General classification of KINETOPLASTA

KINETOPLASTA
TRYPANOSOMOSIS

morphology of parasites, the main species, geographical distribution, epidemiology, the life cycle, pathogenesis and clinical signs, pathology, diagnosis, treatment and control.

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October 4, 2023

Taxonomy of PROTOZOA

Regnum	**EUKARYOTA**	
Subregnum	**PROTOZOA**	
Phylum – Order	**METAMONADA**	– DIPLOMONADIDA *(Giardia)*
	PARABASALA	– TRICHOMONADIDA *(Trichomonas)*
	EUGLENOZOA	– **KINETOPLASTEA – TRYPANOSOMATIDA** *(Trypanosoma, Leishmania)*
	ALVEOLATA	– **COCCIDEA** EIMERIIDA *(Eimeria , Sarcocystis, Toxoplasma)* CRYPTOSPORIDA *(Cryptosporidium)* ADELEIDA *(Hepatozoon)*
		– **HAEMATOZOEA** *(Plasmodium, Babesia, Theileria)*
	CILIOPHORA	– **VESTIBULIFERIDA *(Balantidium)***
	AMOEBOZOA	– ENTAMOEBIDA
	MICROSPORIDIA	– MICROSPORIDA *(Encephalitozoon)* – FUNGI?

Regnum: **EUKARYOTA, PROTOZOA**
Phylum: **EUGLENOZOA**
Subphylum: **KINETOPLASTA**
Class: **KINETOPLASTEA**
Order: **TRYPANOSOMATIDA**
Family: Trypanosomatidae
Genus: ***Trypanosoma*** **Gruby, 1843**
Leishmania **Ross, 1913**

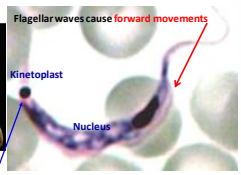
KINETOPLASTEA

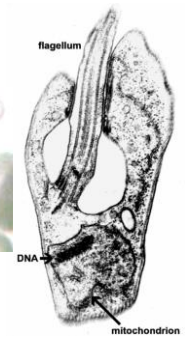
(*kinein* - move; *plastos* – formed)

- Free-living or parasitic protozoa
- 1-2 flagella
- 1/more **kinetoplasts (mitochondrial DNA)**
- Two medically important genera:

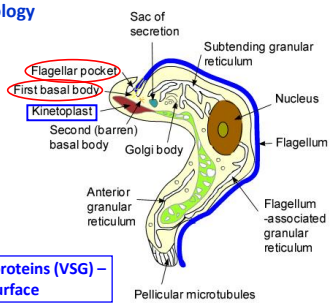
Leishmania - (leishmaniosis)
Trypanosoma – (trypanosomosis)

TRYPANOSOMATIDA: Trypanosomatidae
(*Trypanon* – drill; *soma* – body)





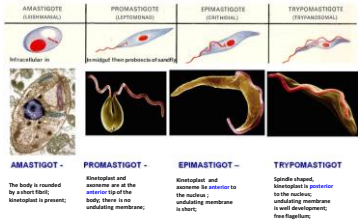
Morphology



Variable glycoproteins (VSG) – cell surface

COMMON PROPERTIES OF KINETOPLASTEA

- **CYCLICAL TRANSMISSION** – **with one exception (mechanical transmission, *T. b. equiperdum*), all have an arthropod vector !**
- **POLY-MORPHYSM** – ability during the life cycle to form stages that are differ in morphology and position of kinetoplast, presence of undulating membrane;



AMASTIGOT - The body is rounded by a short flbril; kinetoplast is present;

PROMASTIGOT - Kinetoplast and axoneme are at the anterior tip of the body; there is no undulating membrane;

EPIMASTIGOT – Kinetoplast and axoneme lie anterior to the nucleus ; undulating membrane is short;

TRYPOMASTIGOT Spindle shaped, kinetoplast is posterior to the nucleus; undulating membrane is well development; free flagellum;

Order: TRYPANOSOMATIDA
Family: Trypanosomatidae
Genus: *TRYPANOSOMA*

Genus: *Trypanosoma*

2 groups

	STERCORARIA	SALIVARIA
Development ending in:	Posterior part of digestive system	Anterior part of DS – proboscis, salivary glands;
Transmission:	By faeces (bugs [Reduviidae], Horse-flies, fleas, ...); infection - contamination	Inoculation into blood (Tse-tse flies - *Glossina*), Horse-flies; exception – *T. b. equiperdum* (coitus/mating)
Location:	INTRACELULAR PARASITES	EXTRACELULAR (plasma, lymph, cerebral liquor, organs of RES;
Reservoirs:	glyptodont, opossum, racoon, pig, dog, cat, man ...	rodents

