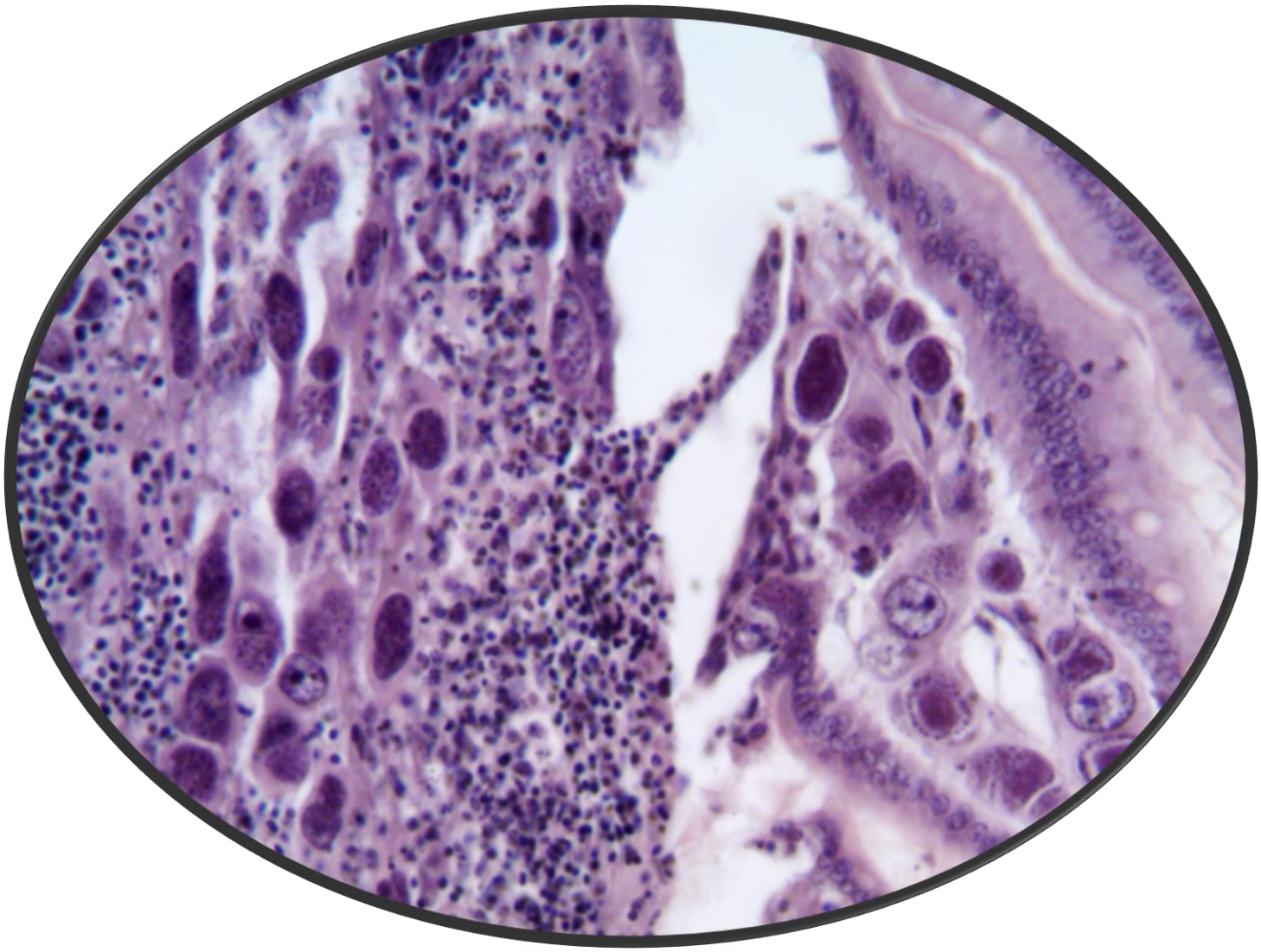
Parasites

part 3 parasites that are protists



Ed Ward MD, Minnesota USA

Origins of this article

I have long been fascinated by weird organisms. During my training I gave talks about spotted fevers and the ticks that spread them. I volunteered in West Africa in 2004, including treating parasites and other neglected tropical diseases. About a decade ago I started using old microscopes as a hobby, and now I contribute to *Micscape* magazine.

My curiosity makes for a long article divided into parts. Protists come this month, and eventually I'll relate my own and other true stories about patients with parasites. I collect vintage slides of parasites, allowing me to illustrate some kinds. I focus on what I find especially interesting and on human parasites, but many of my images show parasites of animals.

Disclaimers

I started out as a little boy and have not fully matured, still thinking creepy crawly things are very interesting.

I am a medical doctor (general internal medicine) but nothing in this article should be used to diagnose or treat medical conditions. Medical Parasitology is a subspecialty full of rare cases and exceptions. The few times I encountered parasites locally, I consulted the US CDC website and the state health department.

If you think you have parasites, consult your doctor. If you live in the USA or Europe having serious parasites is very unlikely, so the doctor will likely dismiss your self diagnosis without testing and offer you $100 of anxiety pills. An alternative healer might happily order $200 of parasite tests and sell you a $200 parasite cleanse you don't need. Serious human parasites are now rare in wealthy nations. Soap, clean water, shoes and indoor plumbing are your best bets against parasites.

Cover page illustration

Eimeria tenella, coccidian parasite *in situ*, chick hemorrhagic colitis, slide by Johns Hopkins School of Hygiene Parasitology, 1948. Coccidia and other parasitic protists have complex life cycles with intracellular asexual and sexual reproduction; many parasites in different stages and intense inflammatory infiltrate seen here. *Eimeria* is an aggressive parasite. 40X objective, image about 300 µ wide

Other illustrations

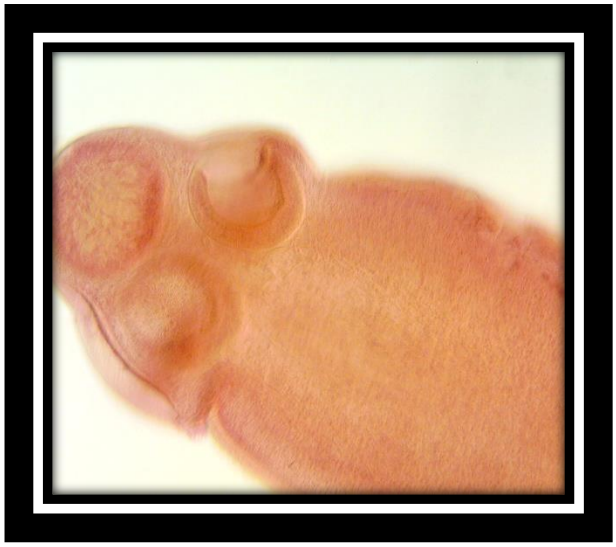
If not noted otherwise, photomicrographs are mine, taken with AO/Reichert microscopes with USB camera. With a 0.5X reducer (added late 2017) my 2.5X objective images are about 5 mm across, the 4X about 3 mm, 10X about 1.1 mm, 40X about 0.3 mm (300 microns), and 100X about 125 microns. Some images adjusted for brightness and contrast. Some patient photos of mine from West Africa are also included.

Contents

Abstract **(pertains to all parasites)**

Life spreads and adapts everywhere it can survive, including inside and outside the bodies of animals. The bodies of animals turned out to be comfy and tasty. Evolution therefore produces many endoparasites (like intestinal worms) and ectoparasites (such as lice). Almost all wild animals have parasites, and did most humans in the past. Although most individuals are not harmed, parasites can injure by heavy infestation or by complications. In poor and tropical areas many people are still harmed and even killed, including over 600,000 annual deaths from malaria. Some parasites can also act as vectors to spread bacteria and viruses that cause Lyme disease, encephalitis, plague and other diseases. Nearly half of humans still have parasites, most commonly helminths (worms) and hidden toxoplasmosis, but they don't make most of us sick. Members of many different branches of life have sometimes become parasitic: especially protozoans, flatworms, roundworms, and arthropods (including ticks, crustaceans, insects). I will discuss three main kinds of parasites of humans: protozoan parasites, worms, and ectoparasites.

The harms of parasites are highest in the tropical and poor areas of the world. We need to continue life saving efforts to control malaria, worms, and other neglected tropical diseases. Still, most of you reading this need not fear parasites. Anxiety about parasites is far more common than parasitic disease in the developed world. Parasites are also part of the balance of nature, which might be hurt if we continue to extinct parasite species faster than we can discover them.



Proteocephalus tapeworm from large mouth bass, slide H Van Cleave, 10X objective, image ~ 1 mm wide

Ctenocephalides felis, cat flea on opossum, slide L Bircham, 10X objective, image ~ 1 mm wide

Chapter 3, Protozoan parasites, Summary

Brief biology

Protozoa/protists are a grab bag of single celled eukaryotes. They are aquatic, and may be free-living or parasitic. Most Their taxonomy remains a mess.

Grouped by rough taxonomic affinities, some important protozoan parasites of humans are:

Super group SAR (see protist tree of life on page 11)

Alveolates

Phylum **Apicomplexa**

Group **Hematazoa** (or class Aconoidasida)

1. *Plasmodium, Babesia*

Deadly malaria is mosquito borne in the tropics; babesiosis is a related tick-borne illness

Group **Coccidia** (or class Conoiddasia)

2. *Toxoplasma,* **3.** *Cryptosporidium*

Toxoplasmosis is cat related; cryptosporidiosis causes diarrhea in people

Super group "excavates"

Discoba

Phylum **Euglenozoa**

Class **Kinetoplastea**

4. *Trypanosoma brucei* and *T. cruzi,* **5.** *Leishmania*

Metomonada

Giardia lamblia and *Trichomonas vaginalis*

Super group Amorphea

Phylum **Amoebozoa** (or **Evosea**)

Class **Archamoebae**

6. *Entameoba*

Class **Discosea**

Acanthamoeba

Parasite glossary: strange words to describe strange relationships

Parasite an organism that lives in or on another and takes nutrients from the host

Endoparasite lives inside of host

Ectoparasite lives on outside of host

Free living not a parasite; makes food or eats it as a predator/scavenger, does not live inside creatures

Parasite load number of parasites per host (affects potential harm of parasites)

Infestation harboring another animal (worm, arthropod) in or on the body (**infection** is microbes in body)

Host a larger organism that harbors a smaller parasitic (potentially harmful) organism

(smaller organisms helpful to, or neutral for a host are beneficial or commensal, not parasitic)

Definitive host organism that harbors adult (sexually reproductive stage) parasites

Intermediate host organism that harbors immature stages (which may reproduce asexually)

Vector an organism (usually intermediate host) that passes a parasitic organism between hosts

Reservoir a population or community of organisms that can permanently harbor a parasite population

Zoonosis a disease transmitted from animals to people; many parasitic diseases are zoonotic

Parasite life cycle a series of stages through which the parasite grows, reproduces and transmits itself

Monoxenous also known as direct parasitism; life cycle requires only a single host species

Heteroxenous indirect parasitism; life cycle requires definitive host plus one or more intermediate hosts

Direct transmission hosts touch each other (sex counts), passing on a free-living life stage (including skin to skin passing lice) or by ingestion of free-living parasite or eggs (i.e. fecal-oral, by food with contaminated dirt)

Indirect transmission from one host to another through an intermediate host (i.e. a vector such as a tick)

Trophic transmission by eating an organism that contains a parasite (i.e. from prey, or uncooked pork or fish)

Iatrogenic transmission by medical care (i.e. malaria from a blood transfusion or organ transplant)

Parasitoid tiny wasps (some are “fairy flies”) whose larva eat a host from inside, eventually killing it

Hyperparasite a parasite of a parasite; i.e. some parasitoid wasps prey on other parasitoid wasps

Parasitic castration some trematode and arthropod parasites gain added resources by neutering the host

Social parasitism i.e. some butterfly larvae mimic ants in shape and smell and are cared for by ant colonies

Kleptoparasitism i.e. stealing food from another species, as do frigatebirds and hyenas

Brood parasitism i.e. cuckoo birds lay eggs in another species nests, to be raised by host parents

Sexual parasitism i.e. male anglerfish attach to a female and shrink to just tiny sperm-making parasites

Part 3

Protozoan Parasites

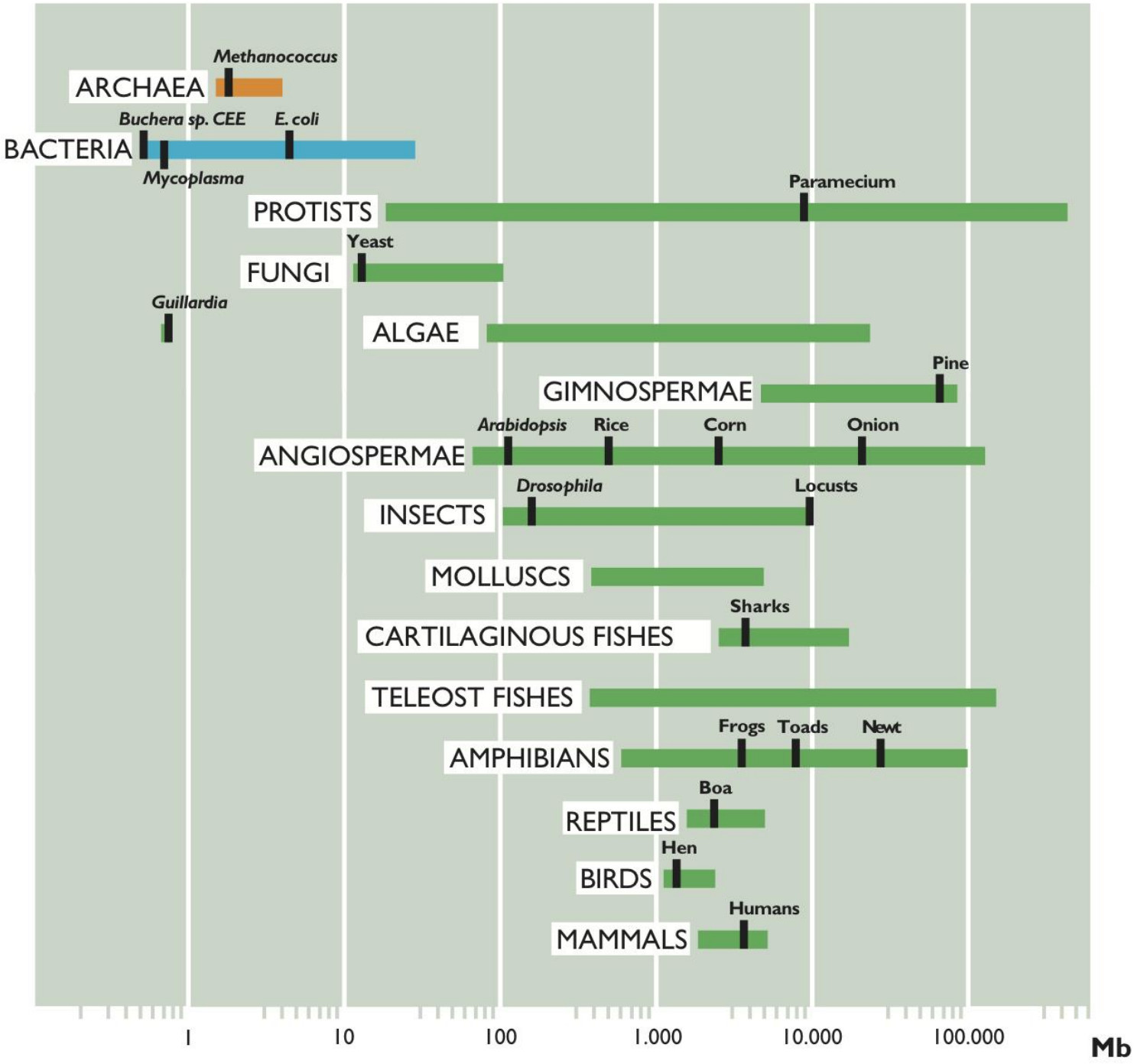
Protozoa is the kingdom of life made up of single celled eukaryotic (with a nucleus) organisms. *Paramecium, Euglena* and other pond water protists are well known to microscope hobbyists. For a long time protists were thought to be single celled animals, as I was taught in my youth. Then we gave them their own Kingdom of life, took it away, then sort of gave it back. Protozoan classification is an ongoing mess. Genomic data from diverse protists confirms the opinion of Michael Angelo, professor pathology at Stanford University that "evolution is not like a scientist with a protractor measuring things out; it's like a drunk guy with duct tape." For protists it is also sometimes difficult to define the line between a pathogen and a parasite.

Protozoan? Protist? Protoctist?

From the time Leuwenhoek saw them in 1764, calling them "animalcules" (little animals) protists were considered mostly little animals, although single celled eukaryotic algae were considered little plants. About a century later multiple biologists realized the single celled aquatic eukaryotes didn't fit neatly into the Animal or Plant Kingdoms, so deserved one of their own. In 1859 Richard Owen proposed Kingdom Protozoa, in 1860 John Hogg named Regnum Primigenum (Protoctista), in 1863 T B Wilson and John Cassin suggested Primalia and in 1866 Ernst Haeckel designated Kingdom Protista. Most biologists didn't accept the idea of a protozoan kingdom, and a century later I still learned about animal (i.e. *Paramecium*) and plant (i.e. *Euglena*) protists in the 1970's. But the idea of a separate Kingdom for single celled eukaryotes was finally accepted by modern biologists around that time, accelerated by the discovery of DNA analysis of relatedness. Most biologists now consider the non-metazoan eukaryotes Kingdom Protozoa, although Protista is also sometimes used, and neither group is monophyletic. (I find protist is easiest to type).

It takes confidence to propose a big new kingdom of life, and several of those who did so were notable characters. Owen was a brilliant biologist and paleontologist, coined the name "dinosaur" and was the first president of the Royal Microscopical Society of London. Both Owen and physician James Paget independently discovered the nematode parasite *Trichinella* in muscle in 1835, but Owen denied Paget's claim. His peers all described Owen as a deceitful bastard. Charles Darwin was the kindest, noting Owen mentored no students. Ernst Haeckel was a gifted German zoologist and artist who unfortunately defended racism with evolutionary arguments. The late US biologist Lynn Margolis (ex-wife of astronomer Carl Sagan) was a proponent of 5 kingdoms of life (including Protoctista). She fought hard for the idea that mitochondria are symbionts inside eukaryotic cells and was vindicated by emerging DNA evidence. Ever the iconoclast, she also denied HIV virus causes AIDS and that 9-11 was a terrorist attack.

Prokaryotes have been evolving for about 3.8 billion years, and about 2 billion years ago an archaea and bacteria got symbiotic to make the first eukaryotic cell, which diversified into all kinds of protists. Although still mostly microscopic, protozoa have more genetic information (in a nucleus) and new battery packs (mitochondria) that bacteria don't. Sea and pond water is full of interesting heterotrophic (eat food) and some photosynthetic protists. Some look like little animals, but they aren't. Life did not become multicellular until about 0.7 billion years ago.



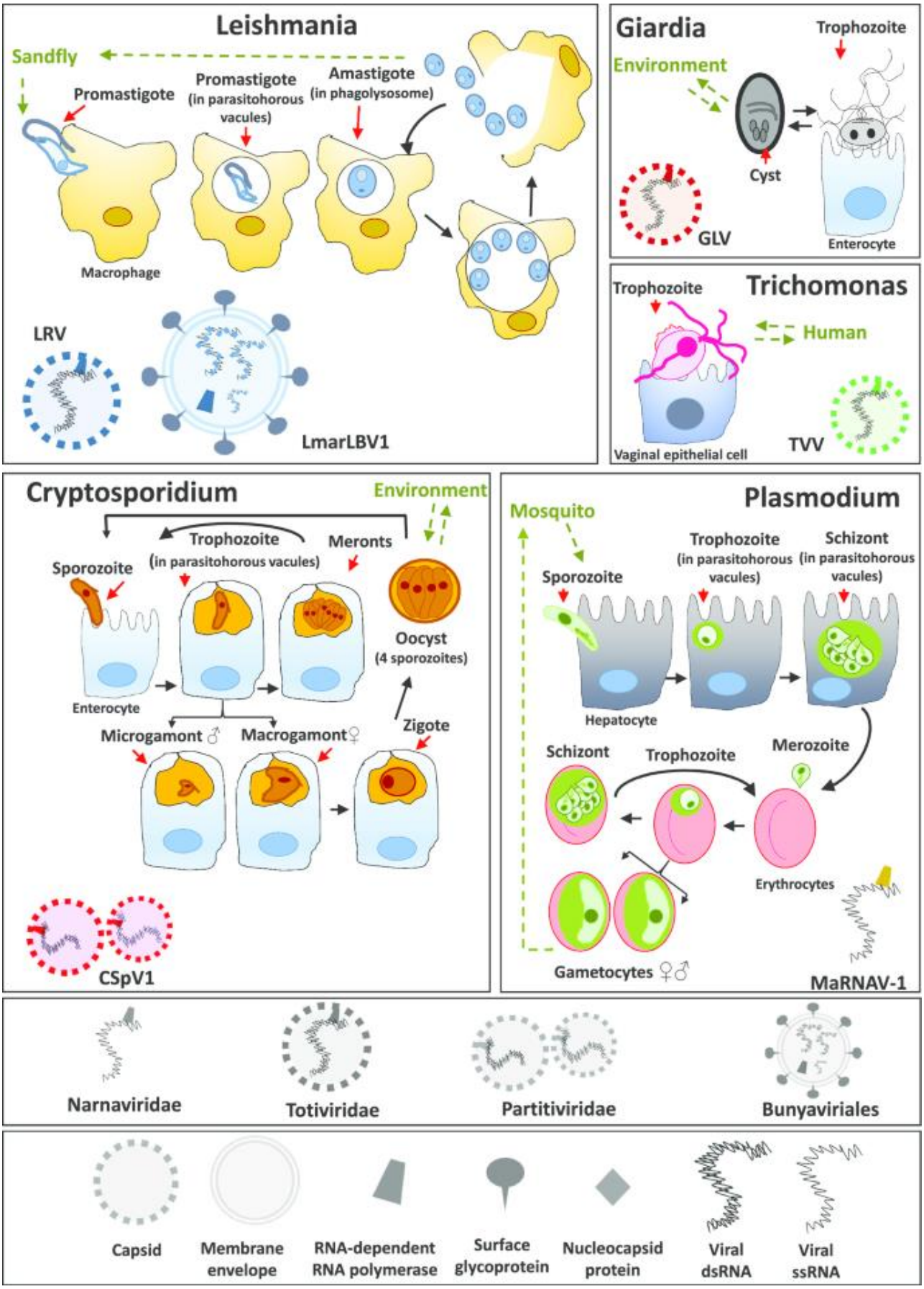
Range of genome size in various groups of organisms, log scaled. Note the diversity of genome sizes in protists. Humans are rather average in genome size for animals and plants. My daughter's pet axolotl has 32 billion base pairs, 10 fold more than me. DNA technology is advancing rapidly. The first 3.2 billion base pair human genome took 13 years to decipher and cost US $2.7 billion. Today a human genome can be read in 1 day for about $500.
Chart from Latorre and Silva, *Mètode Science Studies Journal* 2014.

Metazoans offered a new place for protists to live. Protozoan parasites can infest plants (i.e. *Phytomonas* species flagellates can infect coffee, coconut and oil palm crops) and most if not all animals can also be infested with protozoans. Parasite infections are important in nature, affecting the populations of wild animals in aquatic and terrestrial habitats. Intestinal coccidia, *Toxoplasma* and *Giardia* are major veterinary parasites and can also affect people. Here, I'll focus especially on some of the most important human protozoan parasites.

A rare but scary protozoan pathogen is *Naegleria fowleri,* also known as "the brain eating amoeba", the cause of primary amebic meningoencephalitis. *Naegleria* is a free-living "amoeba" in warmish lakes and hot springs (although it is amoeboid under the microscope it's classified in a separate group). Amoebas are the shape shifting pond water microorganisms many learned about in school and amateur microscopists know well. But if you dip your head into the wrong water *Naegleria* can crawl up through your nose into your brain, causing headache, fever and usually death (in 153 out of all 157 US cases since 1962). *Naegleria* is a bad actor but not a parasite; it is an opportunistic pathogen, a free-living organism that occasionally causes an acute and serious illness. Hungry amoebas eating your most vital organ, that's acutely pathogenic. It differs from a complex parasitic life cycle in the blood and organs of host animals that might persist hidden for years in some cases, like the malaria parasite.

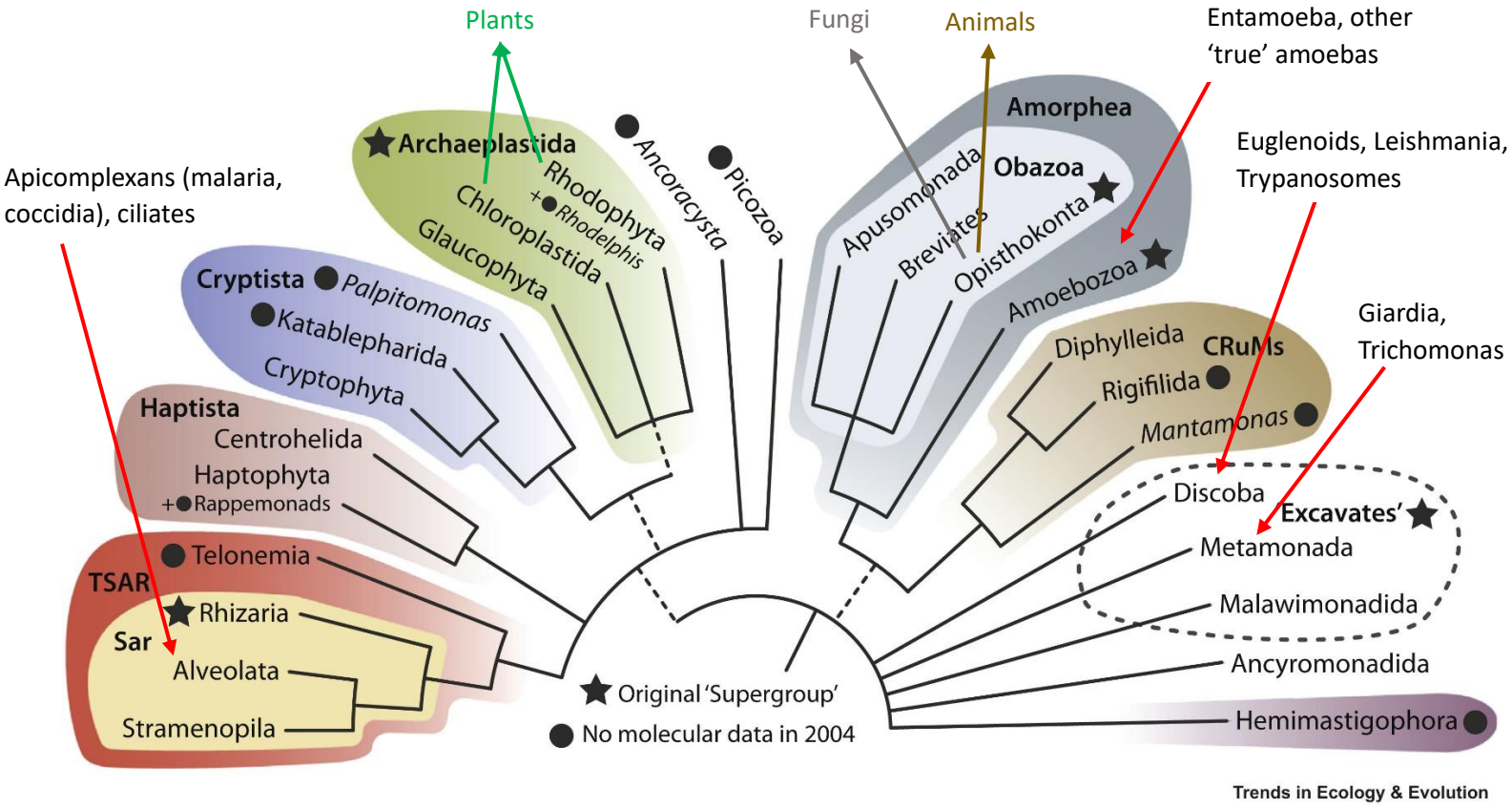
Although single cells, protozoans can carry smaller biological agents. *Acanthamoeba* can perhaps act as a sort of vector, packaging *Legionella* bacteria for delivery to humans, where they cause pneumonia. Presumably protozoans can be infected by bacteria, fungi and other protists but I find few examples. *Paramecium bursaria* is a beautiful free living ciliate with beneficial eukaryotic algal *Chlorella* symbionts providing nutrition for the host. Targeting parasitic protozoa with viruses harmful to them has been considered as a treatment for human parasite infections. Many of these viruses seem harmless or even beneficial (as is true of many viruses) but some harm the host protist or perhaps could be altered to do so.

Free living *Paramecium bursaria* from aquarium with added pond water, Red Wing, MN, US, August 2018. The green "organelles are not chloroplasts but *Chlorella* endosymbionts. A strand of prokaryotic cyanobacteria is seen at left. Image with 10X objective, cropped. *P. bursaria* about 100 microns long, *Chlorella* about 4 microns wide.



