Introductory Parasitology, Bio 328

Guide to slides and preparations, Labs 1 to 3

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Unicellular Parasites

Introduction

Thirty years ago, the Protista was treated as a natural group by many, and opposed in the Eukaryota by Animalia, Plantae and Fungi. The integration of molecular (particularly DNA) data with ultrastructural data reveals that what was referred to as the Protista represents a diverse array of evolutionary lines, each a parallel experiment with unicellular eukaryotic life. Several of the most primitive eukaryotic forms are now known as essentially parasitic e.g. Diplomonadida and Trichomonadida. However, they cannot have been parasitic when they arose because their hosts had not appeared. Free-living ancestors of these lines arose in an essentially anaerobic environment; oxygen was highly poisonous to them. The parasitic habitat, particularly that of the metazoan intestine, provided a refuge in which many of these early eukaryotic forms found asylum as oxygen tensions rose. This idea is supported by the recent finding of what appears to be free-living trichomonads and retortamonads in anaerobic sediments in marshes. For a view on interrelationships among free-living and parasitic Protist taxa, see Cavalier-Smith (1993) [1]

A word about the material: Many of the prepared slides you will be asked to examine are faecal smears: a small amount of faecal material from an infected individual is spread on a coverslip with a toothpick, and then fixed for a few minutes before being further processed (specific stains) and mounted on a slide. In these preparations, the organisms of interest must be sought among much faecal material. Use the high dry objective (40x) to do your searching. Once you are confident you have found a parasite, use oil immersion lens (100x) to study its morphology in detail. A full appreciation of the morphology will only come from examination of a number of different specimens in slightly different orientations. Be patient!

faecal smears

Tissue smears are made by slicing a selected tissue with a scalpel and pressing a coverslip against it. Parasites and cells adhering to the coverslip can then be fixed and stained as in a faecal smear. A

tissue smear

blood smear

[1] Cavalier-Smith, T. 1993. The Kingdom Protozoa and its 18 phyla. *Microbiol Rev*: 57:953–994

blood smear is made by spreading a drop of blood into a thin layer on a slide, air drying, fixing and then staining.

sectioned material

Another type of preparation you will be presented with is the histological section. A piece of tissue is fixed and embedded in paraffin. Thin sections ($5 - 10\mu m$) are cut and mounted on a slide; the paraffin is dissolved away and the preparation stained to reveal nuclei and other cell constituents.

What you are expected to know: You should be able to recognize the organism in question in all of the preparations. You will occasionally be presented with several species belonging to the same genus, and although you may not be required to distinguish among them, you should know whether this is possible, and you should be aware of the criteria used to do so. In addition you should know the life cycle and the biological and/or economic significance of the organism.

Make drawings of each preparation with a pencil (preferably a $2H$). Draw the organism in several orientations if necessary; the "textbook" image is a one in a million example and need not be the one we show you on an exam.

Laboratory 1. Parasitic Flagellates

Slides to study: *Chilomastix mesnili*, slide 6.
Giardia lamblia, slides 7, 8.
Pentatrichomonas hominis, slide 9.
Entamoeba histolytica, slides 1–3.
Entamoeba coli, slides 4–5.

Living material: Material from hind-gut of termites, *Zootermopsis* sp.

Chilomestic mesnilii trophozoite. Note the nucleus at the extreme anterior end of the organism and the cytostome just behind it.

Phylum Retortamonadida

This Phylum is thought to include some of the most ancient eukaryotic forms. Free-living species have been reported from fresh and salt water but are poorly known. Most parasiteic species are parasitic in the intestine where they feed on bacteria. Hosts include mole-crickets, roaches and various vertebrate groups. They lack mitochondria (although *Giardia* has hydogenosomes whihc some believe to be modified mitochondria) and plastids, and they have no golgi apparatus. They possess flagella that are arranged in at least two groups: an anteriorly-directed and a posteriorly-directed group. They feed on bacteria which are taken in through a specialized region, the cytostome, where phagosomes form.

flagellum: pl. flagella

golgi

Class Retortamonadea

Slide 6: *Chilomastix mesnili* is a harmless gut commensal of humans. They multiply by binary fission and transmission is direct involving lemon shaped cyst stages. The organism is $10 - 20\mu m$ long, tear-drop shaped with a pointed posterior end. The nucleus is single, and is located very near the extreme anterior end. The cytostome is visible as oblong pale area at anterior end of the organism. The three anterior flagella are often difficult if not impossible to make out in our preparations.

