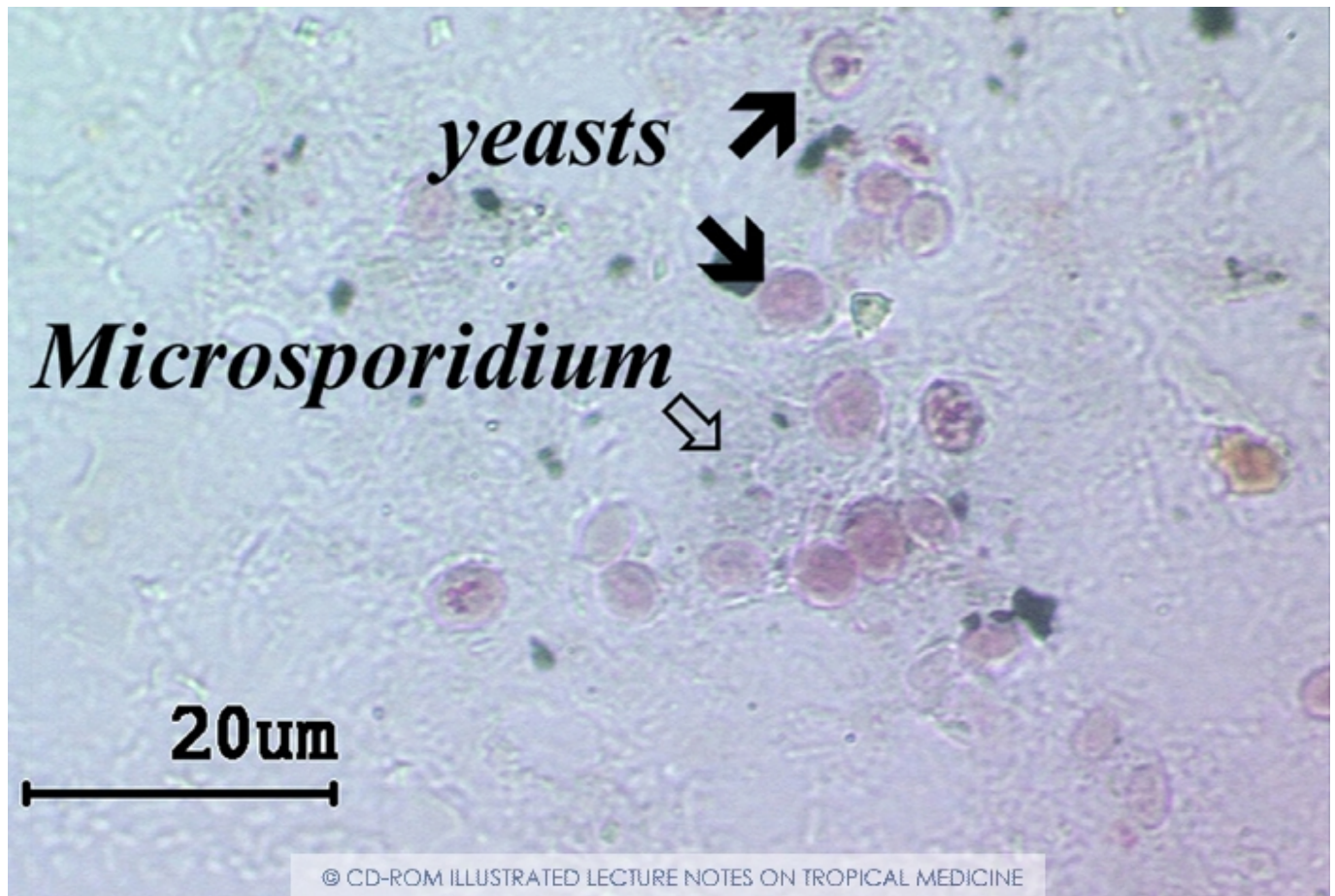
Most human infections are apparently asymptomatic. It is also possible that the diagnosis is systematically missed (data from investigation of routine autopsies). No cases of neurological involvement in human patients are known. Some patients with muscular sarcocystosis develop an eosinophilic myositis. The myositis is characterised by muscle pain, painful mild muscular swelling, mild fever, general weakness, bronchospasms and eosinophilia. This should be differentiated from trichinosis (*Trichinella spiralis*). Eosinophilic fasciitis, toxoplasmosis, polymyositis, dermatomyositis and polymyalgia rheumatica may lead to similar clinical pictures. Diagnosis is made via muscle biopsy. The intact cysts in the muscle generally do not trigger a local inflammatory reaction. Dead and ruptured cysts may cause inflammation. Muscular sarcocystosis can be treated with cotrimoxazole, although its efficacy is not proven. The use of corticosteroids is under discussion but often necessary to control the symptoms of myositis when they are prominent.

Microsporidiosis

General



Microsporidia in muscle of AIDS-patient. Ziehl-stain. Copyright ITM



Microsporidium sp. in faeces. Species identification requires PCR or electron microscopy.
Copyright ITM

Species belonging to the phylum Microspora are called microsporidia. At present more than 140 genera are recognized and 1200 species have been described. These obligate intracellular organisms appear to have separated very early from the eukaryotic family tree. They have true nuclei, but no mitochondria or peroxisomes. Their ribosomes are prokaryote-like (70S). Since the spore wall contains chitin some researchers regard them as aberrant fungi. They are obligate intracellular parasites and are recovered in countless widely varying host groups (insects, fish, rodents, and so on). Species which can parasitise humans are very small (1-2 μm).

