Trypanosoma brucei
(protist: flagellate)

Overview

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). Flagellates are protists that swim using one or more flagella (undulipodia); each arising from a small centriole (basal body, kinetosome) and having a microtubular axoneme core (2+9 configuration). Rather than forming a monophyletic group, flagellates are divided into several disparate groups: metamonads (amitochondriate flagellates), heteroloboseans (amoeboflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). Most kinetoplastids are parasitic in vertebrate or invertebrate hosts (some in plants) whereas the remainder are free-living aquatic organisms. All species are characterized by the possession of extranuclear DNA in the form of a kinetoplast, a unique structure formed by massed DNA (circles or lattice) within the single large mitochondrion near the flagellar basal body. The flagellates reproduce by longitudinal binary fission and parasitic species may have simple monoxenous (one-host) or more complicated heteroxenous (two-host) life cycles involving different developmental stages. Trypanosomes have a single flagellum and they form four main developmental stages: trypomastigotes (with a posterior kinetoplast and an emergent flagellum forming a long undulating membrane); epimastigotes (with an anterior kinetoplast and an emergent flagellum forming a short undulating membrane); promastigotes (with an anterior kinetoplast and a short emergent flagellum, but no undulating membrane); and amastigotes (with a kinetoplast but no emergent flagellum or undulating membrane). Many trypanosome species are parasitic only in insects whereas others are transmitted by invertebrate vectors to a wide range of vertebrate hosts. Three main groups infect the blood and/or tissues of humans and animals causing severe clinical diseases: including the cyclic salivarian trypanosomes which undergo anterior station (foregut) development in the insect vector and are transmitted via saliva to the blood of vertebrate hosts (e.g. tsetse flies transmit *T. brucei* which causes sleeping sickness in humans and nagana in cattle).

Classification:

Domain:	Eukaryota (membrane-bound nucleus)
Supergroup:	Excavata (with conspicuous ventral feeding groove)
Group:	Discoba (diverse group supported robustly by molecular studies)
Phylum:	Euglenozoa (flagella inserted in anterior pocket, some heterotrophs, some autotrophs (with chloroplasts))
Class:	Kinetoplastea (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)
Subclass:	Metakinetoplastina (large polyphyletic group supported by molecular studies)
Order:	Trypanosomatida (parasitic, single anterior flagellum, often forming undulating membrane)
Family:	Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)
Genus:	*Trypanosoma* (vector-borne haemoparasites)
Species:	*T. brucei* (causes sleeping sickness in humans and nagana in cattle)

Parasite biodiversity and host range: Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Flagellated species have one or more flagella with an internal microtubular core (in a characteristic 2+9 configuration comprising 2 single central microtubules and 9 peripheral doublets) anchored to a submembranous protein structure (known variously as a centriole, basal body, kinetosome or blepharoplast). Many types of flagellated cells have been described and recent phylogenetic studies have classified them into several disparate groups: including the metamonads (amitochondriate flagellates), heteroloboseans (amoeboflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). While most flagellated protists are free-living organisms swimming and feeding in aquatic environments, representatives of several groups have developed symbiotic relationships with various hosts; some being endoparasitic in vertebrates (notably anaerobic metamonads in tubular organs, and heterotrophic euglenozoans occurring in blood or tissues), and some being parasitic in invertebrates (alveolates in crustacean tissues) (representatives tabulated below).

