

9 Protozoa

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General information on parasites. A parasite (from the Greek word *parasitos*) is defined as an organism that lives in a more or less close association with another organism of a different species (the host), derives sustenance from it and is pathogenic to the host, although this potential is not always expressed. In the wider sense, the term parasite refers to all organisms with such characteristics. In medicine the term is used in a narrower sense and designates eukaryotic pathogens, which belong to the protozoa (unicellular organisms Chapter 9) and metazoa, including helminths (parasitic "worms," Chapter 10), arthropods (Chapter 11), and some other groups of lower medical significance (Annelida, Pentastomida, not covered in this book). Parasites cause numerous diseases (parasitoses) in humans, some being of extraordinary significance (e.g., malaria). Of practical concern in central Europe are both autochthonous and imported (tropical and travelers') parasitic infections.

A uniform disease nomenclature has been adopted in this book with the sole use of the suffix *-osis* (plural *-oses*)—for example trypanosomosis and not trypanosomiasis. This system, based on the Standardized Nomenclature of Parasitic Diseases (SNOPAD) (originally published in 1988 and recommended by the International Society of Parasitologists) avoids the inconsistent usage of disease names, such as leishmaniasis on the one hand and toxoplasmosis on the other.

A selection of the most important parasitoses is presented in the following chapters. In Table 1.10 (p. 28) zoonoses caused by parasites are listed.

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Parasitic protozoa are eukaryotic, single-celled microorganisms about 1–150 µm in size and enclosed by a trilaminated cell membrane. They possess one, rarely two nuclei (and multinuclear reproductive forms). Reproduction is asexual by binary or multiple fission of the cell, or sexual. The cellular construction of the protozoa is generally the same as in other eukaryotes but they also exhibit some special features. For example, during the course of evolution some protozoa (*Giardia*, *Entamoeba*) have lost the mitochondria secondarily, except several genomic traits that were laterally transferred to the nuclei. The apicoplast present in some species of Apicomplexa (see *Toxoplasma*) is a residual of a former plastid typical for their ancestors. Some protozoa contain specialized organelles, such as glycosomes (exclusively in trypanosomatids), hydrogenosomes (trichomonads and protozoa

Table 9.1 Provisional Classification of the Protozoa Mentioned in the Text

Phylum ■ Subphylum	Class	Order	Genus
Metamonada	Diplomonadea	Diplomonadida Enteromonadida Retortamonadida	<i>Giardia</i> <i>Enteromonas</i> <i>Chilomastix</i> , <i>Retortamonas</i>
Axostylata	Parabasalea	Trichomonadia	<i>Trichomonas</i> , <i>Pentatrichomonas</i> , <i>Dientamoeba</i>
Euglenozoa			
■ Kinetoplastida	Trypanosomatidea	Trypanosomatida	<i>Trypanosoma</i> , <i>Leishmania</i>
Amoebozoa	Lobosea	Amoebida	<i>Entamoeba</i> , <i>Iodamoeba</i> , <i>Endolimax</i> , <i>Acanthamoeba</i> , <i>Hartmanella</i> , <i>Balamuthia</i>
Heterolobosa	Schizopyrenidea	Schizopyrenida	<i>Naegleria</i>
Alveolata			
■ Apicomplexa	Coccidea	Eimeriida	<i>Toxoplasma</i> , <i>Isospora</i> , <i>Cyclospora</i> , <i>Sarcocystis</i> , <i>Cryptosporidium</i>
■ Ciliophora	Haematozoa Piroplasmea Litostomatea	Haemosporida Piroplasmida Vestibuliferida	<i>Plasmodium</i> <i>Babesia</i> <i>Balantidium</i>
Microspora¹	Microsporea	Microsporida	<i>Brachiola</i> , <i>Encephalitozoon</i> , <i>Enterocytozoon</i> , <i>Microsporidium</i> , <i>Nosema</i> , <i>Vittaforma</i>
		Pleistophorida	<i>Pleistophora</i> , <i>Trachipleistophora</i>
Incerta ²			<i>Blastocystis</i>

¹ Closely related to fungi. ² Taxonomy uncertain.

of other groups), and mitosomes (*Entamoeba*) (see under specific protozoan groups). Motile stages of the parasitic protozoa mostly move by means of flagella, cilia, or pseudopodia. Some species produce resistant stages (cysts, oocysts) in which the parasites can survive outside of their hosts for longer periods.

According to current theories, the protozoa are a heterogeneous group consisting of different phyla within the regnum of Eukaryota. The term protozoa has no phylogenetic significance but is still used as a collective name for the various eukaryotic unicellular organisms. The classification of the protozoa is highly controversial. Therefore, all classification systems have to be regarded as provisional (Table 9.1).

Giardia intestinalis

Causative agent of giardiasis, lambliosis

■ *Giardia intestinalis* (syn. *Giardia lamblia*, *G. duodenalis*), a parasite of worldwide distribution, occurs also in Europe with relatively high frequency. It is a parasite of the small intestine of humans that can cause enteritis. Infection occurs by peroral ingestion of *Giardia* cysts. Various species of mammalian animals are reservoir hosts. **■**

Occurrence. *G. intestinalis* has a worldwide distribution with prevalence rates of 2–5% in industrialized countries and very high rates, up to 50%, in developing countries. Children up to the age of five are frequently infected.

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Parasite and life cycle. *Giardia* exists in two morphological forms: a motile vegetative stage, the trophozoite, and a cyst stage. The trophozoites live on the small intestine mucosa (less frequently on the gallbladder mucosa as well). They resemble a pear split lengthwise, are 9–21 µm long and 5–12 µm wide and possess eight flagella, two nuclei—one on each side of the longitudinal axis—and two claw-shaped median bodies (Figs. 9.1 and 9.1a). Their dorsal side is convex, the anterior part of the ventral side forms a concave adhesive disk. Reproduction is by means of longitudinal binary fission of the trophozoites, which are able to produce variant specific surface proteins. *G. intestinalis* produces oval cysts (8–18 × 7–10 µm) with four nuclei, flagella, and claw-shaped median bodies. The cysts (and, less frequently, trophozoites) are excreted in stool. Fig. 9.1 illustrates the life cycle of *G. intestinalis*.

Epidemiology. The genus *Giardia* includes several species (*G. intestinalis*, *G. muris*, *G. agilis*, etc.) that show morphological, biological, and genetic differences. *Giardia* isolates obtained from humans and various species of

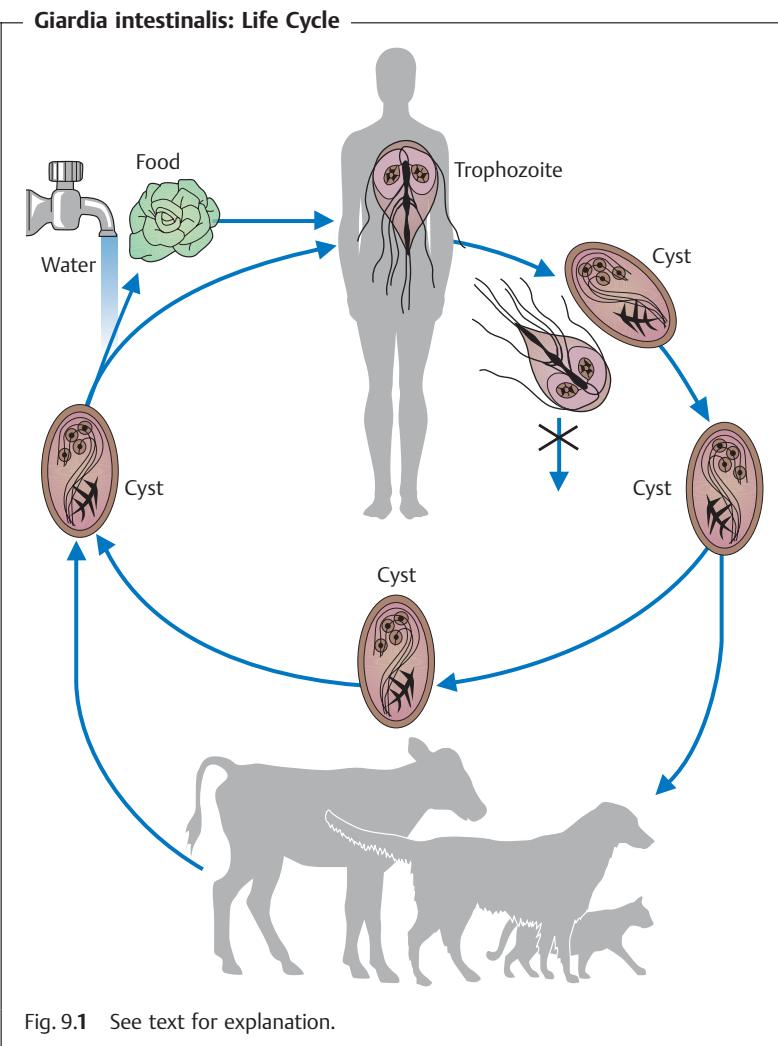


Fig. 9.1 See text for explanation.

domestic and wild mammals are morphologically uniform and correspond to *G. intestinalis*. However, this is a genetically heterogeneous species, i.e., it comprises a number of different genotypes that can be differentiated by means of isoenzymatic and DNA analysis. Several identical genotypes

were found in both humans and domestic animals (e.g., cattle, sheep, dogs). These and other facts support the conclusion that some strains of *G. duodenalis* can be transmitted from vertebrate animals to humans and that giardiasis is a zoonosis. Humans are apparently the most important reservoir hosts and certain mammalian animal species are considered additional sources of infection.

The cysts excreted in stool are responsible for spreading the infection. They remain viable for up to three weeks in moist surroundings at 21 °C and up to about three months in cool water (8 °C). The trophozoites, by contrast, die off soon outside the host. Infection is per os, whereby cysts are transmitted by the fecal-oral route from person to person (within families, kindergartens, between homosexuals, etc.) or in food and drinking water. Numerous epidemic outbreaks of giardioses due to contaminated drinking water have been described in the US and other countries with up to 7000 persons locally involved.

Pathogenesis and clinical manifestations. In the small intestine, *G. intestinalis* can cause inflammation as well as other morphological changes and malabsorption. Gallbladder infections have also been described. The pathogenesis is unclear; new data provide evidence that *Giardia* produce toxinlike proteins.

The course of infection is frequently asymptomatic. The parasite can be eliminated spontaneously within a few weeks; on the other hand, it may persist for years. The ability to produce variable surface proteins may influence elimination and persistence. Patients with symptomatic infections experience chronic and recurrent diarrhea, steatorrhea, and signs of malabsorption as well as upper abdominal pains, vomiting, occasionally fever, and weight loss.

Diagnosis, therapy, and prevention. The standard diagnostic method is stool examination using the SAFC technique to detect cysts and (more rarely) trophozoites (p. 621). Trophozoites can also be found in duodenal aspirate. IFAT and ELISA kits are now also available to detect *Giardia*-specific structural and soluble antigens in stool samples. Nitroimidazole compounds are used for chemotherapy of infections, for instance metronidazole, ornidazole, and tinidazole (see Table 9.5), as well as the benzimidazole compound albendazole and the recently introduced nitazoxanide (nitrothiazole compound). Prophylactic measures are the same as for amebiosis (p. 499). A vaccine induces a reliably protective effect in dogs and cats.

Trichomonas vaginalis

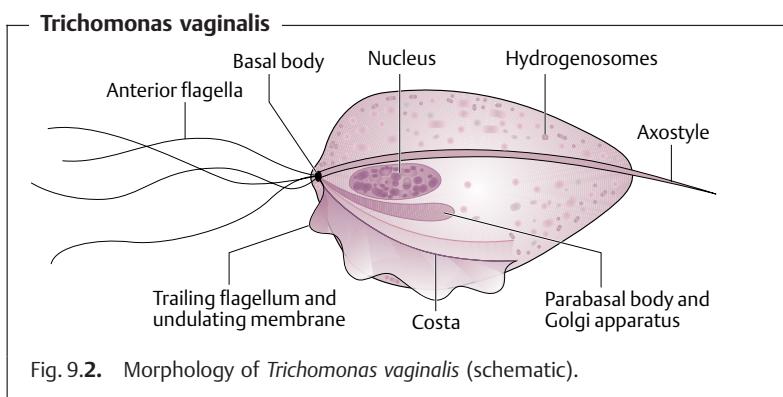
Causative agent of trichomonosis

■ *Trichomonas vaginalis* is a frequent flagellate species that occurs worldwide and is transmitted mainly by sexual intercourse. It causes vaginitis in women and urethritis in men. ■

Occurrence. The number of new cases is estimated at 170 million annually (WHO, 1998). In average populations of developed countries, infection rates are about 5–20% in women and usually below 5% in men.