DNA structure, ultrastructure and lifecycle

Encephalitozoon cuniculi holds the record at present for the smallest eukaryotic genome (<2.9 Mb). Other species known to infect humans are *Brachiola vesicularum*, *Encephalitozoon cuniculi*, *Encephalitozoon hellem*, *Encephalitozoon intestinalis* (previously *Septata intestinalis*), *Enterocytozoon bieneusi*, *Microsporidium africanum*, *Microsporidium ceylonensis*, *Nosema algerae*, *Nosema connori*, *Nosema ocularum*, *Pleistophora sp*, *Trachipleistophora hominis*, *Trachipleistophora anthropophthera* and *Vittaforma corneae* (previously *Nosema corneum*). These organisms have mainly been described in immunodeficient persons.

The parasites have a very characteristic ultrastructure. The organism forms oval-shaped spores with an external exospore (glycoproteins) and an internal endospore (chitin). Within the spore is a coiled spiral tube (polar tube). After it is ingested, the spore is stimulated to protrude this polar tube which then penetrates a host cell. The sporoplasm is then injected via this tube into the cytoplasm of the host cell. Subsequently there is reproduction of the parasite (merogony and sporogony). New spores may infect other neighbouring cells or be passed to the outside world to infect a new host.

Transmission

Transmission is chiefly via the faeco-oral route but much is still uncertain. Possibly transmission is via aerosol for those protista which cause corneal lesions. Transmission via infected water is being investigated.

Clinical aspects

Symptoms will be determined by the anatomical location of the parasites. Disseminated infections, corneal infections (keratitis), intestinal locations etc all occur, almost exclusively in immunosuppressed individuals. In HIV patients with low resistance (CD4 < 100/ μ L) there is often persistent diarrhoea, abdominal pain, loss of weight and sometimes sclerosing cholangiopathy.

Diagnosis

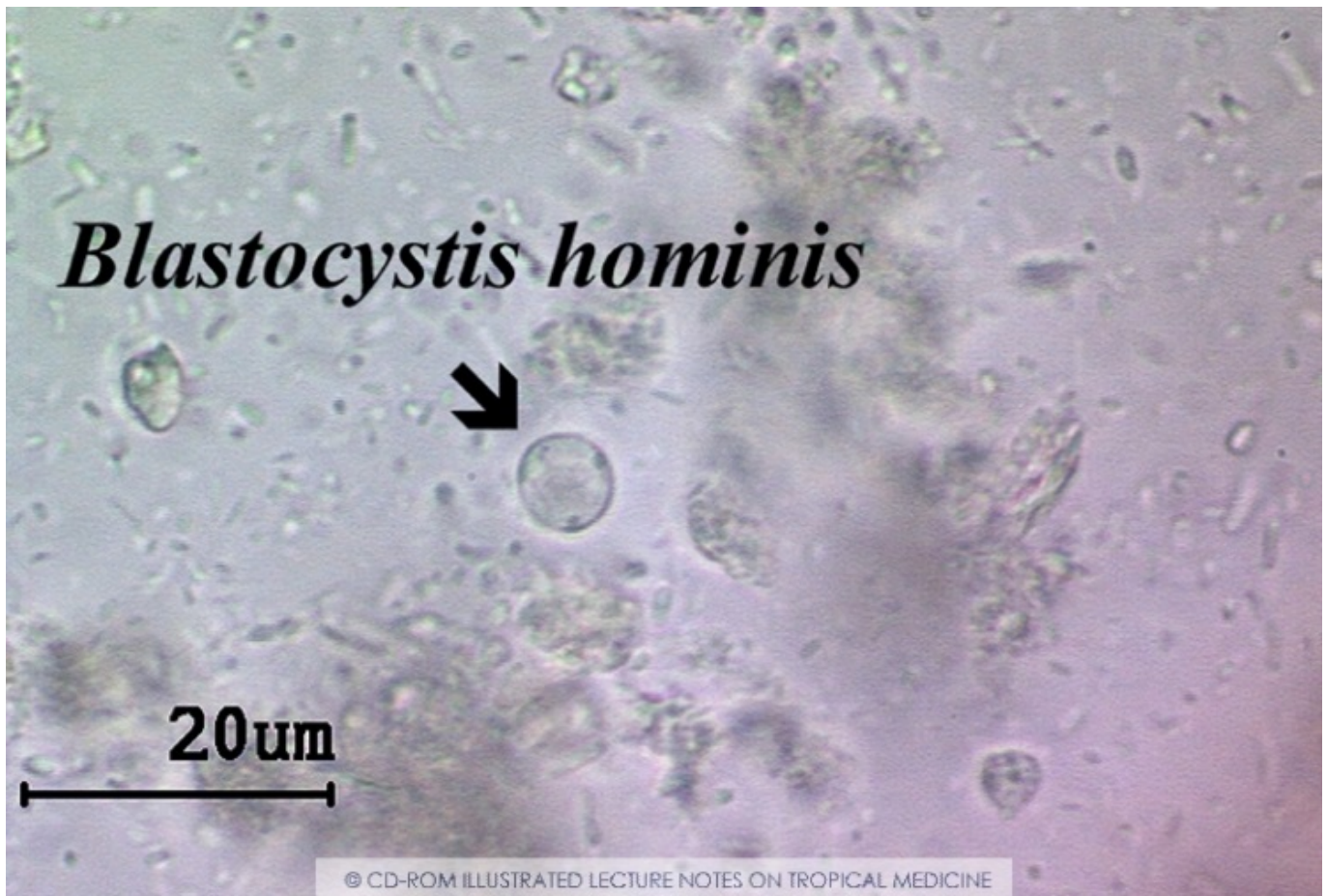
Diagnosis by light microscope (faeces, biopsies, corneal scraping) is often difficult due to the small dimensions of the parasites and the labour-intensive staining techniques. Experience is essential and the parasites must be properly differentiated from fungal spores and bacteria. Electron microscopy is a good technique for species identification, together with PCR but of course this can only be carried out in specialized centres. The organisms can be detected in routine formalin-fixed and paraffin-embedded tissues.