Higher taxonomy	Class or order	Family	Genera	Hosts (tissues)	Trans-mission*
Supergroup: Excavata (with conspicuous ventral feeding groove)					
Group: Metamonad (amitochondriate flagellates with karyomastigonts)					
Phylum: Fornicata (diplomonads)	Order: Diplomonadida (1-2 karyomastigonts)	Hexamitidae (2 karyomastigonts with binary axial symmetry)	*Giardia*	vertebrates (gut)	direct (f-o)
			Hexamita *Spironucleus*	vertebrates (tissues)	direct (f-o, w)
Phylum: Parabasalia (with parabasal body)	Order: Trichomonadida (3-5 anterior flagella plus recurrent flagellum)	Monocercomonadidae (costa absent, most without undulating membrane)	*Histomonas*	birds (gut, liver)	direct (f-o)
			Dientamoeba	vertebrates (gut)	direct (f-o)
		Trichomonadidae (stout axostyle, costa, undulating membrane)	*Trichomonas*	vertebrates (urogenital tract, gut)	direct (f-o, v)
		Cochlosomatidae (anterior adhesive disc)	*Cochlosoma*	birds (gut)	direct (f-o)
Group: Discoba (diverse group supported robustly by molecular studies)					
Phylum: Euglenozoa (flagella inserted in anterior pocket, heterotrophs, autotrophs)	Class: Kinetoplastea (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)	Ichthyobodonidae (flagellar pocket continues as groove)	*Ichthyobodo* (= *Costia*)	fish (gills, skin)	direct (w)
		Parabodonidae (epizoic or endozoic)	*Cryptobia*	fish (gills, skin)	direct (w)
			Trypanoplasma	fish (blood)	indirect (v-b)
		Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)	*Trypanosoma*	vertebrates (blood, tissues)	indirect (v-b)
			Leishmania	vertebrates (blood, tissues)	indirect (v-b)
Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria) (3 groups unified by molecular studies)					
Group: Alveolata (with cortical alveoli)					
Phylum: Dinoflagellata (with unique mesokaryotic nuclei)	Order: Blastodiniales (uninucleate trophonts with chloroplasts)	Oodiniaceae (trophont with rhizoid-like invasive organelle)	*Amyloodinium* *Crepidoodinium* *Piscinoodinium*	fish (skin)	direct (w)
	Order: Syndiniales (multinucleate plasmodial trophonts)	Syndiniaceae (without chloroplasts)	*Haematodinium* *Ichthyodinium*	crustaceans, fish (tissues)	direct (w)
Phylum: Perkinsozoa (parasitic)	Order: Perkinsorida (released trophonts form biflagellated zoospores)	Perkinsidae (incomplete conoid)	*Perkinsus*	gastropods, bivalves (tissues)	direct (w)

*f-o = faecal-oral transmission; v-b = vector-borne transmission, w = water-borne transmission; v = venereal transmission

Euglenozoans comprise a large group of excavates (with ventral feeding groove), most with 1-2 flagella inserted into an anterior pocket. Many species are free-living aquatic autotrophs possessing chloroplasts while others are free-living or symbiotic heterotrophs feeding on solutes, particles and even other organisms. Kinetoplastids are characterised by the possession of a kinetoplast (containing mitochondrial DNA separate from nuclear DNA), a flagellar pocket, basal bodies with three microtubular roots and paraxonemal (paraxial or paraflagellar) rods, and asexual multiplication by longitudinal binary fission. The unique kinetoplast is formed by massed DNA (circles or lattice) usually closely associated with the flagellar basal body (eukinetoplastic) although some species may be polykinetoplastic (with several kinetoplasts) or pankinetoplastic (irregular kDNA) and some mutants even dyskinetoplastic (without a kinetoplast). Two major kinetoplastid groups are recognized: bodonids with two flagella (most being free-living bactivores in aquatic/terrestrial habitats); and trypanosomes with a single flagellum (most being parasites of animals or plants with monoxenous or dixenous life-cycles). Different kinetoplastid assemblages exhibit increasing morphological/ultrastructural complexity in their cellular organization thought to reflect evolutionary grades or clines. Amastigotes are simple non-flagellated cells, choano-, pro- and opistho-mastigotes are flagellated cells with elongate flagella, while epi- and trypo-mastigotes are flagellated cells with undulating membranes. Most kinetoplastids have amastigote and promastigote developmental stages but monoxenous parasites of insects (e.g. *Crithidia*, *Herpetomonas*) do not have more elaborate forms whereas dixenous parasites of plants or animals with invertebrate vectors (e.g. *Trypanosoma, Leishmania*) do have more morphologically complex forms such as epimastigotes and trypomastigotes.

Traditional classification	Molecular classification	Genera	No. spp.	Vertebrate hosts	Transmission (vectors)
F: Trypanosomatidae	SC: Metakinetoplastina F: Trypanosomatidae	*Trypanosoma*	537	mammals, reptiles, frogs, birds, fish	indirect (arthropods, leeches)
		Leishmania	53	mammals, lizards	indirect (sand flies)
F: Bodonidae	SC: Metakinetoplastina F: Parabodonidae	*Cryptobia, Trypanoplasma*	79	fish	direct or indirect (leeches)
	SC: Prokinetoplastina F: Ichthyobodonidae	*Ichthyobodo* (*Costia*)	5	fish	direct

Conventional taxonomic classification systems divide the kinetoplastids into 2 groups: the free-living bi-flagellated Bodonina; and the parasitic uni-flagellated Trypanosomatina. Over 600 species have been described on the basis of multiple phenotypic characters (host occurrence, geographic distribution, vectors, transmission cycles, morphology, development, pathogenicity, culture requirements, etc.). Modern molecular characterization studies, however, have shown that many traditional groups are polyphyletic and composed of numerous clades. Contemporary phylogenetic classifications recognize 2 main lineages: the Prokinetoplastina represented by 2 diverse genera (*Ichthyobodo* biflagellates ectoparasitic on freshwater and marine fishes, and *Perkinsela* (= *Perkinsiella*) aflagellates endosymbiotic (as parasomes or parasome-like organisms (PLOs)) in amoeba *Paramoeba* and *Neoparamoeba*); and the Metakinetoplastina containing 4 groups, including free-living aquatic eu-bodonids (with one genus *Bodo*), free-living neo-bodonids (with 10 genera, including *Rhynchomonas*), free-living or commensal/parasitic para-bodonids (with 5 genera, including *Cryptobia, Trypanoplasma*), and the parasitic trypanosomatids (containing some 39 genera, including *Trypanosoma* and *Leishmania*).