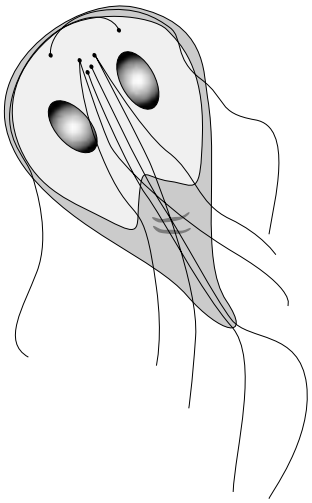
commensal
direct transmission

cytostome

Class Diplomonadea

This group includes parasitic as well as free-living forms, and some genera (e.g. *Hexamita* and *Trepomonas*) contain free-living and parasitic representatives. Like retortamonads, diplomonads lack organelles of symbiotic origin and have no golgi apparatus.

Giardia lamblia is an intestinal parasite of humans and a variety of other mammalian hosts. Trophozoites, feeding stages, feed on bacteria but may occur in high numbers and graze on the mucosal surface eroding the surface and causing malabsorption associated with severe, but bloodless, diarrhea.



Giardia lamblia trophozoite.

trophozoites
hydrogenosomes
undulating membrane
axostyle
endosome

Slide 7: The trophozoite is racket-shaped and consists of 2 sets of organelles arranged as mirror images of one another (hence *diplo*monad). There are four pairs of flagella, posteriorly directed, two anterior nuclei with prominent endosomes, and a prominent anteriorly located adhesive disc.

Slide 8: The cyst is oval. There are 4 nuclei and rudimentary flagella enclosed in a hyaline cyst wall.

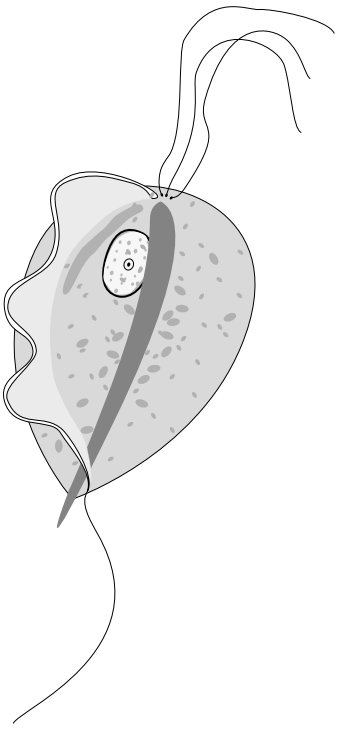
Phylum Parabasalia

Trichomonadida

Trichomonads are essentially anaerobic intestinal parasites although one free-living species is known. They have hydrogenosomes which are presumed to be of symbiotic origin (perhaps a modified mitochondrion), and a golgi apparatus.

Typically, trichomonads are pear shaped, with 3–5 anterior flagella, an undulating membrane and a prominent axostyle. There is no cyst stage. Many species are commensals but some are pathogenic.

Trichomonas vaginalis: Found in the genital and urinary tract of humans. Often asymptomatic in men but can cause severe vaginal itching in women. Transmission is venereal.



Trititrichomonas foetus trophozoite. Note the three anterior flagella and the prominent axostyle.

Trichomonas foetus: This is a widespread cosmopolitan pathogen of cattle. It occurs in the genital system and is spread venereally, typically from bulls to heifers. It attachs mucous membranes and invades the uterus where it causes abortions. Although heifers eventually rid themselves of the infection and become immune, bulls remain infected and act as carriers.

Slide 9: *Pentatrichomonas hominis* occurs in the large intestine of humans, and is nonpathogenic.

Dientamoeba fragilis: A parasite in the intestine of humans suspected as a pathogenic agent in cases of chronic diarrhea. This species lacks visible flagella but ultrastructural studies demonstrate presence of basal bodies and support its inclusion in the Trichomonadida. Nevertheless it resembles and therefore must be distinguished from intestinal amobae. There is no cyst stage and trophozoites commonly contain 2 nuclei; nuclei lack peripheral chromatin and have fragmented, irregularly shaped endosomes. Transmission apparently occurs within the egg of the pinworm *Enterobius vermicularis*.