Treatment

There is still too little known about treatment. Fumagillin is a product originating from *Aspergillus fumigatus* which is used in microsporidiosis of honey bees, has been used topically in keratitis with good results. Other drugs have been used with varying success. Albendazole is effective in infections with *Encephalitozoon intestinalis* and to a lesser extent in *Enterocytozoon bieneusi*. Nitazoxamide possibly has a place in treatment. Improving immunity in HIV patients, e.g. by combination antiretroviral therapy, often leads to remission of the infection. For symptomatic treatment (e.g. in persistent diarrhoea without knowing its cause), loperamide (Imodium®), opioids (laudanum) or even somatostatin analogues may be used. The latter is of course not easily available in developing countries.

Blastocystosis

Blastocystis hominis is a rather common enteric unicellular protista. The parasite colonises chiefly the caecum and to a lesser extent the distal colon.



Blastocystis hominis. Copyright ITM

Biology

Very little is known of the basic biology of this organism, including the life cycle. Several morphological forms have been recognized: ameboid, vacuolar, avacuolar, multivacuolar, granular, cyst. Which of the forms is responsible for transmission is not known.

The vacuolar stage divides, while the amoeboid stage might be invasive and is capable of budding. *B. hominis* forms pseudopods, and ingests bacteria and debris. It reproduces by binary fission or sporulation.

Transmission is faeco-oral through contaminated food or water. There seems to be a large animal reservoir. Its pathogenicity is controversial. Several studies using different methods

and examining different patient groups have reported very variable results, from asymptomatic infection, acute symptomatic infection and chronic symptomatic infection; with abdominal pain, diarrhoea, constipation, irritable bowel, fatigue, skin rash, and other symptoms. The variation in results led to disagreements concerning a possible pathogenic role of *Blastocystis* in humans. Maybe *Blastocystis* has several variants which differ in their pathogenicity or virulence. The pathogenicity might depend on the parasitic load (more than 5 *Blastocystis* per 40x field, but different pathogenic properties of different strains will likely also play a role). Molecular typing has revealed extensive genetic diversity in morphologically identical strains. According to current PCR-based genotype analysis there may be 12 different species which are lumped together under one name. It is possible that additional studies will show that what we call *Blastocystis hominis* will turn out to be a mixture of different microorganisms, a situation similar to the past confusion about the morphologically identical *Entamoeba histolytica*, *E. moshkovskii* and *E. dispar*.

Classically *Blastocystis* is considered to be non-pathogenic and doesn't need treatment. However a treatment can be justified if symptoms are severe in the absence of other pathogens or in immunosuppressed patients (AIDS), or if the parasitic load is very high. If considered necessary, metronidazole/tinidazole or trimethoprim/sulfamethoxazole are used for treatment.

Rhinosporidiosis

Rhinosporidiosis is an infectious disease which occurs in the New World, Europe, Africa and Asia, but is most common in the tropics (India and Sri Lanka). The disease is characterised by slow-growing, painless polyps or tumour-like masses, which are usually found on the nasal mucosa, lachrymal sac, conjunctivae, palate, larynx or penis. Chronic rhinitis and/or epistaxis may occur. Treatment consists of surgical excision, but recurrence can be expected in approximately 10% of patients. No natural reservoir is known. It is also assumed that people become infected by swimming in fresh water lakes or rivers. It is likely that fish or other water creatures are the normal hosts.

Protothecosis

Protothecosis is a rare infection in humans. Infection is more common in cases of immunosuppression (AIDS, leukaemia).

The disease is caused by *Prototheca wickerhamii* and *P. zopfii* (segbwema). These are aerobic unicellular round (*P. wickerhamii*) to oval (*P. zophii*) algae which activity belong to the Chlorococcales [Chlorophyta or green algae]. However they contain no chlorophyll and are colourless. The protista occur in still water, sewage sludge, mud and slime on trees.

Various animals may be infected (cattle, dogs, rabbits, mice, rats, pigs, deer). Humans are infected via traumatic inoculation of the germ into the skin or via infection of an open wound. Infection is usually limited to the skin, where local painless granulomatous hyperkeratotic dermatitis results. Bursitis and tenosynovitis have been described. Indolent olecranon bursitis can be tender. Sometimes there is systemic involvement including cholangitis, chronic meningitis and retinitis. There have been cases of peritonitis after peritoneal dialysis.

Diagnosis is made by biopsy. The pathogens are morphologically similar to mulberries. Confusion with yeasts is possible. For tissue sections a PAS [periodic acid-Schiff] or a Gomori methenamine silver stain are used.

Treatment is surgical with or without amphotericin B. Ketoconazole has frequently been used with success, but requires long-term administration. The possible therapeutic roles of itraconazole and fluconazole need to be better determined.