Trypanosomatids are dixenous (2-host) parasites with indirect transmission cycles between vertebrates and invertebrate vectors. *Trypanosoma* spp. form trypo- and/or a-mastigote stages in the blood/tissues of vertebrate hosts, and epi- or pro-mastigote stages in invertebrate haematophagous vectors. Infections have been found in a range of vertebrate species (mammals, birds, reptiles, amphibians and fish) with many different types of haematophagous invertebrates (leeches, bugs, flies, fleas) implicated as vectors or paratenic hosts. The species found in mammals have been grouped into two Sections (convenient groups without formal taxonomic rank) primarily on the basis of their developmental cycles in their vectors and their modes of transmission. Each Section contains several subgenera, as follows:

Section: Stercoraria (posterior station development in vector, contaminative transmission)

- *T.* (*Megatrypanum*), large trypanosomes with kinetoplast close to nucleus;
- *T.* (*Herpetosoma*), medium trypanosomes with subterminal kinetoplast;
- *T.* (*Schizotrypanum*), small C-shaped trypanosomes with voluminous terminal kinetoplast;

Section: Salivaria (anterior station development in vector, inoculative (some mechanical) transmission);

- *T.* (*Duttonella*), former *vivax*-group, monomorphic forms with large terminal kinetoplast;
- *T.* (*Nannomonas*), former *congolense*-group, small forms with medium marginal kinetoplast;
- *T.* (*Trypanozoon*), former *brucei*-group, pleomorphic forms with small subterminal kinetoplast;
- *T.* (*Pycnomonas*), former *suis*-subgroup, stout monomorphic forms with small subterminal kinetoplast.

Recent molecular phylogenetic studies have validated the separation of mammalian salivarian and stercorarian trypanosomes, but indicated complex placements for trypanosomes from non-mammalian hosts, despite earlier suggestions that they be assigned to separate subgenera (*Trypanomorpha* for those in birds, *Trypanosoma* for those in amphibians, and *Haematomonas* for those in fish). Many clades have been identified, most associated with particular vertebrate or invertebrate hosts, or both, suggesting that 'host-fitting' rather than 'co-speciation' has been the principal mechanism for trypanosome evolution. Comparative studies have found many differences between trypanosomes of aquatic and terrestrial hosts, with several clades found for species in freshwater fishes, marine fishes, amphibians, tortoises and platypuses (all thought to be transmitted by leech vectors). *T. brucei* belongs to the salivarian trypanosomes which exhibit continuous multiplication in the mammalian host and develop in the anterior station in tsetse fly vectors with inoculative transmission (except for *T. evansi* and *T. equiperdum* which are transmitted mechanically by biting flies or by contact). Four salivarian subgenera are recognized: *Duttonella* comprising monomorphic trypanosomes with a large kinetoplast (*T. vivax, T. uniforme*); *Nannomonas* comprising small trypanosomes with a medium-sized kinetoplast (e.g. *T. congolense, T. simiae*); *Pycnomonas* comprising thick monomorphic forms with a small kinetoplast (*T. suis*); and *Trypanozoon* comprising pleomorphic (stumpy to slender) forms with a small kinetoplast (*T. brucei, T. evansi, T. equiperdum*). Species belonging to the former two subgenera develop in the proboscis or midgut of their vectors while those of the latter two subgenera undergo development in the midgut and salivary glands (except for *T. evansi* and *T. equiperdum* which are transmitted mechanically). Three closely-related subspecies of *T. brucei* are found: *T. b. brucei* which is primarily parasitic in native antelopes and other wild ruminants (asymptomatic carriers, trypanotolerant) but infects introduced domestic animals; *T. b. rhodesiense* which causes acute

disease in humans in East Africa; and *T. b. gambiense* which produces a much more chronic disease in humans in West Africa. Other species are also found in ruminants, horses and pigs. Salivarian trypanosomes are confined to tropical Africa, corresponding in distribution with their tsetse fly vectors. Of the ~30 *Glossina* species/subspecies, eight are major vectors of sleeping sickness. The trypanosomes transmitted mechanically by biting flies or by coitus have a more global distribution.