Some viral endosymbionts of parasitic protists. Life stages in the host of *Leishmania spp., Giardia duodenalis, Trichomonas vaginalis, Cryptosporidium spp, and Plasmodium spp*, are depicted with graphical representations of corresponding viral endosymbionts.
Barrow, et al. Viruses of protozoan parasites and viral therapy: Is the time now right? **Virol J** 2020

Later I will go into a little bit of detail on some important human protist pathogens. The first three, malaria, toxoplasma, and cryptosporidium are all apicomplexans, an ancient group of protozoa with a special apparatus to get into animal cells (plants and fungi are even tougher customers, each having a tough cell wall around their cells). Trypanosomes are flagellates, parasitic relatives of the harmless green euglena seen in pond water. Leishmania is also in the same protist phylum of Euglenozoa. Brain eaters are scary but the most medically important amoeba is *Entamoeba histolytica* which can cause bloody diarrhea.



Another recent family tree of eukaryotes. The super groups are all protist groups, with fungi and animals having evolved from choanoflagellates in Opisthokonta (Obazoa, Amorphea) and plants arising from Archeoplastida (with markers of both green and red algae, Chloroplastida and Rhodophyta). "Excavates" remain a polyphyletic group of convenience. A messy family tree, to which I added placement of some protist parasites (red arrows) and the metazoan kingdoms. original image from Burki, et al, New Tree of Eukaryotes Trends in *Ecology & Evolution* 2020

Affinities of some Protist parasites discussed in this paper

Here is a written hierarchical taxonomy to supplement the tree above. Experts don't agree on taxonomy schemes for protozoans, and I will be vague and somewhat outdated here. Note to strict cladists: if birds are dinosaurs, then you are a choanoflagellate protozoan. Genus names italicized.

Domain Eukarya

Kingdom Protozoa

Clade or superphylum Alveolata (flattened sacs under cell membrane)

Clade or superphylum or phylum Apicomplexa (parasites, apical complex)

Class Conoiddasia (the coccidia)

Cryptospordium, Cyclospora, Eimeria, Sarcocystis, Toxoplasma

Class Aconoidasida (or Haemosporidiasina)

Plasmodium, Babesia

Phylum Ciliophora (ciliates)

Balantidium

Clade Amorphea, subclade Evosea (amoebas and relatives)

Phylum Amoebozoa

Entameoba

Group Excavata (a large paraphyletic group)

superphylum Discoba (a clade within Excavata, flagellate, amoeboid, cyst stages)

Phylum Euglenozoa (euglenoids and relatives, some photosynthetic)

Class Kinetoplastea (a group of flagellates with DNA discs in mitochondria)

Leishmania, Trypanosoma

"phylum" Percolozoa (a colorless group in the excavates)

Naegleria

"phylum" Metamonada (amitochrondriate, anaerobic excavates)

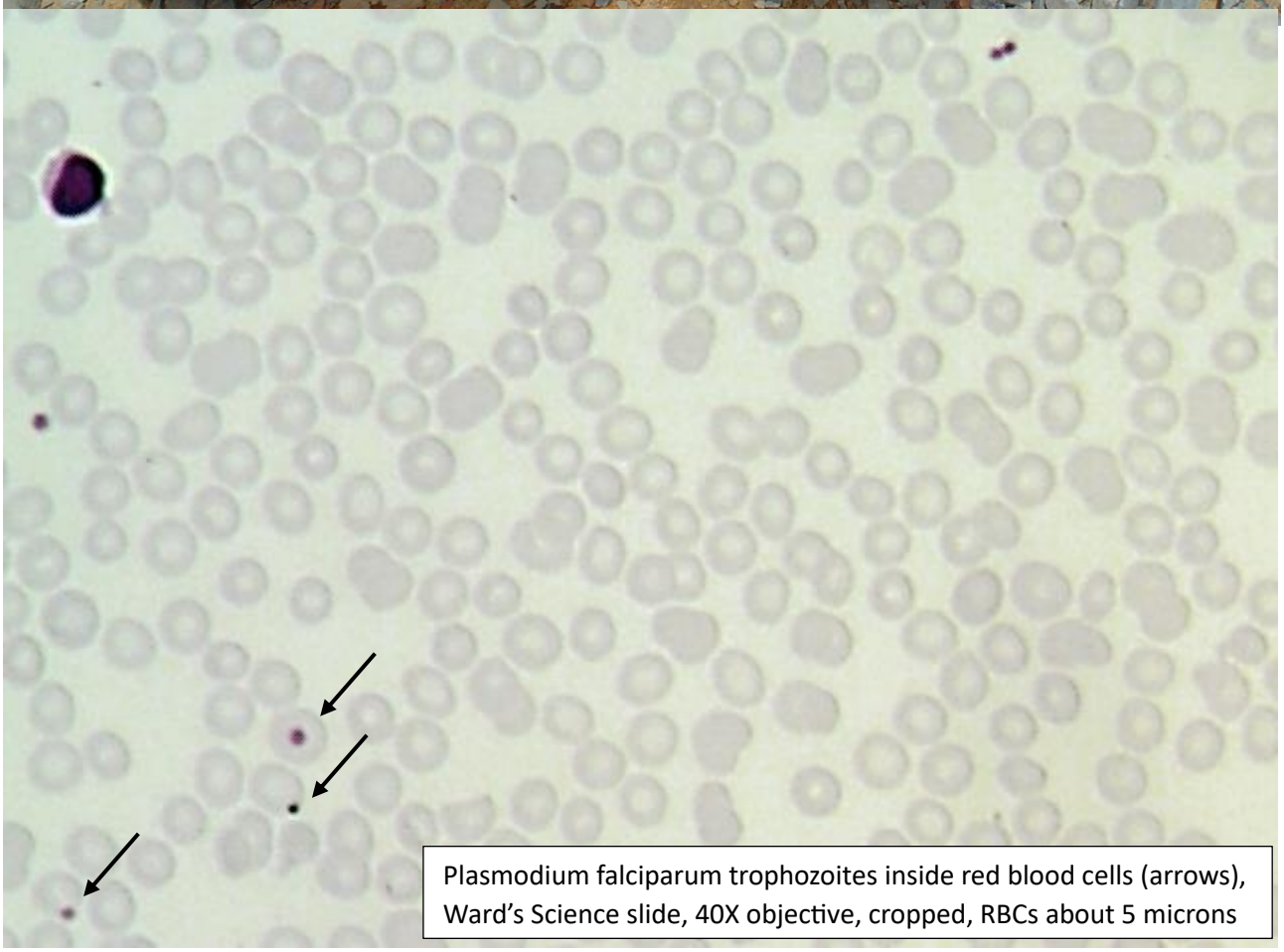
Giardia, Trichomonas

1. Malaria and Babesia

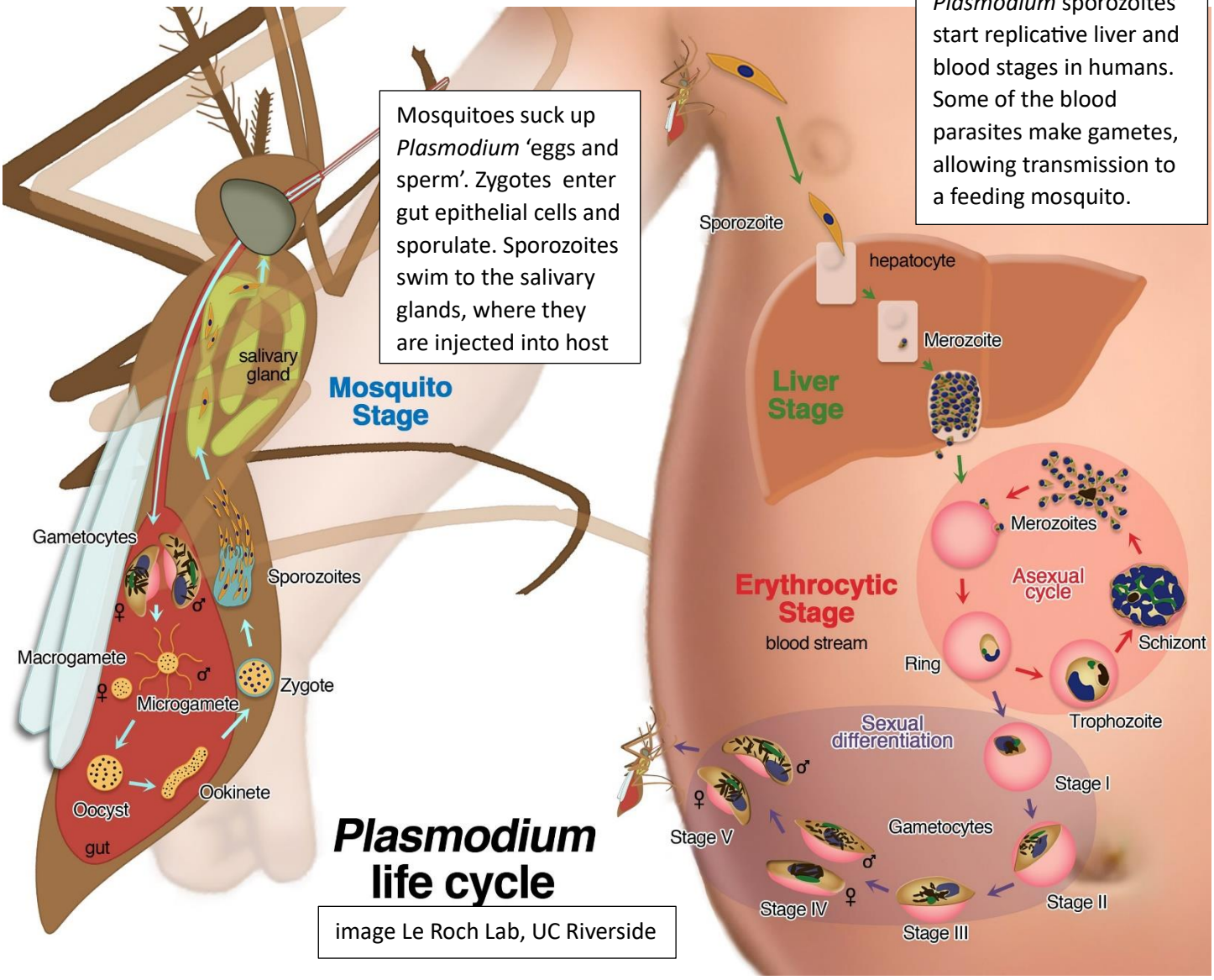
Malaria is the biggest killer of humans among all parasites. It is an ancient scourge of other mammals, reptiles and birds as well. It has defeated armies (Haitian slaves from Africa defeated French soldiers in large part because they were more immune to malaria and yellow fever) and changed the course of history. Around 1800 some US slave owners preferred buying new slaves from Africa because of better malaria resistance. Malaria killed 150 to 300 million people even during the 20th century, 2-5% of all deaths. Not long ago malaria was still killing a million people a year, mostly children and pregnant women in Africa, but through massive international efforts the number of deaths is now down to under 500,000 a year.

Plasmodium and *Babesia* are apicomplexan protists with complex life cycles that include replication in blood. Human malaria is set of diseases caused by 4 species (5 if you include people in Malaysia getting zoonotic macaque monkey malaria) of *Plasmodium* protozoa that have a complex life cycle in humans and genus *Anopheles* mosquitoes. The big killer is *P. falciparum*, which causes fever, headache and chills about 10 to 15 days after the bite of an infected mosquito. In a classic case, chills, fever and sweats recur every 2 to 3 days as trillions of malaria parasites bust out of red blood cells in batches. The urine turns red or black. If not treated quickly malaria can progress to critical multi-organ failure (liver, lungs, brain) and death. Adults in Liberia and other highly endemic areas of Africa get milder illness and seldom die because of partial immunity acquired from having malaria 10 times or more in childhood. African children under 5 and pregnant women (pregnancy turns down the immune system) account for most of the deaths today. (Some African friends worried about a “half way” place in malaria when more adults might die). Travelers from outside Africa or Asia are also at risk for a fatal case because they don’t have immunity. Every year some people die in the United States of malaria after acquiring it during travel, coming home and the doctor not thinking about malaria. If you are traveling to an area with tropical diseases see your doctor, or perhaps even better a travel clinic before you go, to see about preventive medications and vaccines available for some tropical diseases. As bad as falciparum malaria is, at least it is over when you recover. But *P. vivax, P. malariae* and *P. ovale* can have relapsing forms causing intermittent fevers months to years later. I used to see rare World War 2 veterans getting fevers from relapsing malaria acquired in the Pacific Islands, but not for decades now. I have seen miserable kids in Liberia in West Africa with fevers of 41 Celsius (106 F) and chills who cried out in pain when they were drenched with cool water to keep the fever from going higher. Research has shown sponging with water brings down fever more quickly than paracetamol (acetaminophen), but the effect is temporary and maybe not worth the extra suffering. 100 years from now people might marvel at our ignorance in quelling fever, just as we make fun of the doctors who bled their patients to death with leeches 200 years ago. But I digress.

African toddler having his malarial fever reduced with cool water at MSF clinic, Monrovia, Liberia in 2004

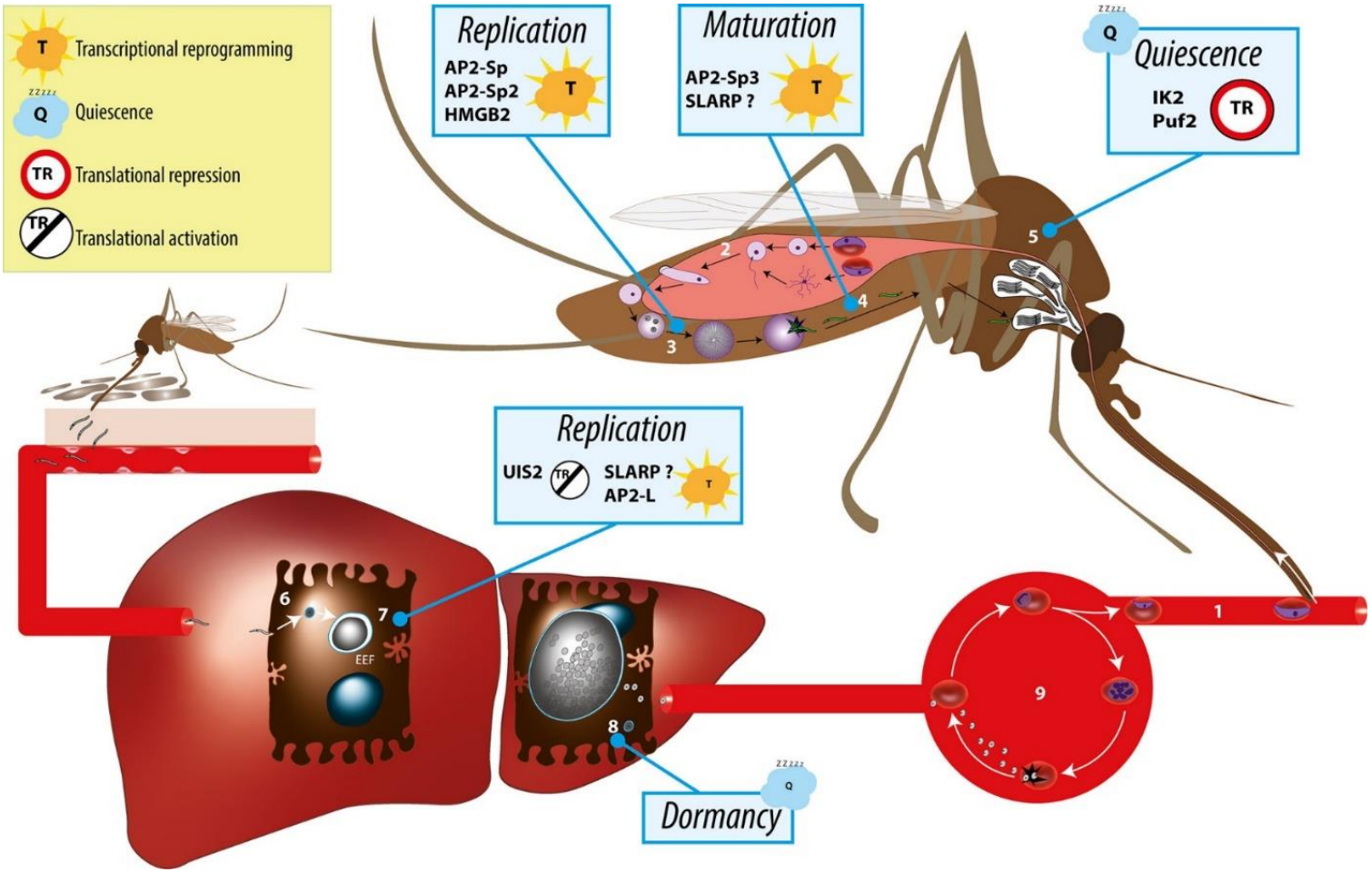


Plasmodium falciparum trophozoites inside red blood cells (arrows), Ward's Science slide, 40X objective, cropped, RBCs about 5 microns

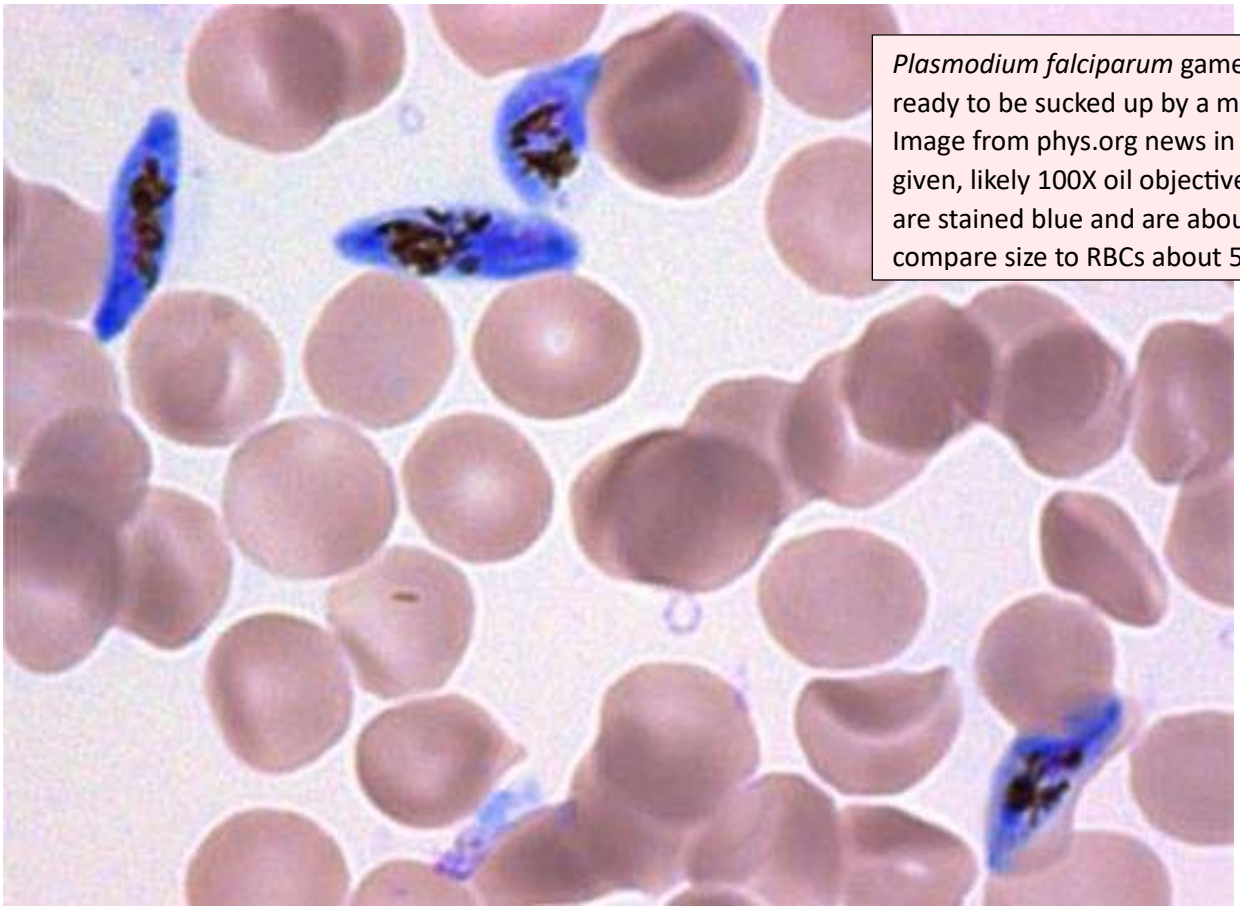


The biology of malaria is amazing. It took generations of researchers to discover, and more research to develop treatments. Adult Africans are immune, so maybe an effective vaccine will be found eventually, if it hasn't been already. But as I noted previously the holy grail of malaria prevention with vaccines may have been delayed by pharmaceutical company greed.

Unlike today's drug company executives, the old time doctors and biologists who figured out malaria were real heroes. The phylum Apicomplexa comprises the biggest group of protist parasites and includes the blood borne *Plasmodium* and *Babesia* along with the coccidia (a large group of spore forming parasites of vertebrate guts including a lot of veterinary and some human pathogens such as *Isospora, Cyclospora, Cystoisospora* <formerly called *Isospora*>, *Eimeria, Cryptospordium, Sarcocystis* and *Toxoplasma*). All apicomplexans are obligate intracellular parasites with complex life cycles featuring both sexual and asexual reproduction, and all apicomplexans have an eponymous conical apical complex with structural and secretory components that assist with cell entry and exit. *Plasmodium*'s apical complex lacks a conoid.

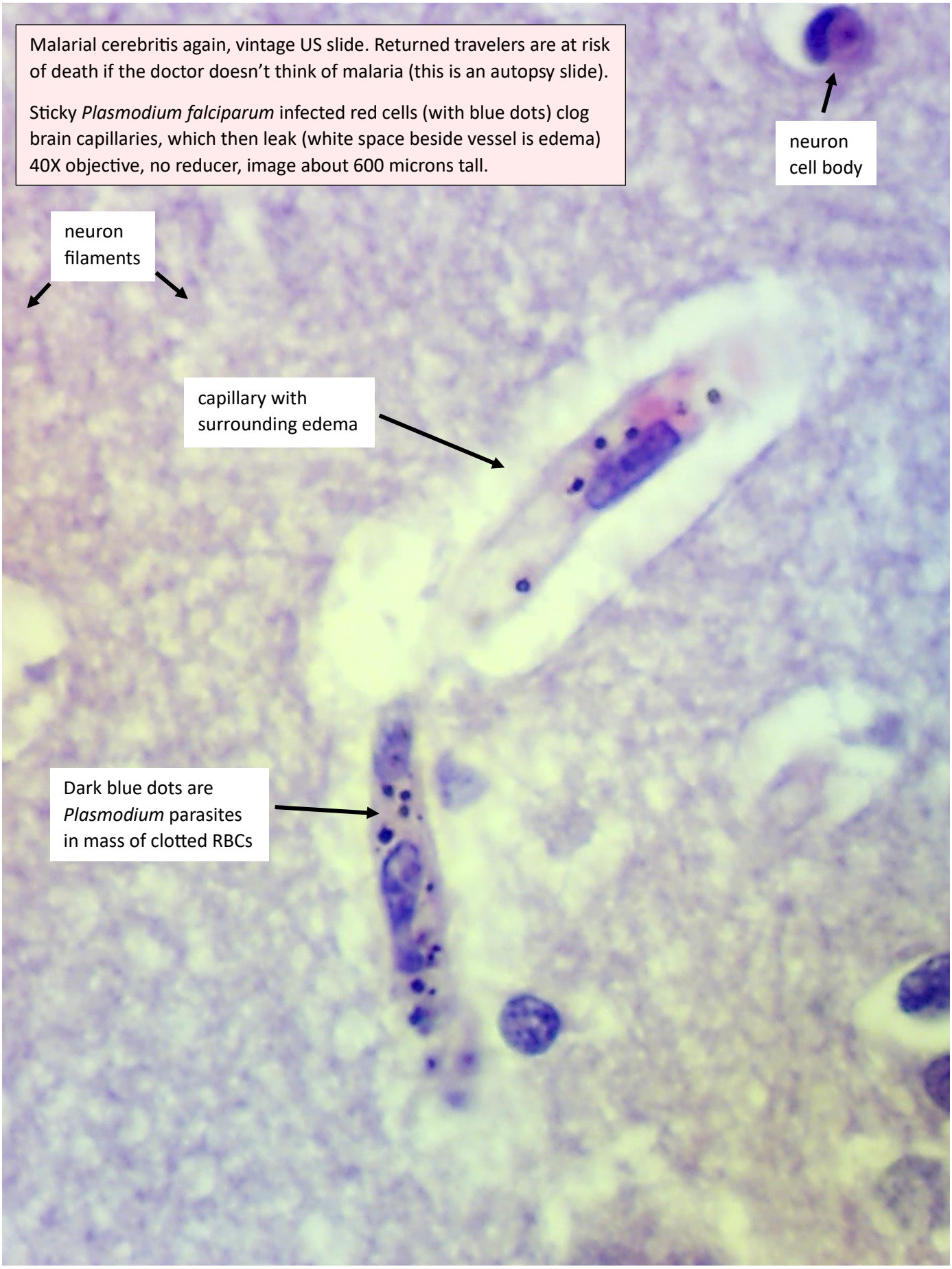


Another overview of *Plasmodium* life cycle noting gene regulation mechanisms (blue text boxes) controlling parasite transmission from mosquito to mammal. Female anopheline mosquito ingests male and female gametocytes (1), which mature in the insect midgut, followed by fertilization (2) forming motile ookinetes that cross the midgut epithelium and transform into oocysts. Oocysts undergo intense replication (3) releasing thousands of sporozoites that swim to the salivary glands and mature into infectious sporozoites (4). Mature sporozoites can persist in a poised state for up to weeks (5), then transmission occurs when the mosquito feeds on a new host. Sporozoites injected in the host skin migrate to the liver, where they invade hepatocytes (6) and transform into replicative exoerythrocytic forms (EEFs) (7). In species such as *P. vivax*, some of the parasites do not replicate immediately but transform into dormant hypnozoites (8). Replication in the liver culminates with the release of thousands of merozoites into the circulation, which invade erythrocytes and initiate the asexual blood stage cycle (9). Figure Briquet et al Preparing for Transmission: Gene Regulation in Plasmodium Sporozoites, *Frontiers Cellular and Infection Microbiology* 2021.

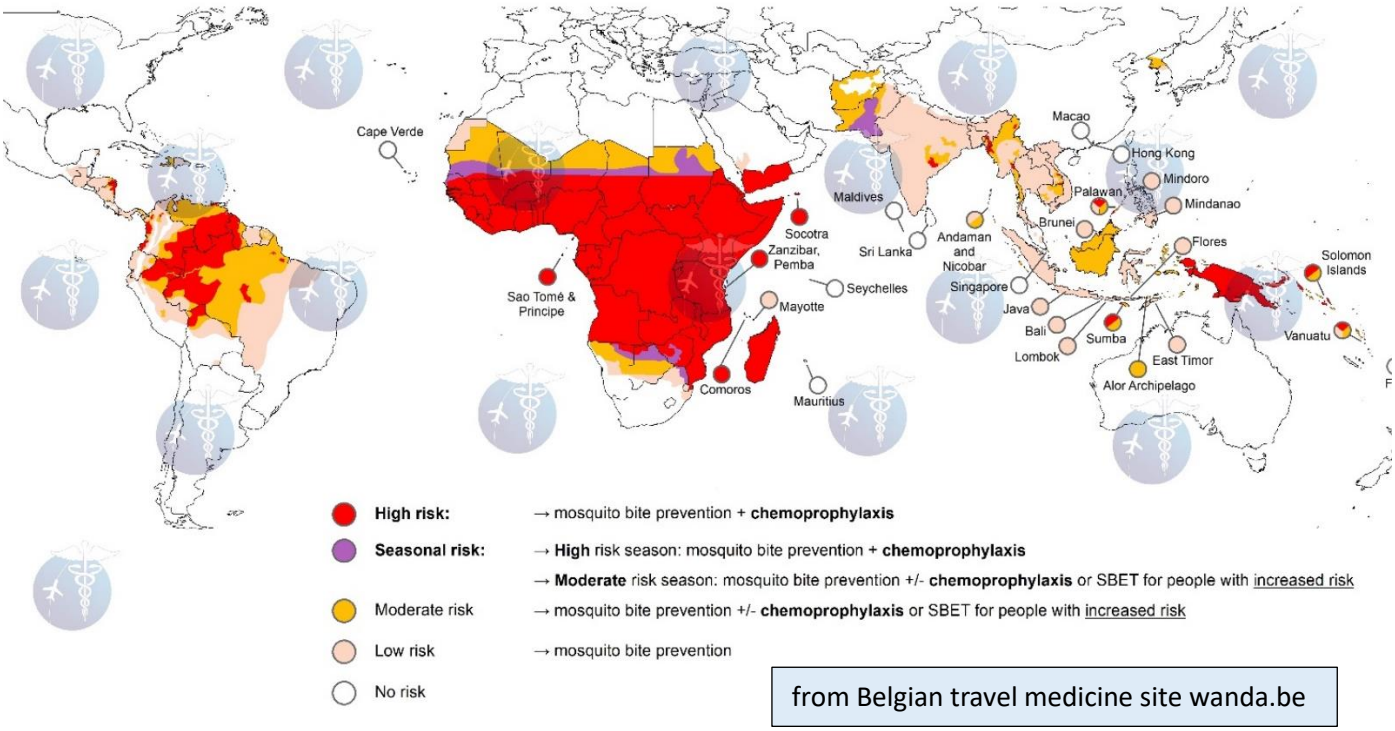


Plasmodium falciparum gametocytes in blood, ready to be sucked up by a mama mosquito. Image from phys.org news in 2014. No details given, likely 100X oil objective. Gametocytes are stained blue and are about 7 microns long; compare size to RBCs about 5 microns wide.