Babesiosis

General

Babesiosis is a zoonotic disease which is triggered by infection with a protozoon of the genus *Babesia*. The disease is also known as piroplasmosis. The order of Piroplasmida belongs to the Apicomplexa (cf. malaria). There are more than 110 species in the genus *Babesia*. Some infect fish, birds, reptiles or mammals. The rodent parasite *Babesia microti* (USA) and the bovine parasites *B. divergens* and *B. bovis* (Europe) cause most infections in humans. Occasionally other species may be responsible for human infections (e.g. the WA1 strain = *B.*

duncani).

Transmission

Voles form the reservoir. Transmission is via the bite of hard ticks such as *Ixodes scapularis* and *Ixodes ricinus*. In the USA larval nymphs of *Ixodes scapularis* feed chiefly on *Peromyscus maniculatus* ("the white-footed deer mouse"). The adult ticks suck blood from deer (cf. Lyme disease). Strangely enough the deer are not infected with *B. microti*. In ticks trans-stadial transmission occurs. The parasite passes from larva to nymph to adult tick. There is no transovarian transmission. Infections in humans are accidental occurrences. After injection of saliva of the tick, the micro-organisms penetrate red blood cells and mature. *Babesia microti* trophozoites undergo asexual reproduction in human blood and divide into two or four merozoites. Infected red blood cells undergo haemolysis. This releases the protista which can then penetrate new red blood cells. Infections via blood transfusions have been described. Transplacental infection may occur.

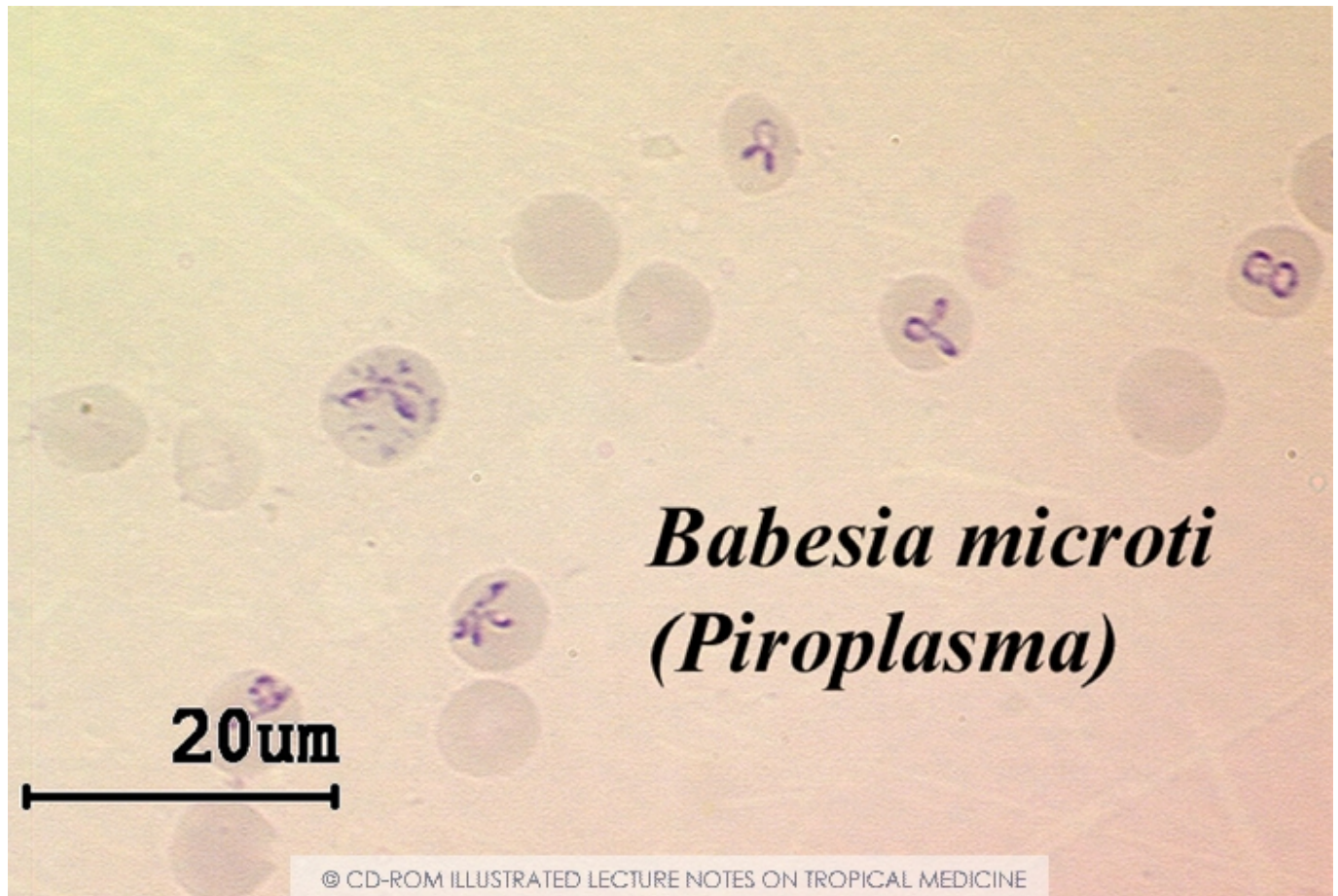
Geographical distribution

Endemic regions in the USA include Massachusetts and New York State with Nantucket Island, Long Island, the coast of Connecticut as well as foci in Georgia, California and Wisconsin. Cases have also been reported from various European countries such as Ireland, Scotland, Sweden, former Yugoslavia, France and Russia. There have been isolated case reports from Africa, Asia and Latin America.