***Trypanosoma* species**	**Mastigote length (µm)**	**Hosts**	**Disease**	**Vectors**	**Distribution**
CYCLIC TRANSMISSION (development within vector)					
SALIVARIA (free flagellum present or absent, kinetoplast terminal or subterminal, posterior end blunt, reproduction in mammalian host continuous, taking place in trypomastigote stages, development in vector (with formation of metatrypanosomes) in anterior station (except in mechanical inoculators), transmission inoculative (except for *T. equiperdum*)					
Subgenus: ***Duttonella*** (trypanosomes of former *vivax*-group, monomorphic forms with free flagellum, rounded posterior end, large kinetoplast usually terminal, development in vector (*Glossina*) in proboscis)					
T. (*D.*) *uniforme*	12-20	Artiodactyla: bovid (cattle, African buffalo, sheep, goat, waterbuck, harnessed bushbuck, sitatunga), giraffid (giraffe)	variable pathogenicity	Diptera: glossinid (*Glossina fuscipes, palpalis*)	East and Central Africa
T. (*D.*) *vivax* [type species] [incl. subspp. *vivax, viennei*] (syn. *T. angolense, bovis, caprae, cazalboui*)	18-31	Artiodactyla: bovid (cattle, African buffalo, sheep, goat, impala, hartebeest, western hartebeest, Lichtenstein's hartebeest, blue wildebeest, topi, common eland, nyala, southern reedbuck, Bohor reedbuck, common duiker, red forest duiker, yellow-backed duiker, Thomson's gazelle, roan antelope, sable antelope, waterbuck, puku, suni, oribi, steenbok, harnessed bushbuck, sitatunga, greater kudu), camelid (camel), suid (warthog); giraffid (giraffe, reticulated giraffe), cervid (white-tailed deer); Perissodactyla: equid (horse, donkey, mule, plains zebra); Carnivora: canid (dog), felid (lion); Rodentia: murid (mouse); Primates: hominid (human)	nagana, souma	Diptera: glossinid (*Glossina austeni, brevipalpis, fuscipes, longipalpis, morsitans, pallidipes, palpalis, swynnertoni, tachinoides, vanhoofi*) plus mechanical transmission by Diptera: tabanid (*Tabanus, Stomoxys* spp.) in New World	tropical Africa
Subgenus: ***Nannomonas*** (trypanosomes of former *congolense*-group, small forms without free flagellum, medium kinetoplast typically marginal, development in vector (*Glossina*) in midgut and proboscis)					
T. (*N.*) *congolense* [type species] (syn. T. *berghei*, *celii*, *dimorphon*, *frobenius*, *mossoense, multiforme* p.p., *nanum*, *pecorum*, *ruandae*, *somaliense*, *urundiense*)	8-24	Artiodactyla: bovid (cattle, African buffalo, goat, sheep, impala, hartebeest, Lichtenstein's hartebeest, common duiker, red forest duiker, yellow-backed duiker, blue wildebeest, topi, Grant's gazelle, Thomson's gazelle, roan antelope, sable antelope, waterbuck, kob, puku, dik-dik, suni, klipspringer, steenbok, southern reedbuck, common eland, nyala, sitatunga, greater kudu), camelid (dromedary), giraffid (giraffe, reticulated giraffe), suid (pig, warthog, red river hog, bushpig); Perissodactyla (horse, donkey, mule, plains zebra);	nagana	Diptera: glossinid (*Glossina austeni, brevipalpis, fuscipes, longipalpis, morsitans, pallidipes, palpalis, swynnertoni, tachinoides, vanhoofi*)	tropical Africa