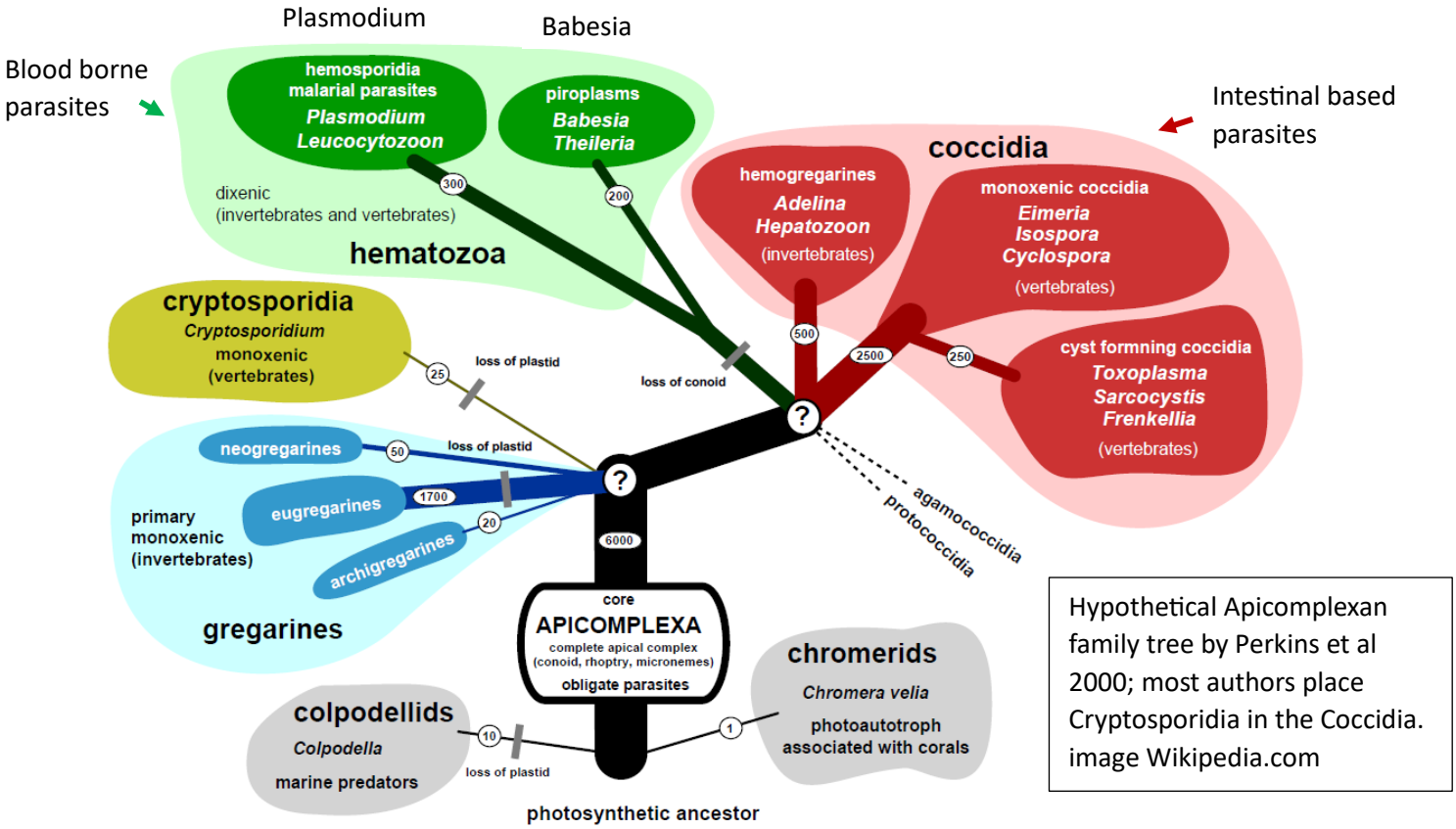
Malaria was historicallly diagnosed by examination of thick blood smears (more red cells on thicker smear meant the viewer was more likely to find the parasite if the parasitic load was low). Malaria diagnosis was one of the most iconic uses of a microscope in medical care. In Liberia, West Africa in 2004 I saw several cases daily of malaria, mostly in kids. I didn't have a microscope. At the Médecins Sans Frontières hospital and clinic we used cheap (from India or South Africa) rapid ELISA (enzyme-linked immunosorbent assay) tests for falciparum malaria. A drop of blood was put on a specially manufactured strip of paper, working like a home pregnacy or home COVID test. If positive we treated cases with artesunate-amodiaquine artemenesin combination therapy (a new anti-malaria drug combined with an old one to prevent resistance from emerging). Because falciparum malaria is potentially fatal we ignored the small chances of serouis side efectrs including agranulocytosis, liver failure and long QT arrythmias. The one time I saw malaria in a returned US traveller we treated with IV chloroquine (back then malaria from the Caribean was still sensitive to the old drug).


Malarial cerebritis again, vintage US slide. Returned travelers are at risk of death if the doctor doesn't think of malaria (this is an autopsy slide).
Sticky *Plasmodium falciparum* infected red cells (with blue dots) clog brain capillaries, which then leak (white space beside vessel is edema) 40X objective, no reducer, image about 600 microns tall.
neuron cell body
neuron filaments
capillary with surrounding edema
Dark blue dots are *Plasmodium* parasites in mass of clotted RBCs

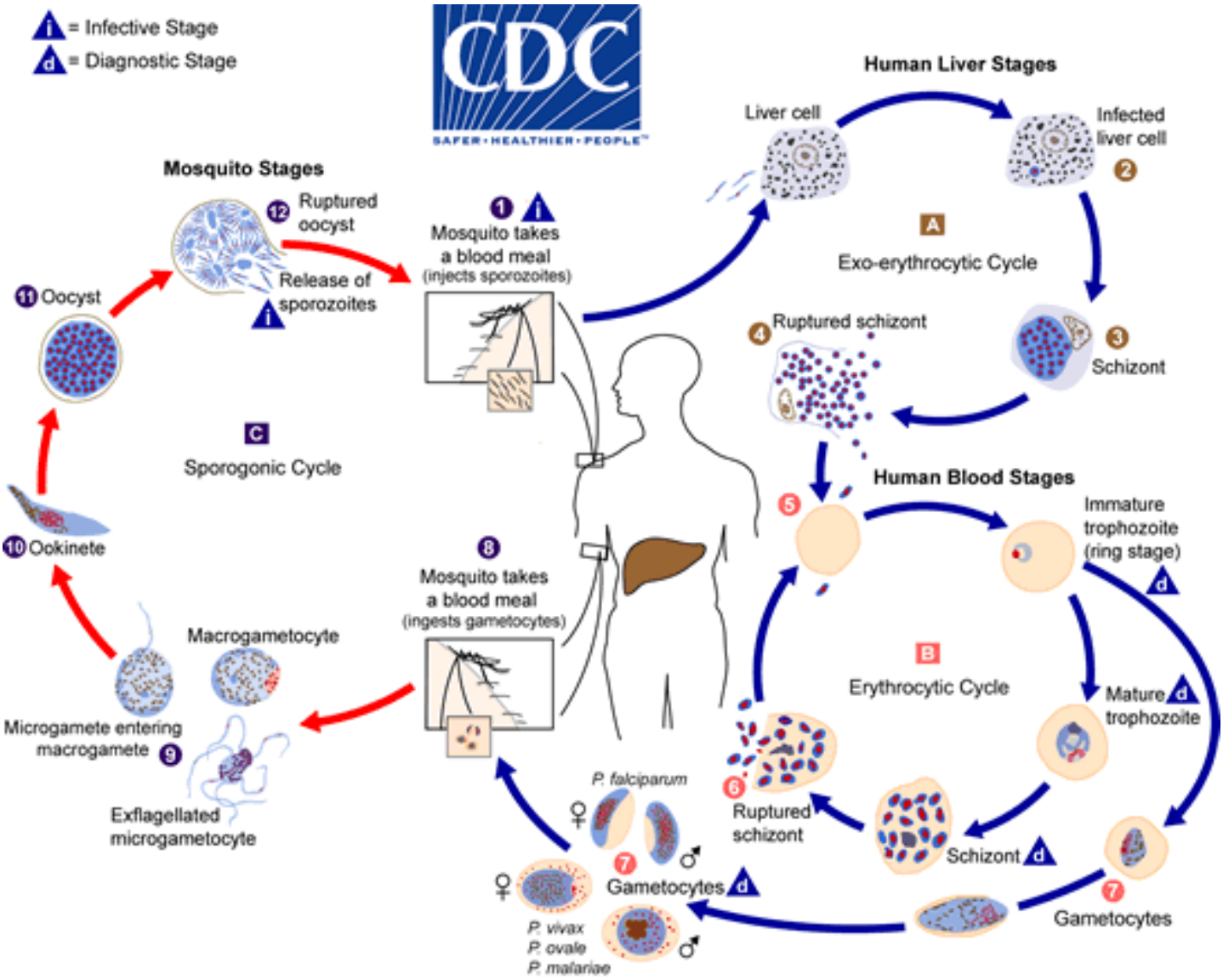
Malaria risk 2023



from Belgian travel medicine site wanda.be



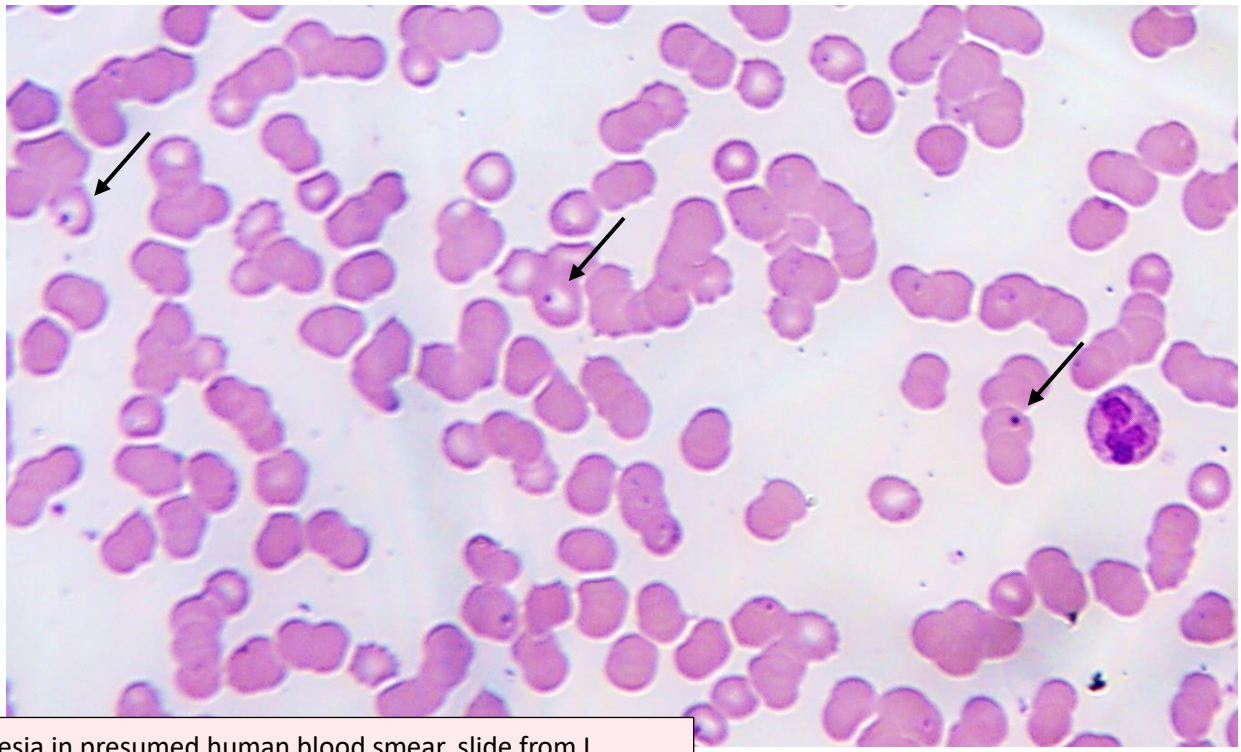
Hypothetical Apicomplexan family tree by Perkins et al 2000; most authors place Cryptosporidia in the Coccidia. image Wikipedia.com



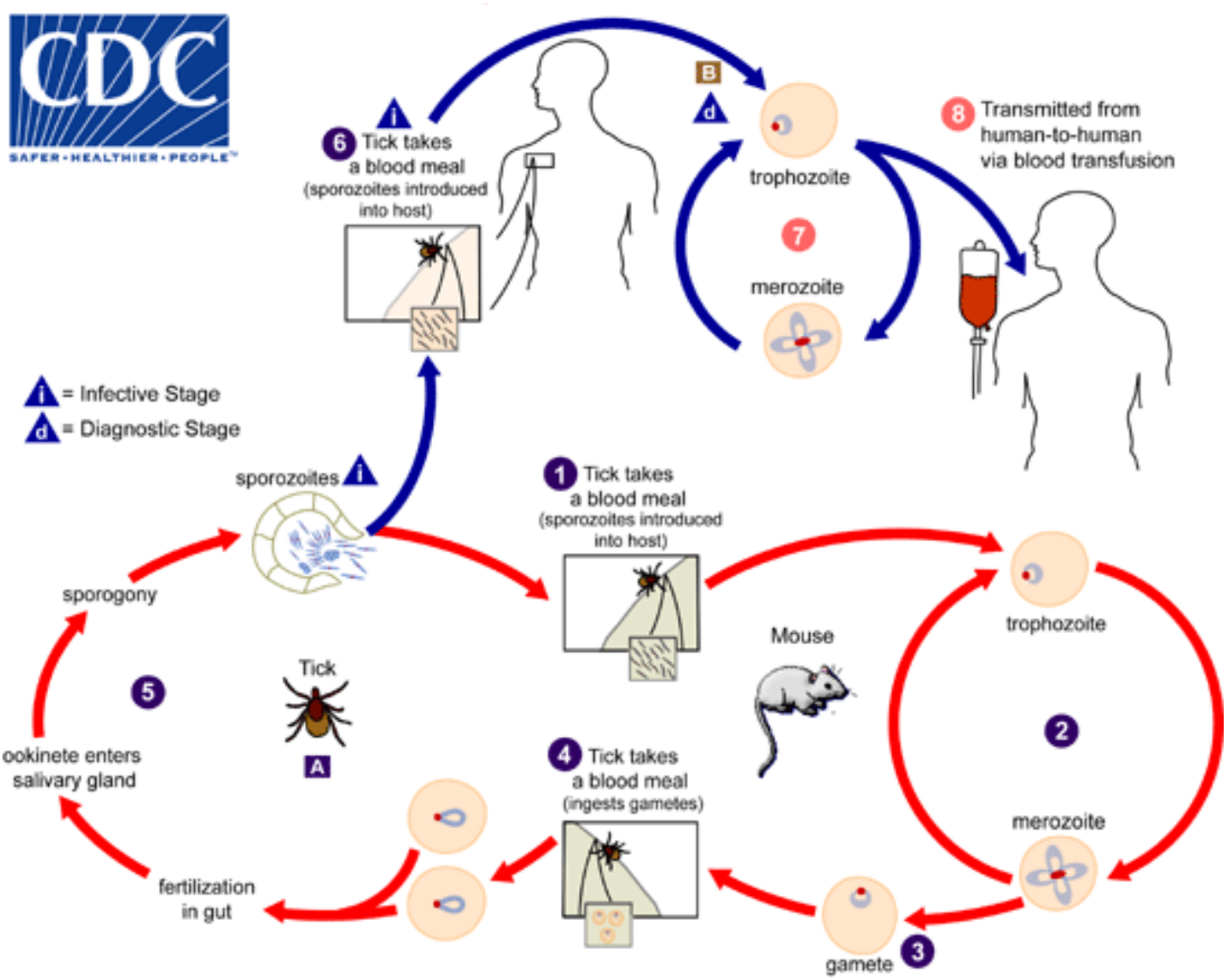
The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host 1. Sporozoites infect liver cells 2 and mature into schizonts 3, which rupture and release merozoites 4. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver (if untreated) and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells 5. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites 5. Some parasites differentiate into sexual erythrocytic stages (gametocytes) 7. Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal 8. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes 9. The zygotes in turn become motile and elongated (ookinetes) 10 which invade the midgut wall of the mosquito where they develop into oocysts 11. The oocysts grow, rupture, and release sporozoites 12, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites 1 into a new human host perpetuates the malaria life cycle.

Babesiosis is caused by a malaria like apicomplexan protist parasite with a blood centric life cycle, most commonly *Babesia microti* (the closely related *Theileria* is a cattle parasite). Babesiosis in the Midwest U.S. is spread by the deer tick, *Ixodes scapularis* (also the vector of Lyme disease, caused by a spirochete bacterial pathogen; Lyme is not a parasite). Babesiosis is a malaria like but milder or asymptomatic illness, with possible fatigue, hemolytic anemia and recurrent fevers coming 1-4 weeks after a tick bite. It can also be spread by blood transfusion.

Apicomplexans, including *Babesia* and the many species of coccidia, have complex asexual and sexual reproduction with many life stages. Stages may include: **sporozoites** (infective haploid stage, which malarial mosquitoes inject into vertebrates), **merozoites** (malaria red blood cell phase, haploid, multiplying asexually by schizogony, may have multiple generations of different numbers and sizes), **gametocytes** (flagellated male **microgametes** and female **macrogametes**), **ookinetes** (fertilized diploid zygotes), **oocysts** (infective coccidian stage with tough thick walled spore containing a zygote and able to survive passage outside host; after sporulation contains two **sporocysts** each holding two sporozoites), **trophozoites** (large, diploid, intracellular, actively feeding stage), **schizonts** (derived from sporozoites or merozoites, divide by fission to produce haploid merozoites), **hypnozoites** (latent resting stage, usually derived from sporozoites, resulting in latent malaria), **bradyzoites** (merozoite derived, latent stage, in toxoplasmosis cysts) and **tachyzoites** (merozoite derived rapidly growing and dividing, in toxoplasmosis pseudocysts). Whew. Was evolution drunk the day it invented protist sex? And then it got lazy and recycled coccidian sporulation to become metazoan meiosis? What's up with the free living ciliate *Tetrahymena* having 7 genders?



Babesia in presumed human blood smear, slide from L Bircham, some erythrocytes infected with trophozoites are arrowed. 40X objective, cropped, RBCs about 5 micron wide

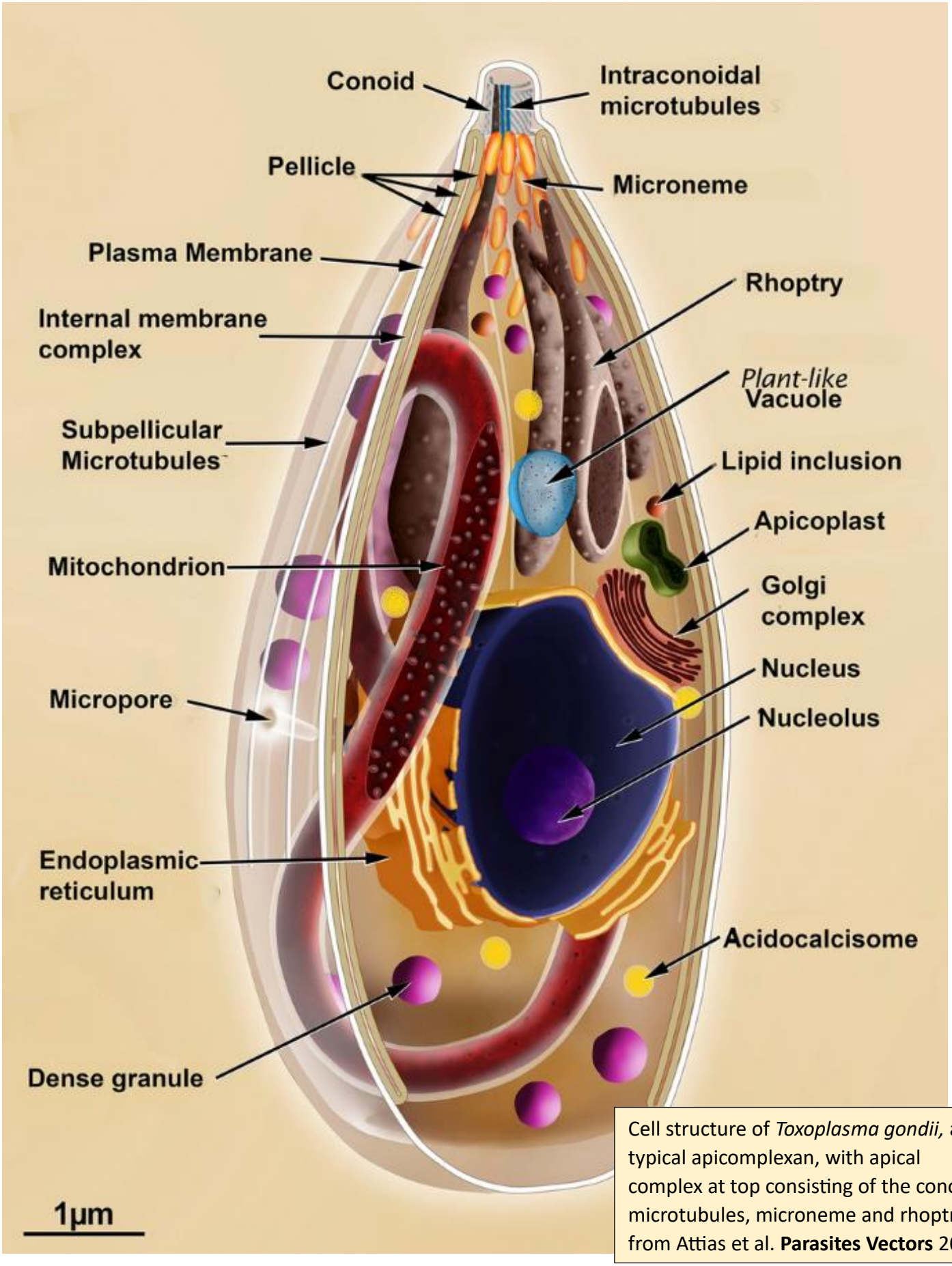


The *Babesia microti* life cycle involves two hosts, which includes a rodent, primarily the white-footed mouse, *Peromyscus leucopus*, and a tick in the genus, *Ixodes*. During a blood meal, a *Babesia*-infected tick introduces sporozoites into the mouse host ❶. Sporozoites enter erythrocytes and undergo asexual reproduction (budding) ❷. In the blood, some parasites differentiate into male and female gametes although these cannot be distinguished at the light microscope level ❸. The definitive host is the tick. Once ingested by an appropriate tick ❹, gametes unite and undergo a sporogonic cycle resulting in sporozoites ❺. Transovarial transmission (also known as vertical, or hereditary, transmission) has been documented for "large" *Babesia* spp. but not for the "small" babesiae, such as *B. microti* Ⓐ. Humans are an incidental host.

2. Toxoplasmosis, again

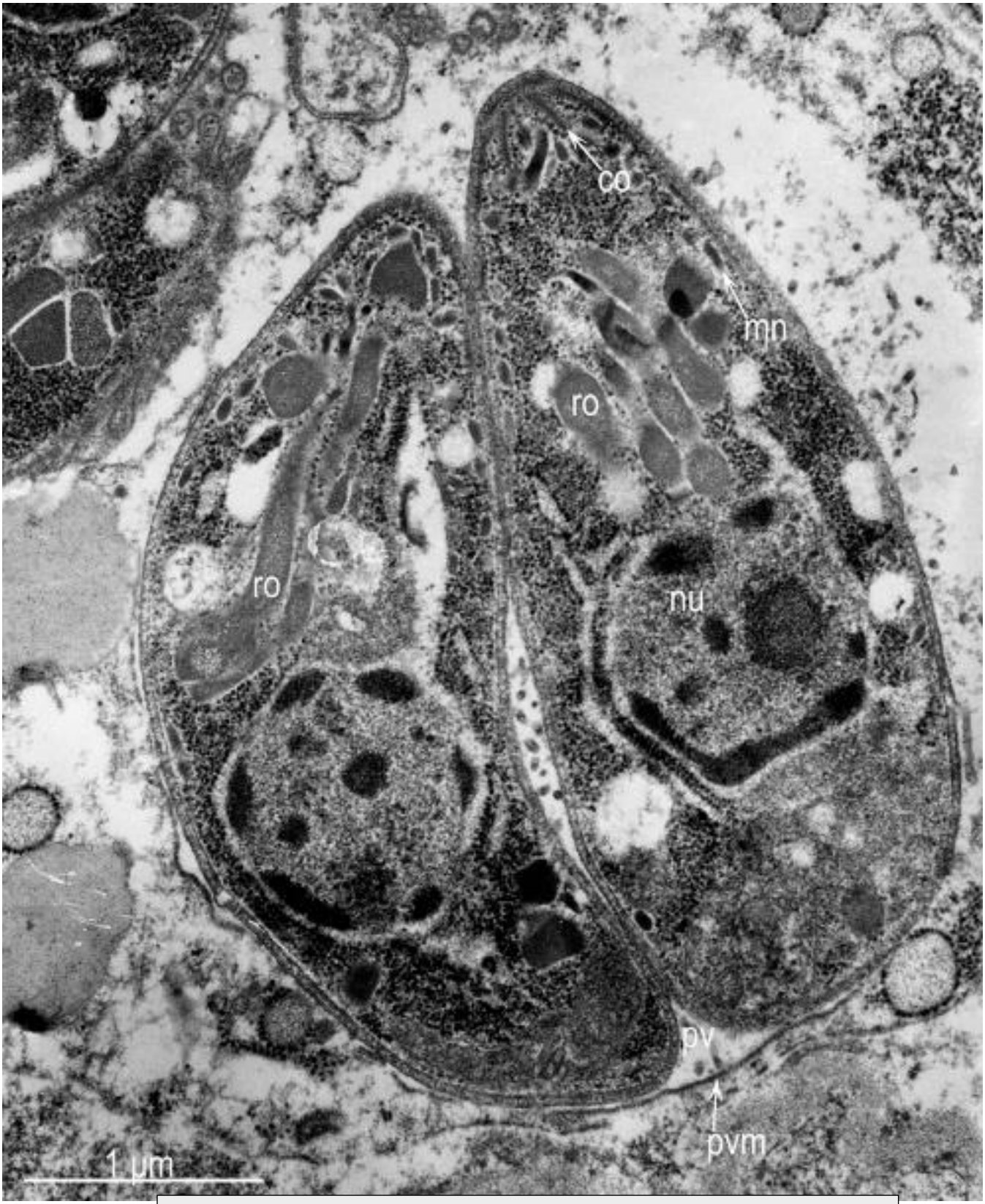
I discussed *Toxoplasma gondii* last time, in relation to the free will of 2 to 4 billion humans who carry its microscopic cysts. A 2019 review of over 150 studies estimated *Toxoplasma gondii* is carried by 26% of the world population, including 61% of Africans, 30% of Europeans, 18% of people in the U.S. and Canada, and 16% of Asians. *Toxoplasma gondii* is an apicomplexan coccidian parasite that reproduces sexually in the intestinal walls of cats. (Coccidia are a group of protozoan parasites of the gut of warm blooded vertebrates, not to be confused with *Coccidioides*, the fungal cause of coccidiomycosis, aka as Valley Fever). The usual life cycle of "toxo" includes rats or birds being preyed on by cats (trophic transmission). *Toxoplasma* moves around the body and in and out of cells by using gliding locomotion and its apical complex (animations are available on YouTube showing the complex life cycle with asexual and sexual phases). All warm blooded vertebrates (i.e. birds and mammals) are potential intermediate hosts. Humans are a dead end for *Toxoplasma*, since we are seldom eaten by cats (although leopards can host *Toxoplasma*). Toxoplasmosis infects more humans than malaria, but causes no obvious illness in the vast majority of hosts, whose immune systems quickly reduce *Toxoplasma* to asymptomatic latency. Still, it is known to manipulate the behavior of rats, its favored intermediate host, and it probably also manipulates human behaviors to some extent, as discussed earlier in part 2 of this article. Toxo can also deform or kill the unborn and badly sicken or kill those with reduced immune function, such as AIDS patients and people getting certain treatments for cancer or arthritis. Most people with latent toxoplasmosis are likely not symptomatic (apart from Danish women allegedly becoming entrepreneurs) and require no treatment. Those who get ill can be treated with a combination of pyrimethamine and sulfadiazine, plus folinic acid. There is no human vaccine against toxoplasmosis, but there is one for sheep. Capitalism can create strange priorities.