Hypermastigida

These abound in the hindgut of termites and cockroaches where they play a role in cellulose digestion. We have supplied you with material collected from the hindgut of local termites. Using vaseline-ringed coverslip preparations, see how many different varieties of organism you can find. Note that in living material it is relatively easy to observe flagella even in very small organisms.

Amoebida, Family Entamoebidae

The term "amoeba" refers to an organism that moves and feeds with the aid of pseudopodia. It is not a reliable taxonomic delimiter since many organisms may have flagella in one developmental stage and become amoeboid in another. However, the Entamoebidae lack flagella (and microtubules, for that matter) at all stages in their development. Furthermore, they do not possess mitochondria although phylogenetic studies suggest this is the result of a secondary loss.

pseudopodia

A number of species occur as parasites in the alimentary system of their hosts. Several species occur in humans; most are harmless commensals but *Entamoeba histolytica* is a highly pathogenic species. Life cycles are similar: trophozoites (feeding stages) live in the alimentary system where they ingest bacteria. Transmission involves specialized multinucleate cyst stages passed in faeces.

Entamoeba histolytica: This species normally feeds on bacteria in the intestine but will also erode the mucous coating of the epithelium and enter the mucosa of the gut causing a typically flask-shaped ulcer. Trophozoites may even be carried by the lymphatics to the liver where they cause further problems.

Slide 1: Note the rounded trophozoites with large lobular pseudopodia and clockface nucleus; the nuclear endosome is typically (but not always) centrally located, and the peripheral chromatin is formed of fine granules. You may see erythrocytes in the cytoplasm, or haemosiderin pigment indicating digested erythrocytes.

Slide 2: In tissue section the trophozoites are often bigger than luminal forms. Note the flattened villi and the sloughed epithelium. Do you see any signs of inflammation? Is this a chronic or acute lesion?

chronic/acute inflammation

Slide 3: Cysts are smaller than trophozoites. They contain 4 nuclei and often chromatoid bars (tightly packaged messenger RNA) with rounded ends.

chromatoid bars

Entamoeba coli: This is another species that occurs in the intestine of humans. It is a harmless commensal. Because multiple infections are common it is important to be able to distinguish between this and pathogenic forms.

Slide 4: Trophozoite distinguished from the above by its coarser chromatin, eccentrically located endosome and the absence of erythrocytes in cytoplasm.

Slide 5: Cyst distinguished by 8 nuclei (mature cyst) and smaller chromatoid bodies often filamentous.

Ciliophora

A number of ciliates are parasites. *Ichthyopthirius* spp. are important pathogens of fish and cause the disease "Ick". *Trichodina* spp. are found on the gills of many fish and can be pathogenic. Rumen ciliates are important contributors to cellulose digestion in cattle and other ruminants.

Balantidium: *Balantidium coli* is the largest protist parasite of humans, up to $100\mu m$ in length. It is generally nonpathogenic but can cause ulceration of the intestinal epithelium followed by secondary bacterial infection.

Slides 29, 30: The trophozoite (slide 29) is oblong and contains a prominent kidney shaped macronucleus. Transmission occurs when the cyst stage (Slide 30) is accidentally ingested.

macronucleus

Laboratory 2. Class Kinetoplastida

Slides to study: *Leishmania*: slide 10 (spleen).
Leishmania: slide 11 (tissue culture).
Trypanosoma lewisi and *T. duttoni*: slide 12 (blood smear).
T. cruzi: slide 13 (blood smear).
T. cruzi: slide 14 (infected heart).
T. brucei, *T. gambiense*: slides 15 & 16 (blood smears).
Cryptobia salmositica: slide 17 (blood smear).

Living material: *Kudoa thrysites* (Myxozoa) from muscle of tubesnout, *Aulorhynchus*.

Class Kinetoplastida

This group belongs to the Phylum Euglenozoa, which arose from early mitochondriate stock. A key acquisition they share with trichomonads and other "higher" eukaryotes is the golgi apparatus, which permits more complex use of carbohydrate-protein combinations. This may have played an important rôle in receptor-mediated host cell entry (e.g. in *Leishmania* and *Trypanosoma cruzi*) and in manipulation of cell surface molecules (in the salivarian trypanosomes).