		Proboscidea: elephantid (African elephant); Carnivora: canid (dog, hyena), felid (lion); Lagomorpha: leporid (rabbit); Rodentia: murid (rat)			
T. (*N.*) *godfreyi*	9-22	Artiodactyla: suid (warthog)	nr	Diptera: glossinid (*Glossina morsitans, pallidipes*)	West Africa
T. (*N.*) *montgomeryi*	10-21	Artiodactyla: bovid (cattle, sheep); suid (pig); Carnivora: canid (dog); Perissodactyla: equid (horse)	nr	Diptera: glossinid (*Glossina morsitans*)	Central Africa
T. (*N.*) *simiae* (syn. *T. ignotum, porci, rhodhaini*)	12-26	Artiodactyla: suid (pig, desert warthog), bovid (cattle); camelid (dromedary); Perissodactyla: equid (horse)	acute porcine trypanosomiasis	Diptera: glossinid (*Glossina austeni, brevipalpis, fusca, fuscipes, fuscipleuris, longipalpis, morsitans, pallidipes. palpalis, tabaniformis, tachinoides, vanhoofi*) plus mechanical transmission by biting flies (*Haematopota*, *Stomoxys*)	tropical Africa
Subgenus: ***Trypanozoon*** (trypanosomes of former *brucei*-group including *brucei*- and *evansi*-subgroups represented by pleomorphic forms (slender, intermediate, stumpy) with or without free flagellum, small kinetoplast subterminal, development in vector (*Glossina*) in midgut and salivary glands, but other species transmitted mechanically without development in vectors (*T. evansi*) or by contact between hosts (*T. equiperdum*))					
T. (*T.*) *brucei brucei* [type species] (syn. *T. anceps, dukei, multiforme p.p., pecaudi, togolense, ugandae*)	11-39	Artiodactyla: bovid (various antelope, cattle, sheep, goat), suid (pig, warthog); Perissodactyla: equid (horse, donkey); Proboscidea: elephantid (African elephant); Carnivora: canid (dog, hyena), felid (cat, lion)	nagana	savanna Diptera: glossinid (*Glossina brevipalpis, fuscipes, longipalpis, morsitans, pallidipes, palpalis, swynnertoni, tachinoides*)	tropical Africa
T. (*T.*) *brucei gambiense* (syn. *T. ugandense, castellanii, hominis, fordii, nepveui, tullochii, rovumense, nigeriense*)	12-35	Primates: hominid (human), cercopithecid (mangabey, white-nosed monkey); Artiodactyla: bovid (cattle, African buffalo, sheep, goat, impala, common wildebeest, black wildebeest, hartebeest, Lichtenstein's hartebeest, topi, roan antelope, sable antelope, waterbuck, puku, oribi, steenbok, southern reedbuck, common duiker, common eland, harnessed bushbuck, sitatunga, greater kudu, kob), camelid (dromedary), suid (pig, desert warthog); Perissodactyla: equid (horse, donkey, mule, plains zebra); Carnivora: canid (dog), hyaenid (spotted hyena), felid (cat, lion), viverrid (genet, civet); Rodentia: murid (rat),	sleeping sickness (chronic form)	riverine/forest Diptera: glossinid (*Glossina fuscipes, morsitans, palpalis, swynnertoni tachinoides*)	West and Central Africa

		caviid (guinea pig), hystricid (porcupine)			
T. (*T.*) *brucei rhodesiense*	12-42	Primates: hominid (humans); Artiodactyla: bovid (cattle, bushbuck, waterbuck, hartebeest, antelope), suid (warthog); Carnivora: canid (dog, hyena), felid (lion); Rodentia: murid (rats)	sleeping sickness (acute form)	savanna Diptera: glossinid (*Glossina morsitans, pallidipes, swynnertoni*)	East and Central Africa
Subgenus: ***Pycnomonas*** (trypanosomes of former *brucei*-group, *suis*-subgroup, represented by stout monomorphic forms with short free flagellum and small subterminal kinetoplast, development in vector (*Glossina*) in midgut and salivary glands)					
T. (*P.*) *suis* [type species]	8-19	Artiodactyla: suid (pig, warthog)	variable pathogenicity	Diptera: glossinid (*Glossina brevipalpis, vanhoofi*)	tropical Africa
Unplaced (but forming a distinct Australian clade)					
T. copemani	30-45	Diprotodontia: phascolarctid (koala), vombatid (wombat), potoroid (potoroo, woylie), macropodid (quokka), peramelid (bandicoot), dasyurid (quoll), phalangerid (brush-tailed possum)	low	Ixodida: ixodid? (*Ixodes australiensis*)	Australia
T. gilletti	42?	Diprotodontia: phascolarctid (koala)	low		Australia
T. vegrandis	7-11	Diprotodontia: phascolarctid (koala), potoroid (woylie), macropodid (grey kangaroo, tammar wallaby), dasyurid (quoll), peramelid (bandicoot); Chiroptera: vespertilionid (wattled bat, long-eared bat), pteropodid (flying fox)			Australia

Parasite morphology: The parasite forms trypomastigotes in vertebrate hosts and epimastigotes in the insect vector. The trypomastigotes (with posterior kinetoplast and emergent flagellum forming an undulating membrane) of the salivarian trypanosome species are pleomorphic in size ranging from 8-42 µm in length by 1-3 µm in width. They occur as elongate slender dividing forms (with long free flagellum) or stumpy non-dividing infective (metacyclic) forms (with no free flagellum). The epimastigotes (with anterior kinetoplast and short undulating membrane) are also variable in size ranging from 10-35 µm in length by 1-3 µm in width.