Parasite, life cycle, and epidemiology. *Trichomonas vaginalis* is a pear-shaped protozoon about 10–20 µm long and 2–14 µm wide (Fig. 9.2). Five flagella emerge from a basal body at the anterior pole, four freely extend forwards and one extends backwards, forming the outer edge of the undulating membrane, which reaches back only just beyond the middle of the cell. An axial rod made up of microtubules (the axostyle) protrudes with its free tip from the posterior end of the cell. The oval cell nucleus lies near the upper pole of the protozoan. Trichomonads are anaerobic protozoa that possess hydrogenosomes, which are specialized organelles producing H₂ as a metabolite.

T. vaginalis colonizes the mucosa of the urogenital tract and reproduces by longitudinal binary fission. Trichomonads do not encyst, although rounded, nonmotile forms are observed which are degenerated stages without epidemiological significance.



Humans are the sole reservoir of *T. vaginalis*. The parasites are transmitted mainly during sexual intercourse. About 2–17% of female neonates born of infected mothers contract a perinatal infection.

T. vaginalis is highly labile outside of a host. Nonetheless, a few trophozoites can survive for up to five hours in the water of nonchlorinated thermal baths and for five minutes to 24 hours in tap water with standard chlorination; they are killed within a few minutes in swimming-pool water with high chlorine concentrations (44 mg/l). It is conceivable that infections could be transmitted by wet bathing suits, sponges, towels, etc. as well as acquired from nonchlorinated thermal baths and poorly maintained swimming pools, but there is no evidence showing that these are significant sources of infection.

Clinical manifestations. In women, *T. vaginalis* primarily colonizes the vaginal mucosa, more rarely that of the cervix. In about 20–50% of cases the infection is asymptomatic, but vaginitis can develop after an incubation period of two to 24 days. The infection results in production of a purulent, thin, yellowish discharge in which trichomonads, pus cells, and bacteria are found. The parasites also enter the urethra in about 75–90% of cases, where they can also cause an inflammation, but only rarely infect the urinary bladder or uterus. Infections in men are for the most part asymptomatic (50–90%), but they may also cause a symptomatic urethritis, more rarely involving the prostate gland and seminal vesicles as well. Infection does not confer effective immunity.

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Diagnosis. A fresh specimen of vaginal or urethral secretion is mixed with physiological saline solution and examined under a microscope for trichomonads. The trichomonads are readily recognized by their typical tumbling movements. The round trichomonad forms, by contrast, are hardly distinguishable from leukocytes. Trichomonads can also be identified in smear preparations following Giemsa staining or in an immunofluorescence test with monoclonal antibodies. The most reliable diagnostic results are obtained by culturing specimens in special liquid media. The “In-Pouch Test System” (BioMed Diagnostics) has proved useful: two flexible plastic chambers containing culture medium for combined microscopic and cultural analysis. Other special methods are based on detection of antigen (ELISA) or DNA (PCR).

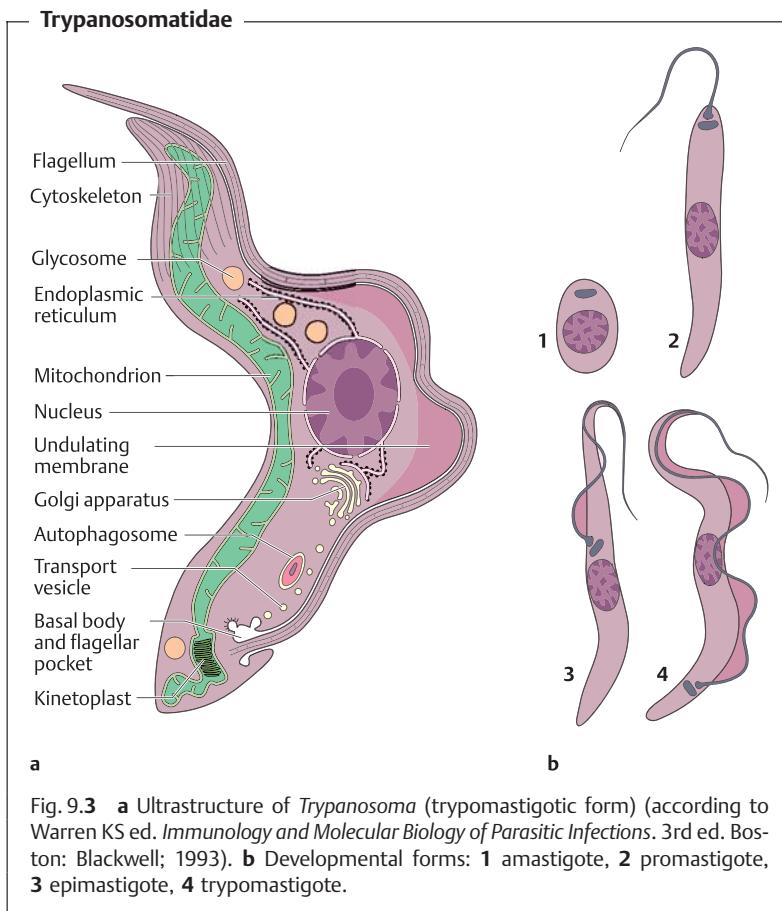
Therapy and prevention. It is always necessary for both sexual partners to receive treatment. Effective nitromidazole preparations for oral application—in women vaginal application—include metronidazole, tinidazole and ornidazole. These substances are contraindicated in early pregnancy. Preventive measures are the same as for other venereal diseases.

Trypanosoma

Causative agents of African trypanosomosis (sleeping sickness) and American trypanosomosis (Chagas disease)

■ *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* cause African trypanosomosis (sleeping sickness) in humans, which presents inter alia as fever and meningoencephalitis. In a chronic form (*T. gambiense*) the disease occurs mainly in western and central Africa, whereas the acute form (*T. rhodesiense*) is predominately distributed in eastern and southeastern Africa. The trypanosomes are transmitted by the bites of tsetse flies (*Glossina*). Antelopes and other wild or domestic animals serve as reservoir hosts of varying significance. *Trypanosoma cruzi*, the causative agent of American trypanosomosis (Chagas disease) occurs in humans and many vertebrate animals in Central and South America. It is transmitted in the feces of bloodsucking reduviid bugs. In recent years, considerable progress has been made in the control of Chagas disease. ■

General. The genus *Trypanosoma* (from *trypanon*: borer and *soma*: body) belongs to the family *Trypanosomatidae* (subphylum *Kinetoplastida*) (Table 9.1). One feature of this family is that various forms develop during the life cycle in vertebrates and vectors (insect) involved. The morphologically differentiated forms include spindly, uniflagellate stages (trypomastigote, epimastigote, promastigote) and a rounded, amastigote form (Fig. 9.3b). The trypomastigote form of the genus *Trypanosoma* has the following characteristic features: a central nucleus, an elongated mitochondrion containing the kinetoplast in its posterior section, an area free of cristae with especially densely packed DNA (Fig. 9.3a). Close to, but outside of the mitochondrion is the base of the flagellum, which originates in the plasmatic basal body. The flagellum is at first enclosed by the flagellar pocket, and then emerges onto the surface of the organism and runs to the anterior end of the organism as a pulling flagellum. The flagellar adheres locally to the cell surface so that an “undulating membrane” is folded out during movement—visible under a light microscope. Special organelles of the kinetoplastids are the membrane-enclosed glycosomes, which contain glycolytic enzymes. The cell is enclosed by an elementary membrane, which in the bloodstream stages is covered by a surface coat or glycocalyx (see below). Spiral microtubules forming a cytoskeleton are arranged along the inner cell membrane (see Fig. 9.3a for more cell organelles). In the epimastigote and promastigote forms, the kinetoplast and base of the flagellum are near the nucleus or more toward the anterior end. In the amastigote form, a reduced flagellum is visible by electron microscopy, but it does not emerge onto the cell surface (Fig. 9.3b).



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Fig. 9.3 **a** Ultrastructure of *Trypanosoma* (trypomastigotic form) (according to Warren KS ed. *Immunology and Molecular Biology of Parasitic Infections*. 3rd ed. Boston: Blackwell; 1993). **b** Developmental forms: **1** amastigote, **2** promastigote, **3** epimastigote, **4** trypomastigote.

Trypanosomatidae multiply by longitudinal binary fission. In *Trypanosoma brucei brucei* (see below) there is evidence of genetic exchange during development within the vector (sexual reproduction).

Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense

Causative agents of African trypanosomosis (sleeping sickness)

Parasite species and occurrence. The causative agents of sleeping sickness are considered to be a subspecies of *Trypanosoma brucei* and therefore their taxonomically correct designations are *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. In the following text they will be designated as *T. gambiense* and *T. rhodesiense*. Morphologically, these two subspecies differ neither from one another nor from *Trypanosoma brucei brucei*, one of the causative agents of the nagana in domestic animals that does not infect humans. These subspecies can be differentiated by means of biological criteria (e.g., host specificity, sensitivity to human serum) as well as isoenzymatic and DNA analysis.

Sleeping sickness occurs only in sub-Saharan Africa in regions between 14° north and 20° south latitude where the vectors (tsetse flies) are endemic (Fig. 9.4). Currently between 300 000 and 500 000 persons are infected in the heterogeneously distributed endemic areas in 36 African countries. The official numbers of new cases diagnosed annually (1999: 45 000) are unrealistically low because of the large numbers of unregistered cases (WHO, 2000). In some areas, sleeping sickness has occurred in increased,

Distribution of Sleeping Sickness in Africa



Fig. 9.4 West of the dotted line preponderance of *Trypanosoma gambiense*, east of the line *T. rhodesiense* (according to WHO Tech. Ser. 881, Geneva: World Health Organization; 1998).

even epidemic proportions in recent years. The risk of infection for travelers staying briefly in endemic areas is low, but infections do occur on a regular basis.

Life cycle. *T. gambiense* and *T. rhodesiense* parasitize extracellular in the blood plasma or in other body fluids of vertebrates (Fig. 9.5). The trypomastigote forms are pleomorphic in human blood (Fig. 9.6): with increasing parasitemia they transform to slender, 25–40 µm-long forms with the flagellar tip extending beyond the anterior end which reproduce by longitudinal binary fission. With decreasing parasitemia, they appear as short, “stumpy” approximately 12–25 µm long forms without a free flagellar end. These forms do not divide in blood but are infective for *Glossina* (tsetse flies). Under a light microscope in a Giemsa-stained blood smear the trypanosomes present as spindly organisms with a central nucleus, a kinetoplast at the posterior end (both stained violet) and an undulating membrane (Fig. 9.5). The cell surface of the bloodstream forms is covered with a uniform layer (about 10–15 nm thick) of a specific glycoprotein, which can be replaced by another glycoprotein. These glycoproteins are denominated as variant specific surface antigens (VSSA), the expression of which is coded by about 1000 genes; they form the basis of the organisms' antigen variation (see below).

The trypanosomes taken up by *Glossina* (tsetse flies) when they suck blood from an infected host go through a complex developmental and reproductive cycle in the insects lasting 15–35 days (Fig. 9.6). The resulting (metacyclic) stages can then be inoculated into the skin of a host with the fly's saliva. Infected *Glossina* can transmit the trypanosomes throughout their entire lifespan (up to six months).