Toxoplasma is a coccidian, one of a large number of apicomplexan protozoa that complete their life cycles (consummated with sex) in vertebrate guts, including man and our domestic animals. Apicomplexans are thus well studied because of their veterinary and medical impacts. Light and later electron microscopes revealed tiny structural details (see science art next page) inside these miniscule parasites (sometimes about 2-3 microns wide, still a little bigger than most bacteria). Although parasitic now, apicomplexans were likely originally free living and photosynthetic. Ribosomal RNA suggests the group is 1 billion years old, youngsters compared to 3.8 billion year old bacteria and archaea but possibly older than the first plants, fungi and animals. The emergence of metazoans provided a new habitat, inside their bodies. Apicomplexans probably became parasitic several different times.

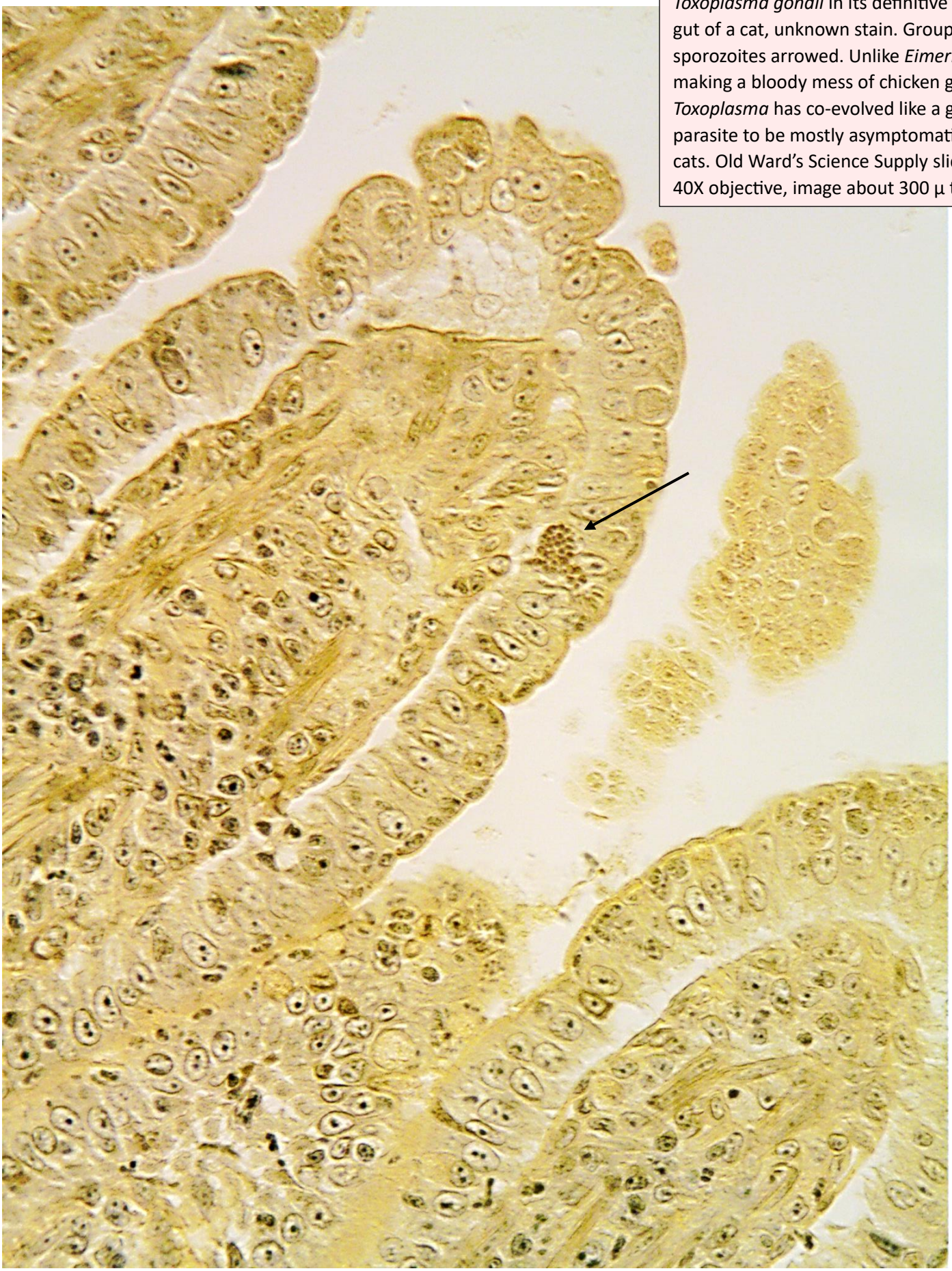


Cell structure of *Toxoplasma gondii,* a typical apicomplexan, with apical complex at top consisting of the conoid, microtubules, microneme and rhoptry, from Attias et al. **Parasites Vectors** 2020

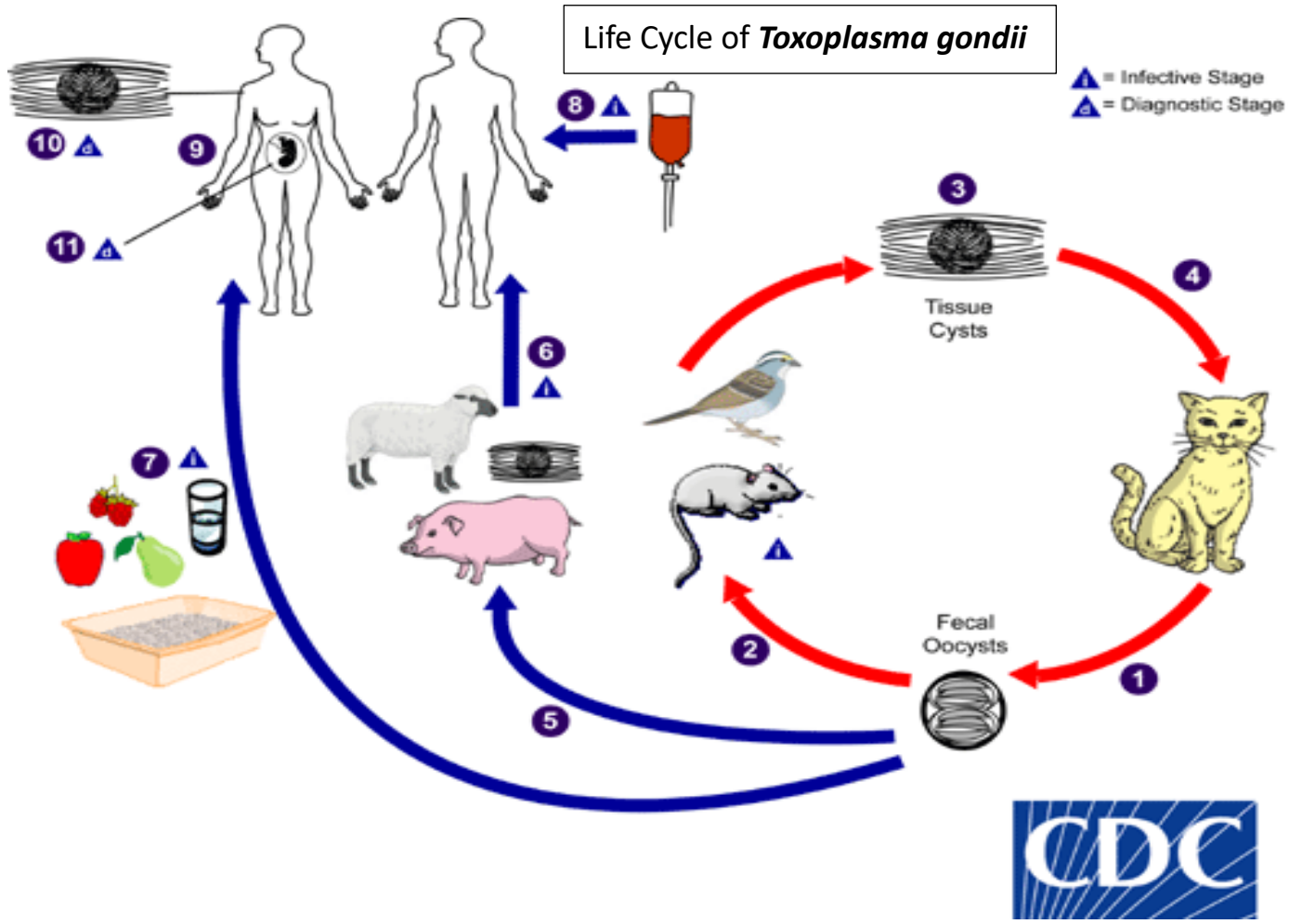
This science art is based largely on electron microscopy imaging, a powerful tool for understanding subcellular structure. Compare electron micrograph next page.



Transmission Electron Microscopy *Toxoplasma gondii* in lung of a bar-shouldered dove. Two tachyzoites enclosed in parasitophorous vacuolar membrane (pvm). Conoid (co) at top, micronemes (mn), rhoptries (ro), and nucleus (nu).
Rigoulet et al 2014 Toxoplasmosis in a bar-shouldered dove (Geopelia humeralis) from the Zoo of Clères, France. **Parasite** 21, 62, image at Wikipedia



Toxoplasma gondii in its definitive host, gut of a cat, unknown stain. Group of sporozoites arrowed. Unlike *Eimeria* making a bloody mess of chicken guts, *Toxoplasma* has co-evolved like a good parasite to be mostly asymptomatic in cats. Old Ward's Science Supply slide. 40X objective, image about 300 µ tall



The only known definitive hosts for *Toxoplasma gondii* are members of family Felidae (domestic cats and their relatives). Unsporulated oocysts are shed in the cat's feces 1. Although oocysts are usually only shed for 1–3 weeks, large numbers may be shed. Oocysts take 1–5 days to sporulate in the environment and become infective. Intermediate hosts in nature (including birds and rodents) become infected after ingesting soil, water or plant material contaminated with oocysts 2. Oocysts transform into tachyzoites shortly after ingestion. These tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites 3. Cats become infected after consuming intermediate hosts harboring tissue cysts 4. Cats may also become infected directly by ingestion of sporulated oocysts. Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment 5. Humans can become infected by any of several routes:

- Eating undercooked meat of animals harboring tissue cysts 6.
- Consuming food or water contaminated with cat feces or by contaminated environmental samples (such as fecal-contaminated soil or changing the litter box of a pet cat) 7.
- Blood transfusion or organ transplantation 8.
- Transplacentally from mother to fetus 9.

In the human host, the parasites form tissue cysts, most commonly in skeletal muscle, myocardium, brain, and eyes; these cysts may remain throughout the life of the host. Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained biopsy specimens 10. Diagnosis of congenital infections can be achieved by detecting *T. gondii* DNA in amniotic fluid using molecular methods such as PCR 11.

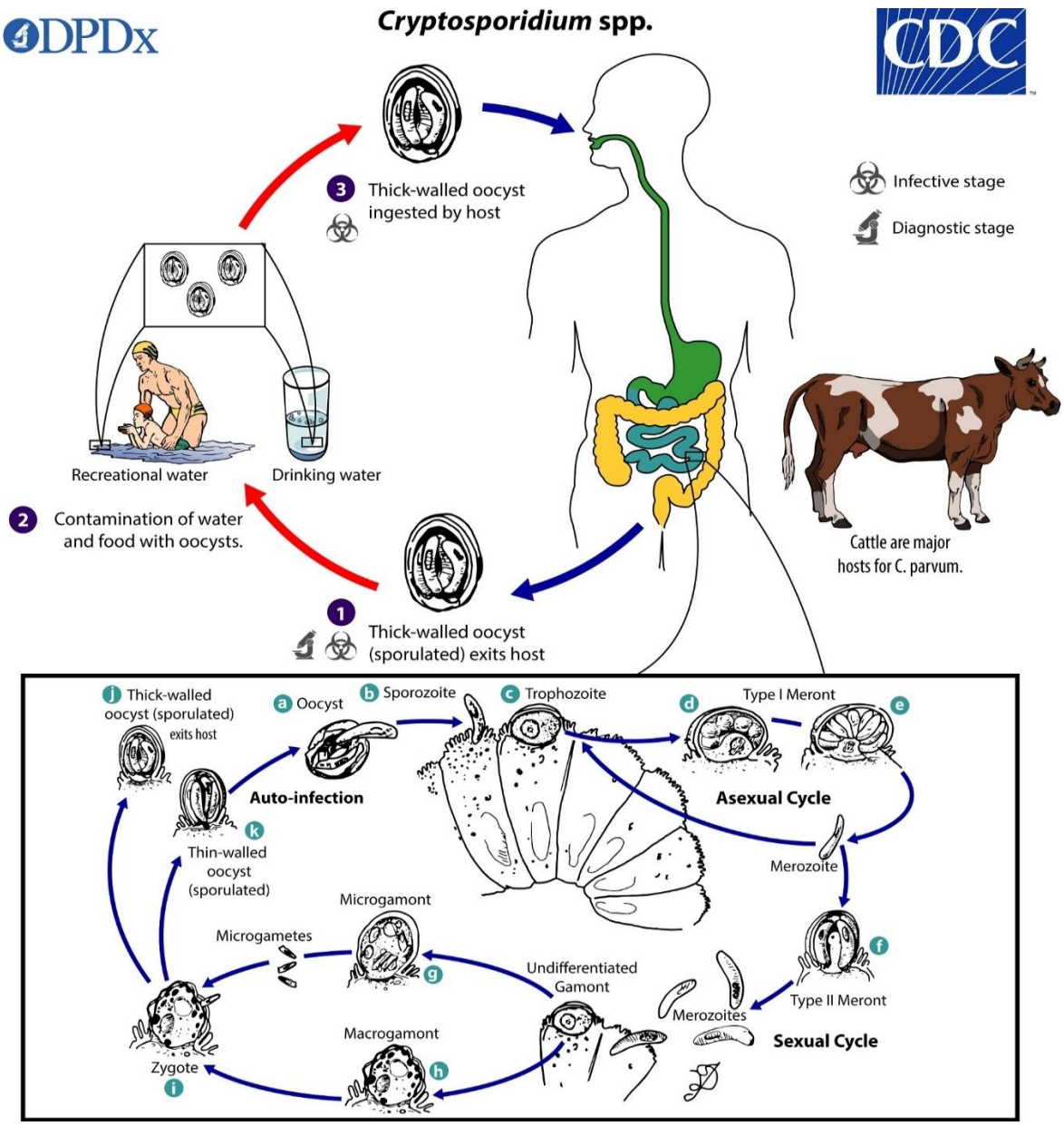
3. Cryptosporidiosis

Cryptosporidium is another apicomplexan gut targeting coccidian (differing from other apicomplexans in having lost a plant derived plastid involved with lipid synthesis). Humans usually get the disease, cryptosporidiosis, from acquiring *C. parvum* (harbored by cattle) or *C. hominis* (which has sex only in our guts, making humans the definitive host) from contaminated water or food. People less commonly pick up dog or cat or mouse "crypto" as *Cryptosporidium* has many species affecting animals. (This crypto is not to be confused with the yeast *Cryptococcus* that causes meningitis in the immunocompromised).

Cryptosporidiosis can cause dysentery (severe and sometimes bloody diarrhea) and less commonly, respiratory disease. I have seen a number of cases of diarrhea, which lasts up to 2 weeks in most people but can go on for months or years in the immunocompromised. Years ago, I saw some AIDS patients wasted away to human skeletons with cryptosporidiosis. In healthy people cryptosporidiosis resolves spontaneously and no treatment is needed. In the immunocompromised, cryptosporidiosis can be difficult to get rid of even with treatment (nitazoxanide), and they are also prone to *Cryptosporidium* disseminating to the lungs, bile ducts, pancreas and blader.

Because of its infectious, thick-walled oocyst (cyst full of eggs) *Cryptosporidium* can be resistant to chlorine and difficult to eliminate from swimming pools and water supplies. There have been many large outbreaks over the years related to public pools and water supplies, the biggest sickened 400,000 people in Milwaukee, Wisconsin in 1993. Imagine all the diarrhea…

Cryptosporidium mature schizonts attached to microvilli, releasing merozoites – scanning electron microscopy by D Stenzel at parasite.org.au/pugh-collection



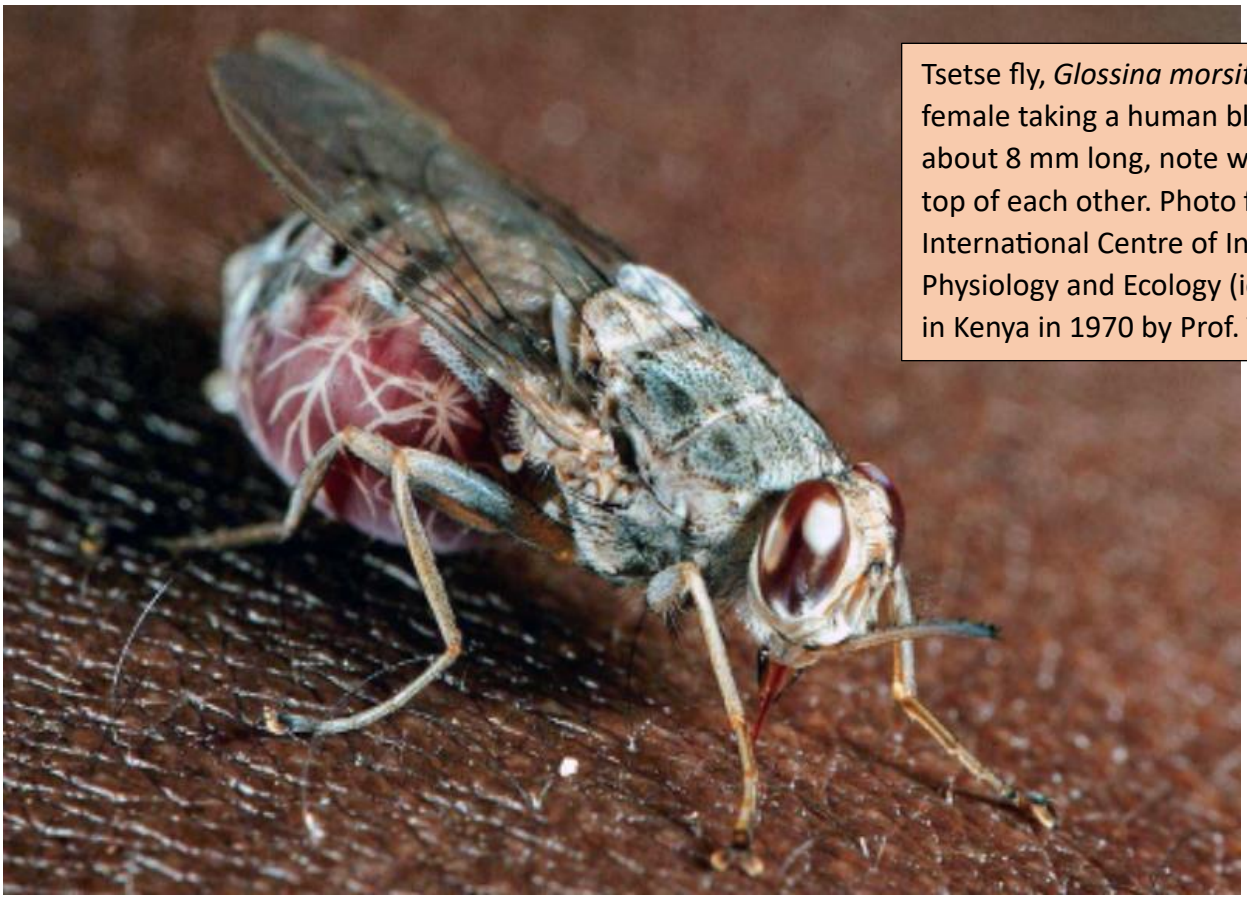
Sporulated oocysts, containing 4 sporozoites, are excreted by the infected host through feces 1 (and possibly other routes such as respiratory secretions). Transmission of *Cryptosporidium* spp. occurs mainly through ingestion of fecally contaminated water (e.g., drinking or recreational water) or food (e.g., raw milk) or following direct contact with infected animals or people 2 . Following ingestion (and possibly inhalation) by a suitable host 3 , excystation a occurs. The sporozoites are released and parasitize the epithelial cells (b , c) of the gastrointestinal tract (and possibly the respiratory tract). In these cells, usually within the brush border, the parasites undergo asexual multiplication (schizogony or merogony) (d , e , f) and then sexual multiplication (gametogony) producing microgamonts (male) g and macrogamonts (female) h . Upon fertilization of the macrogamonts by the microgametes (i) that rupture from the microgamont, oocysts develop and sporulate in the infected host. Zygotes give rise to two different types of oocysts (thick-walled and thin-walled). Thick-walled oocysts are excreted from the host into the environment j , whereas thin-walled oocysts are involved in the internal autoinfective cycle and are not recovered from stools k . Oocysts are infectious upon excretion, thus enabling direct and immediate fecal-oral transmission. Extracellular stages have been reported, but their relevance in the overall life cycle is unclear.

4. Sleeping sickness and Chagas disease

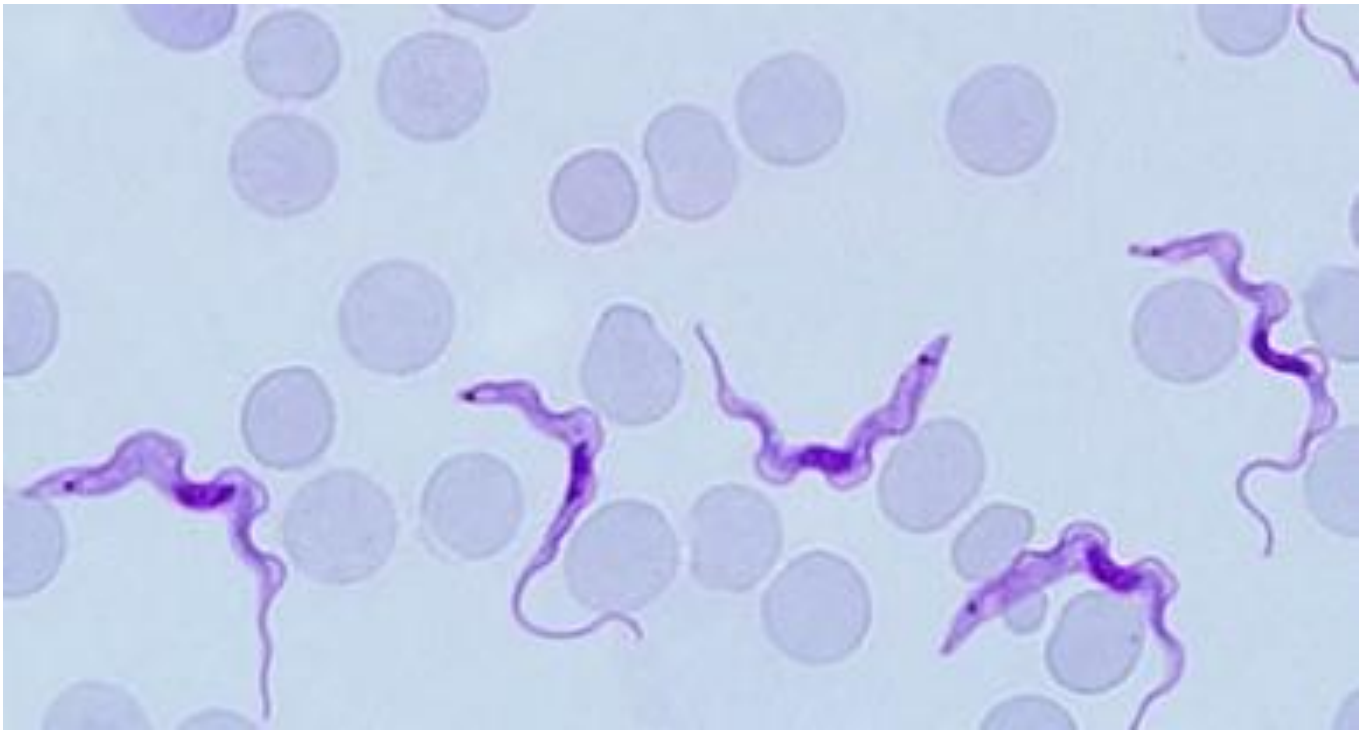
Trypanosoma is a genus of parasitic flagellate protist, in the phylum Euglenozoa (or in the kingdom or superphylum Discoba; there are many competing protists taxonomy schemes). Yes, either way it's related to the free-living *Euglena*, the beautiful green with red eyespot protozoan you find swimming gracefully in a drop of pond water with its whiplike flagellum. Trypanosomiasis is two important human parasitic diseases caused by *Trypanosoma spp*.: "sleeping sickness" in Africa and Chagas disease in the Americas.

African trypanosomiasis or sleeping sickness is caused by 2 subspecies of *Trypanosoma brucei* spread by the bite of a tsetse fly. *Trypanosoma brucei* was discovered in South African cattle sick with "nagana" in 1894 by British Army Medical Corps Captain David Bruce. In 1898 the disease was found in humans, and the trypanosome was seen in humans during an epidemic in Gambia in 1901. Unlike the previously discussed parasitic protists, *Trypanosoma brucei* has no intracellular life stages; it swims freely in blood, lymph or spinal fluid. Sometimes a chancre (painful sore) develops at the site of an infected bite before the person otherwise becomes sick. African trypanosomiasis has two stages of illness: a first hemolymphatic stage (weakness, intermittent fevers, cervical adenopathy, headache) and a second neurological stage (daytime sleepiness, apathy or aggressiveness, tremor, hallucinations). Sleeping sickness is curable with medication but if not treated leads to coma, organ failure and death. More common *Trypanosoma brucei gambiens* causes chronic sleeping sickness in West Africa (with intermittent fevers for years before death), while *Trypanosoma brucei rhodesiense* causes acute sleeping sickness in East And south Africa (which can progress to death in a matter of months). Humans are the main host reservoir for *T. brucei gambiens*, whereas domestic cattle and wild animals (including occasional giraffes, lions, warthogs and zebra) host *T. brucei rhodesiense.* The biting tsetse fly *Glossina morsitans* is the vector for both forms of African trypanosomiasis. Control efforts have been successful in fighting this terrible disease. In 2020 fewer than 700 combined cases (85% *T. b. gambiens*) were reported to the WHO.

Definitive diagnosis of African trypanosomiasis depends on seeing the flagellate parasite under the microscope in a body fluid, often a lymph node aspirate but sometimes blood or CSF. Some rapid serology tests are now available, but are too insensitive for routine use. Treatment is vital and depends on stage of infection and subspecies of parasite. Pentamidine, fexinidazole or nifurtimox plus eflornithine might be used for treatment of *T. brucei gambiense* infections; These drugs are available in the United States. Suramin and melarsoprol are used to treat first and second stage *T. brucei rhodesiense* infections, respectively. Expert consultation is required.