The Kinetoplastida comprises two subgroups, the predominantly free-living bodonids, and the parasitic trypanosomatids. Members of the former group possess two flagella; this has been secondarily reduced to a single flagellum in trypanosomatids. Kinetoplastids are parasitic in plants and animals. Many species occur in the digestive tract of insects. The group is characterized by a mitochondrion that is single and extends throughout body as a tube; a prominent swelling of mitochondrion near base of flagella, the kinetoplast, contains the DNA.

kinetoplast

Bodonids: This is essentially a free-living group; they occur in soils rich in organic material where they feed on bacteria. A few are predators and some are parasitic. *Cryptobia* spp. (= *Trypanoplasma*) occur in the reproductive tract of mollusks and the blood system of fishes. Life cycles may be direct or indirect depending on the species.

Slide 17 : *Cryptobia salmositica.* This species is parasitic in the blood and on the body surface of salmonid fish. Transmission may occur by leech vectors but direct transmission is also possible. Note the highly varied shape of the parasites in the blood smear. Note also the nucleated erythrocytes. Only in mammals do erythrocytes lose their nuclei at maturation.

Trypanosomatids: Trypanosomatids occur in the intestinal tract of invertebrates (typically insects) and in the blood and other tissues of vertebrates. Parasites of insects are transmitted directly by oral/faecal contamination; those of vertebrates are usually transmitted by a vector. Most species pass through a number of developmental stages during the course of the life cycle, distinguished on the basis of flagellar structure and the relative positions of the kinetoplast and the nucleus. Learn how to distinguish among amastigote, promastigote, and trypomastigote. Class material belongs to two genera, *Trypanosoma* and *Leishmania.*

amastigote
promastigote
trypomastigote

Trypanosomes: Trypanosomes parasitize all classes of vertebrates, and most species occur in blood or extracellular tissues. One group, the *Trypanosoma cruzi* group, also occurs intracellularly. Most have low pathogenicity but a few cause some of the most important diseases of livestock and man.

Typically, life cycles are indirect, with transmission mediated by a vector (insects in terrestrial hosts, leeches in aquatic hosts). In the vector there is development as well as reproduction (cyclopropagative) and the culmination of this phase is the production of metacyclic forms, infective to the next host.

vector

Two types of development in the vector are recognized: anterior station and posterior station. Anterior station development involves division of trypomastigotes in the midgut followed by migration forward into upper digestive tract (e.g. salivary glands). Metacyclic trypomastigotes are passed to the next host when the vector feeds. Anterior station development occurs in leech transmitted parasites of fish and amphibians, and in the salivarian trypanosomes transmitted by Tse tse flies (*Glossina*).

anterior station
posterior station

Posterior station involves development in the hindgut with eventual transformation into epimastigotes and metacyclic trypomastig-

otes which are passed with the vector faeces. This form of development and transmission occurs in trypanosomes of terrestrial hosts with the exception of the salivarian forms.

Mammalian trypanosomes have been divided into subgenera based on their biological characteristics. You will examine species belonging to three subgenera:

(1) salivarian trypanosomes, *Trypanosoma* (*Trypanozoon*), including *T. brucei*, a pathogen of livestock, as well as *T. rhodesiense* and *T. gambiense*, which are important pathogens of man; (2) *T. cruzi*, a South American member of the subgenus *T.* (*Schizotrypanum*), pathogenic to man; (3) *T.lewisi* or *T. duttoni*, subgenus *T.* (*Duttonella*), non pathogenic rodent parasites: the former occurs in rats, the latter in mice, both transmitted by fleas.

Slides 15—16: The salivarian[2] trypanosomes are agents of human sleeping sickness, but are probably more important as causes of death and morbidity in livestock throughout much of equatorial Africa. The vector is the Tse tse fly (*Glossina* spp.) and diseases caused by these trypanosomes kept European man out of Africa until the end of the last century largely because no beasts of burden could survive in the fly belt. These forms are recognized by their blunt posterior end.

sleeping sickness

Tse tse fly

Slide 14: *T. cruzi*: This is a thin section of heart muscle from a rat. First examine the section under low power (10×) to familiarize yourself with the tissue. The muscle cells are multinucleate and striated. Furthermore, heart muscle cells may be branched. Under higher power you will recognize the tiny amastigote stages that fill the cytoplasm of infected muscle cells. Some of these preparations are startling in the extent of the damage. Recall that one of the principal ailments associated with Chagas' disease is chronic heart disease. Heart problems in these patient's are only partly due to direct effects of parasites. There is also an element of autoimmunity that develops.