Trypanosoma brucei rhodesiense

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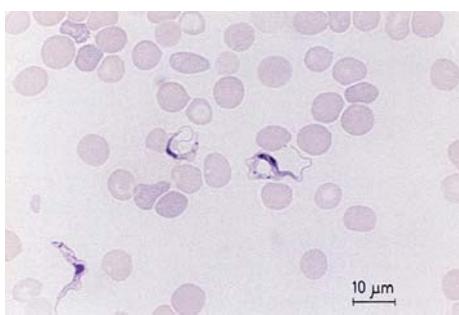
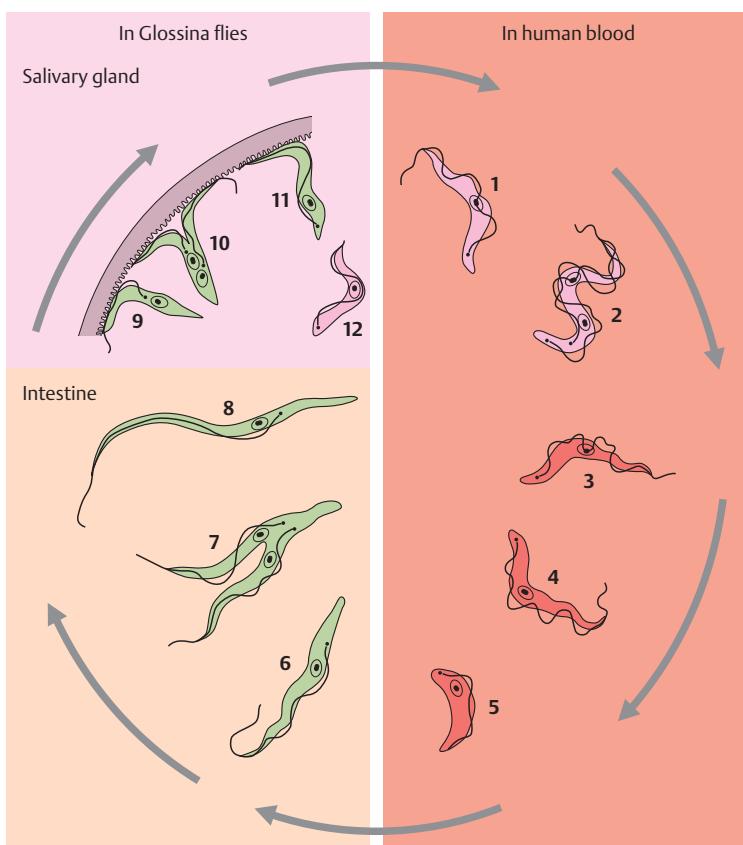


Fig. 9.5 Giemsa staining of a blood smear preparation.

Trypanosoma gambiense and rhodesiense: Life Cycle

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Fig. 9.6 Human blood: 1 trypomastigote, slender form with variant specific surface antigen (VSSA); 2 binary fission form; 3, 4 slender forms with other VSSA type; 5 short ("stumpy") form.

Glossina intestine: 6–8 procyclic forms without VSSA (reproduction by longitudinal fission).

Salivary gland of Glossina: 9, 10 epimastigote forms on epithelium; 11 trypomastigote form without VSSA; 12 trypomastigote form with VSSA (metacyclic form). (According to Vickerman K, Barry JE, in Kreier JP, Baker JR, eds. *Parasitic Protozoa*, Vol. 2, San Diego: Academic Press; 1992: 94.)

Epidemiology. There are epidemiological differences between *T. gambiense* and *T. rhodesiense* (Table 9.2), the main one being that *T. rhodesiense* persists in a latent enzootic cycle in wild and domestic animals and is normally transmitted by *Glossina* from animal to animal, more rarely to humans. *T. gambiense*, on the other hand, is transmitted mainly from human to human by the tsetse flies, although various animal species have also been identified as reservoir hosts for *T. gambiense* strains.

Clinical manifestations. Sleeping sickness is, in the initial phase, a febrile, generalized disease with lymphadenopathy and is later characterized by meningoencephalitic symptoms. The infection runs a two-stage course: the febrile-glandular or hemolymphatic stage 1 and the meningoencephalitic stage 2. The difference is therapeutically significant. In stage 1, the trypanosomes multiply in the tissue fluid at the inoculation site. Within 2–4 days an inflammatory, edematous swelling can develop—the primary lesion or “trypanosome chancre,” which then disappears within about three weeks. Within a period of approximately two weeks the trypanosomes enter the bloodstream and lymphatic system. Later, in the second stage, they also invade the central nervous system. Table 9.3 summarizes further details of the disease.

Table 9.2 Epidemiological Differences between *Trypanosoma gambiense* and *T. rhodesiense*

Parameter	<i>T. gambiense</i>	<i>T. rhodesiense</i>
Distribution:	Western and central Africa	Eastern and central Africa
Vector:	<i>Glossina palpalis</i> group: Moist biotopes	<i>G. morsitans</i> group: Savanna biotopes
Sites of transmission:		
■ Frequently focal:	At rivers, lakes, watering holes, etc.	—
■ Less localized:	In moist forest areas	Savannas
Dominant cycle:	Human → human	Wild and domestic ruminants, other wild animals → humans
Reservoir hosts (for certain <i>Trypanosoma</i> strains)	Pigs, cattle, sheep, dogs, a small number of antelope species	Antelope species, cattle, sheep, goats, warthogs, lions, hyenas, dogs, etc.

Table 9.3 Infection Course and Clinical Manifestations of Sleeping Sickness

Stage, course and symptoms	<i>T. gambiense</i>	<i>T. rhodesiense</i>
1st stage: Febrile-glandular or hemolymphatic phase		
■ Trypanosome chancre	In Africans: <5% In Europeans: approximately 20%	Approximately 50%
■ Onset of parasitemia:	2–3 weeks p.i.	1–2 weeks p.i.
■ Type of parasitemia:	Low-level, intermittent	High-level, often persistent
■ Parasitemia-associated symptoms:	Fever, chills, headache, joint and muscle pain, transitory edemas, weight loss, generalized lymphadenopathy (swelling of lymph nodes in neck = Winterbottom's sign); cardiac dysfunction (especially in <i>T. rhodesiense</i> infections), anemia, thrombocytopenia, raised serum IgM	
Course:	Chronic (also acute in persons without immunity)	
2nd stage: Meningoencephalitic phase		
■ Penetration of trypanosomes into CNS:	4–6 months p.i. or later	Frequently after only a few weeks
■ Symptoms:	Signs of progressive meningoencephalitis, epileptiform convulsions, later somnolence, apathy, coma. Pleocytosis in cerebrospinal fluid, raised total protein and IgM levels.	
Duration of disease, both stages	Months to >6 years	Rarely >3–7 months

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Pathogenesis and immunology. The course of the infection is characterized by successive waves of parasitemia caused by antigenic variation in successive trypanosome populations (see above). Parallel to an increasing parasitemia, IgM antibodies are produced that are directed against a certain variant specific surface antigen (VSSA) of the trypanosomes, whereupon they eliminate the segment of the parasite population bearing this VSSA. The parasitemia then declines, but the trypanosomes with a different VSSA multiply, whereupon specific antibodies are once again produced. Antigen variation is one of a number of strategies to circumvent host defenses (immunoevolution). About the time when one VSSA variant of trypanosomes is being eliminated from the body, the concentrations of IgG antibodies rise and immune complexes form.

Many factors contribute to the pathogenesis of sleeping sickness, among them the activation of kallikrein, kinin, complement, and the coagulation system by circulating immune complexes (resulting in increased vascular permeability, edema, hemostasis, tissue hypoxia, tissue damage, disseminated intravasal coagulation), in addition anemia, deposition of immune complexes in the kidneys and other organs, immunosuppression, endocrinial disturbances, and CNS damage. The trypanosomes cause CD8⁺ T cells and macrophages to produce IFNγ and TNF. IFNγ stimulates trypanosomes to multiply. TNF contributes to immunosuppression and may initiate tissue damage.

Diagnosis. Important diagnostic tools include direct detection of the trypanosomes in the blood, lymph node aspirate and, in cerebral forms, in the cerebrospinal fluid (Fig. 9.5). Trypanosomes can be detected in native blood preparations, in Giemsa-stained thin smears or in thick blood films (p. 622). Since low-level parasitemias are often present, concentration methods may be required, e.g., microhematocrit centrifugation, anion exchange chromatography, or the QBC technique (p. 531). Other methods are cultivation and mouse inoculation tests (suitable for *T. rhodesiense*). Analysis of lymph node aspirate has a high diagnostic value in infections with *T. gambiense*. To confirm or exclude CNS infections obtain a cerebrospinal fluid sample, centrifuge it, and examine the sediment for trypanosomes. Antibodies in the bloodstream can be detected using various techniques (p. 625). The card agglutination trypanosomosis test (CATT) has proved valuable in epidemiological surveys. Indicators of a stage 2 infection include presence of trypanosomes and/or raised leukocyte numbers and elevated concentrations of protein and IgM in cerebrospinal fluid.

Therapy. Medical treatment of sleeping sickness is highly problematical, since only a small number of effective drugs are available, serious side effects are fairly frequent and drug-resistant trypanosomes are to be expected. In stage 1, *T. gambiense* infections are mainly treated with pentamidine, whereas *T. rhodesiense* infections are treated with suramin. These drugs are not effective in the second stage (cerebrospinal fluid-positive cases), so that the arsenic compound melarsoprol, a relatively toxic substance, must be used in these cases. The worst side effect of this substance is a potentially lethal encephalopathy observed in 1–10% of patients treated with melarsoprol. Eflornithine is used for treating the late stage of the *T. gambiense* infection. Treatment of sleeping sickness victims should be entrusted to specialists if possible.

Prevention and control. Use individual prophylactic measures to protect against the diurnally active (!) *Glossina* flies. It is very important that tourists wear clothing that covers the skin as much as possible and treat uncovered skin with repellents (see Malaria, p. 535). They should also inspect the inte-

rior of cars for tsetse flies and spray with insecticides. *Glossina* flies are targeted by insecticide sprayings in preventive programs. More recently, the flies are also being caught in insecticide-charged traps using attractant colors and odors.

Trypanosoma cruzi

Causative agent of American trypanosomosis (Chagas disease)

Occurrence. Human Chagas disease is endemic in Central and South America and is caused by *Trypanosoma cruzi* (discovered in 1908 by Chagas). This parasite circulates in endemic sylvatic foci between vertebrates and insects (reduviid bugs), the latter transmitting it to humans. Until a few years ago, the endemic area of Chagas disease extended from Mexico to southern Argentina. In recent years parasite transmission to humans has been reduced or prevented in some countries (Argentina, Brazil, Chile, Paraguay, Uruguay) by control measures. The number of infected persons is currently estimated to be 16–18 million (WHO, 2000).

Causative agent and life cycle. In the natural cycle, the reduviid bugs ingest trypomastigote forms of *T. cruzi* in bloodmeals from infected hosts (vertebrate animals, humans). In the intestine of the vector, the parasites convert into intensively multiplying epimastigote stages, and later into trypomastigote forms that are excreted in feces after six to seven days. At subsequent bloodmeals, infected reduviids excrete droppings from which the trypomastigotes infect the host through skin lesions (e.g., lesions of bug bites) or the mucosa (e.g., conjunctiva).

Once in the human body, the parasites are phagocytosed by macrophages or invade other cells, mainly muscle cells (heart, skeletal, or smooth musculature) as well as neuroglial cells. Within the cells, they transform into amastigote forms (1.5–4.0 µm) and multiply by binary fission. Cells filled with up to 500 parasites are called “pseudocysts.” After about five days the parasites develop into the epimastigote form and then the trypomastigote form and return to the bloodstream, whereupon the cell infection cycle is repeated.

The *T. cruzi* organism in the blood of infected hosts (vertebrate animals, humans) is 16–22 µm long. It has a pointed posterior end and a large kinetoplast. Multiplication does not take place in the blood.

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Epidemiology. The bloodsucking bugs of the family Reduviidae find a hiding place for the day and quest for food at night. The natural habitats of these insects are nests, animal dens, and other places frequented by vertebrate animals whose blood provides their sustenance. Some species of reduviids (e.g., *Triatoma infestans*, *Rhodnius prolixus*, *Panstrongylus megistus*) have invaded domestic habitats (also in urban areas!) and are typically found in simple human domiciles. Potential carriers of *T. cruzi* include over 150 species of wild

and domestic mammals. The most important in epidemiological terms are dogs, cats, rodents, chickens, opossums, and armadillos. Aside from the reduviid vector, *T. cruzi* can be transmitted between humans by blood transfusions, diaplacental infection, or organ transplants.

Clinical manifestations and pathogenesis. Some infected persons react to entry of the parasite into the skin or conjunctiva with a local, inflammatory dermal reaction (chagoma) or conjunctivitis with eyelid edema (Romaña sign). The following symptoms are observed in the acute phase, which follows an incubation period of seven to 30 days: fever, edema, lymph node swelling, hepatomegaly, splenomegaly, myocarditis, and, less frequently, meningoencephalitis. Beginning about eight to 10 weeks after the acute phase the infection turns to an inapparent phase: serum antibodies are detectable, as are parasites in 20–60% of cases (by means of xenodiagnosis). Clinical manifestations of the chronic phase, often starting 10–20 years after the acute phase, are cardiopathy (cardiomegaly, 30% of cases), digestive tract damage (megaeosophagus, megacolon, etc., 6%), and neuropathies (3%).

The important pathogenic processes include immunologically induced destruction of ganglial cells in the autonomic nervous system that have adsorbed *T. cruzi* antigen (resulting in dysfunction and organomegaly in various organs) and inflammatory processes, especially in the myocardial tissues, probably the result of autoimmune reactions. Inapparent *T. cruzi* infections can be reactivated by AIDS.