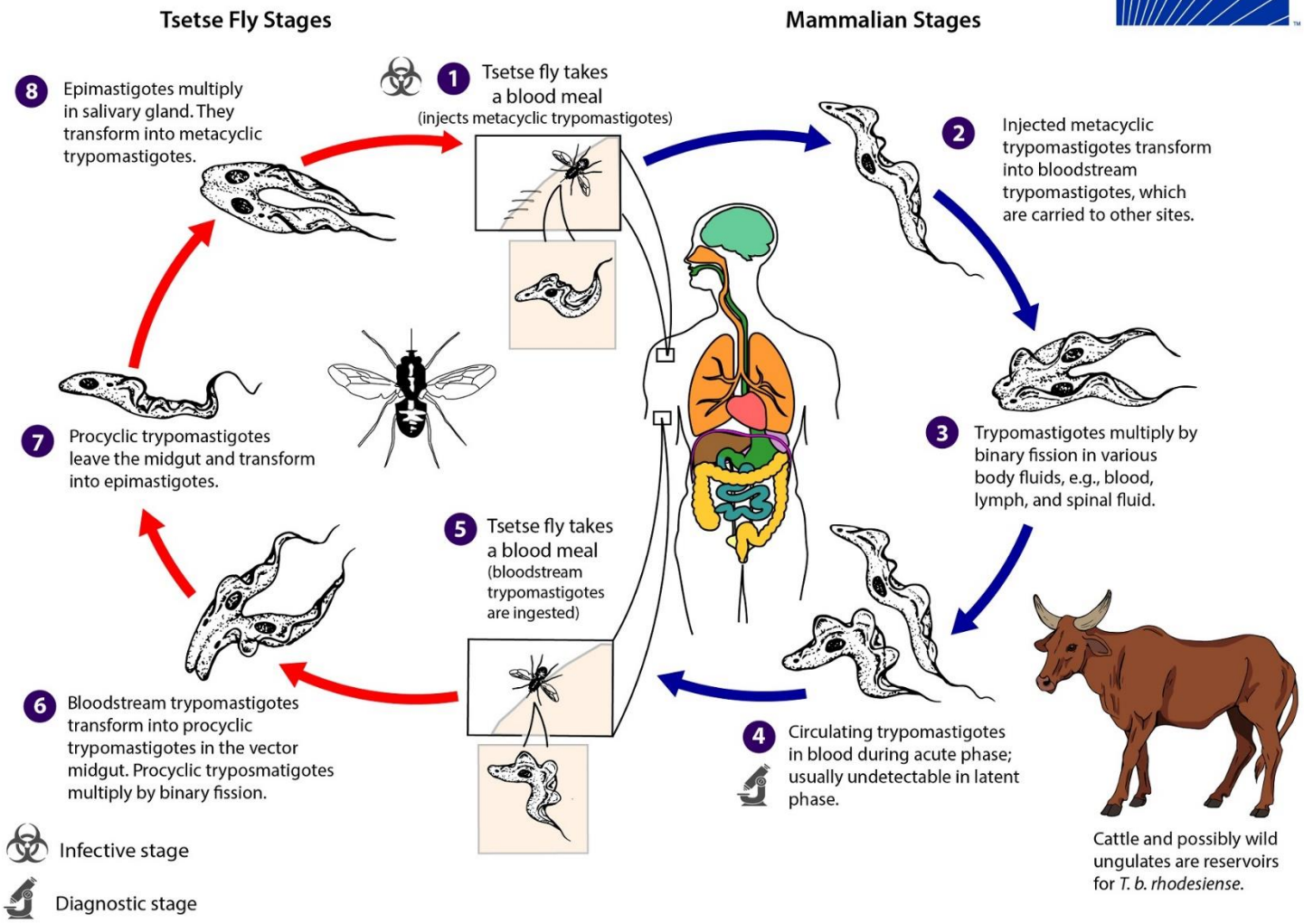
Tsetse fly, *Glossina morsitans,* pregnant female taking a human blood meal. Fly about 8 mm long, note wings folded on top of each other. Photo from International Centre of Insect Physiology and Ecology (icipe), founded in Kenya in 1970 by Prof. T R Odhiambo.



Trypanosoma brucei rhodesiense in a Giemsa-stained blood smear. No other details given. Trypanosomes appear about 30 microns long. Likely 100X objective. Image DPDx at CDC.gov

African Trypanosomiasis

Trypanosoma brucei gambiense & Trypanosoma brucei rhodesiense



During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream (1). Inside the host, they transform into bloodstream trypomastigotes (2), are carried to other sites throughout the body, reach other body fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission (3). The entire life cycle of African trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host (4), (5). In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission (6), leave the midgut, and transform into epimastigotes (7). The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission (8). The cycle in the fly takes approximately 3 weeks. Rarely, *T. b. gambiense* may be acquired congenitally if the mother is infected during pregnancy.

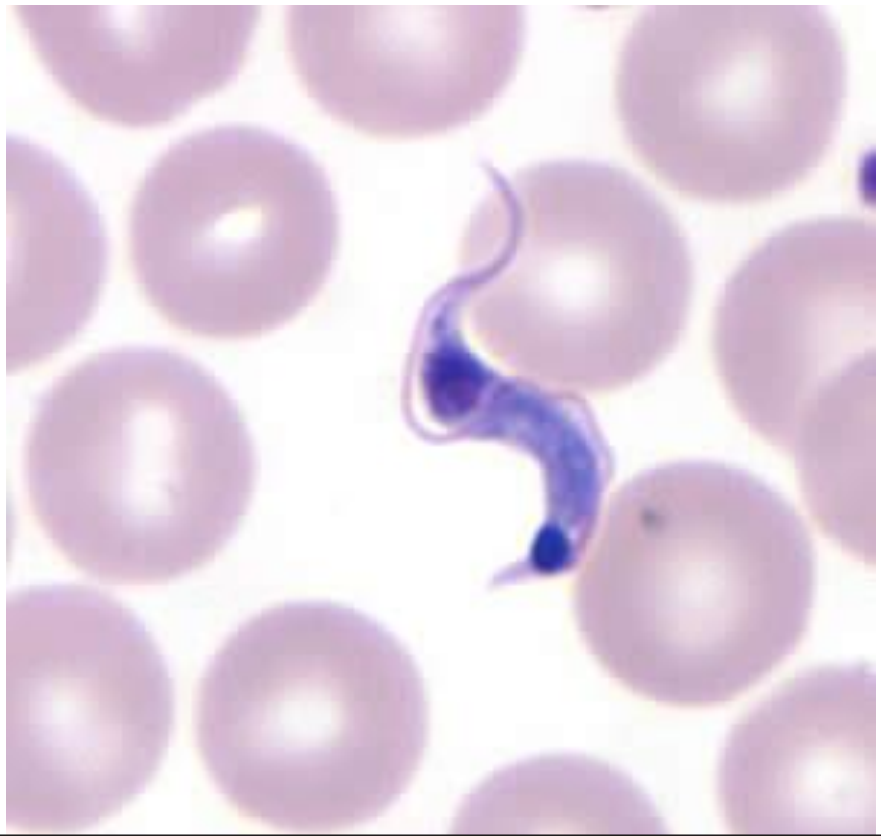
American trypanosomiasis or Chagas disease is caused by *Trypanosoma cruzi* transmitted by the bite of a reduviid bug. Brazilian public health doctor and researcher Carlos Chagas found a new trypanosome in the blood of a febrile 2 year old girl in 1909. He also found trypanosomes inside reduviid bugs he noticed in people's huts while investigating a malaria outbreak. He published his findings of the disease and its cause, but acceptance was slow, both in Brazil and abroad. Over the years it was found that the disease now named for Chagas is quite variable.

T. cruzi is endemic throughout Latin America from the extreme southern US to Chile, and Chagas noted an association with rural poverty. The CDC estimates as many as 8 million people in Mexico, Central America, and South America have Chagas disease, most of whom do not know they are infected. American trypanosomiasis has acute and chronic phases, both of range from asymptomatic to life threatening in severity. *Trypanosoma cruzi* is most often transmitted by triatomine bugs also called kissing or reduviid or assassin bugs. These "true bugs" (insects in order Hemiptera) live in thatched huts and become vectors while feeding on the blood of sleeping victims. Transmission also occurs directly in many possible ways: from kids rubbing insect feces into their eyes (causing Romaña's sign, swelling of one eyelid), vertically from a pregnant woman to her baby, iatrogenic from blood transfusion or organ transplant, from eating uncooked food that is contaminated with bug feces, or from accidental laboratory exposure. Charles Darwin may have acquired Chagas disease from collecting bugs.

The **acute phase** of illness lasts a few weeks to months and may have no symptoms at all or nonspecific symptoms: fever, fatigue, aches, headache, anorexia, diarrhea. Immunocompromised patients might have severe acute infection. After the acute phase patients are usually asymptomatic for decades but trypanosomes continue to live in the body and eventually some patients enter a **chronic phase** with sometimes incurable heart or intestinal problems. Due to fibrosis and degeneration of nerves provoked by the parasite, the heart, esophagus or colon can become massively enlarged, causing heart failure, cardiac arrythmias, intractable vomiting and severe constipation. Complications include death.

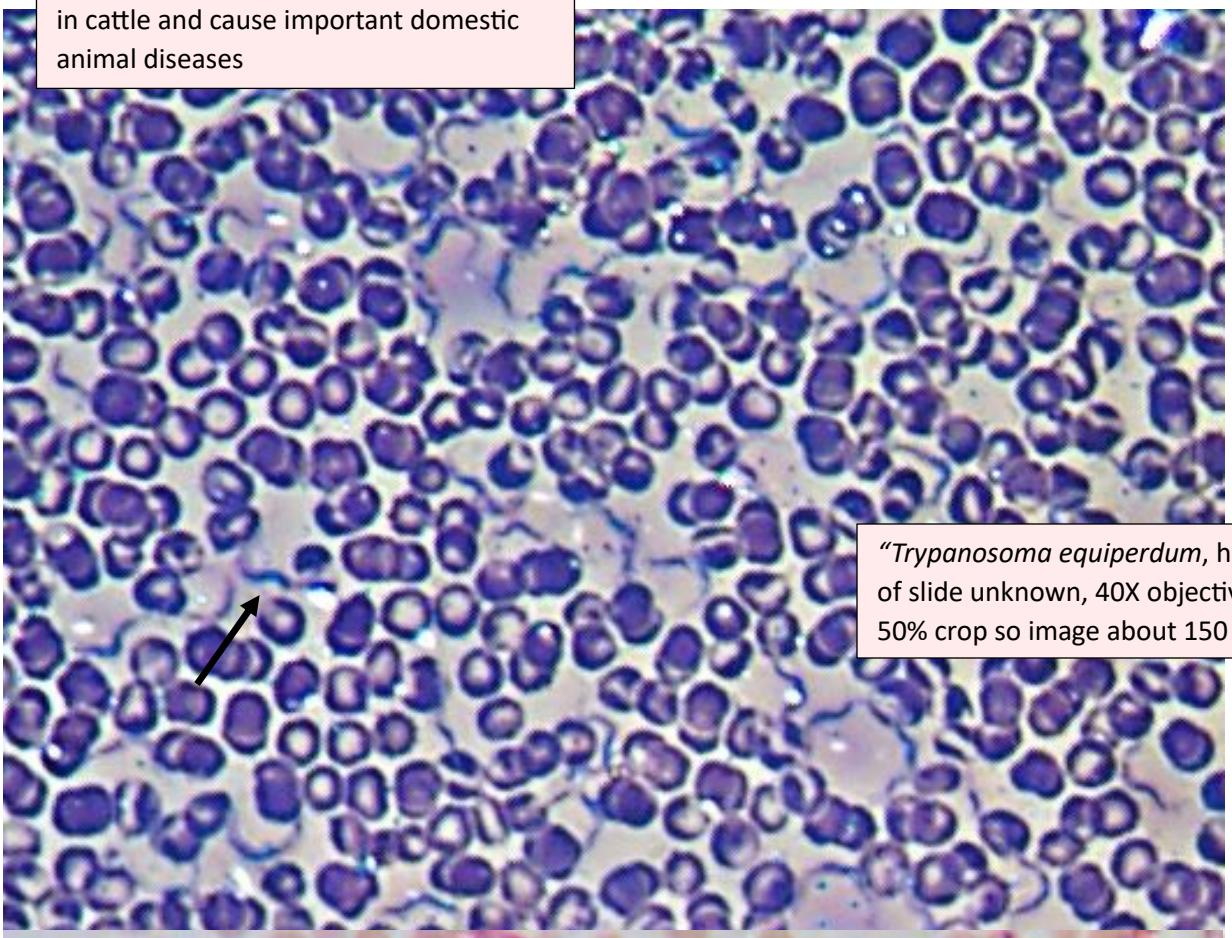
During the acute phase diagnosis is by old fashioned microscopy of blood looking for trypanosomes. Serology tests are used during the chronic phase, but don't distinguish whether or not the patient is ill from chronic infection. This is done by cardiac and GI imaging and testing. Antiparasitic drug therapy is recommended to all with acute or reactivated Chagas disease, especially the immunocompromised. Treatment might be with nifurtimox or benznidazole, with expert consultation. Patients over 50 with chronic disease might also by offered antiparasitic treatment after considering the risks of the disease versus the risks of the drugs. The damage to the patient may already be done, and the side effects of antiparasitic drugs might be more dangerous. Although not cures, there may be life prolonging pharmaceutical treatments for heart failure and helpful drugs for gastrointestinal symptoms.

Triatoma infestans. triatomine or "kissing" bug. A big (25 mm 1 inch) micropredator, it lives in crevices of shoddy houses and feeds nocturnally on blood. Several closely related bugs (insect order Hemiptera) are also vectors of Chagas disease. Image D Dörge, Goethe University at phys.org

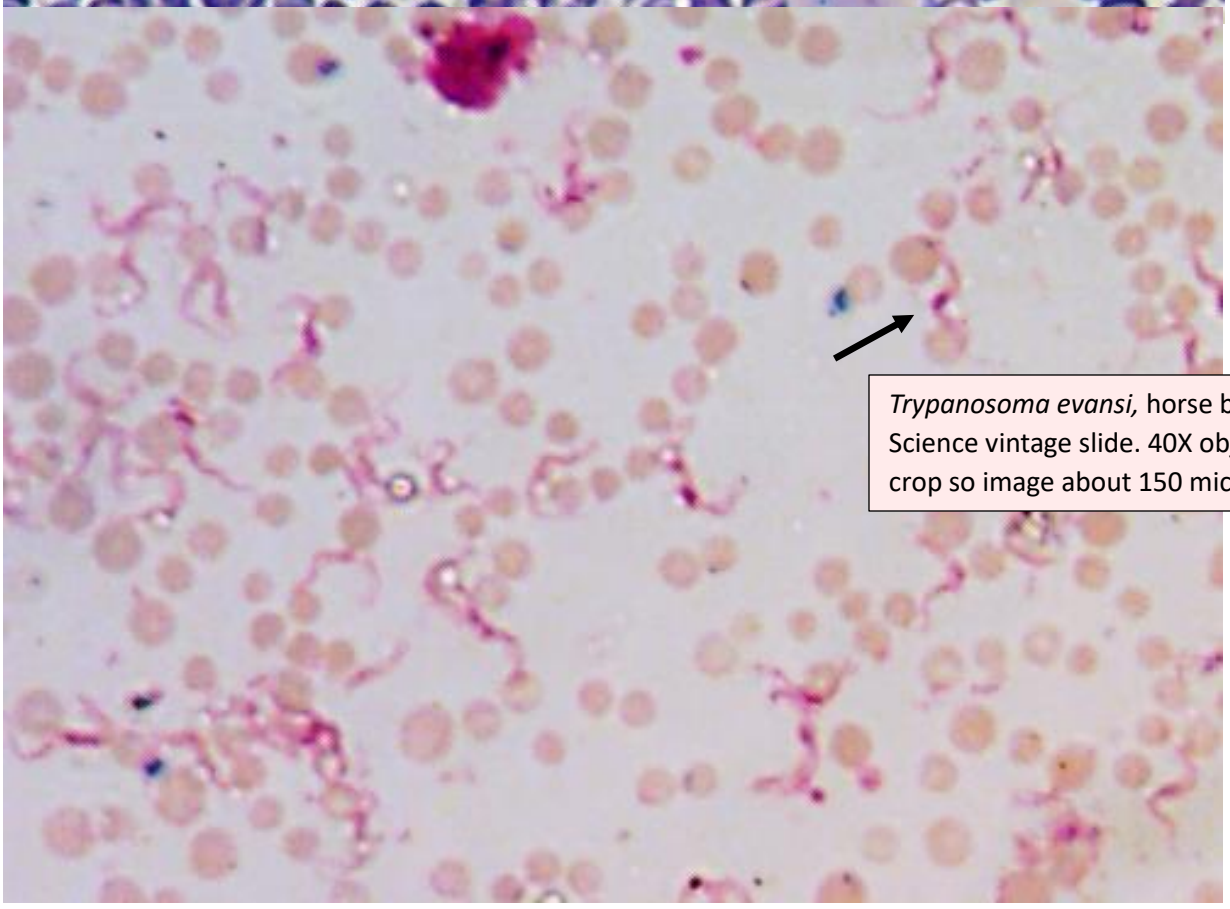


Trypanosoma cruzi presumed in human blood, likely cropped from image by 100X objective, result near limit of conventional light microscope. With RBCs about 4 to 7 microns the protist is likely about 15 microns long (if straightened out). Great image, shows attached flagellum with undulating membrane, nucleus (larger, upper) and kinetoplast (lower). Image from CDC.gov

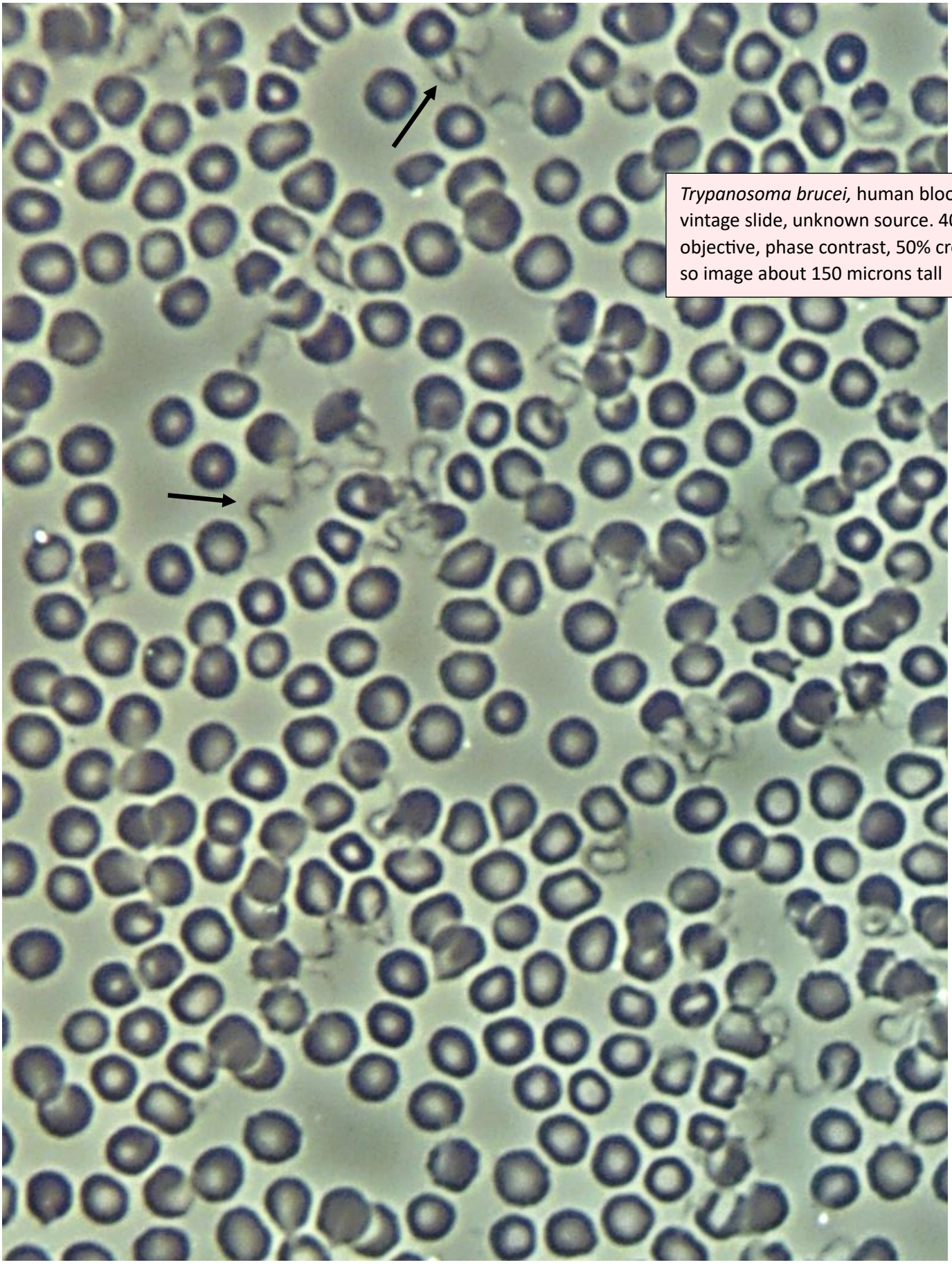
Trypanosome parasites were first found in cattle and cause important domestic animal diseases



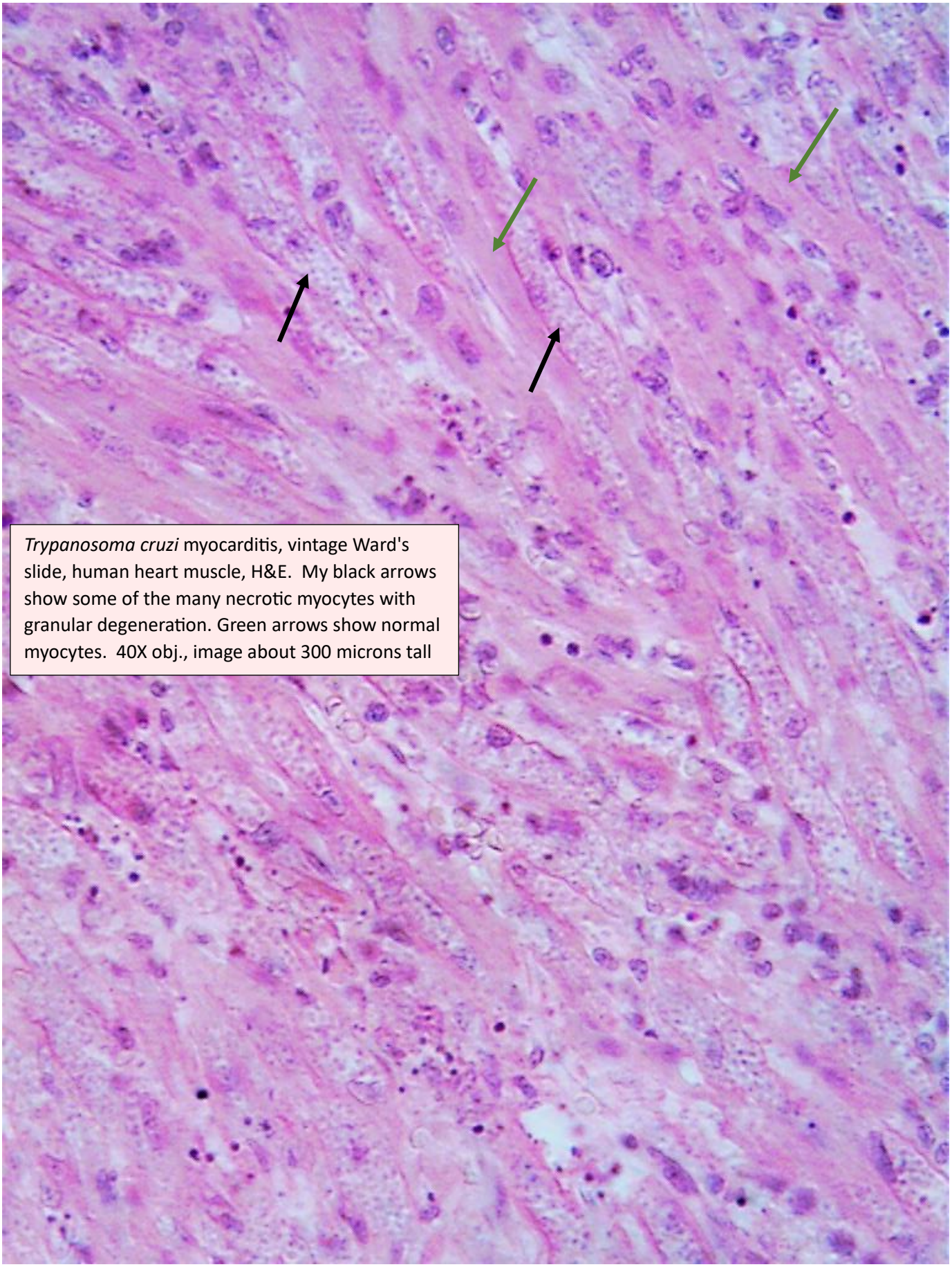
"Trypanosoma equiperdum, horse blood", origin of slide unknown, 40X objective, phase contrast, 50% crop so image about 150 microns wide



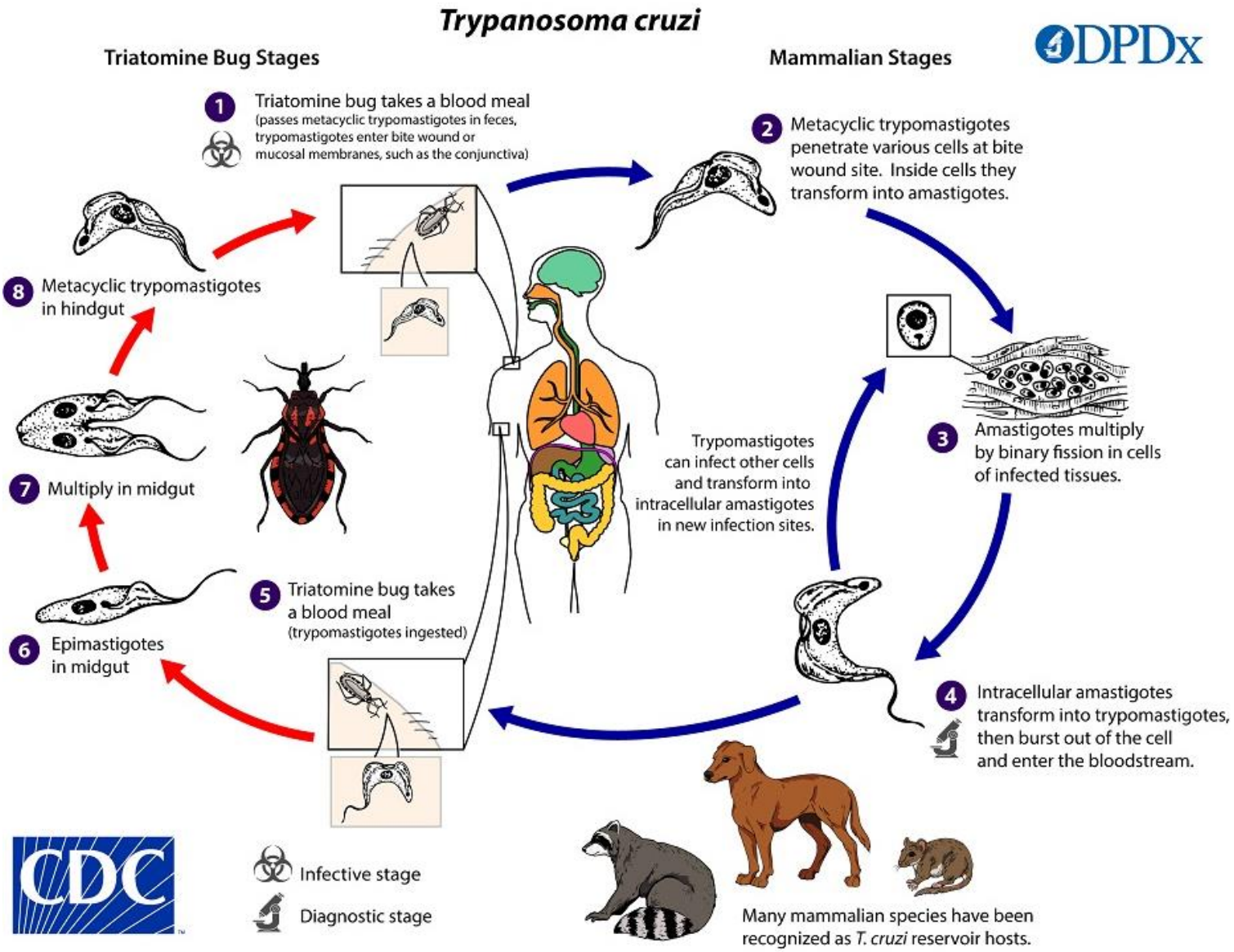
Trypanosoma evansi, horse blood, Ward's Science vintage slide. 40X objective, 50% crop so image about 150 microns wide



Trypanosoma brucei, human blood, vintage slide, unknown source. 40X objective, phase contrast, 50% crop so image about 150 microns tall



Trypanosoma cruzi myocarditis, vintage Ward's slide, human heart muscle, H&E. My black arrows show some of the many necrotic myocytes with granular degeneration. Green arrows show normal myocytes. 40X obj., image about 300 microns tall