Chagas' disease

[2]Salivarian trypanosomes are also referred to as African Trypanosomes. Although they are largely African in their distributions, they represent only one small group of African trypanosomes and the name serves only to distinguish the group from *T. cruzi* and its allies that infect man and other animals in South America.

Slide 13: Find the trypomastigote stage in the blood smear. These often die in a "question-mark". The kinetosome is very large and the posterior end, little of which extends beyond the kinetosome, is sharply pointed.

kinetosome

Slide 12: *T. lewisi* and *T. duttoni* are parasites of rats and mice respectively. Note the prominent kinetosome, and the long pointed posterior end. The vector is the flea, *Nosopsyllus fasciatus*, and development is posterior station. Transmission may occur either by ingestion of the infected flea or by contamination of the flea bite with infected faeces.

Leishmania: These are important pathogens of man throughout the tropics and subtropics. The vectors are sandflies, Subfamily Phlebotominae. Amastigotes, ingested by the fly, transform to promastigotes in the midgut and multiply, blocking the gut of the fly. Soon they back up into the pharynx and buccal cavity from where they contaminate the bite and gain access to the vertebrate host, mammals and lizards. The parasites are intracellular parasites of macrophages and can occur in any tissue. Some forms are restricted to the skin around the site of the bite; others invade the cartilaginous tissues of the face (mucocutaneous form) and still others disseminate througout the body (visceral leishmaniasis). The latter form of the disease is characteristic of *L. donovani* and is by far the most severe.

cutaneous form

mucocutaneous form

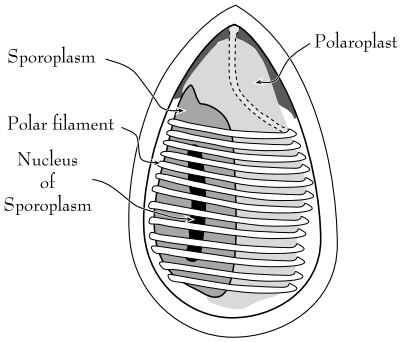
visceral form

Older textbooks recognize three species as important in man : *L. donovani*, *L. tropica* and *L. brasiliensis*. However, it is now believed that there are many genetically distinct forms each with particular epidemiological characteristics and disease implications. Epidemiologically, species differ in their reservoir hosts (hosts that maintain the parasite in the wild), and in how man acquires the disease. For example, in some forms, the disease is maintained largely in man and transmission flow is commonly man—fly—man, whereas in others man more frequently acquires disease from a fly by way of dogs or wild reservoirs.

reservoir host

Slide 10: This is a tissue smear from the spleen. In it you will see macrophages infected with the tiny amastigotes.

Slide 11: These are cultured forms, promastigotes, that mimic development in the vector.



Spore of the microsporean *Thelohania*

Microsporea

Phylogenetic analyses based on rDNA suggest that Microsporea are among the most ancient Eukarote lineages; however, other molecular analyses suggest that they are offshoots of fungus. All microsporea are parasitic and infective stages are equipped with a hollow spiral filament which is extruded and used to enter the cell; the parasite travels through this narrow tube into the host cell cytoplasm. No specific receptors are thought to be involved. Some species (e.g. *Encephalitozoon cuniculi*) show little host or tissue specificity, entering a broad array of mammalian (more rarely birds) hosts via the intestinal epithelium but spreading subsequently to other tissues. Microspora are widespread in insects, especially aquatic stages; a number of genera are hyperparasitic on gregarine parasites of insects. Other hosts include platyhelminths, molluscs, annelids, crustacea and echinoderms. Some are important pests—e.g. *Nosema apis* of honeybees, *Loma salmonis* and *Glugea* spp. in fish farms.