Diagnosis. In the acute phase, trypanosomes are detectable in peripheral blood at the earliest one to two weeks after infection (thick blood films, centrifugation in hematocrit tubes, blood cultures; sensitivity 60–100%). In the chronic phase, detection of the parasites by conventional means is no longer reliable (sensitivity <10%). Tools for detection of low-level parasitemias include xenodiagnosis (from *xenos*, foreign: reduviids free of trypanosomes are allowed to suck the blood of persons in whom an infection is suspected or suck patient blood through a membrane; after a few weeks, the reduviids are examined for trypanosomes) or specific DNA detection by PCR. The apathogenic species *Trypanosoma rangeli* must be taken into consideration in differential diagnosis. Serological methods are also available that can be diagnostically useful in the chronic phase in particular (Table 11.5, p. 625).

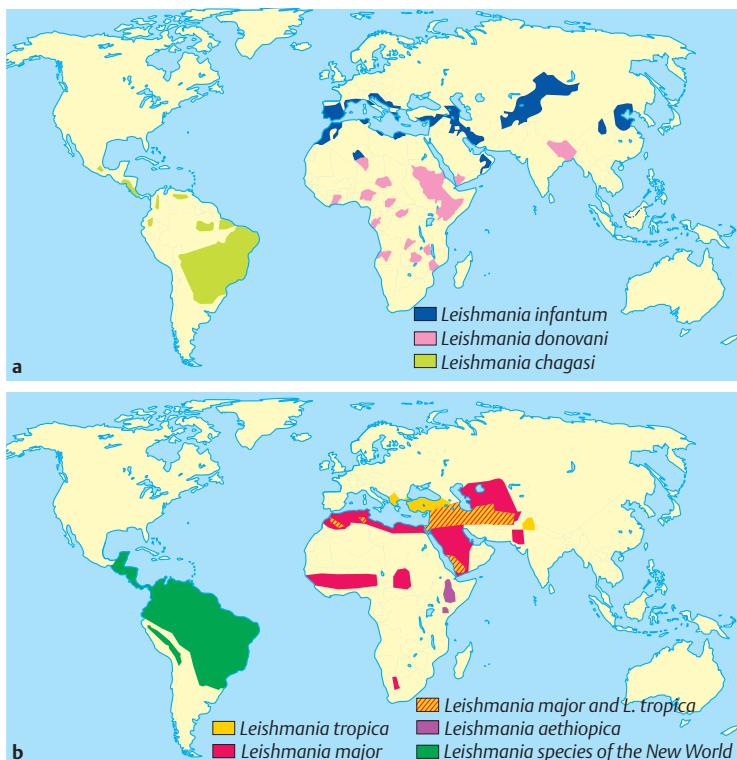
Therapy and prevention. In the early phase of an infection, cure rates of 80% have been achieved with nifurtimox and benznidazole. Both of these preparations frequently cause side effects. Preventive measures concentrate mainly on vector eradication with insecticides, improvement of living conditions, individual protection from reduviid bites with mosquito nets (see Malaria, p. 535), and measures to prevent transfusion and transplantation infections.

Leishmania

Causative agent of leishmanioses

- Leishmanias are transmitted by sandflies (Phlebotomidal) and cause the following main forms of leishmanioses in warm regions: visceral leishmanioses (VL), cutaneous leishmanioses (oriental sore) (CL), and mucocutaneous leishmanioses (MCL). In Central Europe, leishmaniosis is of significance as an imported disease and as an HIV-associated infection.

Distribution of Leishmanioses



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Fig. 9.7 **a** Visceral leishmaniosis, **b** cutaneous and mucocutaneous leishmanioses (according to Bryceson ADM, in Cook GC, ed. *Manson's Tropical Diseases*. 20th ed., London: Saunders; 1996: 1217–1219).

Occurrence. Various forms of leishmanioses occur in the warmer regions of 88 countries in Asia, Africa, Europe (Mediterranean countries!), and Latin America (Fig. 9.7). The annual number of new cases is estimated at 1.5–2 million (0.5 million VL, 1–1.5 million CL and MCL). Both geographic distribution and case numbers are reported to be on the increase (WHO, 2000).

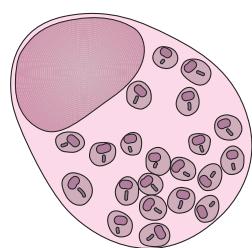
Parasites and life cycle. The many (about 15) species of the genus *Leishmania* pathogenic to humans do not show morphological differences. They can be differentiated on the basis of biological criteria, laboratory analyzes (mainly isoenzyme patterns and DNA analysis), the different clinical pictures, and epidemiological facts (Table 9.4, p. 495).

In humans and other vertebrates, leishmanias parasitize in mononuclear phagocytic cells (macrophages, monocytes, Langerhans cells) in the amastigote form. The Giemsa-stained organisms are recognizable under a light microscope as round-to-oval cells 2–5 µm in diameter with a nucleus and a small, rod-shaped kinetoplast (Fig. 9.8). A rudimentary flagellum, a single mitochondrion and other cell organelles are also rendered visible on the electron microscopic level (see also *Trypanosoma*).

The leishmania species are transmitted by female mosquitoes of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World) known as "sandflies" (Fig. 9.9 and 11.1). The amastigote stages of the parasite ingested by the insect with a blood meal are transformed in its intestine into slender, flagellate promastigote forms 10–15 µm long, which multiply and migrate back into the proboscis. At tropical temperatures this process takes five to eight days. When infected sandflies take another bloodmeal the promastigote forms are inoculated into a new host (humans or other vertebrates). In the

Leishmanias

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a

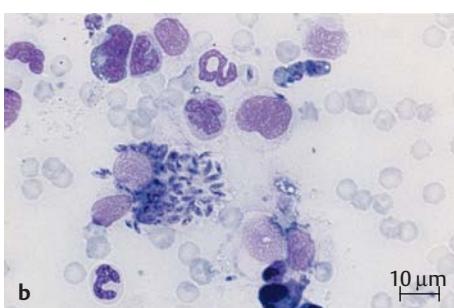


Fig. 9.8 **a** Leishmanias in a macrophage. **b** *Leishmania infantum* in a bursting macrophage; Giemsa staining of a bone marrow smear.

Table 9.4 Selected Forms of Leishmanioses in Humans
 (L.: subgenus *Leishmania*, V.: subgenus *Viannia*)

Visceral leishmanioses¹

Main localization:	Internal organs, less often skin.
Incubation:	In most cases 3–6 months, also: several weeks to years
Primary symptoms:	Fever, splenomegaly, hypergammaglobulinemia, progressive anemia, leucopenia etc.
■ <i>L. (L.) donovani</i> :	Asia: India, Bangladesh, southern Nepal. Mainly in adults. Reservoir hosts ² : humans. Vectors: <i>Phlebotomus</i> species.
■ <i>L. (L.) donovani</i> :	Africa: Mainly Sudan, Ethiopia, Kenya. Reservoir hosts: humans, dogs (<i>Felidae</i> , rodents?) ² . Vectors: <i>Phlebotomus</i> species.
■ <i>L. (L.) infantum</i> :	Mediterranean region (Iberian Peninsula to Turkey, northern Africa), Middle East and central Asia, China. In children and adults; in adults cutaneous manifestations as well: Reservoir hosts: humans, dogs, wild <i>Canidae</i> . Vectors: <i>Phlebotomus</i> species.
■ <i>L. (L.) chagasi</i> ³ :	Central and northern South America. Mainly in youths. Reservoir hosts: humans, dogs, fox species, (opossum?). Vectors: <i>Lutzomyia</i> species.

Cutaneous leishmanioses (oriental sore)

Main localization:	Skin.
Incubation:	Weeks to months.
Primary symptoms:	On skin accessible by <i>Phlebotomus</i> species, development of solitary or multiple, dry, later possibly ulcerating papules; rarely spread to lymph vessels and nodes. Healing with scarification. Solid immunity is conferred by infections with <i>L. major</i> and <i>L. tropica</i> .
■ <i>L. (L.) major</i> :	Northern Africa, Middle East, Sahel Zone, western Asia. Incubation: up to 2 months. “Moist,” (= “rural,” or “zoonotic”) form. Rapid growth of cutaneous lesion, later ulceration and healing within 6 months. Reservoir hosts: rodents. Vectors: <i>Phlebotomus</i> species.
■ <i>L. (L.) tropica</i> :	Mediterranean region, southwestern Asia to India. Incubation: 2–24 months. “Dry,” (= “urban,” or “anthroponotic”) form. Development of lesions and persistence longer than with <i>L. major</i> . Reservoir hosts: humans. Vectors: <i>Phlebotomus</i> species.

Table 9.4 Continued: Selected Forms of Leishmanioses in Humans

- *L. (L.) aethiopica*: Ethiopia, Kenya. Cutaneous leishmaniosis. Reservoir hosts: rock hyrax and bush hyrax. Vectors: *Phlebotomus* species.

American cutaneous and mucocutaneous leishmanioses

- | | |
|--|---|
| Main localization: | Skin, mucosa. |
| Primary symptoms: | Skin changes similar to oriental sore. Some forms tend to spread to mucosa and cause severe tissue destruction. |
| ■ <i>L. (L.) mexicana</i> -complex: | Southern US (Texas), parts of Central America and northern South America. Various <i>Leishmania</i> subspecies. Destructive cutaneous form. Reservoir hosts: woodland rodents. Vectors: <i>Lutzomyia</i> species. |
| ■ <i>L. (V.) braziliensis</i> complex: | Parts of Central and South America. Various <i>Leishmania</i> subspecies. Mucocutaneous form ("espundia"). Reservoir hosts: woodland rodents, sloth, opossum. Vectors: <i>Lutzomyia</i> species. |
| ■ <i>L. (V.) peruviana</i> : | Peru (Andes). Cutaneous form ("uta"). Reservoir hosts: dogs. Vectors: <i>Lutzomyia</i> |

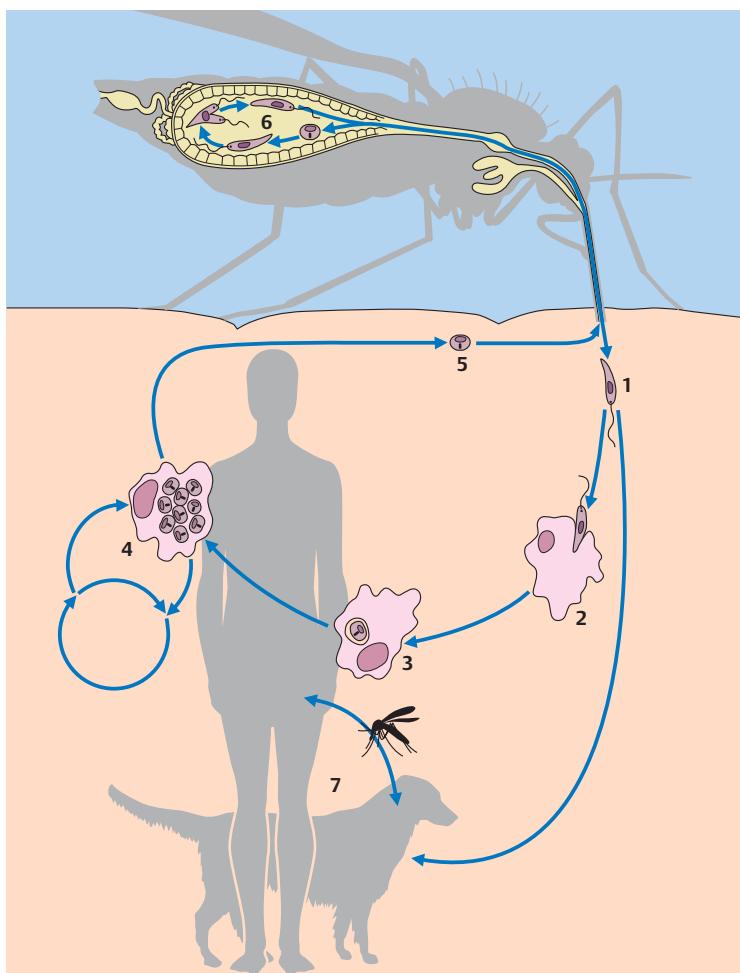
¹ Kala (Hindi) azar (Persian) = black disease (due to hyperpigmentation of skin caused by the infection) is the name given in India to the infection caused by *L. (L.) donovani*.

² Reservoir hosts: Epidemiologically significant hosts from which vectors can transmit parasites to humans. In parentheses with question mark: significance questionable.