Clinical aspects

Asymptomatic infection may persist for months or years. If symptomatic, the first symptoms occur after an incubation period of one to two weeks. Malaise, tiredness, fever, headache, nausea and abdominal pain, myalgia and joint pain are early but non-specific symptoms. The body temperature may rise to 40°C. Hepatosplenomegaly with haemolysis and jaundice, haemoglobinuria, mild neutropenia and thrombocytopenia follow. In severe cases ARDS [acute respiratory distress syndrome] with shock may develop. Infections may have a dramatic course in asplenic persons, chiefly in the European forms.

Diagnosis



Babesia microti. Copyright ITM

Diagnosis is made from a blood smear stained with Giemsa. The parasitaemia is generally 1 to 10%. Sometimes the mature parasite is in the form of a clover leaf: a so-called tetrad or Maltese cross. The intra-erythrocytic dimension of the merozoite is 1 to 2.5 μm . It is pear-shaped, oval or round. The circular appearance means that *Babesia* is often confused with *Plasmodium falciparum*, but malaria pigment cannot be detected. There are also no gametocytes or malaria schizonts. In *Babesia* infections, large parasites may contain a central white vacuole, which is not present in malaria. Serological tests and DNA analysis may help in diagnosis.

Treatment

Quinine is the drug of choice, 650 mg TDS plus clindamycin 600 mg TDS or 1.2g BD IV for 7 to 10 days. Children receive 25 mg quinine/kg/day. Atovaquone (750 mg BD) and azithromycin (500 mg on day 1, then 250 mg daily) are also used and this combination is better tolerated. Exchange transfusion may be considered if there is life-threatening parasitaemia. A blood transfusion may be life-saving. Remember that ticks can be infected with more than one pathogen. In endemic regions co-infection with *Borrelia burgdorferi*, certain *Rickettsia*, *Anaplasma*, *Ehrlichia* or viral pathogen must be considered.

Prevention

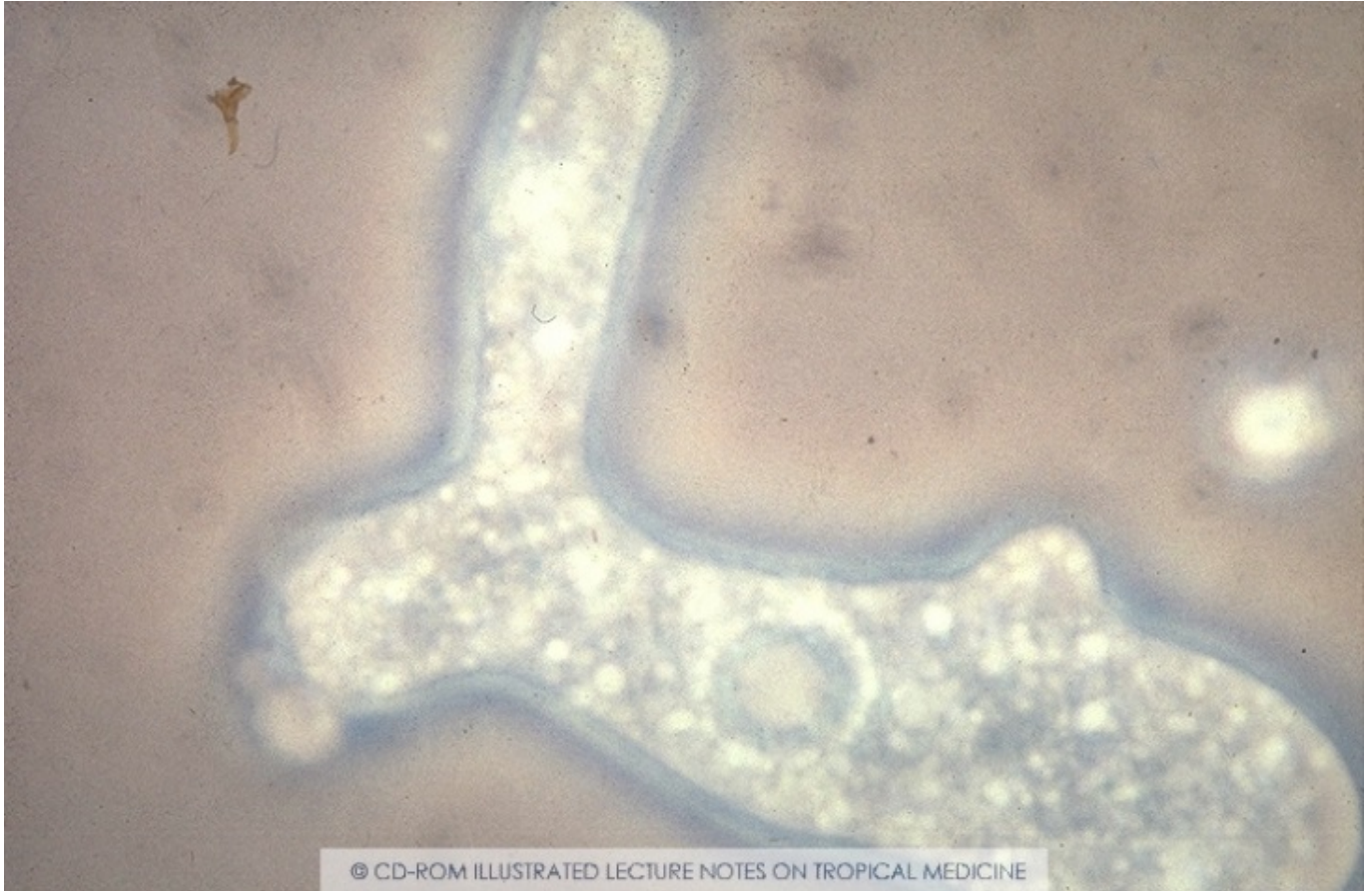
Asplenic persons should avoid endemic regions and pay extra attention to tick prevention (proper clothes, repellent containing at least 30% DEET, permethrine and physical inspection after walking).