Site of infection: The parasites undergo anterior station development in their invertebrate vectors and salivarian transmission (via vector saliva) to vertebrates where trypomastigotes are found extracellularly in the blood and lymph (including lymph nodes and spleen) although they may invade the central nervous system and other tissues. Some 13 salivarian species have been described in 82 mammalian species belonging to 27 families in 9 orders (artiodactylans, perissodactylans, proboscideans, carnivores, lagomorphs, rodents, marsupials, chiropterans, and primates). Their dipteran vectors have so far included 13 species of tsetse flies in which parasite development occurred.

Pathogenesis: The disease in humans is known as Old World (African) trypanosomiasis or human African trypanosomiasis (HAT). Although there are many regional common names given depending on the parasite subspecies and hosts involved, the disease is often called sleeping sickness in humans, and nagana in animals. Parasites injected into the host by the insect vector first cause an inflammatory reaction characterized by a localized tender reddish swelling (known as a chancre). Trypanosomes then multiply in the plasma and interstitial fluid causing acute to subacute febrile illness. *T. b. gambiense* is responsible for most infections in humans (~90%) causing Gambian or West African sleeping sickness, while *T. b. rhodesiense* is generally considered a zoonotic disease with humans as accidental hosts but developing Rhodesian or East African sleeping sickness. *T. b. rhodesiense* infections in humans usually cause acute systemic disease over several weeks with haemolymphatic involvement, swollen lymph nodes, fever and rapid weight loss. *T. b. gambiense* usually causes chronic disease over several months with neurological involvement, meningoencephalitis, lethargy and coma (hence sleeping sickness). A classic sign of *T. b. gambiense* infection is the enlargement of the cervical lymph glands at the back of the neck (known as Winterbottom's sign). Parasite development occurs in cyclic waves moderated by host immune responses. Trypanosomes have a glycoprotein coat on the outer surface of the cell membrane which is highly antigenic and leads to the production of host antibodies which act, together with complement, to lyse parasites. Trypanosomes, however, repeatedly change the molecular arrangement of the coat so some individuals avoid immune destruction and divide to produce a new wave of infection. This antigenic variation is under genetic control and while synthesis of successive variant surface glycoproteins does not occur in a fixed sequence, it is not entirely random. The repeated cycles of host antibody production and parasite destruction leads to cyclic fevers, macroglobulinaemia, microvascular damage, coagulopathy, and perivascular inflammation. There are often two stages of disease: an early haemo-lymphatic stage I with nonspecific symptoms such as fever, anaemia, headache, and lethargy; and a later meningo-encephalitic stage II when parasites have penetrated the blood-brain barrier (within weeks for *T. b. rhodesiense* or up to years for *T. b. gambiense*) causing meningoencephalitis, severe headaches, altered circadian rhythms (night-time insomnia and day-time somnolence), muscle twitches, ataxia, weight loss, psychiatric problems (confusion, irritability, anxiety, hallucinations, delirium), seizures, coma and death. In animals, the clinical course of *T. b. brucei* infections depends on the susceptibility of the host species. Horses and dogs are particularly susceptible and may succumb within 2-3 weeks Cattle and pigs are more refractory to disease and may survive for several months. Clinical signs include anaemia, fever, oedema and progressive paralysis. Native animal species (antelope and other wild ruminants) are trypanotolerant and may act as asymptomatic carriers. Humans have been shown to resist infections by other *Trypanosoma* spp. by serum-mediated lysis of the parasites; involving apolipoprotein (apoL1) lytic factors and haptoglobin-like protein (hpr) receptors. *T. b. rhodesiense* avoids lysis through the expression of a serum resistance-associated (*sra*) gene that neutralizes the lytic activity of apoL1. *T. b. gambiense* does not contain the *sra* gene, but resists lysis possibly by the reduced expression of the hpr receptor.

Developmental cycle and mode of transmission: All salivarian trypanosomes are transmitted by tsetse fly vectors (*Glossina* spp.). Metacyclic trypomastigotes ingested during feeding transform into procyclic trypomastigotes in the midgut. These stages migrate through gut membranes and invade the salivary glands over 12-30 days where they transform into epimastigotes which undergo anterior station development to produce infective metacyclic trypomastigotes in the proboscis which are injected during feeding (inoculative transmission). Infections can be transmitted to new susceptible hosts over the entire lifespans of infected flies (around 3 months). Disease distribution mirrors that of the tsetse fly vectors, but is nonetheless patchy with discrete foci, and cases outside the tsetse fly belt are usually imported. Tsetse flies bite and feed sporadically during the day and rest in shaded sites. They find hosts by following carbon dioxide expiratory plumes from animals and use visual cues to land on appropriate feeding sites. Infections by *T. b. gambiense* account for around 95% of human cases and occur in tropical forest of West and Central Africa (separated by the Great Rift Valley) where they are mainly transmitted by tsetse flies belonging to the *palpalis* complex (*G. palpalis, G. fuscipes, G. tachinoides*). Infections by *T. b rhodesiense* occur in savannah areas of East and Southern Africa where they are transmitted by tsetse flies belonging to both the *morsitans* complex (*G. morsitans, G. pallipes, G. swynnertoni*) and the palpalis complex (*G. fuscipes, G. tachinoides*). In addition to vector-borne transmission, there are occasional reports of infections being transmitted horizontally via blood transfusions, or vertically via transplacental passage from mother to foetus. Several species in the New World are also thought to undergo mechanical transmission by tabanid flies (*Tabanus, Stomoxys, Haematopota*).