³ *L. chagasi* and *L. infantum* have many similarities. It is therefore assumed that *L. infantum* was imported to South America in dogs from Europe.

host, they bind host components to their surface (IgM, complement, erythrocyte receptor) and, thus equipped, couple to macrophage receptors. They are then phagocytosed and enclosed in a phagolysosome, where they are protected from the effects of lysosomal enzymes inter alia by substances in their cell membrane. The promastigotes quickly (within 12–14 hours) transform into amastigote stages, which are finally surrounded by a parasitophorous vacuole within the phagolysosome and reproduce by binary fission. The amastigote forms are then released in a process resembling exocytosis and can infect new cells.

Clinical manifestations and immunology. The most important human forms of leishmanioses are summarized in Table 9.4. It is important to note that in CL and MCL the parasites generally remain restricted to the skin or skin and mucosa. CL lesions may persist for long periods, but tend to heal spontaneously, whereas a greater tendency to destructive changes is seen in MCL infections. By contrast, in VL the leishmania organisms can invade the entire

Leishmania infantum: Life Cycle

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Fig. 9.9 1 Inoculation of promastigote stages by sandfly; 2 ingestion of parasites by phagocytes (Langerhans cells, dendritic cells, macrophages); 3 amastigote form in parasitophorous vacuole of a macrophage; 4 reproduction of amastigote forms in a macrophage; 5 ingestion of amastigote forms by sandfly with blood meal; 6 transformation into promastigote form and multiplication in insect; 7 dog as reservoir host.

mononuclear phagocytic system in various organs (spleen, liver, lymph nodes, bone marrow, blood monocytes, etc.), causing infections that are normally lethal without treatment.

Basic research using animal models has provided an explanation for these differences: the course of an infection is apparently dependent on the activation of various T lymphocyte subpopulations by *Leishmania* antigens. Activation of TH1 cells involves production of IFN γ , which activates macrophages that exert a protective effect by killing *Leishmania* organisms by means of a nitric oxide-mediated mechanism. On the other hand, when TH2 cells are activated large amounts of IL-4 and IL-10 are produced, which inhibit NO activity, thus reducing or even preventing elimination of the parasites. Production of antibodies is also greatly increased, but they do not play a significant role in immune protection. Findings in patients are in accordance with these interpretations: in CL, high concentrations of IFN γ were found, but in severe cases of VL the levels of IL-4 and IL-10 were raised and IFN γ concentrations were low. The situation is similar in severe forms of MCL. It would appear that the cell-mediated immune response in CL protects efficiently, but the immune response in advanced VL and some forms of MCL is more or less suppressed. In cases where the immune defenses are additionally weakened by AIDS, a latent *Leishmania* infection may be activated and take a fulminant symptomatic course. In endemic regions, the risk to acquire a *Leishmania* infection is increased for AIDS patients by 100–1000 times. Most of the cases of AIDS-associated leishmaniasis (about 50%) registered to date (1990–1998) were reported from areas in which *L. infantum* is endemic in southwestern Europe (Italy, France, Spain, Portugal) (others from India, Africa, Latin America, etc.) (WHO, 1999). Besides *L. infantum*, coinfections with other *Leishmania* species have also been found in AIDS patients (e.g., *L. donovani*, *L. braziliensis*, *L. tropica*).

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Epidemiology. Table 9.4 refers briefly to the epidemiology of this disease. In central Europe, leishmaniasis deserves attention as a travelers' disease, especially the VL imported from Mediterranean countries. Major VL epidemics have occurred recently in various parts of the world, e.g., in southern Sudan with 100 000 deaths in a population of <1 million (WHO, 2000).

Diagnosis. An etiological diagnosis of VL is made by means of direct parasite detection in aspirate material from lymph nodes or bone marrow (in HIV patients also in the enriched blood leukocyte fraction) in Giemsa-stained smears (uncertain!), in cultures (in which promastigotes develop) or using PCR. Cultivation and PCR have about the same high level of sensitivity. Antibodies are detectable in nearly all immunocompetent patients (around 99%), but 40–50% of HIV-coinfected patients are seronegative (Table 11.5, p. 625).

Diagnosis of a cutaneous leishmaniasis is usually based on clinical evidence. Etiological verification requires direct parasite detection (see above)

in smears or excised specimens from the edges of the skin lesions. More reliably, the parasites can be detected by cultivation or PCR. Serological antibody tests are positive in only a small proportion of cases.

Therapy and prevention. Treatment of VL is usually done with pentavalent antimonials (meglumine antimonate, sodium stibogluconate) pentamidine, or amphotericin B. The recurrence rate is relatively high, especially in HIV patients. Miltefosine, a newly developed and well tolerated antitumor alkyl-phospholipid for oral application, has proved effective against VL. Various forms of CL (for instance *L. major* and *L. tropica*) can be influenced by injecting antimonial preparations into the lesions; mucocutaneous leishmaniasis (*L. braziliensis*) is treated systemically with antimonials (see above, amphotericin B, or pentamidine). An effective chemoprophylaxis has not yet been developed. It is therefore important to prevent Phlebotome bites with fine-meshed, insecticide-impregnated “mosquito nets” (p. 535). Control of the vectors involves use of insecticides and elimination of breeding places.

Entamoeba histolytica and Other Intestinal Amebas

Causative agents of amebosis (entamebosis, amebiasis)

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Of the various amebic species that parasitize the human intestinal tract, *Entamoeba histolytica* is significant as the causative agent of the worldwide occurring entamebosis, a disease particularly prevalent in warmer countries. The vegetative stages (trophozoites) of *E. histolytica* live in the large intestine and form encysted stages (cysts) that are excreted with feces. The infection is transmitted by cysts from one human to another. The trophozoites of *E. histolytica* can penetrate into the intestinal wall and invade the liver and other organs hematogenously to produce clinical forms of amebosis, most frequently intestinal ameboses (amebic dysentery) and hepatic amebosis (“amebic liver abscess”). Diagnosis of an intestinal infection is primarily confirmed by detection of the parasites in stool. If an invasive, intestinal or extraintestinal infection with *E. histolytica* is suspected, a serological antibody test can also provide valuable information. Morphologically, *E. histolytica* is indistinguishable from the apathogenic *Entamoeba dispar* (collective term for both species: *E. histolytica/E. dispar* complex).

Occurrence. In endemic areas in Africa, Asia, and Central and South America up to 70–90% of the population can be carriers of *E. histolytica/E. dispar*, in the USA and Europe about 1–4%. Worldwide the annual number of new cases is estimated at 48 million, with about 70 000 lethal outcomes (WHO, 1998).

Parasites. The causative agent of amebiosis is the pathogenic *E. histolytica*. This species is morphologically identical with the apathogenic *E. dispar*. They can be differentiated by means of zymodeme and DNA analysis and with monoclonal antibodies. The two species occur in the form of trophozoites (vegetative stages) and cysts (Figs. 9.10 and 9.11c).

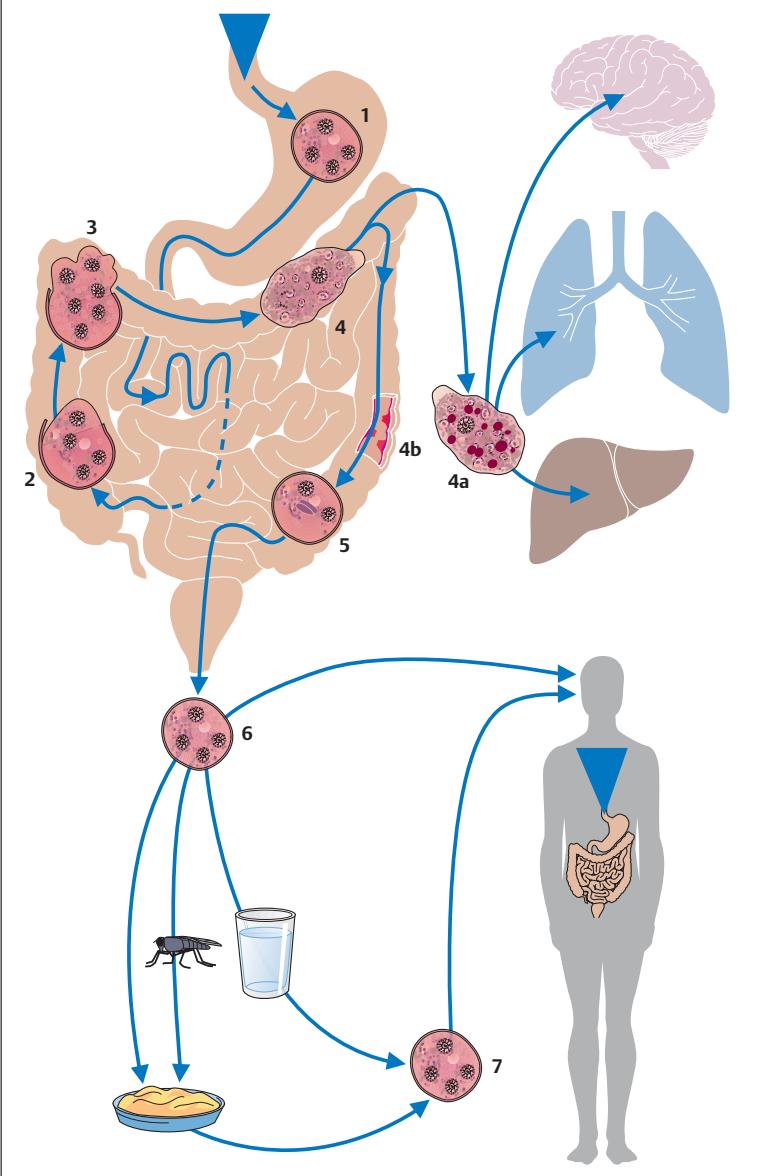
- The **trophozoites** of *E. histolytica* are cells of variable shape and size (10–60 µm) that usually form a single, broad pseudopod (protrusion of cell membrane and cytoplasm) that is often quickly extended in the direction of movement. Stained preparations of the genus *Entamoeba* show a characteristic ring-shaped nucleus with a central nucleolus and chromatin granula on the nuclear membrane. Trophozoites that have penetrated into tissues often contain phagocytosed erythrocytes.
- The spherical, nonmotile **cysts** (10–16 µm) have a resistant cyst wall. At first each cyst contains a uninucleate ameba, with glycogen in vacuoles and the so-called chromidial bodies, which are cigar-shaped. The nucleus divides once to produce the binuclear form and later once again to produce the infective tetranuclear cyst (Fig. 9.11c). The cysts are eliminated in the stool of infected persons, either alone or together with trophozoites.

Life cycle and pathogenesis. The cycle of *E. histolytica* is shown in Fig. 9.10.

- **Symptomatic intestinal amebosis.** Following peroral ingestion of a mature *E. histolytica* cyst, the tetranuclear ameba is released, divides to produce four or eight uninucleate trophozoites, which then continue to multiply and encyst (Fig. 9.10). The trophozoites colonize the large intestine mucosa or lumen. Their potential for invading and destroying tissue is high and is based on the following characteristics and processes: adhesion of trophozoites to intestinal cells by means of surface lectins, killing of cells with pore-forming peptides (amebapore, types A–C) and dissolution of the extracellular matrix by cysteine proteases. This enables the amebas to penetrate into the intestinal wall, where they multiply and cause pathological changes (necrotic foci, ulcers, inflammatory reactions) (see below).
- **Asymptomatic intestinal amebosis.** This condition is usually caused by *E. dispar*, less frequently by *E. histolytica*. Characterizing *E. dispar* as “apathogenic” is not entirely accurate, since these organisms can cause slight intest-

Fig. 9.10 1 Cyst of *E. histolytica*, following peroral ingestion, in stomach; 2 ameba emerging from a cyst; 3 dividing stage of ameba; 4 uninucleate trophozoites result from division; 4a invasive stage with phagocytosed erythrocytes, extraintestinal; 4b lesions in the intestinal wall; 5 the amebas encyst; 6 cysts excreted with feces and different transmission routes; 7 peroral ingestion of cysts. ▶

Entamoeba histolytica: Life Cycle



inal lesions in experimental animals. *E. dispar* adheres to host cells in very much the same way as *E. histolytica*, but it produces only very small amounts of amebapore A and B and none of the particularly potent type C at all. *E. dispar* is lacking several genes that code for certain cysteine proteases. Also, the activity of certain proteases in *E. dispar* is greatly reduced compared to *E. histolytica*.