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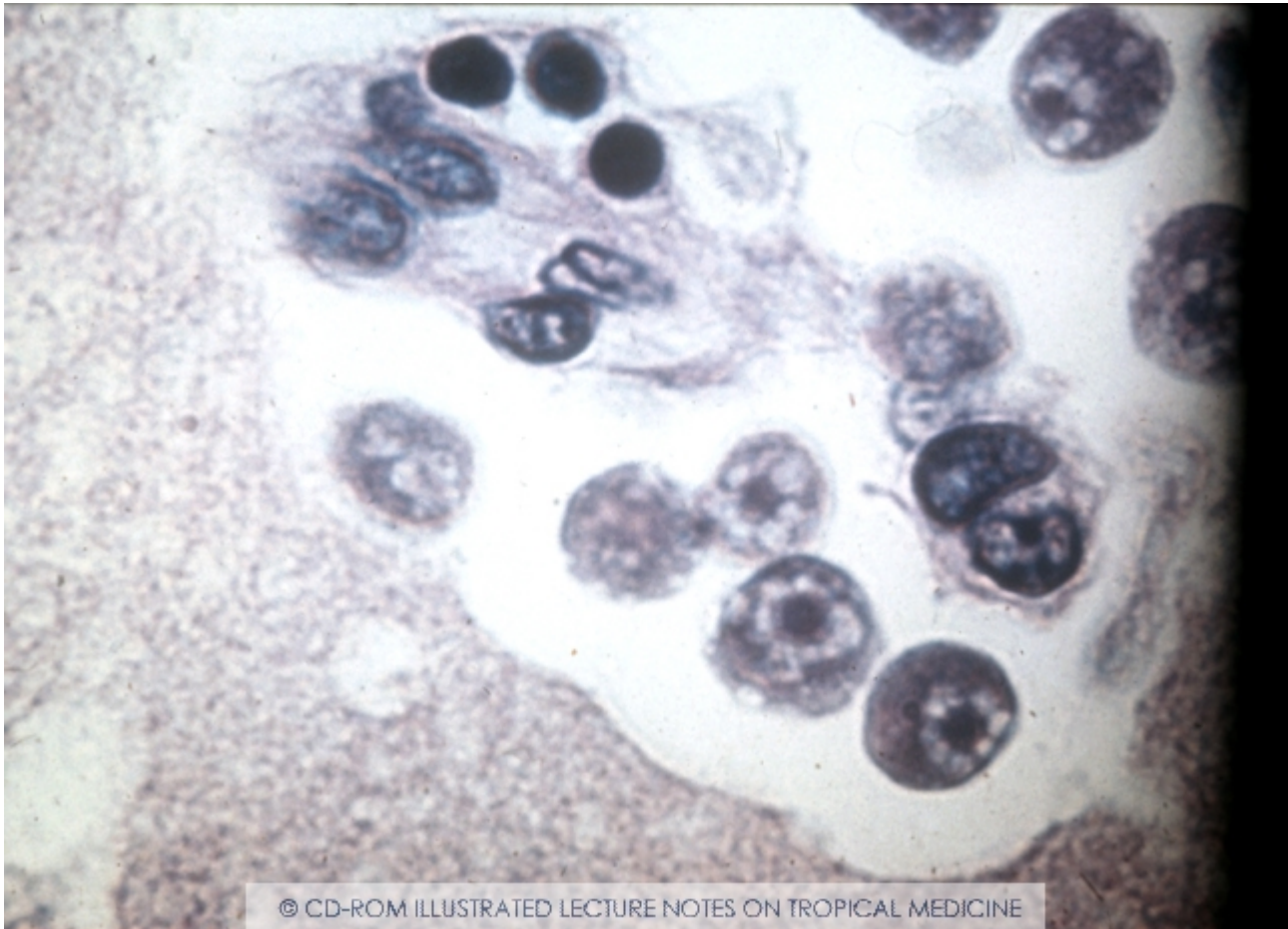
Free-living amoebae

Saprophytic amoebae from water, silt and wet soil which belong to the genera *Naegleria*, *Acanthamoeba*, *Balamuthia* and *Sappinia* are cosmopolitan and potentially pathogenic. They seldom cause infection although underreporting is probable. In industrialised countries with a moderate climate these amoebae prefer fresh water with a temperature higher than average, such as public swimming pools and warm waste water from factories or power stations. This suggests that these amoebae must be widely distributed in a tropical environment.