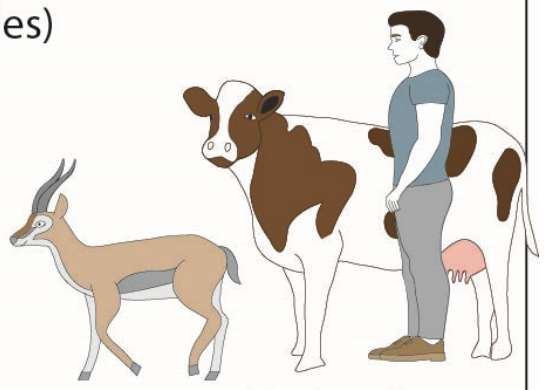
Differential diagnosis: Infections were conventionally diagnosed by the direct detection of parasites in blood, bone marrow, lymph or cerebrospinal fluid by microscopic examination before or after centrifugation. Infections by *T. b. rhodesiense* are often characterized by high parasitaemia, while those by *T. b. gambiense* exhibit intermittent cyclic parasitaemia. The routine examination of wet blood films or stained blood smears is best supplemented by various concentration techniques, such as microhaematocrit tube centrifugation, mini-anion-exchange centrifugation, buffy coat extraction, or fluorochrome staining. It is important that the kinetics (time course) of infection be determined as the treatments for stage I and II diseases are radically different (drugs for stage I cannot cross the blood-brain barrier and those for stage II are extremely toxic). Confirmation of stage II disease may be afforded by examination of cerebrospinal fluid obtained by lumbar puncture for parasites, leucocytes and sometimes increased protein content. *In vitro* cultivation has proven difficult and *in vivo* inoculation into laboratory animals yields variable results. More recently, a variety of immunoserological tests have been developed to detect host antibodies using fluorescent, agglutination or enzyme markers. Card-agglutination and dot-spot tests are available for field use. Molecular characterization techniques utilizing polymerase chain reaction (PCR) amplification of parasite DNA have yielded good results in species/strain differentiation using particular sequences (e.g. serum resistance-associated (*sra*) gene that enables *T. b. rhodesiense* to survive in humans, *T. b. gambiense*-specific glycoprotein (TgsGP), glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) as well as small subunit (SSU), large subunit (LSU) and internal transcribed spacer (ITS) regions of ribosomal DNA).

Treatment and control: Historically, arsenical drugs have been used to treat trypanosomiasis despite major toxicity problems (causing up to 5% mortality in patients). A combination of suramin and tryparsamide was used to treat African sleeping sickness for decades, and mapharside was widely used in experimental trypanosomiasis. At present, only five drugs are used to treat human infections. Suramin and pentamidine are used to treat stage I infections by *T. b. rhodesiense* and *T. b. gambiense* in humans respectively, while eflornithine is used alone or in combination with nifurtimox to treat stage II infections by *T. b. gambiense*, and melarsoprol to treat stage II infections by *T. b. rhodesiense.* Drug discovery projects are investigating new formulations for the treatment of infections in humans (e.g. fexinidazole, benzoxaboroles) as well as mechanisms involved in drug bioavailability and brain penetration (e.g. dicationic diamidines). Drugs that are routinely used to treat trypanosomiasis in animals include diminazine aceturate (berenil), isometamidium, homidium bromide, homidium chloride or pyrithidium bromide in ruminants and pigs, quinapyramine sulphate or suramin in horses and camels, and quinapyramine methylsulphate or isometamidium in dogs. Prevention involves avoiding being bitten by tsetse flies, but this can be difficult as they are persistent daytime feeders and can bite through thin clothing. Control measures based on vector eradication (using insecticidal sprays (ground or aerial spraying), fly traps, clearing vegetation or release of sterile male flies) and managing wild game reservoirs of infection (by fencing, culling or creating wildlife corridors) have proven partially effective. Some recent success has been recorded in breeding trypanotolerant domestic livestock (e.g. Ndama cattle). The widespread adoption of various control programs in the tsetse belt of Africa led to a decline in the incidence of human infections several decades ago, but a breakdown of those programs resulted in a resurgence of infections in the late twentieth century. Renewed efforts over the last decade have reduced the prevalence of disease and it is hoped that sustainable programs will help control disease for the 60 million people considered to be at risk of infection.