Extraintestinal amebosis. *E. histolytica* can disseminate to other organs from the intestinal wall, most particularly to the liver (Fig. 9.10). As a result of the destruction of parenchymal cells, small necrotic foci, so-called abscesses, form and gradually become larger and can even affect major portions of the organ. Bacteria are involved in only about 5% of cases, so that the inflammatory reactions at the edges of the foci are usually mild. The decomposing lesion contains a brownish or yellowish, puslike liquid, in most cases bacteriologically sterile, later becoming a necrotic mass; amebas are often only detectable in the transition zone between the lesion and intact hepatic tissue. Liver abscesses sometimes perforate into the pleural space or lung; less often a hematogenous dissemination of amebas results in an invasion of the spleen, brain, and other organs. Cutaneous amebosis most frequently occurs in the perianal area, associated with rectal changes.

Epidemiology. Humans are the reservoirs for *E. histolytica* (rarely also: monkeys, dogs, cats). The infection is due to transmission of mature cysts with contaminated foods (fruit, vegetables), drinking water or fecally contaminated hands. Flies and cockroaches can function as intermediaries by carrying cysts from the feces of an excretor to foods. In contrast to the vegetative forms, the cysts are quite resistant in a moist environment (i.e., they survive at 28–34 °C for about eight days, at 10 °C for about one month); under conditions of desiccation and temperatures exceeding 55 °C they are quickly killed. The amounts of chlorine normally added to drinking water are insufficient to kill the cysts. Monkeys have been shown to be hosts of *E. histolytica* and *E. dispar*.

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Clinical manifestations. Clinical symptoms can develop as early as two to four weeks after infection with *E. histolytica* or after asymptomatic periods of months or even years.

Intestinal forms

— **Asymptomatic intestinal form.** *E. histolytica* can colonize the intestinal mucosa, reproduce, and persist for long periods without becoming invasive or causing any changes. The apathogenic *E. dispar* is more frequent than *E. histolytica*, so that most asymptomatic infections are probably caused by the former. Trophozoites, and more frequently cysts, of the *E. histolytica/E. dispar* type are excreted, antibodies to *E. histolytica* antigens are usually not found in serum.

- The **invasive intestinal form** results from the invasion of the intestinal wall by the pathogenic *E. histolytica* and reflects large intestine disease. The intestinal parts affected (colon, cecum, rectum, sometimes terminal ileum) show either circumscribed or more expanded lesions of varying intensity, ranging from edematous swelling and reddening to pinhead-sized foci with central necrosis or larger, bottle-shaped ulcers extending deep into the intestinal wall with swollen edges and large decomposing foci. The ulcers sometimes perforate into the peritoneal cavity. Healing processes with scar formation may reduce the intestinal lumen; pronounced inflammatory processes can lead to a tumorlike thickening of the intestinal wall (ameboma). The **acute disease** usually begins with abdominal discomfort and episodes of diarrhea of varying duration, at first mushy then increasing mucoid, including blood-tinged, so-called “red currant jelly stools” in which amebas can be detected, including trophozoites containing erythrocytes. In such cases, antibodies are usually present in serum. The symptoms may abate spontaneously, but fairly often a recidivating **chronic colitis** develops that can last for months or even years.

Extraintestinal forms

- Extraintestinal forms develop because of hematogenous dissemination of *E. histolytica* originating in the intestine. The most frequent form is the so-called **“liver abscess,”** which may develop in some infected persons. Only about 10% of patients with liver abscesses are also suffering from amebic colitis; coproscopic methods often do not reveal amebas in stool. The liver abscess causes remittent fever (sometimes high), upper abdominal pain, liver enlargement, elevation of the diaphragm, general weakness, and other symptoms. Large liver abscesses that are not treated in time are often lethal. Antibodies are detectable in most cases (around 95%) (see also Diagnosis). **Other forms** of extraintestinal amebosis are much rarer and include involvement of the lungs, brain, and skin.

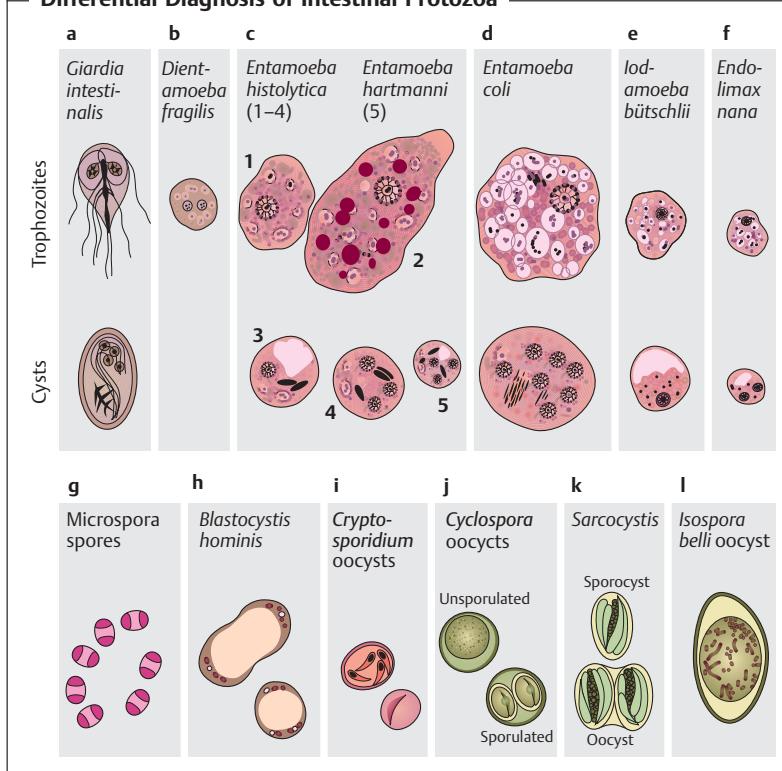
Immunity. Reinfections are possible since sufficient immunity is not conferred in the course of an infection. Antibodies are usually detectable in serum in invasive intestinal and extraintestinal amebosis caused by *E. histolytica*.

Diagnosis

Intestinal amebosis

- **Coproscopic diagnosis.** For diagnosis of intestinal amebosis a body-warm stool specimen must be fixed without delay in SAF solution and examined microscopically following laboratory processing (p. 621). A single stool analysis has a statistical sensitivity of only 50–60%, but this can be raised to 95% by examining stool specimens from three consecutive days. Since *E. histolytica* and *E. dispar* are morphologically indistinguishable, a finding is classified as *E. histolytica/E. dispar complex*.

Differential Diagnosis of Intestinal Protozoa



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- Differential diagnosis.** It is important to differentiate the *E. histolytica/E. dispar* complex from intestinal epithelia, granulocytes, macrophages, and fungi as well as from other, apathogenic, intestinal protozoa (amebas: *Entamoeba coli*, *E. hartmanni*, *E. polecki*, *Iodamoeba bütschlii*, *Endolimax nana*; flagellates: *Dientamoeba fragilis*, *Enteromonas hominis*, *Chilomastix mesnili*, *Pentatrichomonas hominis* (Fig. 9.11). *D. fragilis* is classified by some authors as potentially pathogenic. *Blastocystis hominis* is frequently found in stool samples (Fig. 9.11h): this intestinal inhabitant is considered a fungus or a protozoon; some authors ascribe it a certain significance as causative agent of diarrhea. It is important to remember that a number of drugs reduce the excretion of intestinal protozoa.

◀ Fig. 9.11

a *Giardia intestinalis* (pathogenic):

trophozoites: $9-21 \times 5-12 \mu\text{m}$;
cysts: $8-14 \times 8-10 \mu\text{m}$ (see also p. 478).

b *Dientamoeba fragilis* (apathogenic or facultatively pathogenic):

trophozoites: $5-15 \mu\text{m}$, 3/4 of stages with two nuclei, the rest with one;
karyosome consisting of four to six granules;
cysts: none.

c *Entamoeba histolytica* (pathogenic):

trophozoites: small form (1) $10-20 \mu\text{m}$, with *Entamoeba* nucleus and small number of vacuoles containing bacteria; larger form (2) $20-60 \mu\text{m}$, with phagocytosed erythrocytes; (3) cysts: $10-16 \mu\text{m}$, one to four nuclei. Here binuclear cyst with glycogen vacuole and cigarshaped chromidial bodies and (4) a tetranuclear cyst.

Entamoeba dispar (apathogenic): morphologically identical with *E. histolytica* (see text).

Entamoeba hartmanni (5) (apathogenic): similar to *E. histolytica*, but smaller.
Trophozoites and cysts approximately $3-10 \mu\text{m}$.

d *Entamoeba coli* (apathogenic):

trophozoites: with *Entamoeba* nucleus, $10-50 \mu\text{m}$, in most cases numerous vacuoles containing bacteria and particles.Cysts: $15-25 \mu\text{m}$, one to eight nuclei, chromidial bodies slender, splinter-shaped.

e *Iodamoeba butschlii* (apathogenic):

trophozoites: $6-20 \mu\text{m}$, nucleus with large karyosome, either centrally located or contiguous with the nuclear membrane.

Cysts: $5-18 \mu\text{m}$, one nucleus, rarely two.

f *Endolimax nana* (apathogenic):

trophozoites: $6-15 \mu\text{m}$, nucleus with karyosome.

cysts: usually oval, $8-12 \mu\text{m}$ long.

g *Microsporidia* (pathogenic):

spores: very small (!), $1-3.5 \mu\text{m}$ long depending on species, oval shape; spores often not stained homogeneously by chromotropic staining according to Weber (see also Fig. 9.20c, p. 539).

h *Blastocystis hominis* (facultatively pathogenic?):

single cells: $5-20 \mu\text{m}$.

i *Cryptosporidium* species (pathogenic):

oocysts: $4-5 \mu\text{m}$ (see also Fig. 9.14a, p. 516).

j *Cyclospora cayetanensis* (pathogenic):

oocysts: $4-5 \mu\text{m}$, spherical, unsporulated in fresh stool; after sporulation two sporocysts with two sporozoites each (see also Fig. 9.14a, p. 516).

k *Sarcocystis* species (pathogenic):

sporocyst: $14 \times 9 \mu\text{m}$; oocyst: $20 \times 13 \mu\text{m}$.

l *Isospora belli* (pathogenic):

oocyst: $20-33 \times 10-19 \mu\text{m}$.

- **Differentiation of *E. histolytica* and *E. dispar*.** A new type of PCR is now used in specialized laboratories that facilitates direct detection of these amebic species in stool specimens as well as a differential diagnosis.
 - **Detection of coproantigen.** *E. histolytica* antigen can be detected in stool specimens using an ELISA based on monoclonal antibodies with high levels of sensitivity and specificity.
 - **Serological antibody assay.** Antibodies can be detected serologically in 95–100% of patients with amebic liver abscess. This is also frequently the case in invasive intestinal amebosis caused by *E. histolytica*. On the other hand, antibodies are produced far less often in *E. dispar* infections. When stages of the *E. histolytica/E. dispar* complex are detected in stool, the presence or absence of serum antibodies can be used in differential diagnosis of invasive vs. noninvasive intestinal amebosis.
- **Extraintestinal amebosis.** This type of amebosis is diagnosed with the help of clinical methods (ultrasound, computer tomography, etc.) and serological antibody detection (see above).

Therapy. Nitromidazole derivatives are effective against symptomatic intestinal and extraintestinal forms of amebosis. On the other hand, amebicides with only luminal activity are effective against asymptomatic intestinal amebosis (e.g., diloxanide furoate) (Table 9.5). A new drug against intestinal amebic infections is nitazoxanide. Besides chemotherapy, other measures may also be required, e.g., surgery and symptomatic treatment for liver abscesses.

Prevention. Travelers to endemic areas should decontaminate drinking water by boiling or filtering it (e.g., with Katadyn filters), not eat salads, eat only fruit they have peeled themselves and exercise caution when it comes to changing their diet. Chemoprophylactic drugs are not available.

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Table 9.5 Chemotherapy in Amebosis (examples)

Group of amoebicides*	Active substance	Indication
Luminal amebicides	Diloxanide furoate Paromomycin Nitazoxanide	Asymptomatic cyst excretors, follow-up treatment of invasive intestinal form
Systemic amebicides	Nitroimidazoles: Metronidazole Ornidazole Tinidazole	Invasive intestinal and extra- intestinal forms

*Application per os

Naegleria, Acanthamoeba, and Balamuthia

Causative agents of naegleriosis, acanthamebosis, and balamuthiosis

Free-living ameba of the genera *Naegleria*, *Acanthamoeba*, and *Balamuthia* have the potential to infect vertebrates and to cause diseases in humans. The morphological characteristics of these amoebas include: nucleus with large karyosome, lack of chromatin granules at the nuclear membrane (see *Entamoeba*). Trophozoites: *Naegleria fowleri* (15–30 µm) with wide pseudopods, produces flagellated stages in water; *Acanthamoeba* spp. (24–56 µm) with fingerlike protrusions ("filopods"); *Balamuthia mandrillaris* (12–60 µm) has irregularly branched pseudopods. All of these genera produce cysts.