Myxozoa

The phylogenetic affiliations of the Myxozoa are unclear but it has been postulated that they represent a degenerate line of coelenterates; the polar capsule with its polar filaments that characterize the "spore" stage bears a remarkable resemblance to nematocysts of this latter group. Myxozoa are important pathogens of fish. *Myxobolus cerebralis* is nonpathogenic in its normal host, brown trout (*Salmo truttae*), but damages the axial skeleton of rainbow trout, *Oncorhynchus mykiss* giving rise to a syndrome known as whirling disease, where fish swim in circles. The species causes large losses in hatcheries throughout the world.

polar capsule

Slide 26: *Leptotheca ohlmacheri* occurs in frog kidneys. Examine the slides under low power to understand the tissue architecture. Find the glomeruli. You will find the spores in the collecting tubules of the kidney.

Kudoa thrysites. This organisms forms pale cyst like lesions about the size and shape of rice grains in muscle of many fish species. Parasites release a non-specific protease upon death of the host and heavy infections destroy the texture of the meat making it unsuitable for eating. *Kudoa* is an important problem in Hake on the West Coast. You are provided with material from tubesnout, *Aulorhynchus*, a distant relative of seahorses and pipefish. Dissect one of the muscle cysts and place a bit of the material in the cyst in a drop of fish saline (0.80% NaCl). Cover with a vaseline ringed coverslip and study spores under the microscope.

Laboratory 3. Phylum Apicomplexa

Slides to study: *Monocystis*: slide 18.
Eimeria stiedai: slide 19 (bile ducts).
Sarcocystis: slides 27 & 28 (muscle).
Haemogregarina stepanowi: slide 20 (blood).
Plasmodium vivax: slide 22 (blood).
P. malariae: slide 23 (blood).
P. falciparum: slide 24 (blood)
Plasmodium cathemerium: slide 21 (blood).
Haemoproteus: slide 25 (blood).

Living material: *Monocystis* from the seminal vesicles of earthworms.

Apicomplexa

This is the most diverse group of parasites in terms of host distribution, life cycle and tissue site inhabited in the host. The nearest free-living relatives are dinoflagellates. There is mounting molecular evidence that this group has close affiliations with plants; apicomplexans have recently been shown to possess modified chloroplasts. The characteristic morphological feature of the group is the apical complex, a combination of structures at the anterior end of infective stages that is used to enter cells. Some free-living predators, like *Spiromonas* seem to be much closer to apicomplexans than to dinoflagellates and use their apical complex to penetrate their prey which are other one-celled organisms.

apical complex

All Apicomplexan life cycles follow the same pattern. Infection typically involves a cyst, or oocyst, containing sporozoites which invade the host and develop into trophozoites. These may (typical) or may not undergo a process of multiple fission, called schizogony, resulting in merozoites which reinfect cells, transform into trophozoites and initiate schizogony again. Eventually trophozoites develop into gamonts (gamogony) which develop as microgametocytes (male) or macrogametocytes (female). Fertilization occurs and the zygote undergoes meiosis, followed by a variable number of mitotic divisions (sporogony) to produce sporozoites.

sporozoite
merozoite
schizogony
gamogony
sporogony

Slide 18: *Monocystis* spp. parasitize the seminal vesicles of earthworms. This is a disheartening slide. Don't bother with it. Examine living material if at all possible. There is no schizogonic phase in the life cycle: sporozoites form trophozoites in sperm mother cells in the earthworm; these grow and eventually associate as pairs in a common cyst and develop into gamonts (syzygy). Hundreds of zygotes are formed in this common cyst and these become thick shelled oocysts each containing 8 sporozoites. These are released when the worm dies or via the genital pore.

syzygy

Slide 19: *Eimeria stiedai* is a parasite of rabbits and developmental stages occur in the bile ducts in the liver. The life cycle is direct and transmission occurs when rabbits accidentally ingest oocysts contaminating their food supply. Study the section under low power to appreciate the architecture of the liver. Learn to distinguish the liver parenchyma from the bile ducts. Then examine the bile ducts and search for stages of the parasite. Trophozoites are round inclusions in the biliary epithelium. Macrogametocytes are large oval bodies with peripheral red-staining granules. Microgametocytes are fewer in number and more diffusely stained than macrogametocytes. Schizonts require careful searching to find. They contain 6–20 banana shaped merozoites. Oocysts are large oval bodies with a thick wall. Many are free in the lumen of the bile duct.

oocyst

Eimeria sp. occur in a wide variety of birds and mammals. A related genus, *Isospora*, can be distinguished by its sporulated oocyst.