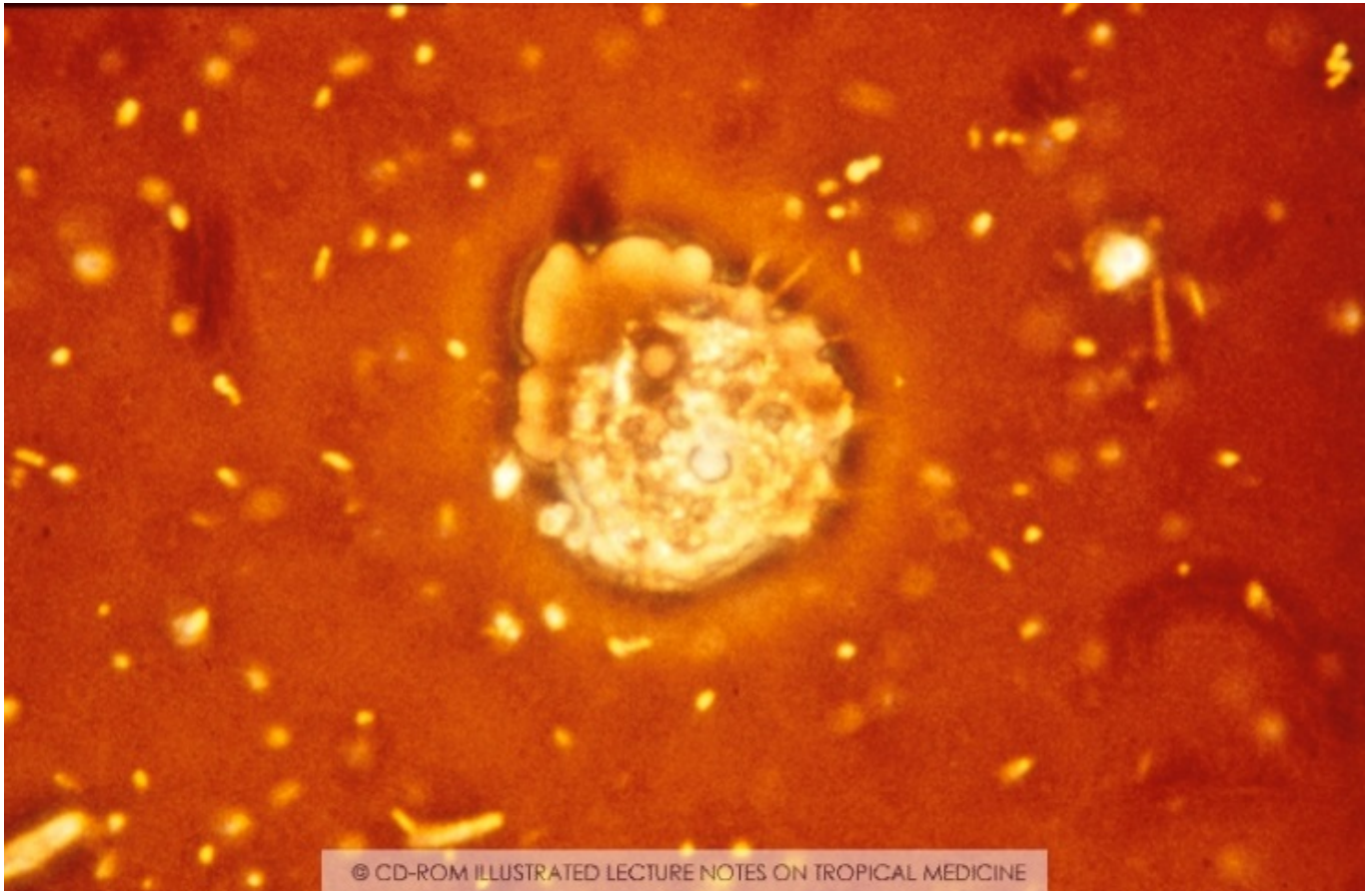
Naegleria fowleri



Naegleria fowleri trophozoite, one of the free-living amoebae.



Naegleria fowleri amoebae. Copyright ITM



Acanthamoeba sp. Notice the typical thorn-like projections (acanthopoda). Copyright ITM

Historical note

Culberston et al in 1958 were the first to launch the concept that free-living soil and water amoebae could cause disease in humans. In order to feed trophozoites form a kind of “cell-mouth”, called an amoebostome. This is quite spectacular in electron microscopic pictures. In addition to phagocytosis via these food cups, contact-mediated cytolysis occurs. When flagella develop, e.g. after transfer from culture or from tissue to water, they sprout at the broad blunt end. This change to the flagellate form can take 2-20 hours. The flagellate form does not divide, but is motile. When the flagella are lost, the amoeboid form is regained and the parasite resumes asexual reproduction. Cysts measure 7-15 μm , but are absent from human tissue, in contrast with *Acanthamoeba* infections.

Infection with *Naegleria fowleri* is the consequence of bathing or swimming in contaminated freshwater ponds or lakes at quite high temperatures, such as fresh water lakes in the summer (e.g. southern USA) ponds, rivers and hot springs. Sampling of such warm water has indicated that *N. fowleri* is commonly present in such environments. The infection follows penetration of water into the nasal cavities. From there the lamina cribriforma of the ethmoid bone is penetrated, probably through phagocytosis of the olfactory epithelium. Via the first cranial nerve, the infection spreads to the lowermost part of the frontal cerebral lobes. Extensive tissue damage follows. The amoebae reproduce rapidly in the cerebrospinal fluid. There is virtually no inflammatory reaction. Haemorrhagic necrosis of the base of the brain, cerebral cortex and the olfactory lobes develops.

The incubation time is 2 to 15 days. Early in the infection, upper respiratory distress, severe headache, sore throat, runny or stuffy nose, altered smell and taste occur. Fever, vomiting and neck stiffness follow. Mental confusion and coma occur after 3 to 6 days. Most infections are lethal. A high index of suspicion is needed for diagnosis since CSF findings are very similar in acute bacterial meningitis. A history of bathing in surface water during the previous two weeks is significant. The disease closely resembles acute bacterial meningitis and is known as primary amoebic encephalitis (PAM).

In clinical practice, most cases will be diagnosed only at autopsy (immunofluorescence and immunoperoxidase techniques).