Naegleria. The causative agent of primary amebic meningoencephalitis (PAM) is *Naegleria fowleri*. This species occurs worldwide in bodies of freshwater, especially warm water in swimming pools, storage containers or thermally polluted lakes and rivers, etc.

Infection of humans occurs by the nasal route with water containing trophozoites, i.e., during a swim or shower. The amoebas migrate from the olfactory epithelium along the nerve tracts into the CNS and cause, after an incubation period of two to seven (rarely as long as 15) days a hyperacute to acute meningoencephalitis that usually has a lethal outcome. The infection occurs mainly in children and youths. Sporadic occurrence is reported from all continents. Treatment with amphotericin B has been successful in a small number of cases.

Acanthamoeba. Potential human pathogens in this genus include *Acanthamoeba culbertsoni* and several other species. These amoebas occur worldwide in soil, sand, dust (also house dust), air, and water (also in tap water). The cysts can survive for several years in a dry state and disseminate with dust. Inhalation of cysts with dust is considered to be one of the main transmission routes. Acanthamebas are frequent colonizers of human nasal mucosa and have been isolated from the oral mucosa, skin lesions, and the cornea. From the portal of entry, they can spread hematogenously to the CNS and other organs.

Acanthamoeba infections in humans frequently take an asymptomatic course. Keratitis is observed occasionally, especially in contact lens wearers. Diagnosis: culturing of amoebas from conjunctival and contact lens rinsing liquids. Prevention: use only sterile lens rinsing liquid. In rare cases of generalized infection, *Acanthamoeba* can cause granulomatous amebic encephalitis (GAE) as well as granulomatous lesions in the lungs and other organs, especially in immunodeficient patients. *Balamuthia mandrillaris* can also cause GAE.

Toxoplasma gondii

Causative agent of toxoplasmosis

■ *Toxoplasma gondii* is the causative agent of a zoonosis that occurs worldwide with high prevalences (up to 80% depending on region and age). Humans are infected by ingesting oocysts excreted by the definitive hosts (cats) or by eating unprocessed meat containing *Toxoplasma* cysts. If a woman contracts toxoplasmosis for the first time during pregnancy, diaplacental transmission of the pathogen to the fetus is possible with potential severe consequences (for example malformations, eye damage, clinical symptoms during childhood). There is, however, no risk to the fetus from mothers who had been infected before their first pregnancy and have produced serum antibodies (about 35–45%). Latent infections can be activated by immunodeficiencies (e.g., in AIDS patients) and may result in cerebral or generalized symptomatic toxoplasmosis. Serological surveillance in pregnant women is important to prevent prenatal infections.

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Occurrence. *T. gondii* occurs worldwide. The low level of host specificity of this organism explains its ready ability to infect a wide spectrum of warm-blooded vertebrate species (for example humans, sheep, pigs, cattle, horses, dogs, cats, wild mammals, bird species). It is estimated that approximately one-third of the world population is infected with *T. gondii*, although prevalences vary widely depending on age and region. According to a seroepidemiological study in Switzerland (published in 1995) of 4000 persons aged one to 70, an average of 52% was infected with seroprevalence rates in different age groups as follows: one to nine years: 24%, 20–39 years: 43%, and 40–70 years: 69%. Of 9000 pregnant women, 46% were infected. Women of childbearing age groups in other European countries showed either lower or higher seroprevalences. High prevalences were also observed in various animal species (see Epidemiology p. 514).

Parasite. Various strains of *T. gondii* differ in virulence and certain biological and genetic characteristics. The life cycle of *T. gondii* includes various stages:

■ **Tachyzoites** (endozoites) (Fig. 9.12a, b) are proliferative forms that reproduce rapidly within a parasitophorous vacuole in nucleate host cells by means of endodyogeny (endodyogeny: formation of two daughter cells from a mother cell by endogenous budding). The tachyzoites are sickleshaped (*toxon*: bow) cells about 4–7 µm long and 2–4 mm wide. An apical complex is located at the anterior pole, consisting of several components, including the conoid (a conical structure of spirally arranged microtubuli), a pole ring complex, the rhoptries, and micronemes (Fig. 9.12b). The apical complex

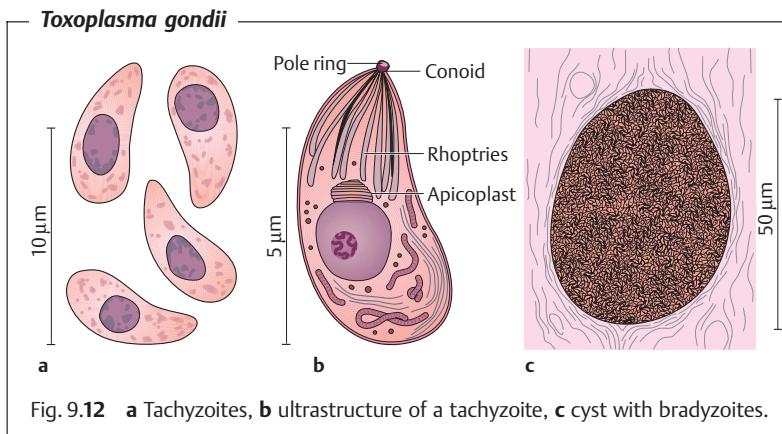


Fig. 9.12 **a** Tachyzoites, **b** ultrastructure of a tachyzoite, **c** cyst with bradyzoites.

contributes to parasite penetration into host cells. The nucleus is located in the posterior half of the cell. More recent investigations have revealed that *Toxoplasma* and several other apicomplexan protozoa (e.g., *Plasmodium*, *Sarcocystis*) contain, in addition to the chromosomal and mitochondrial genomes, a further genome consisting of circular DNA (35 kb) localized in a special organelle called the apicoplast. This organelle, usually located anterior to the nucleus and close to the Golgi apparatus, is surrounded by several membranes and possesses outer and inner membrane complexes. There is evidence that the apicoplast has evolved from the plastids of endosymbiotic green or red algae. The function of the apicoplast remains uncertain but is indispensable for the host cell and may be a new and specific target for chemotherapy. Tachyzoites also multiply in experimental animals and cell cultures. This stage is highly labile outside of a host and usually does not survive the stomach passage following ingestion.

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■ **Bradyzoites (cystozoites)** are stages produced by slow reproduction within the cysts ($4\text{--}8 \times 2\text{--}4 \mu\text{m}$). The cysts develop intracellularly in various tissues (see below), have a relatively resistant wall, grow as large as 150 µm, and can contain up to several thousand bradyzoites (Fig. 9.12c). They have a long lifespan in the host. Humans and animals can be infected by peroral ingestion of meat containing cysts (Fig. 9.13).

■ **Oocysts** are rounded and encysted stages of the organism, surrounded by a resistant cyst wall, and are approx. $9 \times 14 \mu\text{m}$ in size. They are the final product of a sexual reproductive cycle in the intestinal epithelia of *Felidae*. They contain a zygote and are shed in feces (Fig. 9.13). Sporulation takes place

within two to four days, producing two sporocysts with four sporozoites each. Sporulated oocysts are infective for humans and animals.

Life cycle. The developmental cycle of *T. gondii* involves three phases: intestinal, external, and extraintestinal (Fig. 9.13).

■ The **intestinal phase** with production of sexual forms (gamogony) takes place in enterocytes of definitive hosts. Only domestic cats, and several other felid species of little epidemiological significance, can function as definitive hosts for *T. gondii*. Only extraintestinal development is seen in intermediate hosts (pigs, sheep, and many other animal species) as well as in dead-end hosts (humans). Following primary infection of a cat with *Toxoplasma* cysts in raw meat, asexual reproductive forms at first develop in the small intestine epithelium, with sexually differentiated stages and oocysts following later. The oocysts are shed in feces after a prepatent period of three to nine days. When cats are infected with sporulated oocysts, the prepatent period is extended to 20–35 days because in these cases the intestinal development of *Toxoplasma* is preceded by an extraintestinal asexual reproduction (see below). Oocyst shedding lasts for only a few days to a maximum of three weeks, but can be highly intensive (up to 600 million oocysts per cat during the patent period!).

■ **External phase.** Oocysts excreted in cat feces sporulate at room temperature within two to four days, rendering them infective. Kept moist, they remain infective for up to five years and are not killed by standard disinfectant agents. They die within a few minutes at temperatures exceeding 55 °C.

■ **Extraintestinal phase.** This phase follows a peroral infection with oocysts or cysts and is observed in intermediate hosts (dogs, sheep, pigs, other vertebrates, birds) and dead-end hosts (humans), as well as in the definitive hosts (cats). Starting from the intestine, the *Toxoplasma* organisms travel in blood or lymph to various organs and multiply in nucleate host cells, especially in the reticulohistiocytic system, in musculature, and in the CNS. Repeated endodyogenic cycles produce as many as 32 individual daughter cells in the expanding host cell before it bursts. The tachyzoites thus released attack neighboring cells.

These processes result in focal necroses and inflammatory reactions in affected tissues. Generalization of the infection can lead to colonization of the placenta and, about three to four weeks later, infection of the fetus. Cysts that elicit no inflammatory reactions in the near vicinity are produced early in the course of the infection. Such cysts (tissue cysts) are found above all in the CNS, in the skeletal and heart muscles as well as in the retina, the uterine wall, and other organs. They can remain viable for years without causing noticeable damage to the host.

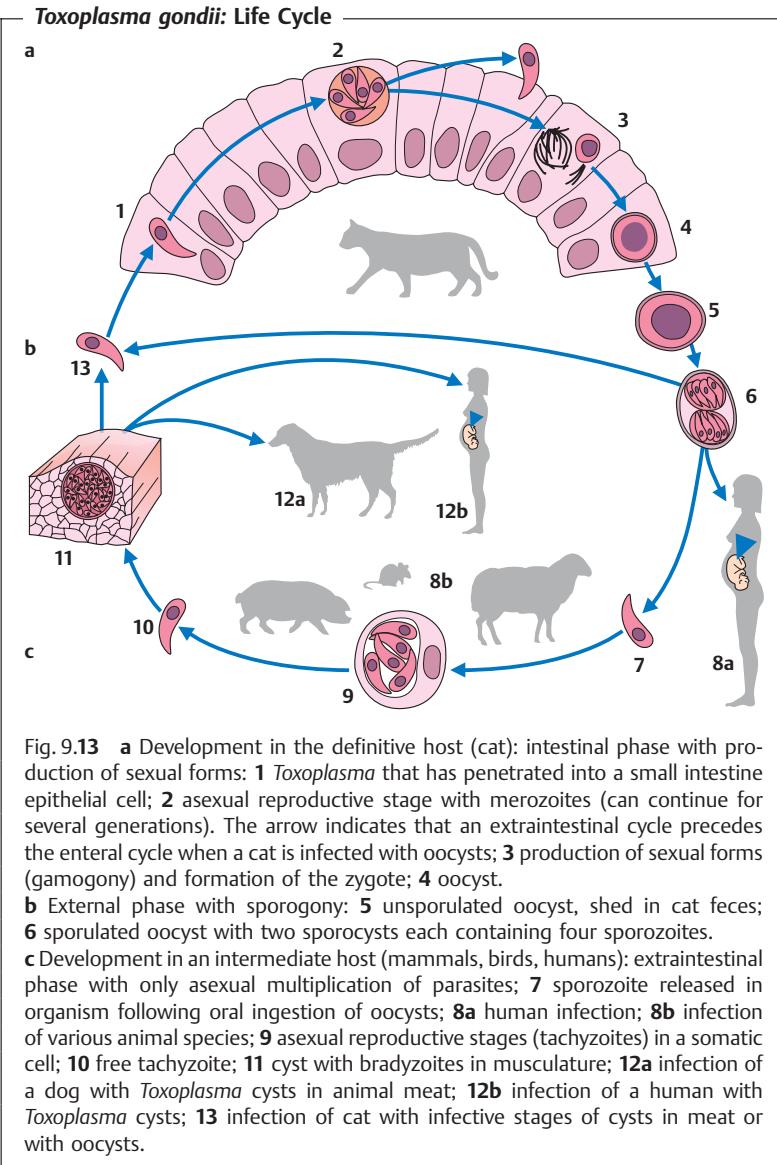


Fig. 9.13 **a** Development in the definitive host (cat): intestinal phase with production of sexual forms: **1** *Toxoplasma* that has penetrated into a small intestine epithelial cell; **2** asexual reproductive stage with merozoites (can continue for several generations). The arrow indicates that an extraintestinal cycle precedes the enteral cycle when a cat is infected with oocysts; **3** production of sexual forms (gamogony) and formation of the zygote; **4** oocyst.

b External phase with sporogony: **5** unsporulated oocyst, shed in cat feces; **6** sporulated oocyst with two sporocysts each containing four sporozoites.

c Development in an intermediate host (mammals, birds, humans): extraintestinal phase with only asexual multiplication of parasites; **7** sporozoite released in organism following oral ingestion of oocysts; **8a** human infection; **8b** infection of various animal species; **9** asexual reproductive stages (tachyzoites) in a somatic cell; **10** free tachyzoite; **11** cyst with bradyzoites in musculature; **12a** infection of a dog with *Toxoplasma* cysts in animal meat; **12b** infection of a human with *Toxoplasma* cysts; **13** infection of cat with infective stages of cysts in meat or with oocysts.