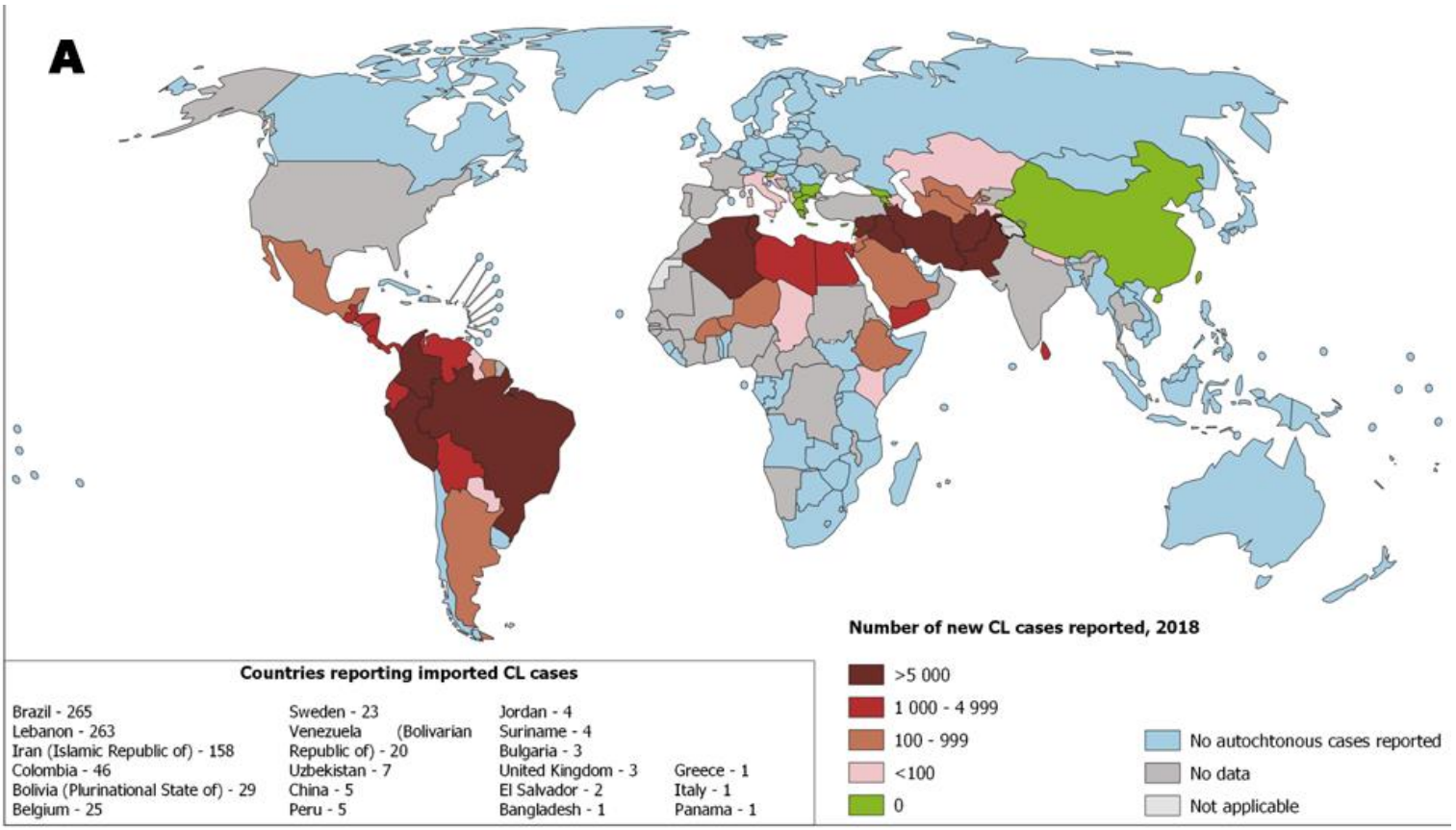


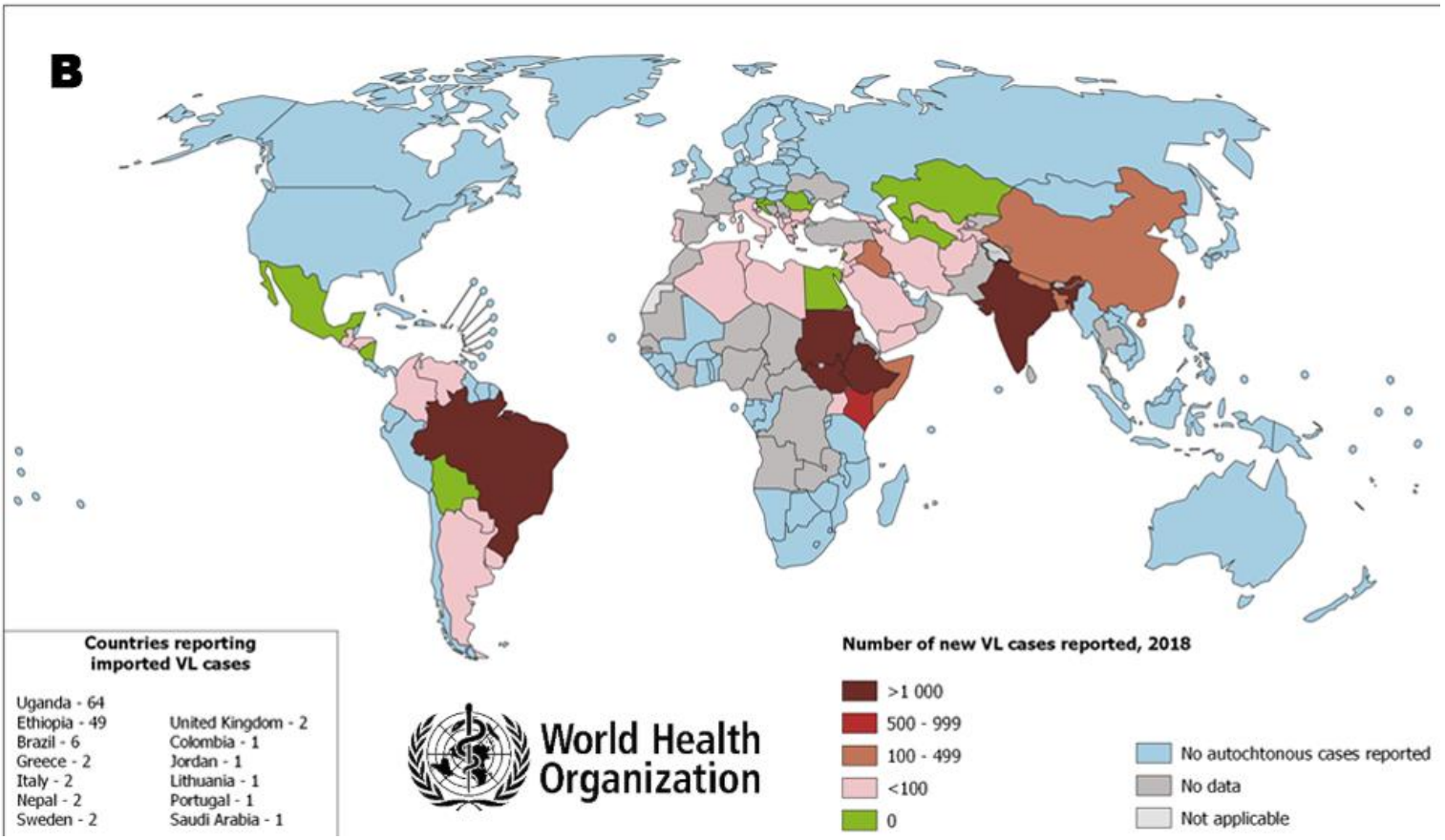
Infected triatomine insect vector (“kissing” bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the bite wound or intact mucosal membranes, such as the conjunctiva 1 . Inside the host, the trypomastigotes invade cells near the site of inoculation, where they differentiate into intracellular amastigotes 2 . The amastigotes multiply by binary fission 3 and differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes 4 . Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The “kissing” bug becomes infected by feeding on human or animal blood that contains circulating parasites 5 . The ingested trypomastigotes transform into epimastigotes in the vector’s midgut 6 . The parasites multiply and differentiate in the midgut 7 and differentiate into infective metacyclic trypomastigotes in the hindgut 8 . Other less common routes of transmission include blood transfusions, organ transplantation, transplacental transmission, and foodborne transmission (via food/drink contaminated with the vector and/or its feces).

5. Leishmaniasis

21 species of flagellate protozoan parasites in genus *Leishmania* are spread to humans by about 31 species of biting sand flies and can cause scarring chronic skin sores and sometimes fatal visceral (internal organ) infections. Some of the 21 species are grouped by tribe including the *L. donovani* complex of 3 species, *L. mexicana* complex with 3 main species and the subgenus *Viannia. Leishmania* protists have been found in 100 million year old Cretaceous amber, and a leishmaniasis like deforming disease was described by ancients and noted by Spanish conquistadors. In 1900 British army pathologist William Boog Leishman was in India, and discovered oval parasites in the enlarged spleen of a deceased soldier who wasted away. He was able to infect and see the parasite again in a rat and published his findings in 1903. Today leishmaniasis is endemic in parts of about 90 countries in the tropics, subtropics, and southern Europe. In 2018 the WHO thought about 12 million people were living with *Leishmania* parasites. About 0.7 to 1.2 million new cases of cutaneous leishmaniasis are diagnosed annually. The more serious visceral forms have dropped from over 400,000 to less than 100,000 new annual cases in recent years, per the CDC. In some places people are the main reservoir of *Leishmania* (anthroponotic transmission), so treating patients helps to prevent spread, whittling away at the endemic disease (regularly occurs in a location) .

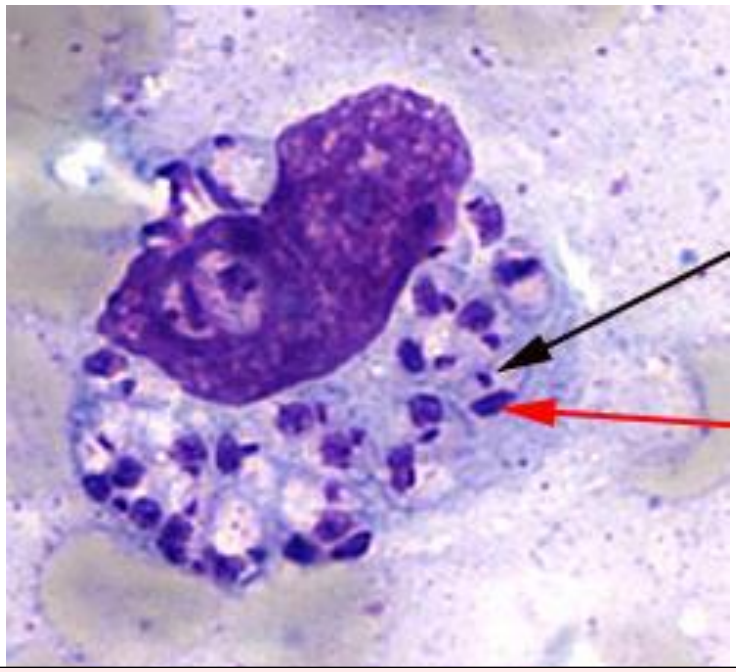
Leishmania is an obligate intracellular parasite that requires phlebotomine sand flies as vectors. The parasite especially infects monocytes/macrophages (our garbage collectors) in the reticuloendothelial system (in liver, bone marrow, spleen, lymph nodes). Disease may be only **cutaneous** (or occasionally mucosal) with chronic sores starting at fly bites. The sore often looks “volcanic” with a crater surrounded by raised edges. Scabs and swollen lymph nodes are possible. It may hurt at first but chronic lesions often lose sensation. The dreadful sores can be destructive around the mouth and nose. **Visceral leishmaniasis** (aka kala-azar) is fatal if left untreated in over 95% of cases, as in Dr. Leishman’s case number 1. Patients often have fever, weight loss, hepatosplenomegaly, adenopathy, and severer anemia (with also low platelets and leukocytes). Kala-azar can greatly complicate HIV infection. Microscopic pathology may show microgranulomas and donovani bodies, but commonly is not definitive, so diagnosis may require special cultures of flagellated promastigote forms or special serologic or molecular tests. Kala-azar needs treatment but the drugs are toxic. Liposomal amphotericin B, miltefosine, pentamidine, fluconazole, paromomycin, or antimony (heavy metal) salts might be considered by an expert. Similar harsh drugs might be considered to help healing of cutaneous disease and prevent visceral spread, but again expert consultation is required.





WHO maps of the occurrence of A Cutaneous and B Visceral Leishmaniasis in 2018

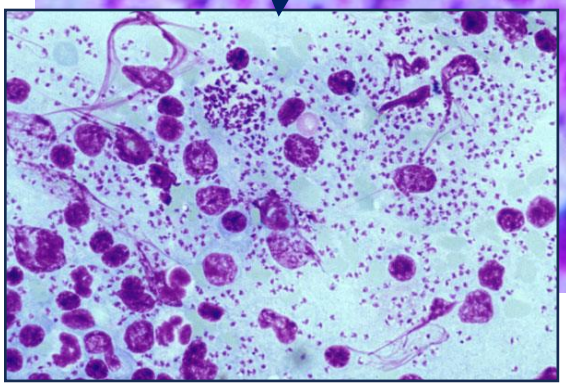
The vectors of human leishmaniasis are infected female sandflies of *Phlebotomus spp*. in the Old World and *Lutzomyia spp*. in the New World. Sand flies are tiny insects (Diptera: Psychodidae), 1.5-3.5 mm in length, with hairy wings (air is viscous at small scale), black eyes and long legs. Image of feeding sand fly from CDC/ Frank Collins/James Gathany at isglobal.org

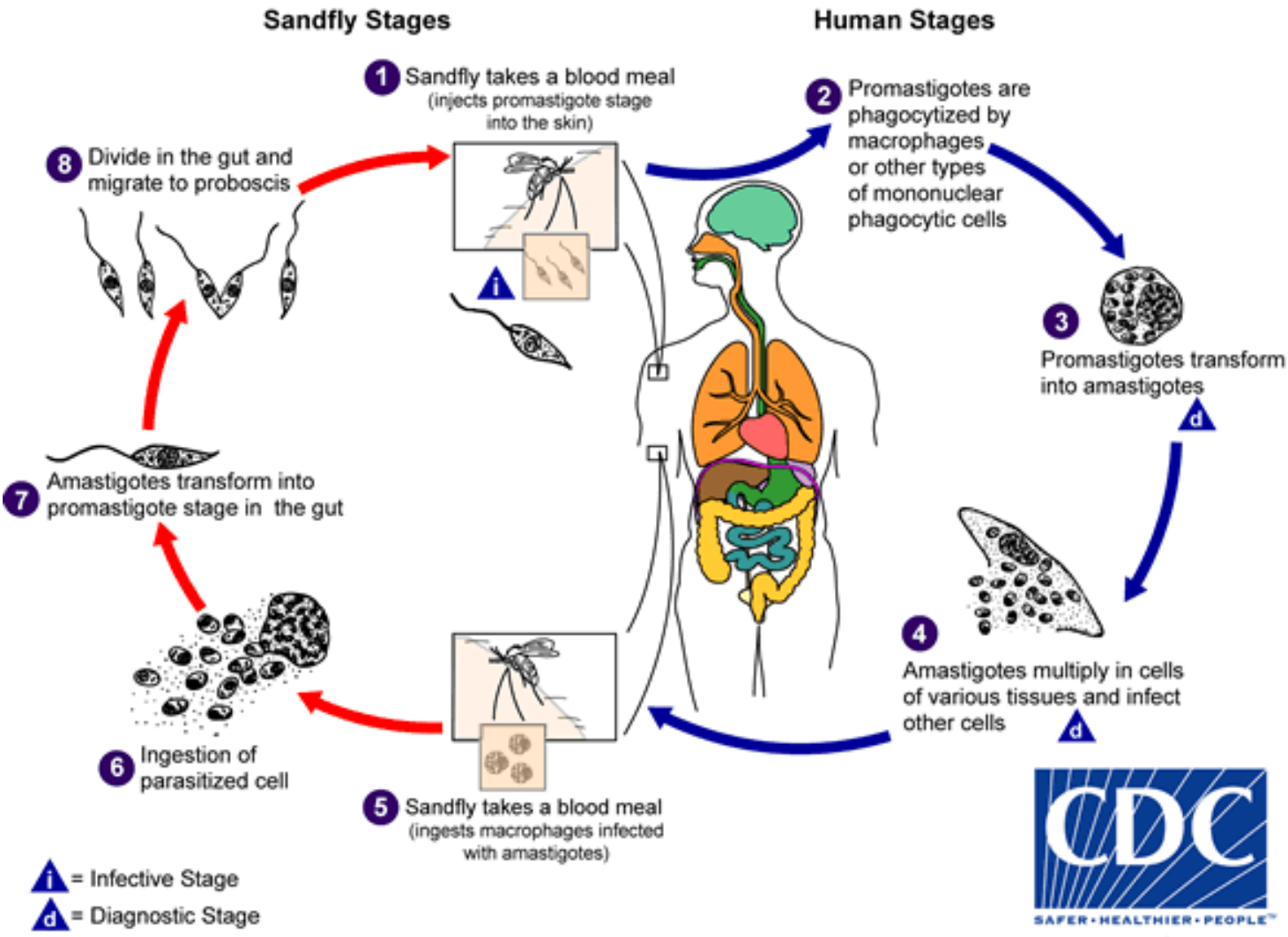


Stained bone marrow specimen from a patient with visceral leishmaniasis—showing a macrophage containing multiple *Leishmania* **amastigotes** (the tissue stage of the parasite). Note that each amastigote has a **nucleus** (red arrow) and a rod-shaped **kinetoplast** (black arrow). Diagnosis is not confirmed if both structures are not seen.
Image at CDC.gov, presume a very good 100X objective, cropped to show detail

"Leishmania donovani, liver smear"*,* typed label, from box of 59 varied parasite slides in circa 1950 Bakelite box. Donovani bodies are 2-5 micron ovoid amastogotes but should be intracellular; these tiny spots don't appear so, but may have spilled from the preparation of specimen. 40X objective, 50% crop so image about 150 microns tall

Compare spleen smear of Chinese boy infected with *Leishmania infantum* (Giemsa) at pathos223.com



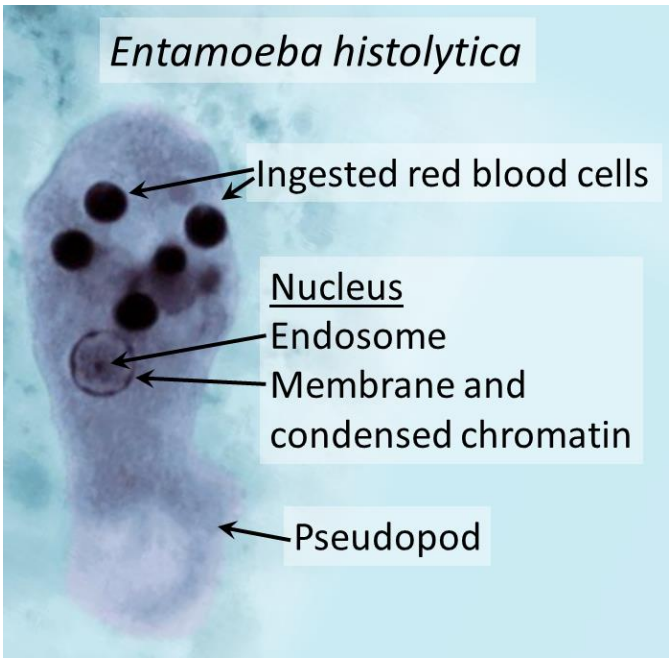


Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies. The sandflies inject the infective stage (i.e., promastigotes) from their proboscis during blood meals ❶. Promastigotes that reach the puncture wound are phagocytized by macrophages ❷ and other types of mononuclear phagocytic cells. Promastigotes transform in these cells into the tissue stage of the parasite (i.e., amastigotes) ❸, which multiply by simple division and proceed to infect other mononuclear phagocytic cells ❹. Parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous or visceral leishmaniasis results. Sandflies become infected by ingesting infected cells during blood meals (❺, ❻). In sandflies, amastigotes transform into promastigotes, develop in the gut ❼ (in the hindgut for leishmanial organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus), and migrate to the proboscis ❽

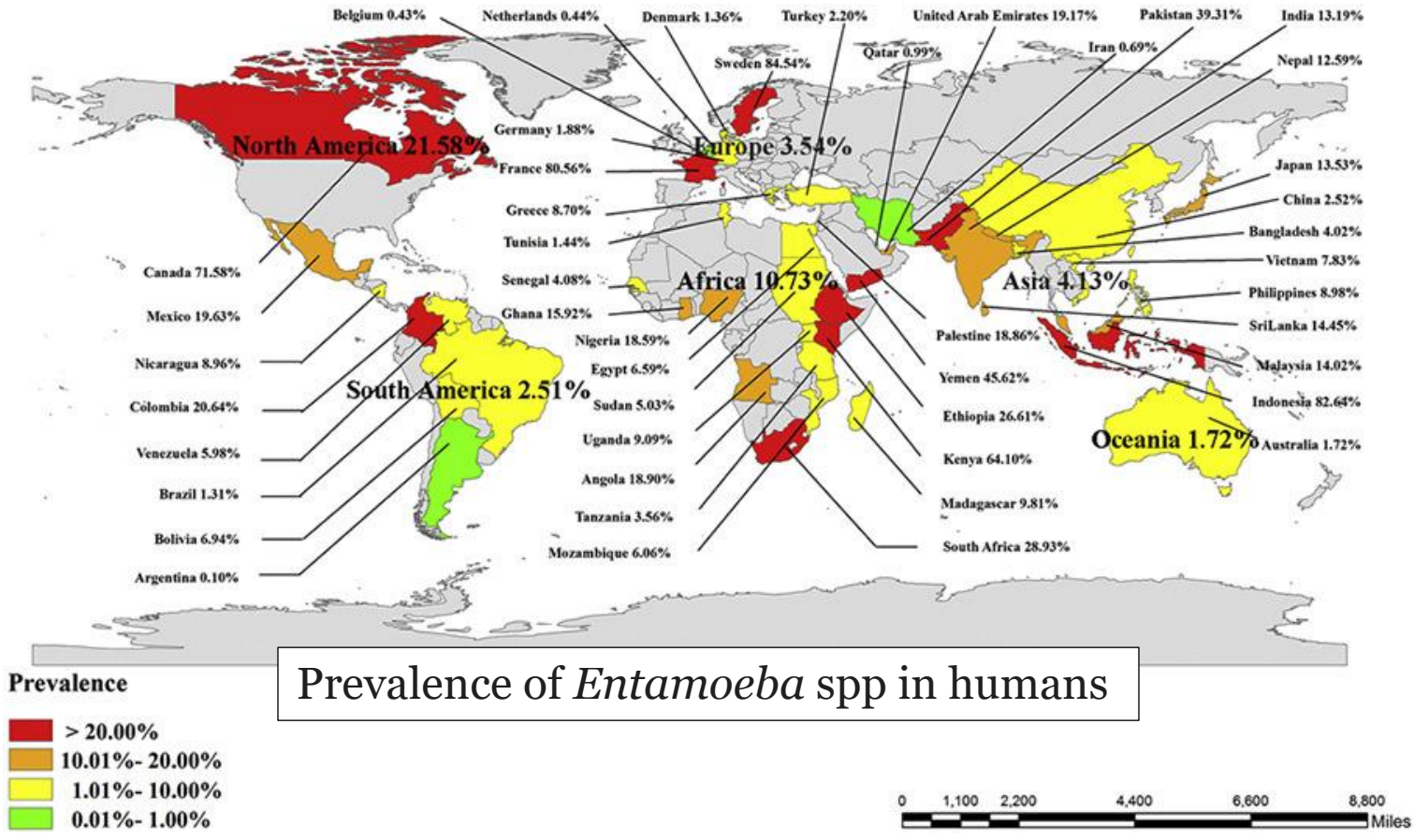
6. Amoebiasis

There are a few other amoebic infections of people but the only common one is amoebic dysentery caused by *Entamoeba histolytica*. It is thought as many as 50 million people get sick from amoebic dysentery yearly, mostly in places with poor access to clean food and water. Annual deaths have fallen from 100,00 to 40,000 annually in recent decades. Most cases are asymptomatic, but more than 10% of patients get mild to severe (bloody with fever and abdominal pain) or life threatening diarrhea. *Entamoeba histolytica* is anaerobic so prefers the low oxygen conditions of the colon. The amoebas can sometimes invade the mucosa, causing micro abscesses and bleeding in the colon and may ingest (eat) your red blood cells. Rare cases are complicated by toxic megacolon, or hepatic amoebic abscesses (which can sometimes spread to chest or brain). As with other parasites, amoebas hit poor, malnourished children and the immunosuppressed (including HIV, pregnant) harder.

Entamoeba has trophozoite active forms with typical amoeboid motion with pseudopods ("fake feet") and *Entamoeba* can also form inactive, resistant cysts which it uses to transfer from host to host. Diagnosis is usually by microscopic examination of poop but is complicated by about 70% of patients not showing *Entamoeba histolytica* in their poop, and by most people carrying harmless commensal or beneficial species of amoebas which look very similar: *Entamoeba coil, E. dispar, E. hartmanni E. moshkovskii, E. Bangladeshi, E. polecki, Chilomastix mesnili,* and *Iodamoeba buetschlii .* Yes, the normal human biome includes protists (as well as bacteria, archaea, viruses, fungi, and often animals <asymptomatic worms and mites>). Durin amoebic infection with *H. histolytica* blood tests may show eosinophilia and anemia, and imaging done for abdominal pain could show abscesses. In resource rich medical systems, EIA (enzyme immunoassay) or a stool PCR panel (looks for DNA or RNA of 22 bacteria, viruses and protists simultaneously) and colonoscopy might be done to rule out other more common causes of severe colitis. Ironically, little parasite testing is done in the most parasite harmed areas of the world because of severe poverty there. My hospital's $200 GI panel would be about 2 months of average worker pay in those places. If *Entamoeba histolytica* dysentery is diagnosed, it may be treated with metronidazole or tinidazole followed by paromomycin, diiodohydroxyquin, or diloxanide, but seek expert consultation at your Public Health Department. Amoebas are not in the top 50 causes of GI problems of my US patients, and I don't think of them at work. If you just got back from a 2 month jungle trek in the Congo, and then get sick, make sure the doctor knows about your potential exposures.

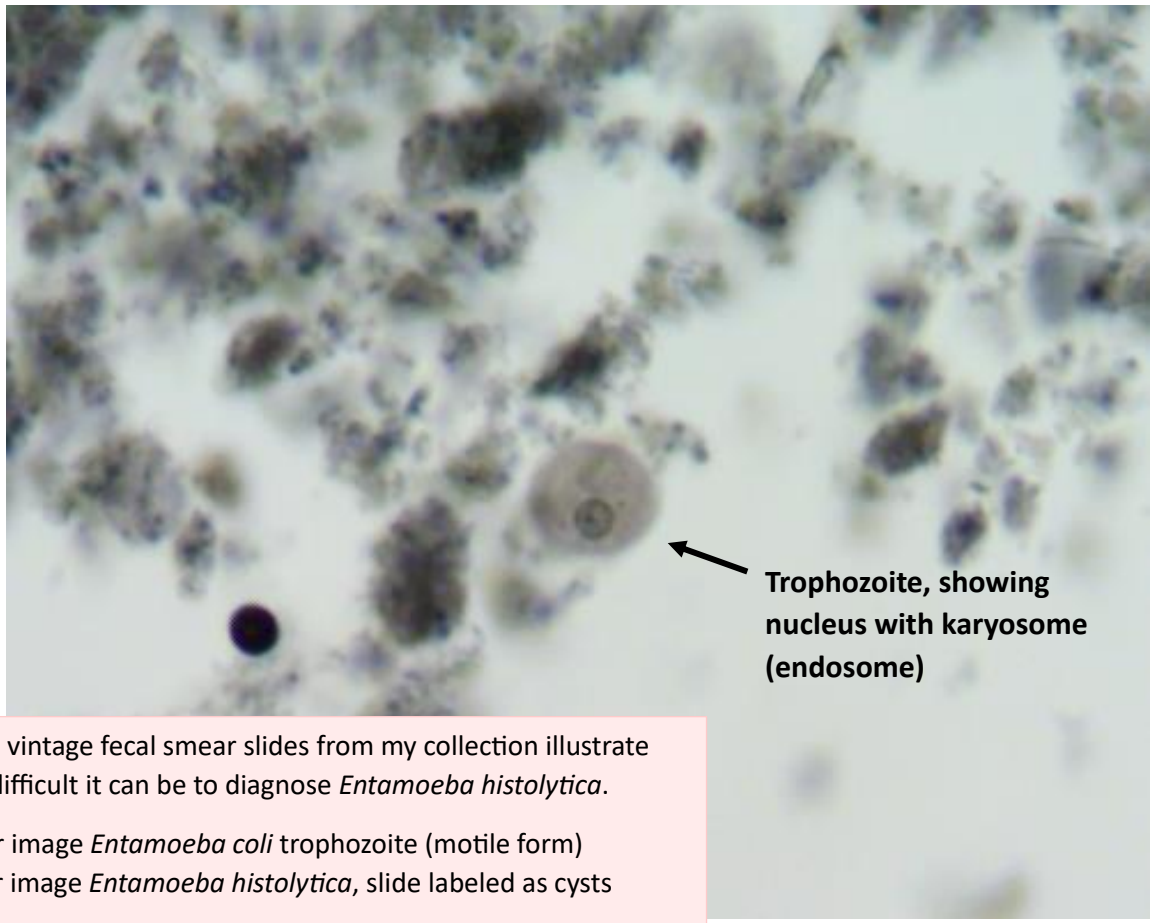


Trichrome-stained *Entamoeba histolytica* trophozoite, containing phagocytized erythrocytes seen as dark, round inclusions. Anatomic features labelled by Dr. Mikael Häggström
Magnification not specified but likely 100X objective.
Note RBCs are about 5 micron wide
image Wikipedia/CDC, Dr. Melvin, Dr. Greene



Prevalence of *Entamoeba* spp in humans

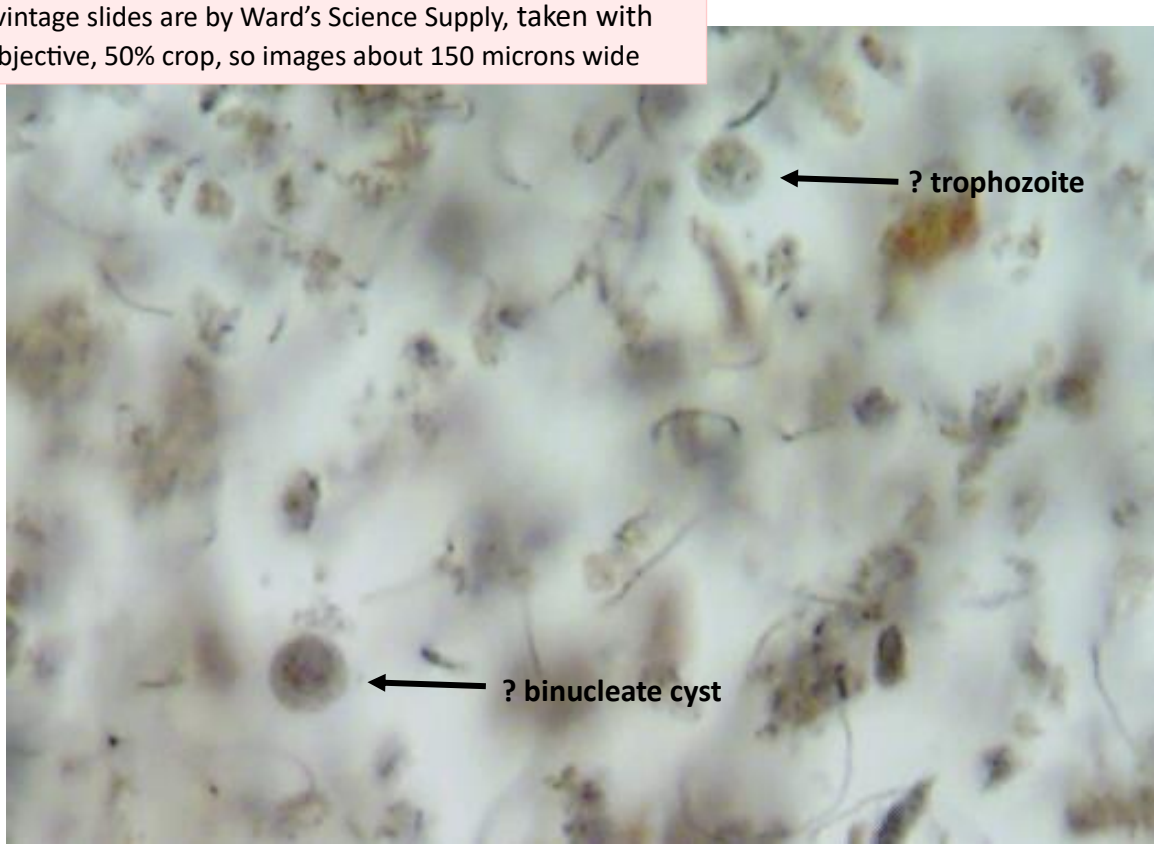
Much information on parasites (and internet "fact" in general) is clearly wrong. This graphic says 85% of Swedes and 72% of Canadians but only 4% of Bangladeshis have *Entamoeba*. Bullshit. Some north Europeans that got bloody diarrhea after travelling got a parasite test. Poor rice famers in the tropics frequently get amoebiasis but can't afford testing.
Map from Cui, Li et al Molecular epidemiology, evolution, and phylogeny of Entamoeba spp. *Infection, Genetics and Evolution* 2019



These vintage fecal smear slides from my collection illustrate how difficult it can be to diagnose *Entamoeba histolytica*.