Immediate chemotherapy is required for survival. *N. fowleri* is sensitive to amphotericin B. Combination treatment with amphotericin B, miltefosine, miconazole, rifampicin, azithromycin, chloramphenicol and/or ketoconazole has been used. Specialised advice is absolutely required.

Balamuthia mandrillaris



Balamuthia mandrillaris infection with important skin lesion. Copyright Alexander von Humboldt Institute, Peru

It is not clear how humans get infected, but transmission via swimming in contaminated surface water is one possibility. The pathogen has also been isolated from a potted plant in a home. Infection with this amoeba causes peri-orbital swelling and ulceration, followed by symptoms of granulomatous meningo-encephalitis, in both immunocompetent and immunocompromised persons. Symptoms include headache, nausea, vomiting, fever, visual disturbances, dysphagia, seizures and hemiparesis. Both trophozoites and cysts are found in CNS tissues. Differentiation with *Acanthamoeba* is difficult when using only simple light microscopy. Electron microscopy, immunofluorescence testing and histochemistry are needed for definite species identification. In vitro studies show that *B. mandrillaris* is susceptible to pentamidine. Ketoconazole, propamidine, miltefosine, 5-flucytosine,

clotrimazole, sulfadiazine, fluconazole and clarithromycine have all been used in treatment of patients. Treatment is not standardised yet. Experience in Peru has shown that prolonged administration of itraconazole 400 mg/day (adults) can be useful.

Acanthamoeba sp.

Acanthamoeba sp are free-living protista which occur in numerous places (water, dust, waste). Several species have been described: *A. castellani*, *A. culbertsoni*, *A. polyphaga*, *A. healyi*, *A. astronyxis*, *A. hatchetti*, *A. rhysodes*, *A. griffini*, *A. quina*, *A. lugdunensis*.

Acanthamoeba species are responsible for several clinical problems: (1) granulomatous amoebic encephalitis, (2) keratitis, (3) disseminated lesions, including skin ulcers, but also lesions in adrenals, kidneys, liver, spleen, thyroid....

1. Granulomatous amoebic encephalitis (GAE): unlike with *Naegleria*, infection of the central nervous system progresses slowly and occurs where there is immunosuppression or in the course of a severe general illness. Infections are more common in AIDS patients with a low CD4-count. Generally it presents as a subacute meningo-encephalitis with signs of a brain abscess and develops in two to three weeks (range 7 days – 5 months). The cerebral hemispheres tend to be involved with an inflammatory exudate covering the cortex, granulomatous necrosis of the brain parenchyma and thrombosed blood vessels. Such infections can mimic malignancies, fungal infections or abscesses. In AIDS patients, the differential diagnosis with cerebral toxoplasmosis can be very difficult.

2. Keratitis. This is more common than cerebral inflammation. The amoebae may infect small wounds of the cornea and then trigger a dangerous ulcerative keratitis which may develop into painful uveitis with hypopyon, scleritis and panophthalmitis. *Acanthamoeba* keratitis should be considered in the differential diagnosis of uveitis in AIDS patients. Initially this diagnosis is often missed and the lesion is considered to be a herpetic or fungal keratitis. Infection can follow corneal trauma (e.g. corpus alienum). The number of cases has grown in recent years as the result of increased use of contact lenses and the practice of rinsing these with tap water, as a result this is a cosmopolitan infection. It is likely that bacteria in the biofilm on dirty contact lenses constitute a good source of nutrition for the amoebae. The amoebae are often scarce in corneal smears. Culture is possible on nonnutrient agar plates

with an overlay growth of *Esch. coli* or *Pseudomonas aeruginosa* bacteria on which the trophozoites feed. Sometimes the diagnosis is made purely on anatomopathological grounds, e.g. during a cornea transplantation.

3. Other locations. Abscesses in other locations and granulomatous skin lesions in which histological investigations show amoebae, have also been observed. Skin lesions are more common in AIDS patients. Hard erythematous papulonodular lesions or non-healing indurated ulcers may be the first sign.

The optimal approach for GAE management is uncertain; therefore, combination regimens are preferred over single-drug regimens. An empiric treatment could be a combination of miltefosine, fluconazole, and pentamidine isethionate. Trimethoprim-sulfamethoxazole, metronidazole and a macrolide (azithromycin or clarithromycin) can be added to this regimen as well. Single cerebral lesions should be resected if possible.

Treatment of *Acanthamoeba* keratitis employs a combination of propamidine isethionate eye drops (Brolene®), topical neomycin, polyhexamethylene biguanide collyre (Lavasept®) and/or topical chlorhexidine (Hibitane®). Brolene® available in Great Britain is an antiseptic which is moderately toxic for the corneal epithelium. The use of topical steroids is controversial but probably beneficial. Oral itraconazole is probably also active. Topical miconazole is sometimes also used. Pentamidine (a diamidine related to propamidine) is being evaluated. Chronic refractory cases may require corneal transplantation. Unresponsive cases may require enucleation.

COMPARISON	
<i>Naegleria fowleri</i>	<i>Acanthamoeba</i> sp
Amoebic form with lobate pseudopodia; Flagellate form (two flagella)	No flagella, filiform sharp pseudopodia
Cysts not present in tissue; they are small and smooth	Cysts can be found in tissues; large and wrinkled with a double wall

Culture requires living cells (bacteria or cell culture) No growth if NaCl concentration > 0.4%	May grow without bacteria; not affected by NaCl 0.85%
Smaller than Acanthamoeba; dense endoplasm; less distinct nuclear staining	Large round, less endoplasm; more distinct nucleus

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