Immunity. Various *Toxoplasma* antigens induce humoral and cellular immune responses which, following a primary infection, result in antibody production and inhibition of tachyzoite multiplication. Toxoplasmas then “escape” from the immune defense system by encysting (immuno-evasion). This enables them to persist in a latent state for many years in immunocompetent hosts, at the same time maintaining an immune status that confers protection from new infections due to the continuous presentation of antigens.

The relevant immune defense mechanisms include mainly cellular mechanisms and production of IFN γ . Bradyzoites can also migrate out of cysts (without cyst rupture), are then, however, locally inactivated in immunocompetent persons, sometimes leading to formation of satellite cysts. In cases of cellular immune deficiency, this control system is lacking and the latent infection progresses to become an acute, manifest toxoplasmosis. Similarly, latent *Toxoplasma* infections in AIDS patients are usually activated and turn symptomatic when the CD4 $^{+}$ cell count falls below 200/ μ l.

Pathogenicity and clinical manifestations. Focal necrotic, inflammatory and immunopathological processes are the basis of the pathogenesis and varied clinical manifestations observed in toxoplasmosis. Cases are differentiated as to time of acquisition, i.e., postnatal and prenatal infections.

Forms of Postnatal *Toxoplasma* Infection

■ **Primary infection in immunocompetent persons.** This is the most frequent form without clinical manifestations, recognizable by the specific serum antibodies. The infection can persist for the life of the host, and it may exacerbate in response to immunosuppression. Subacute cervical lymphadenitis occurs in about 1% of infected persons.

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■ **Primary infection during pregnancy.** This may cause prenatal infection of the fetus and thus become a significant threat (see prenatal toxoplasmosis p. 513).

■ **Primary infection in immunosuppressed persons.** In cases of immune deficiency (with significant disturbance of CD4 $^{+}$ and CD8 $^{+}$ cell functions) or immunosuppressant therapies (e.g., in organ transplantations) the infection gives rise to febrile generalized illness with maculopapulous exanthema, generalized lymphadenitis, necrotizing interstitial pneumonia, hepatosplenomegaly, myocarditis, meningoencephalitis, eye damage, and other manifestations. There is a high rate of lethality if left untreated.

■ **Reactivation toxoplasmosis in cases of immune deficiency.** Local and generalized reactivation of a *Toxoplasma* infection originating from tissue cysts. Cerebral manifestations are the most frequent (up to 40% of patients

in full-blown AIDS stage), for example, with multiple coagulative necroses, small-focus hemorrhages, and surrounding edema. Other organ systems are affected more rarely (in about 15% of cases), e.g., myocardium and lungs.

Prenatal Toxoplasmosis

Occurrence. The prenatal fetal infection occurs only in mothers who contract their *primary* (first!) infection with toxoplasmas during pregnancy! There is no risk of prenatal fetal infection in women having a latent infection with serum antibodies at conception.

Incidence. The rate of verified primary infections in pregnant women was estimated in Germany, Austria, and Switzerland in 1995 at 0.5–0.7%. A prenatal fetal infection is to be expected in some of these cases, whereby the infection risk for the fetus is lower in the first trimester than later in the pregnancy. The frequency of toxoplasmosis in newborns (prenatal toxoplasmosis) is between 0.1 and 0.3% in various European countries. The rate is lower in some countries, e.g., in Austria (0.01%), where monitoring examinations for pregnant women have been obligatory for a number of years (for details see Aspöck, 2000, p. 660).

Possible consequences of prenatal infection:

- 10% clinically severe cases, 85% of these with brain damage (e.g., hydrocephalus, intracerebral calcifications), 15% perinatal deaths,
- 15% milder symptoms (99% chorioretinitis, 1% brain damage),
- 75% subclinical cases (15% no damage, 85% chorioretinitis).

Children in this last group appear to be clinically normal at birth, but signs of brain and eye damage, as well as other symptoms, may manifest later in infancy and early childhood.

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Diagnosis. In **immunocompetent adults**, toxoplasmosis is normally diagnosed serologically by detection of parasite-specific IgG and IgM antibodies (Table 11.5, p. 625). IgM antibodies can be detected as early as one week after the primary infection, peak within two to four weeks, then drop to below the detection limit within a few weeks; in some cases persistence at low titers lasts longer. IgG antibodies appear somewhat later, peak after two to four months and persist for many years. A high or rising IgG titer with contemporaneous detection of IgM indicates an acute primary infection. The isolated cases of **ocular toxoplasmosis** normally cannot be diagnosed by serological methods.

Serological findings are often not reliable indicators in **immunodeficient patients** due to reduced antibody production. The cerebral form of the infection seen frequently in reactivated toxoplasmosis is therefore usually diagnosed by means of clinical imaging methods.

In **prenatal diagnostics**, PCR is seeing increasing use for direct detection of pathogens in the amniotic fluid. Detection of *Toxoplasma* DNA with this method is a reliable sign of fetal infection and demands a chemotherapeutic or other response accordingly. Diagnosis of **prenatal toxoplasmosis** in neonates is difficult, but highly important. Since IgG antibodies are transmitted from mother to child diaplacentally, detection of them in the child cannot serve as a definitive diagnostic indicator. IgM is only present in about 50% of prenatally infected children. In suspected cases, the blood or cerebrospinal fluid should be examined using the PCR.

Therapy. The following cases require treatment: acute or subacute, symptomatic infections in children and adults as well as symptomatic or asymptomatic primary infections in pregnant women. In an acute primary infection during pregnancy, the risk of infection for the developing fetus can be eliminated by starting chemotherapy immediately. Several different therapeutic schemes are recommended for this indication. For example, spiramycin daily for four weeks from diagnosis to the end of the 15th week of gravidity, and in the period beginning with the 16th week of gravidity pyrimethamine daily for four weeks together with sulfadiazine and folic acid. The recommendations also vary for treatment of toxoplasmosis in AIDS cases, e.g., pyrimethamine/sulfadiazine or pyrimethamine/clindamycin.

Epidemiology and prevention. Humans become infected by peroral ingestion of raw meat containing cysts or by uptake of sporulated oocysts. *T. gondii* is also transmitted diaplacentally and by transplantation of infected organs to uninfected recipients.

In Europe about 1–6% of domestic cats are oocyst excretors. Sheep and goats are frequently infected with toxoplasmas; infection prevalences of pigs have decreased significantly in pig farms with high hygienic standards as demonstrated in recent studies. Cattle have always been considered rare carriers, but recently in Switzerland *Toxoplasma* DNA was found using PCR in 1–6% of the animals examined ($n = 350$). The epidemiological significance of these findings has not yet been clarified. Certain wild animal species (e.g., wild boars) are relatively frequently infected, not so horses and chickens. Milk and eggs are not considered sources of infection.

Toxoplasma cysts remain viable and infectious in meat for up to three weeks at 4 °C. Deep-freezing to -20 °C kills bradyzoites within three days, heating to 70 °C is lethal to them within a few minutes. *Toxoplasma* oocysts show considerable environmental resistance, but can be killed rapidly by heat (70 °C).

Pregnant women should eat only meat that has been thoroughly heated or deep-frozen. Close contact with cats should be avoided. Cat litter boxes in the house should be cleaned out daily and flushed with boiling water (wear rubber gloves!). Cats can be fed canned (boiled) meat to protect them from

infection. Of particular importance in prevention of prenatal toxoplasmosis is prophylactic serological monitoring, with one check per trimester as is obligatory in, for example, Austria (Aspöck, 2000 p. 660).

Isospora

Causative agent of isosporosis

The causative agent of human isosporiosis is *Isospora belli*. After peroral ingestion of sporulated oocysts and release of sporozoites, further development (schizogony, gamogony) takes place in the epithelium of the upper small intestine, leading finally to oocyst formation. In AIDS patients, encysted sporozoites have been found in various extraintestinal organs (lymph nodes, liver, gallbladder, spleen).

I. belli can cause severe clinical symptoms, especially in AIDS patients, for example persistent diarrhea, steatorrhea, cholecystitis, weight loss, and fever. Diagnosis is made by detection of unsporulated oocysts (20–30 µm long) in stool (Fig. 9.11l, p. 504) or of developmental stages in intestinal biopsies. High-dosed cotrimoxazole is the recommended therapy.

Cyclospora cayetanensis

Causative agent of cyclosporosis

Parasite and occurrence. *Cyclospora cayetanensis* was first identified as an apicomplexan parasite (family Eimeriidae) in 1994. The parasite occurs in various countries on all continents with generally low prevalences and with seasonal fluctuations, in children and adults, and also in AIDS patients.

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Morphology and life cycle. Infection per os with sporulated oocysts in food or drinking water. Developmental stages in duodenal and jejunal enterocysts, probably two generations of schizonts; following gamogony formation of spherical oocysts 8–10 µm in size. Prepatency about one week; oocysts are shed unsporulated in feces, then sporulate outside of host within five to 12 days to become infective. The sporulated oocysts contain two sporocysts with two sporozoites each (Fig. 9.11j, p. 504).

Clinical manifestations. Villus atrophy, cryptic hyperplasia, and inflammatory changes in the intestinal mucosa. Incubation about one week, self-limiting diarrhea in immunocompetent persons (lasts for about two to three weeks) with loss of appetite, flatulence, and malaise, usually nonfebrile; the diarrhea may persist for months in immunodeficient patients.

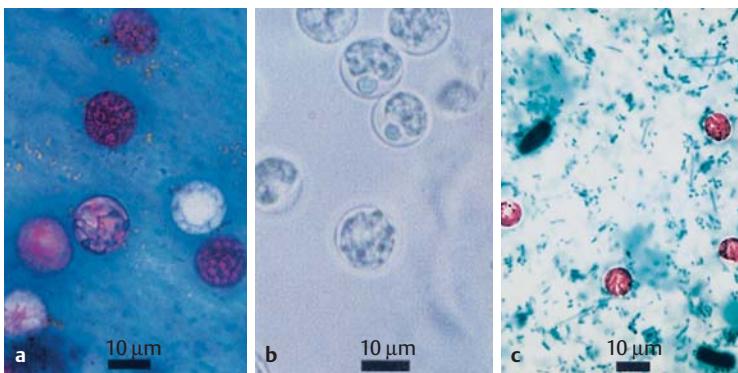
Cyclospora and Cryptosporidium

Fig. 9.14 **a** Oocysts of *Cyclospora cayetanensis* in stool smear, modified Ziehl-Neelsen staining. **b** Oocysts of *Cyclospora*, unstained, after isolation from stool. **c** Oocysts from *Cryptosporidium parvum* in stool smear. Staining as in **a**. (**b** and **c** from: *Bench Aids for the Diagnosis of Intestinal Parasites*. Geneva: WHO; 1995.)

Diagnosis and therapy. Detection of oocysts in stool specimens using concentration methods or in stained stool smears (for instance modified Ziehl-Neelsen staining or modified carbol-fuchsin staining). *Cyclospora* oocysts are easily confused with the oocysts of cryptosporidia (Fig. 9.14); they show autofluorescence in UV light and no reaction with monoclonal antibodies to *Cryptosporidium*. The drug of choice is cotrimoxazole.

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Sarcocystis

Causative agent of sarcocystosis

Parasites, life cycle, and epidemiology. *Sarcocystis hominis* and *S. suisomnis* are known as human intestinal parasites. Infection results from ingestion of raw or insufficiently heated meat from cattle or pigs, which frequently contains muscle cysts of these species. In the small intestine, bradyzoites are released from the muscle cysts. The bradyzoites undergo gamogony without an asexual reproductive phase in the lamina propria of the intestine. This process produces thin-walled oocysts that sporulate in the intestinal wall. Once the frail oocyst wall has burst, free sporocysts containing four sporozoites each are excreted with stool in most cases. The sporozoites are infectious for intermediate hosts. Prepatent periods of 14–18 and