Slides 27—28: *Sarcocystis* spp. have an oocyst stage very similar to that of *Isospora*. The life cycle is indirect involving a carnivore and an herbivore host. In the carnivore, developmental stages similar to those of *E. stiedai* occur in the gut. Oocysts passed in the faeces contaminate the soil and are picked up by the herbivore. Sporozoites enter the tissues and are carried throughout the body. They enter muscles and divide to form pseudocysts the size of a rice grain and containing thousands of merozoites. Carnivores are infected when they ingest the flesh of an infected prey or by accidental ingestion of oocysts.

Haemogregarines: These are parasites of the blood of frogs, lizards and turtles, and are transmitted by leeches or mites. Related species

occur in fish.

Slide 20: *Haemogregarina stepanowi* is a parasite of turtles. Note the *U*-shaped trophozoite. Schizogony and gamogony occur in the blood of the vertebrate host. Fertilization and sporogony occur in the intestine of leech vector; sporozoites migrate to the salivary glands and are transmitted when the vector feeds.

Malaria: This group includes the most pathogenic parasites of man. The word *malaria* literally means bad air and reflects the ancient belief that the disease was contracted by breathing bad air (swamp gas). Human parasites belong to one of four species *Plasmodium vivax*, *P. ovale*, *P. malariae* and *P. falciparum*, all transmitted by various species of *Anopheles*. You do not have to learn to distinguish these species based on the blood smear. Be aware, however, that this can be done and know the sorts of characters that are used.

Life cycle: Sporozoites in the salivary glands of the vector are introduced into the vertebrate host during feeding. These travel via the lymphatics and blood system to the liver where they enter a liver cell, transform into trophozoites, and undergo a series of schizogonic cycles (exoerythrocytic phase). At some point, merozoites enter erythrocytes and undergo another series of schizogonic cycles (erythrocytic phase). Schizogonic cycle are synchronized and occur at regular intervals causing a recurrent succession of fever and chills. Eventually, merozoites enter erythrocytes, forming trophozoites that develop into macro- and microgametocytes. These must be picked up by the vector to continue development. In the vector, the microgametocyte exflagellates, and the macrogametocyte is fertilized developing into a motile zygote, the ookinete. The ookinete penetrates into the stomach of the vector and develops into an oocyst in which thousands of sporozoites develop. These travel to the salivary glands where they await transmission to the next host.

lymphatics

exoerythrocytic phase

erythrocytic phase

macro/micro gametocytes

ookinete

Species that cause malaria in man have no reservoir hosts. This means that to contract malaria, you must be in an area inhabited by man.

Slide 21: *Plasmodium cathemerium* is a parasite of birds trans-

mitted by mosquitoes of the genus *Aedes*. How do you know this slide does not come from a mammal host?

Slide 22: *Plasmodium vivax* can be recognized by its variably-shaped ring-stage. Schizonts contain about 16 merozoites and the infected cell is enlarged and contains Schuffner's dots. The disease caused by this organism is mild and known as benign tertian malaria (fever paroxysms typically recur every 48 h)

ring stage

tertian malaria

Slide 23: In *Plasmodium malariae* the trophozoites often pass through a band form where they stretch across the cytoplasm of the infected cell. Schizonts produce 8 merozoites. Infected cells often contain haemozoin (dark or amber colored granules resulting from the breakdown of haemoglobin). The disease caused by this organism is severe and known as quartan malaria (fever paroxysms recur every 72 h).

quartan malaria

Slide 24: *Plasmodium falciparum* has very neat ring stage trophozoites and multiply infected cells are common. Schizonts are rare in the peripheral blood. Gametocytes are crescent shaped. The disease caused by this organism is severe and known as malignant tertian malaria (fever paroxysms every 48 h). It is this species that kills the vast majority of humans that die of malaria.

Haemoproteus: *Haemoproteus* spp. occur in birds and lizards and are vectored by biting flies.

Slide 25: *H. columbae* is a parasite of pigeons transmitted by Hippoboscidae flies. The trophozoites are oblong and wrap around the nucleus of the erythrocyte. Pathogenesis is mild.