Upper image *Entamoeba coli* trophozoite (motile form)
Lower image *Entamoeba histolytica*, slide labeled as cysts

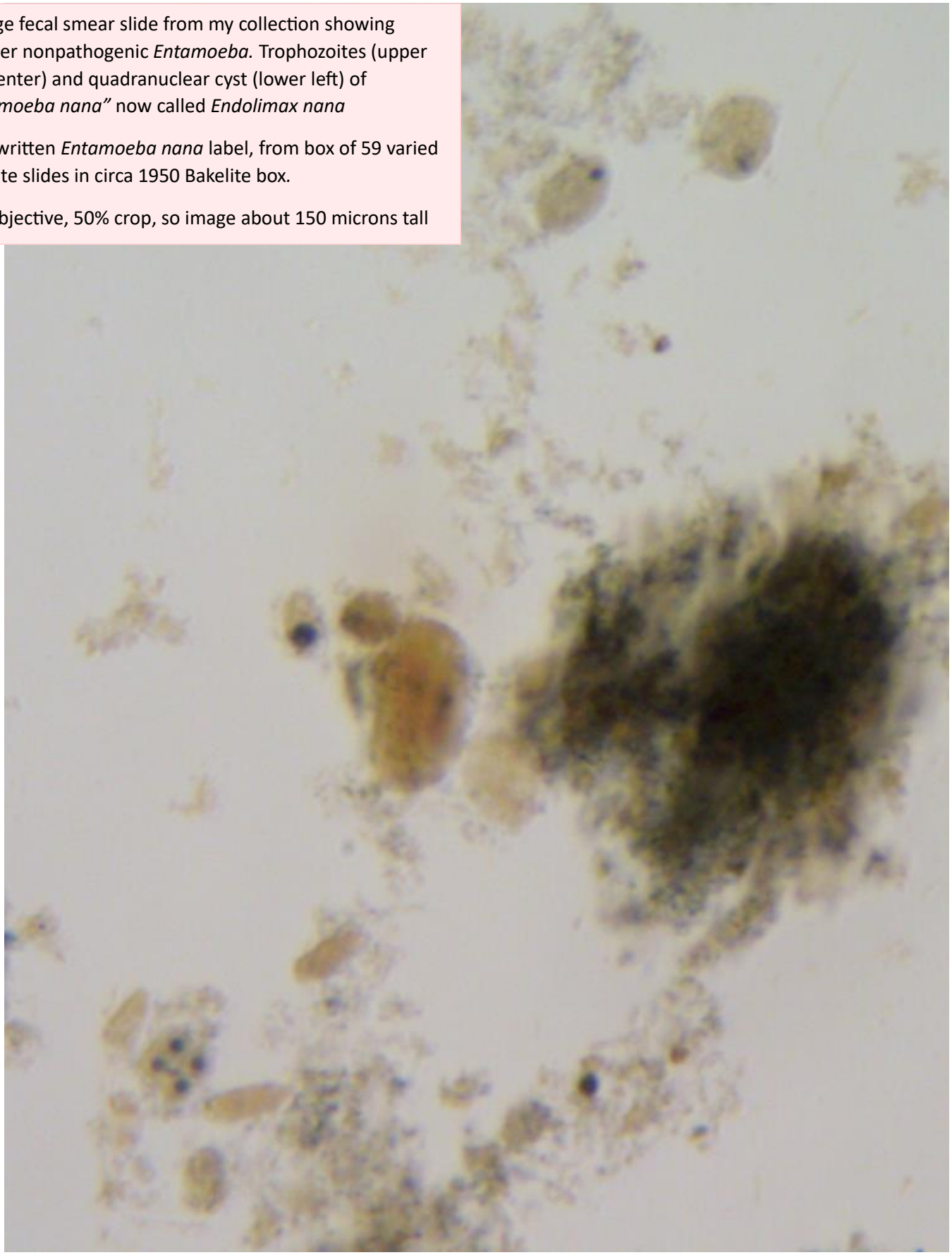
Both vintage slides are by Ward's Science Supply, taken with 40X objective, 50% crop, so images about 150 microns wide

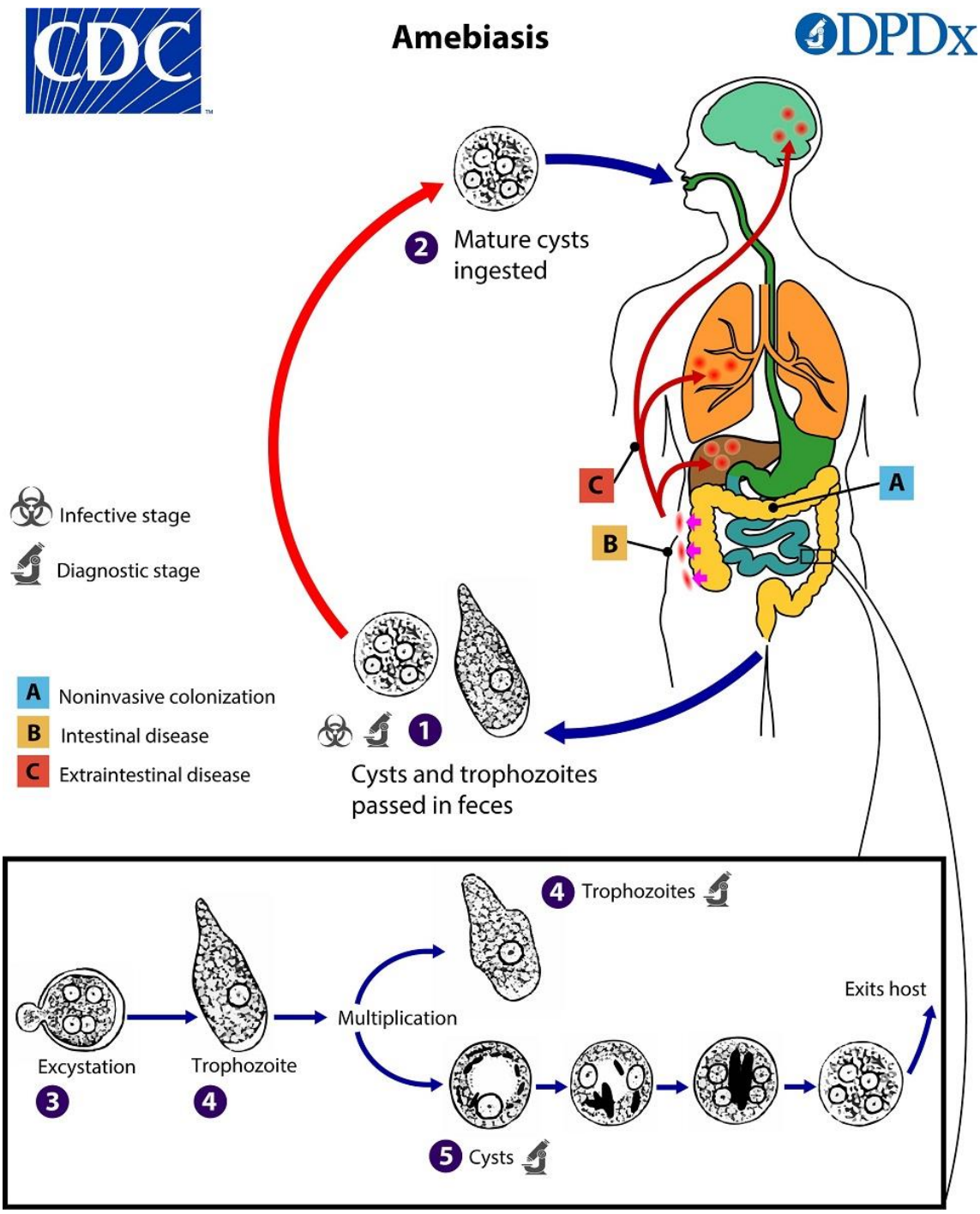


Vintage fecal smear slide from my collection showing another nonpathogenic *Entamoeba.* Trophozoites (upper and center) and quadranuclear cyst (lower left) of "*Entamoeba nana"* now called *Endolimax nana*

Handwritten *Entamoeba nana* label, from box of 59 varied parasite slides in circa 1950 Bakelite box.

40X objective, 50% crop, so image about 150 microns tall

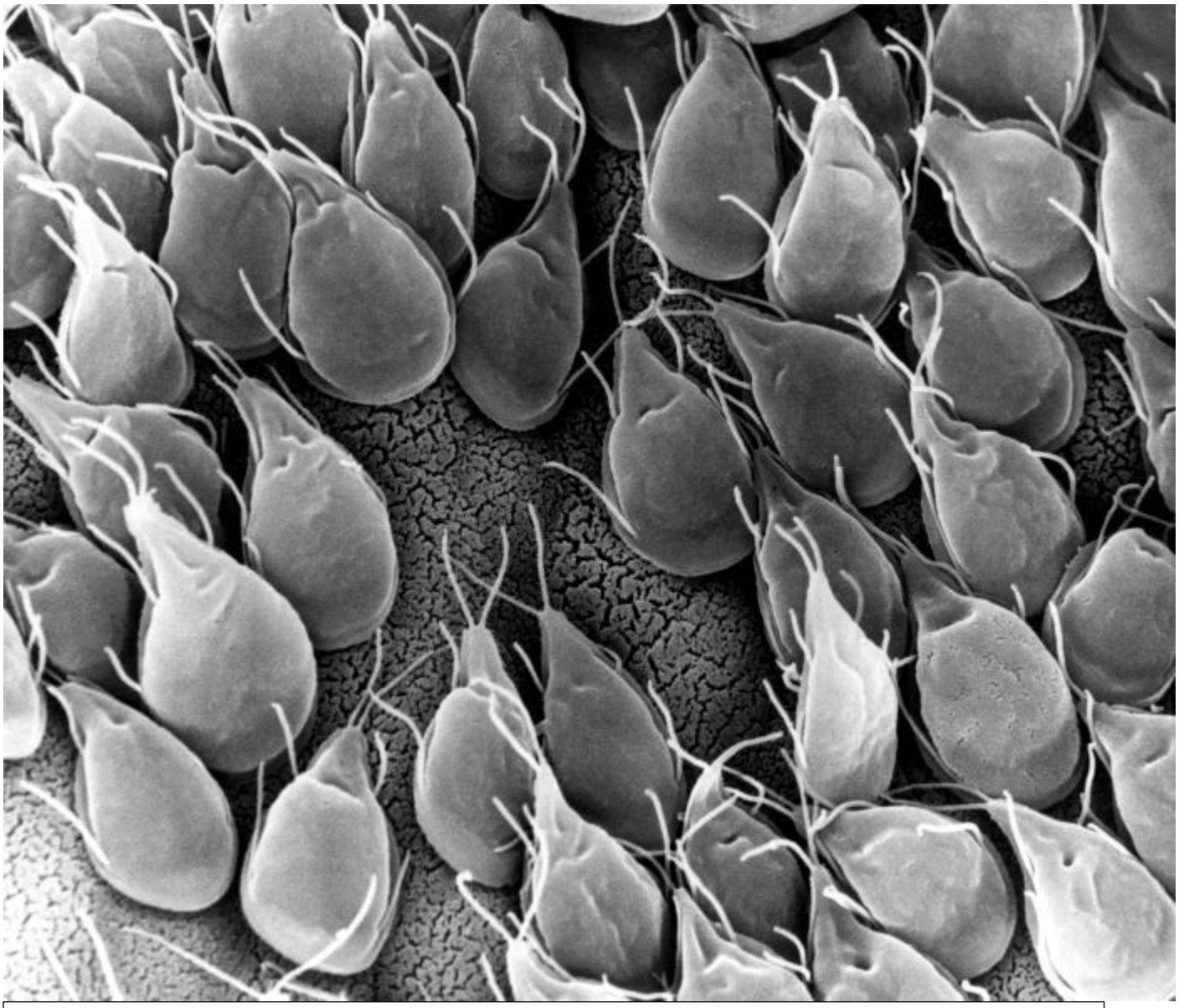




Cysts and trophozoites passed in feces (1). Cysts are typically found in formed stool; trophozoites are typically found in diarrhea. Infection with *Entamoeba histolytica* occurs via ingestion of mature cysts (2) from fecally contaminated food, water, or hands. Sexual contact may also pass infectious cysts. Excystation (3) occurs in the small intestine and trophozoites (4) are released, which migrate to the large intestine. Trophozoites may remain confined to the intestinal lumen (A: noninvasive infection) with individuals continuing to pass cysts in their stool (asymptomatic carriers). Trophozoites can invade the intestinal mucosa (B: intestinal disease), or blood vessels, reaching extraintestinal sites such as the liver, brain, and lungs (C: extraintestinal disease). Trophozoites multiply by binary fission and produce cysts (5), and both stages are passed in the feces (1). Cysts can survive days to weeks in the external environment and remain infectious in the environment due to the protection conferred by their walls. Trophozoites passed in the stool die rapidly in outdoor environment.

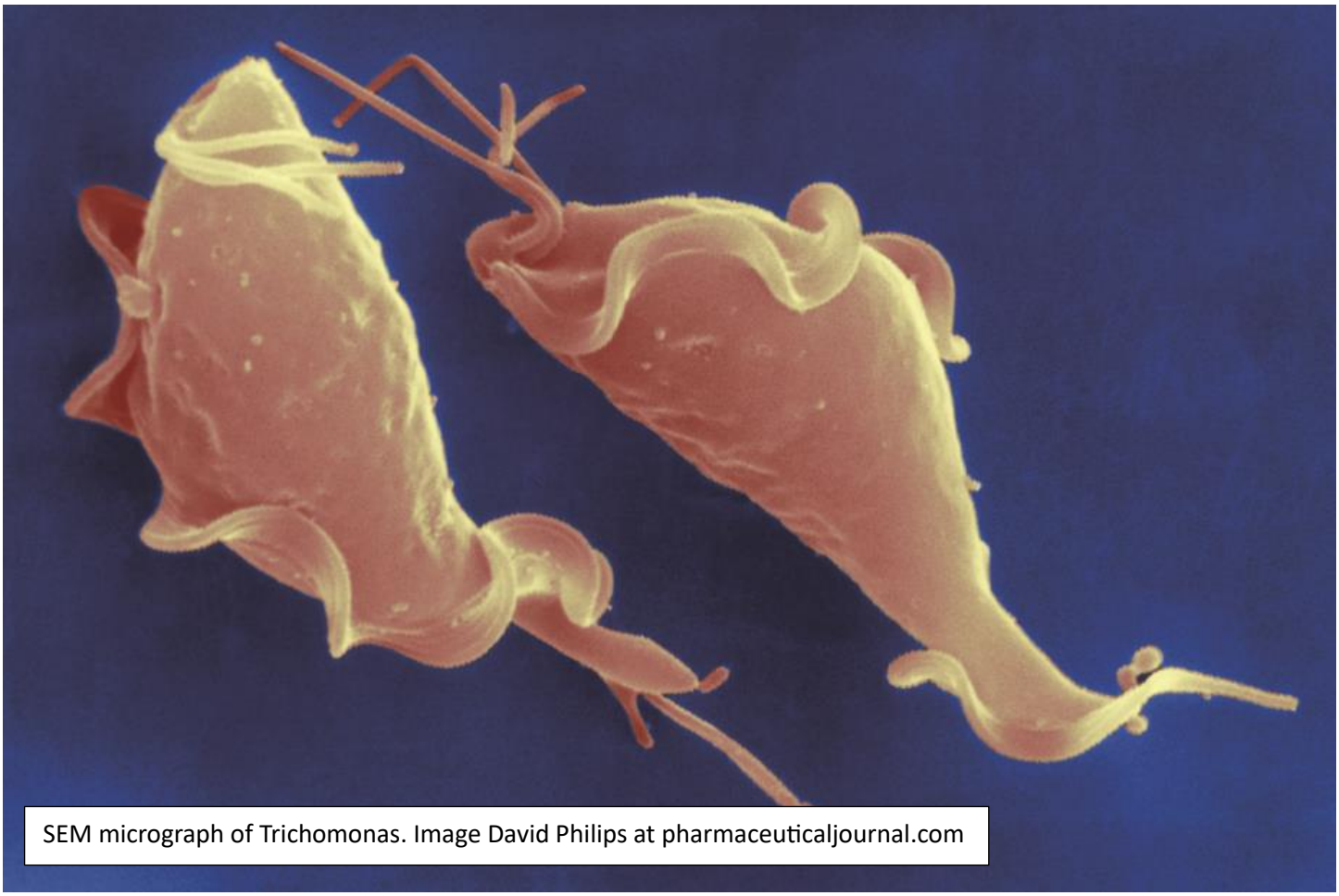
7. some other parasitic protist diseases of note

Giardiasis is an intestinal infection caused by the anaerobic diplomonad protozoan ***Giardia lamblia,*** first seen by the grandfather of microbiology, Antonie van Leeuwenhoek, in 1681. It is water borne and can cause epidemics of diarrhea in wilderness campers drinking from streams or lakes. Fecal microscopy with direct fluorescent antibody testing (DFA) is the modern diagnostic test. In my 20th century medical practice I diagnosed it several times with "the string test". In the evening the patient tapes a 3 foot long piece of string to their cheek and swallows the other end, which migrates to the duodenum where many *Giardia* hang out. In the morning I pull out the string, noting bile staining which tells where the string was in duodenum. I squeeze a drop form thar=t section onto a slide and add a coverslip. The microscope reveals oval shaped swimming flagellate protists, and the diagnosis is made. The diarrhea is relieved by treatment with metronidazole.



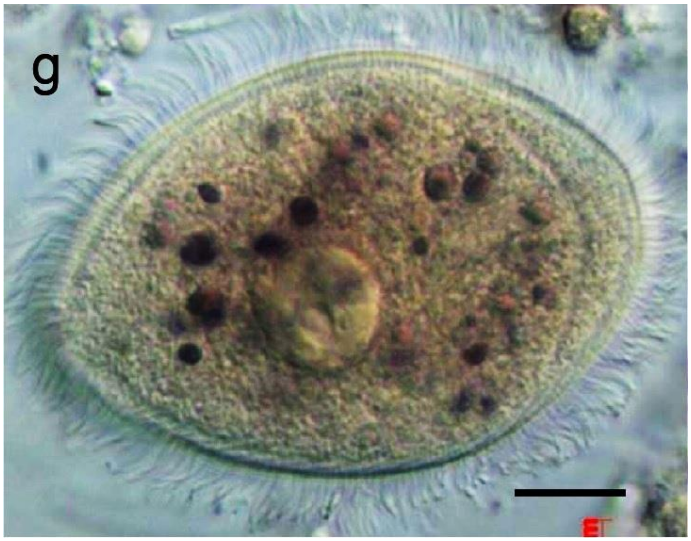
SEM micrograph of the small intestine of gerbil heavily infested with Giardia. Image Wikipedia/ S Erlandsen

Trichomonas is another anaerobic flagellate parasitic protist. Like *Giardia* it lacks functional mitochondria (so no Krebs cycle or electron transport chain) and instead uses hydrogenosomes to ferment pyruvate to carbon dioxide and hydrogen. It can be sexually acquired (but not always) and causes a vaginal discharge. Diagnosis was historically by "sniff test" (fishy odor) and wet mount microscopy of vaginal discharge. I found it fun to find vigorously swimming little beasties on the slide. Like giardiasis, trichomoniasis is easily treated with metronidazole.

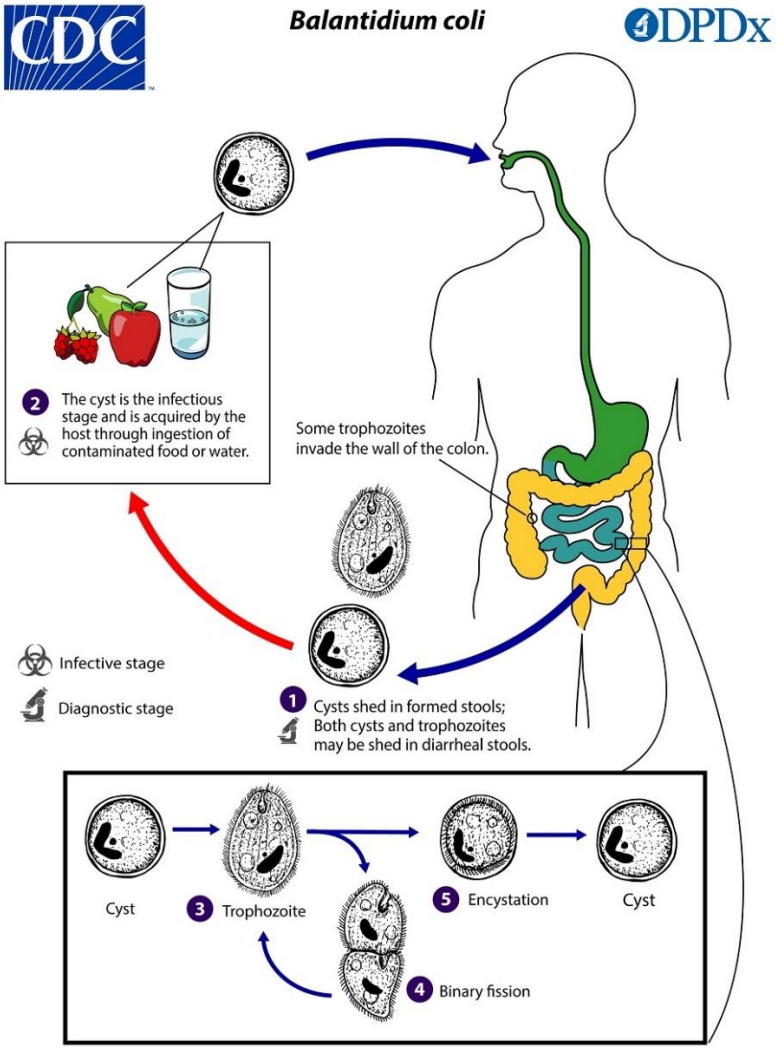


SEM micrograph of Trichomonas. Image David Philips at pharmaceuticaljournal.com

Balantidium coli is the only ciliate human parasite I know much about. (Strange, since ciliates look so complicated and high functioning compared to other pond water protozoans). Rare in the US, *Balantidium* can cause diarrhea in travelers to the tropics and in rare cases abdominal pain and perforated colon (ouch!). Treatable with tetracycline or metronidazole.



Balantidium coli, one of the few ciliates that infest humans. Enteric parasite from monkey host, Tai National Park, Côte d'Ivoire 5 micron bar, *B coli* about 25 µ across image at Wikipedia, original Kouassi et al



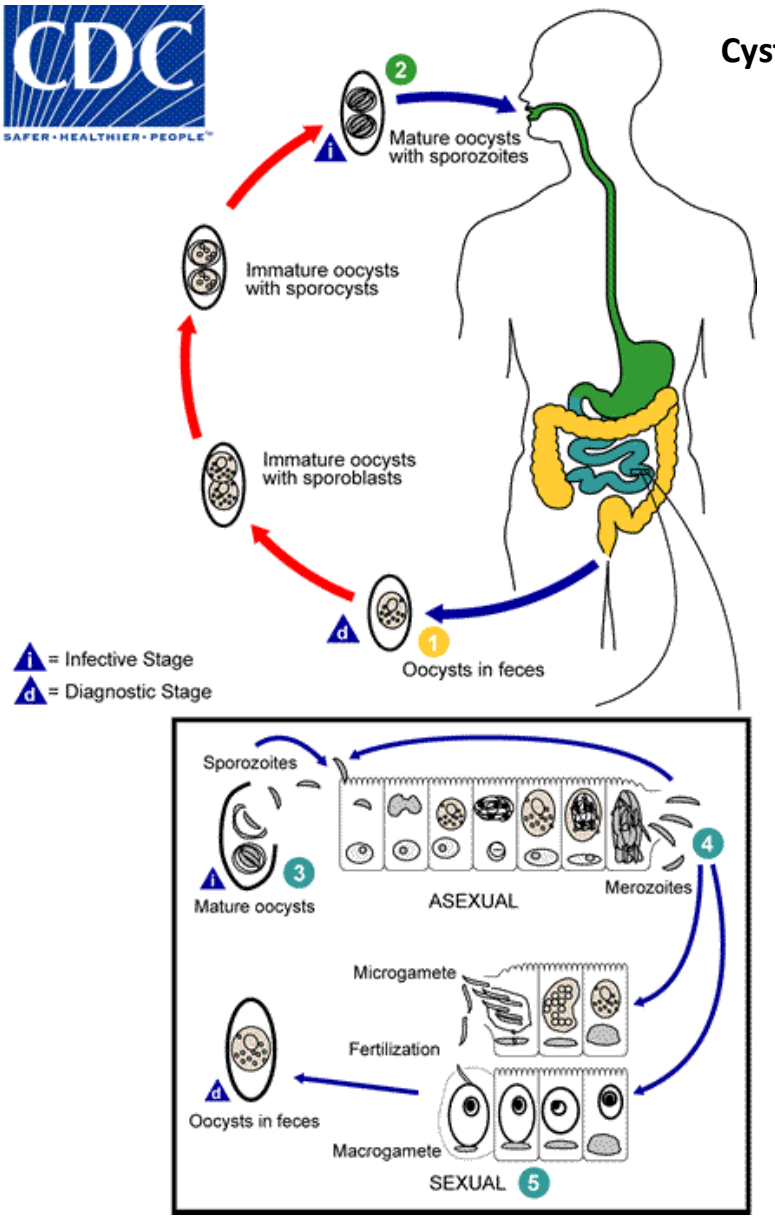
Cysts are the stage responsible for transmission of balantidiasis (1). The host most often acquires the cyst through ingestion of contaminated food or water (2). Following ingestion, excystation occurs in the small intestine, and the trophozoites colonize the large intestine (3). The trophozoites reside in the lumen of the large intestine and appendix of humans and animals, where they replicate by binary fission, during which conjugation may occur (4). Trophozoites undergo encystation to produce infective cysts (5). Some trophozoites invade the wall of the colon and multiply, causing ulcerative pathology in the colon wall. Some return to the lumen and disintegrate. Mature cysts are passed with feces. Pigs are a commonly a reservoir.