Trypanosoma
(mammalian 'salivarian' species)
(e.g. *T. brucei*)

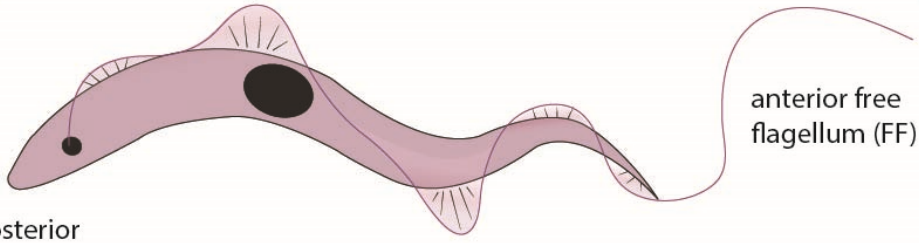
blood
(cyclic fevers, macroglobulinaemia, coagulopathies, encephalitis (ngana, sleeping sickness))

division by longitudinal binary fission



Vertebrate Hosts
(mammals, esp. humans, cattle)

long undulating membrane (UM)



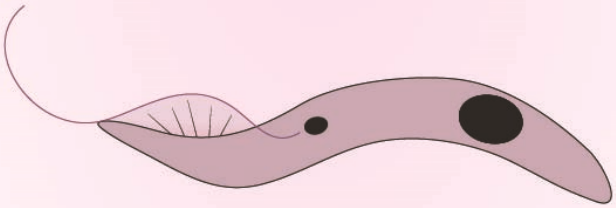
anterior free flagellum (FF)

posterior kinetoplast (K)

trypomastigote (8-45 µm)

inoculative (salivarian) transmission
(infective stages injected with saliva)

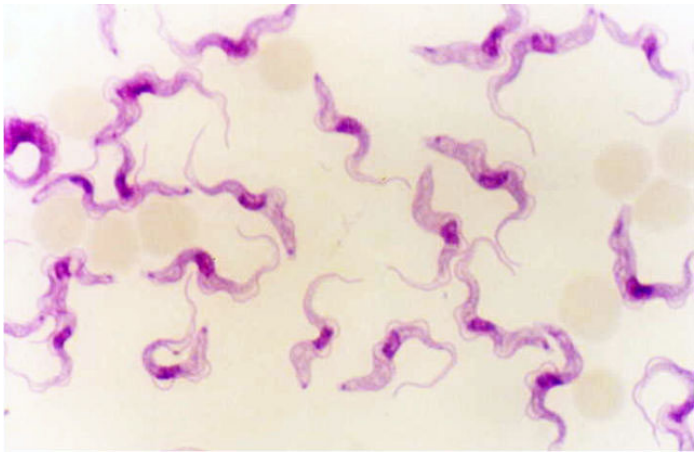
ingested with bloodmeal



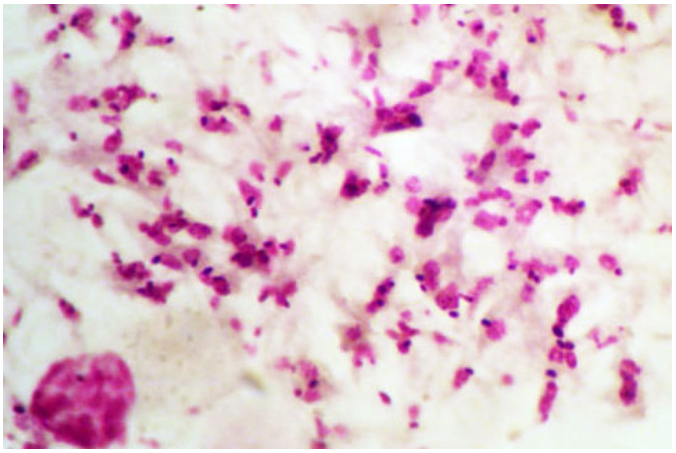
epimastigote (10-35 µm)
[anterior K, short UM, FF]

Invertebrate Hosts
(tsetse fly vectors)
(anterior alimentary tract)

vector-borne transmission



Trypanosoma brucei trypomastigotes in cow blood



Trypanosoma brucei epimastigotes in tsetse fly

Glossina tstetse fly vectors for *Trypanosoma brucei*