Cystoisospora belli (formerly *Isospora belli*) is a coccidian apicomplexan that causes diarrjheal disease in the tropics. ***Sarcocystis hominis*** is another coccidial disease seen mostly in the tropics. Both these parasites often have pig reservoirs.

Acanthamoeba spp. are widespread free living amoebas in water and soil. They can rarely cause serious opportunistic eke (keratitis) skin and brain infections in people.

As was *Pneumocystis*, **microsporidia** including *Enterocytozoon bieneusi, Anncaliia spp, Microsporidium spp, Trachipleistophora, Nosema ocularum, Pleistophora ronneafiei, , Vittaforma corneae,* and *Tubulinosema acridophagus,* were long considered protists parasites but turned out to be in the Fungal Kingdom.

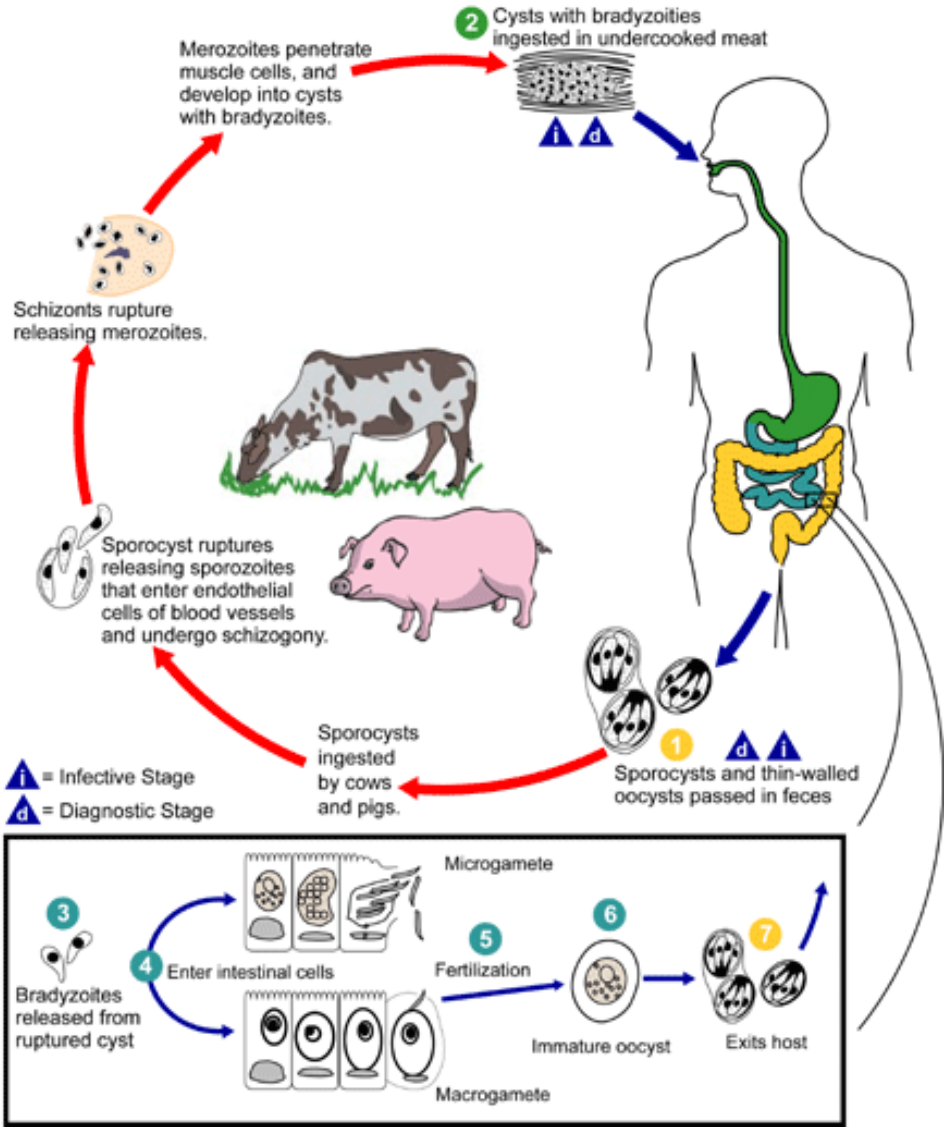
Other protist parasites of humans are rarely seen. New ones will be discovered in the future.



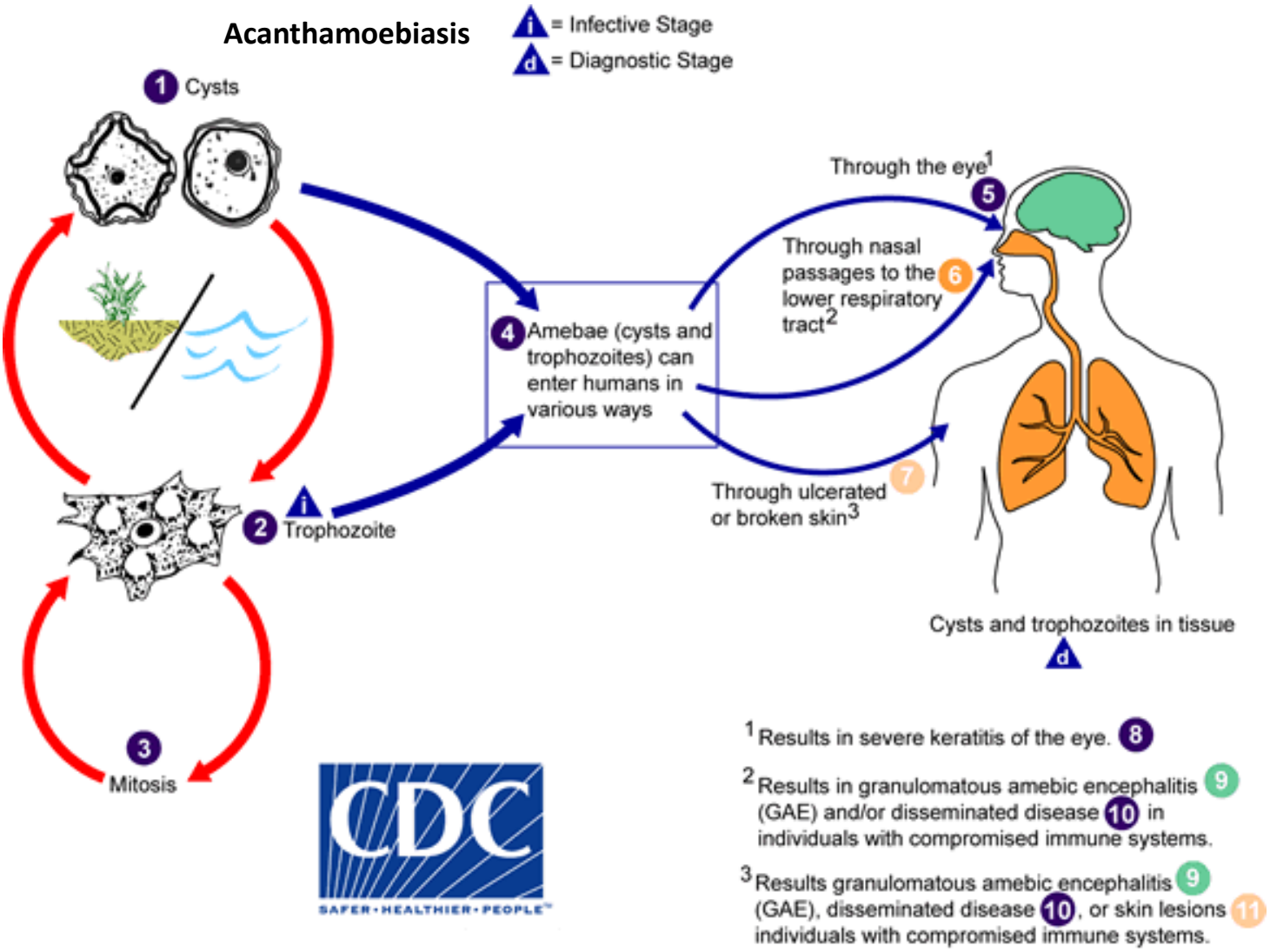
Cystoisosporiasis

At time of excretion, the immature ***Cystoisospora belli*** oocyst contains usually one sporoblast (more rarely two)[1]. In further maturation after excretion, the sporoblast divides in two (the oocyst now contains two sporoblasts); the sporoblasts secrete a cyst wall, thus becoming sporocysts; and the sporocysts divide twice to produce four sporozoites each[2]. Infection occurs by ingestion of sporocysts-containing oocysts: the sporocysts excyst in the small intestine and release their sporozoites, which invade the epithelial cells and initiate schizogony[3]. Upon rupture of the schizonts, the merozoites are released, invade new epithelial cells, and continue the cycle of asexual multiplication[4]. Trophozoites develop into schizonts which contain multiple merozoites. After a minimum of one week, the sexual stage begins with the development of male and female gametocytes[5]. Fertilization results in the development of oocysts, excreted in the stool[1].

Sarcocystosis



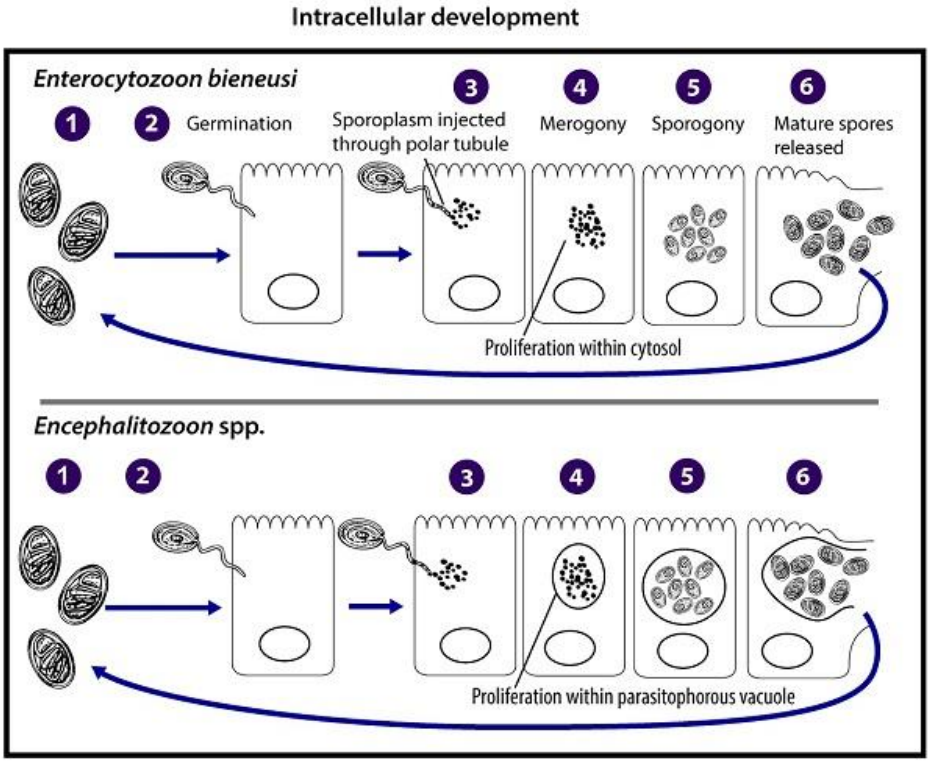
Both sporulated oocysts (containing two sporocysts) and individual sporocysts can be passed in stool 1. Sporocysts contain four sporozoites and a refractile residual body. Sporocysts ingested by the intermediate host (cattle for *S. hominis* and pigs for *S. suihominis*) rupture, releasing sporozoites. Sporozoites enter endothelial cells of blood vessels and undergo schizogony, resulting in first-generation schizonts. Merozoites derived from the first-generation invade small capillaries and blood vessels, becoming second-generation schizonts. The second generation merozoites invade muscle cells and develop into sarcocysts containing bradyzoites, which are the infective stage for the definitive host 2. Humans become infected when they eat undercooked meat containing these sarcocysts. Bradyzoites are released from ruptured cysts in the small intestine 3 and invade the lamina propria of the intestinal epithelium 4. There, they differentiate into macro- and microgametocytes. Fusion of male and female gametes 5 results in the formation of oocysts 6. Oocysts sporulate in the intestinal epithelium and are shed from the host in feces 7. Due to the fragile nature of the oocyst wall, individual sporocysts may also be detected in feces.

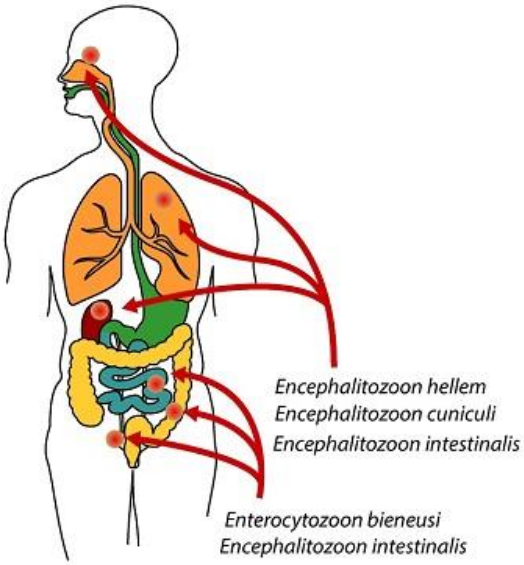


Acanthamoeba spp. , are commonly found in lakes, swimming pools, tap water, and heating and air conditioning units. *Acanthamoeba culbertsoni*, *A. polyphaga*, *A. castellanii*, *A. astronyxis*, *A. hatchetti*, *A. rhysodes*, *A. divionensis*, *A. lugdunensis*, and *A. lenticulata* are implicated in human disease.

Acanthamoeba spp. have been found in soil; fresh, brackish, and sea water; sewage; swimming pools; contact lens equipment; medicinal pools; dental treatment units; dialysis machines; heating, ventilating, and air conditioning systems; mammalian cell cultures; vegetables; human nostrils and throats; and human and animal brain, skin, and lung tissues. Unlike *N. fowleri*, *Acanthamoeba* has only two stages, cysts **(1)** and trophozoites **(2)**, in its life cycle. No flagellated stage exists as part of the life cycle. The trophozoites replicate by mitosis (nuclear membrane does not remain intact) **(3)**. The trophozoites are the infective forms, although both cysts and trophozoites gain entry into the body **(4)** through various means. Entry can occur through the eye **(5)**, the nasal passages to the lower respiratory tract **(6)**, or ulcerated or broken skin **(7)**. When *Acanthamoeba* spp. enters the eye it can cause severe keratitis in otherwise healthy individuals, particularly contact lens users **(8)**. When it enters the respiratory system or through the skin, it can invade the central nervous system by hematogenous dissemination causing granulomatous amebic encephalitis (GAE) **(9)** or disseminated disease **(10)**, or skin lesions **(11)** in individuals with compromised immune systems. *Acanthamoeba* spp. cysts and trophozoites are found in tissue.

Microsporidia



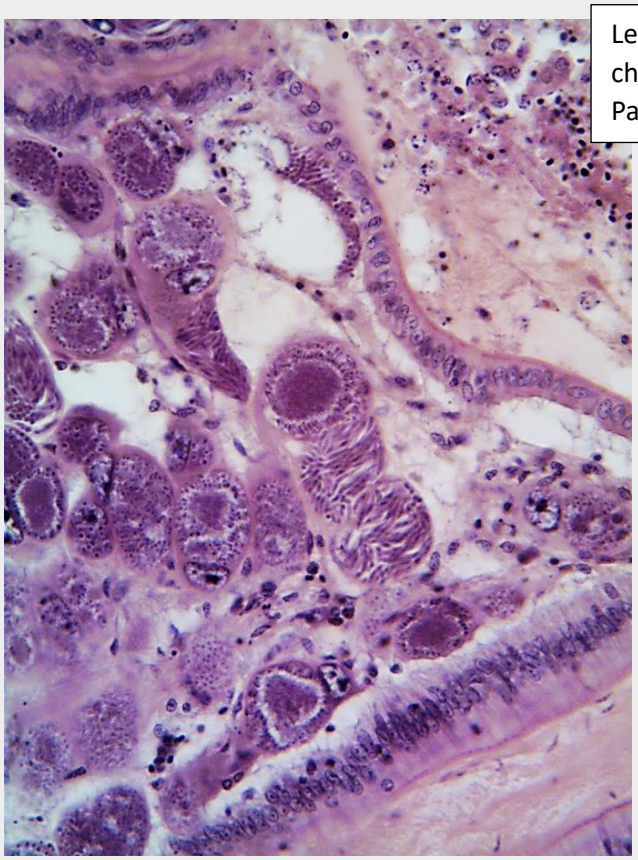


Microsporidia are a group of unicellular intracellular parasites closely related to fungi, although the nature of the relation to the kingdom Fungi is not clear. Historically, they were considered protozoa. Microsporidia are characterized by the production of resistant spores that vary in size (usually 1—4 µm for medically-important species). They possess a unique polar tubule or polar filament and have degenerated mitochondria called mitosomes and lack a Golgi apparatus. More than 1400 species belonging to over 200 genera have been described as parasites infecting a wide range of vertebrate and invertebrate hosts. At least 15 microsporidian species have been identified as human pathogens.

The infective form of microsporidia is the resistant spore, which can persist in the environment for months (1) The spore then germinates, rapidly everting its polar tubule which contacts the eukaryotic host cell membrane (2) . The spore then injects the infective sporoplasm into the host cell through the polar tubule (3) . Inside the cell, the sporoplasm enters the proliferative phase marked by extensive multiplication via merogony (binary fission or multiple fission), creating meronts (4) . The location of this developmental stage within the host cell varies by genus; it can occur either in direct contact with the host cell cytosol (*Enterocytozoon, Nosema*), inside a parasitophorous vacuole of unknown origin (*Encephalitozoon*), in a parasite-secreted envelope (*Pleistophora, Trachipleistophora*), or surrounded by the host cell endoplasmic reticulum (*Endoreticulatus, Vittaforma*) (5) . Following the proliferative phase, meronts undergo sporogony in which the thick spore wall and invasion apparatus develop, creating sporonts and eventually mature spores when all organelles are polarized. When the spores increase in number and completely fill the host cell cytoplasm, the cell membrane is disrupted and spores are released to the surroundings (6) . These free mature spores can infect new cells thus continuing the cycle.

Parasitology and the "Father of Pathology"

Intestinal worms were seen by the ancients and Leuwenhoek saw *Giardia* with his microscope in 1681. But Rudolph Virchow, who discovered cellular pathology, may have been the first to understand a complex parasitic life cycle. His work with *Trichinella* about 1880 earned Virchow the monikers Father of Public Health and Father of Veterinary Pathology. He was the first to show humans can acquire diseases from animals, which he called zoonosis, the term still used today. He was a founder of the "One Health" idea, stating "between animal and human medicine, there is no dividing line—nor should there be".



Left: coccidian protozoan **endoparasite** *Eimeria tenella,* in chick gut, 1948 slide from "J Hopkins School of Hygiene Parasitology", 40X objective, image ~200 microns wide

Ectoparasite: *Haematopinus suis,* hog louse, ~4mm long insect, modern slide by L Bircham, 4X objective, stitched

Parasite Basics

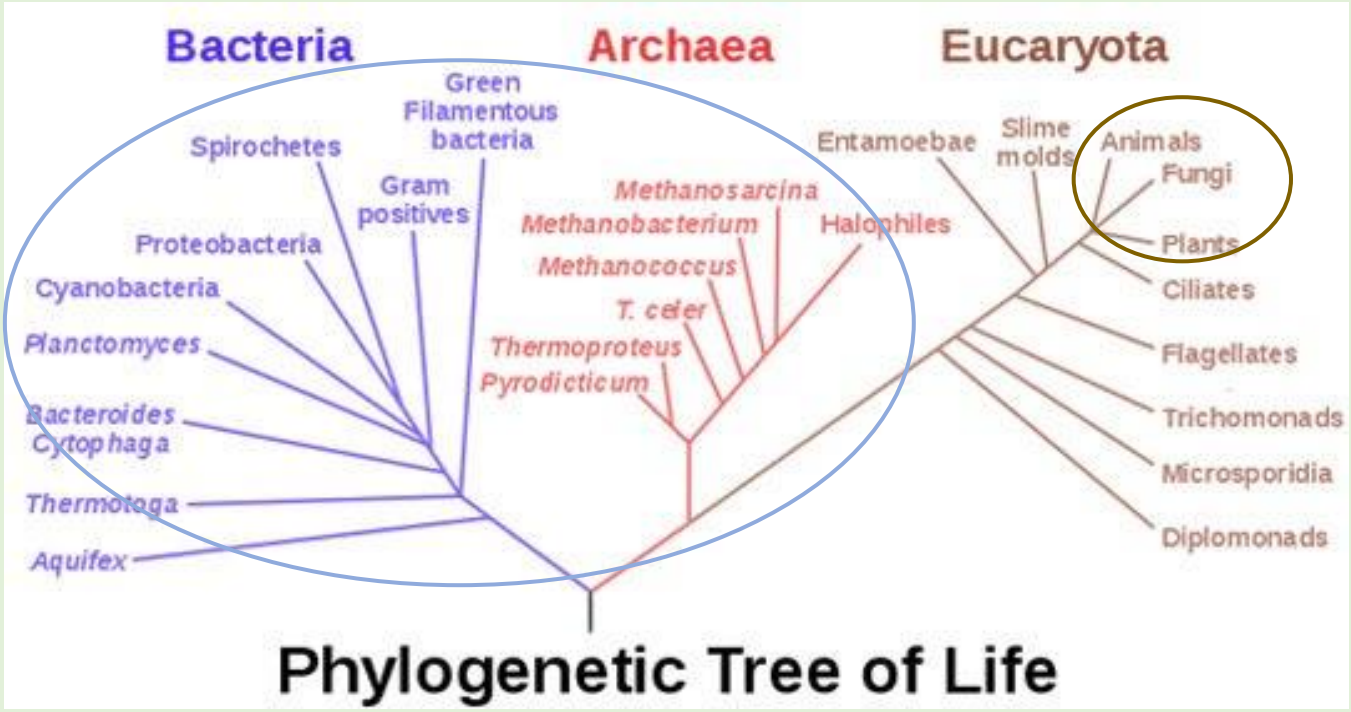
Life adapts to everywhere it can survive, including inside and outside the bodies of other organisms. Evolution thus produces endoparasites (like intestinal protists) and ectoparasites (like lice). Some parasites give a false appearance of being experts in anatomy, often travelling between different organs and different animal species at different life stages. Most wild animals carry parasites, and most humans used to have them, although most individuals are not harmed. In poor and tropical areas many people are still harmed and killed, including over 600,000 annual deaths from malaria. Some members of many different branches of life have become parasitic, including protozoans, flatworms, roundworms, arthropods (ticks, some crustaceans and insects). Ectoparasites can act as vectors to spread the bacteria and viruses that cause Lyme disease, viral encephalitis, typhus and plague. Parasites can also harm people by heavy infestation or complications. Nearly half of humans may have parasites, most commonly helminths (intestinal worms) although they don't make most of them sick. The burden of parasites is highest in the tropical and poor areas of the world. We need to continue life saving efforts to control malaria, worms, and other neglected tropical diseases. Still, most of you reading this need not fear parasites. Anxiety about parasites is far more common than parasite disease in the developed world. Parasites outnumber non-parasites and are part of the balance of nature.

Basics of Evolution

In 1859 evolution was a new theory, laid out in clear, logical and humble detail by Charles Darwin in his book *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. Darwin had gathered thousands of his own and others' observations about the varieties of life, but at that time known fossils were few and the mechanisms of inheritance were unknown (actually Czech monk Gregor Mendel discovered the laws of genetics in pea plants in the 1860's but no one knew of it). Evolution is now a proven fact: millions of new bits of evidence show life slowly evolved into a myriad of different forms on planet earth. Evolution remains also a powerful and changing (all science is subject to modification by additional information) central theory in biology that makes sense of new evidence from biologic and medical research. I see evolution in action in the hospital as bacteria evolve to be resistant to commonly used antibiotics, and the COVID-19 virus evolved to be more docile.

Darwin's theory is amazing in that upon careful reflection it seems inevitable. People create different breeds of dogs, pigeons and other domesticated animals through selective breeding. Darwin showed nature does much the same thing. It can be observed that all organisms come from the reproduction of previous organisms, that there are differences between individuals in a group (i.e. some are faster or slower) and that parents reproduce imperfect copies of themselves (we now know genes are shuffled and sometimes mutate). Darwin noticed that there is a struggle in nature for organisms to survive and produce offspring. In real circumstances (gazelles being chased by cheetahs for example) survival is not just random but favors certain bodily abilities (i.e. running faster) so the next generation comes from selected (faster) surviving parents and so each generation becomes slightly adapted in particular ways. Over deep time (the earth is now known to be about 4.55 billion years old) a single cell became all the amazing life on the planet today, from bacteria to *Paramecium* to grass to mushrooms to worms to you. Every living thing becomes fined tuned for its way of life, making life look like it was designed. Yet there was no designer, just the logical results of how natural life processes (based on chemistry, physics and math) worked out.

Most life is prokaryotic: very tiny, no nucleus, the Bacteria and Archaea, on left. All Metazoans (multicellular organisms) are in the small circle on right. Between are Protists, bigger than bacteria, but mostly microscopic, nucleated single celled life. Carl Woese used ribosome RNA data for the tree.



Phylogenetic Tree of Life

Privileged to be parasite-free

Most people reading this article don't need to worry much about parasites personally, as they are probably living in privileged times and places.

Since the origin of *Homo sapiens* in Africa about 300,000 years ago, most people harbored potentially harmful parasites in and on their bodies. Lice and intestinal worms were nearly universal. Then a combination of industrial and social revolutions starting almost 300 years ago greatly improved health and comfort for most people today. If you are reading this then it is likely you have safe water and food supplies, shoes, indoor plumbing, window screens, floor boards and a solid roof, all diminishing the chances of worms burrowing into your feet or being swallowing in contaminated water, and of exposures to mosquitoes and reduviid bugs. Good living standards, scientific research and public health measures have probably eliminated the most significant human parasites from your part of the world.

Great strides continue to made fighting parasites and poverty in the world. The WHO estimates intestinal worm infestations dropped from 60% to 25% of all humans so far in this century. Global median annual income more than doubled between 2000 and 2019 from $1325 to $2759 (with the mean about $12000 in 2019, and yours is likely higher). Global life expectancy increased 6+ years between 2000 and 2019 from 66.8 to 73.4 years average (even as life expectancy in the US began to decline during the same timeframe).

But the global gains in well being are far from being evenly distributed. Severe inequalities make averages (means) deceptive when almost half of the world's total wealth is held by the top 1%, and the bottom half divvies up just 0.74%. Most people are poor and live in the "majority world" (a newer term for what we also call the third or developing world) and they are still lacking in money, health and governance. Without all the luxuries we take for granted, the parasites they suffer from are just a small part of the unfair miseries (wars, famines, and imprisonment without trial if they complain about the dictator) borne by the powerless majority. 4.3 billion people live in 95 countries under authoritarian regimes today.

Its normal to feel bad about this sorry state of affairs. For some readers the best way to worry about parasites is by helping out people with parasites who have little way to help themselves. You might consider a charitable donation to Oxfam, Against Malaria Foundation or Deworm the World.

It's also perfectly fine to feel grateful for the cosmic lottery you've won. Average *Micscape* readers are often males in rich European or North American countries. You likely know a European language and have computers and microscopes. You may be privileged by your race, gender, citizenship (in a former colonial power), and your political and economic systems. You were likely born in a country with the full modern liberal package: democracy, good schools, free speech and press, universal health care (not in US) and private property. You weren't born in medieval times, living an average 30 miserable years with lice and worms. And be extra thankful you weren't reincarnated this time around into a caterpillar being hormonally manipulated and eaten alive from the inside by dozens of parasitoid wasp larvae.

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Micscape is a high quality website hosted in the UK and made great by contributors from around the globe. *Micscape* Magazine always has lots of good information for amateur microscopists wanting to learn more about how to do it yourself.

For 2024 I offer *Micscape's* readers a series of articles about parasites, illustrated in part from my slide collection.

I am incurably curious about parasites and keep thinking I should know more. The internet makes it easy to learn more, so my articles are always longer than I intended at first.

Just look at the interesting pictures if you want. Don't be freaked out by parasites. They are everywhere but seldom cause harm in the developed world. I celebrate our global north privilege again on page 55.

Some people are real experts and know much more than I do on these subjects. I would be pleased to have any mistakes or misunderstandings corrected.

I am Ed Ward in the state of Minnesota, USA.
Your comments are always welcomed, my email is eward1897 AT gmail DOT com

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