***Trypanosoma* species: SALIVARIA GROUP**
Animal **A**frican **T**rypanosomosis = **Nagana**

Trypanosoma spp. Distribution (D)	Disease; hosts; pathogenicity	Vectors; mode of transmission
T. congolense D: Sub-Saharan Africa	**Nagana** Bovinae**, **Equidae, Camelidae***, antelopes, giraffe	***Glossina***: cyclic
T. vivax D: Sub-Saharan Africa	**A**nimal **A**frican **T**rypanosomosis; **Nagana;** Bov***, Equidae, Camelidae***, antelopes, giraffe	***Glossina***: cyclic
T. vivax D: Central & South America	inapparent infection of domest. Rumin; Equidae*	**haematophagous flies**: non-cyclic
T. brucei brucei D: Sub-Saharan Africa	**Nagana** **Eq, Camelidae***, Car**, Bovinae, Suis, antilopes	***Glossina***: cyclic
T. brucei evansi D: Africa, Middle East, Eurasia, Latin America	**Surra** **Eq, Camelidae***, Car***,** Bovinae, elephants **	**Tabanidae,** other flies, vampire bats: **non-cyclic;** carnivores: **peroral**

SALIVARIA GROUP

T. brucei equiperdum D: Asia, Africa, Latin America, Eastern Europe	**Durine** **Eq**	**Coitus; non-cyclic; direct transmission**

Trypanosoma brucei brucei

↓

Parasite of animals

↓

Lysed by human blood serum
„***trypanosome lytic factor***“

Trypanosoma species: **SALIVARIA GROUP**
Human **A**frican **T**rypanosomosis = **Sleeping sickness**

Trypanosoma spp. Distribution (D)	Disease; hosts; pathogenicity	Vectors; mode of transmissiion
T. brucei rhodesiense D: Sub-Saharan Africa (eastern and suthern)	**Sleeping sickness - acute** humans***, antilopes (bushbuck, impala), zebra, giraffe, lion, hyena ...	***Glossina***: cyclic
T. brucei gambiense D: Sub-Saharan Africa (western and central)	**Sleeping sickness - chronic** humans***, Pig, cattle, dog, antelopes	***Glossina***: cyclic

SALIVARIA GROUP

Life cycle (*Trypanosoma*)

IN VECTOR — **IN VERTEBRATE**

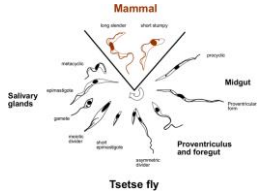
NAGANA

- *T. vivax*
- *T. congolense*
- *T. simiae*

monomorphic

- *T. brucei brucei* -

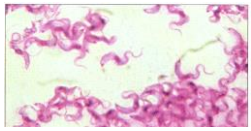
pleomorphic – longer slim and shorter „stumpy" forms



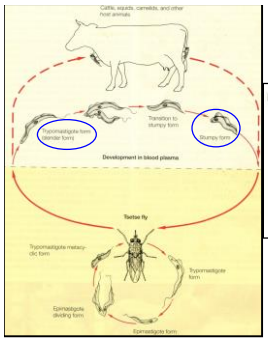
Tsetse fly

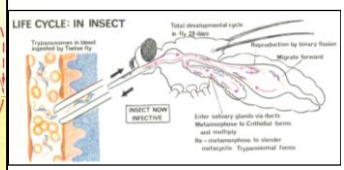
Glossina spp. – „tse – tse fly" – biology

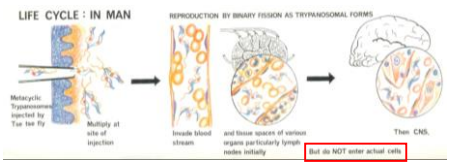
- females are viviparous, lifespan takes about 14 weeks
- after first blood meal they copulate, from fertilized eggs in uterus developed larvae, which hatch 2 times (ten days)
- females produce 8–12 larvae during their life-span
- larvae turns into pupa in 30–60 min.
- imago leave pupa in 35 days




larva, prepupa a pupa







AFRICAN ANIMAL TRYPANOSOMOSIS (AAT) NAGANA – SURRA, ...

African Animal Trypanosomosis causes serious economic loses in livestock from:

- anaemia
- generalised enlargement of lymph nodes
- loss of condition
- fever
- progressive emaciation
- FATAL if untreated

Trypano-tolerant breeds

- **West African taurine breeds:** N´Dama, Bauole, Muturu, Somba, Dahomey
- **East African zebu breeds:** Orma Boran, Massai zebu
- **Small ruminants:** West African dwarf sheep and goats, East African goat

N´Dama

Pathogenesis and clinical signs

- Chronic trypanosomosis caused *T. brucei brucei* , *T. congolense* or *T. vivax*;
- Disease occurs about 7-10 days after the infection;
- **Temperature of the body rises** and the heart and breathing frequencies increase;
- Continuous slow worsen of health with **permanent loss of fitness**;
- The animal is clearly sick with a variable appetite;
- In the initial stage, diarrhoea may be present;
- The animal becomes **anaemic** (the visible mucosa is pale);
- Very often there is an increased secretion of tears (**lacrymation**);
- **Emaciation** is associated with weakness, inability to stand, severe anaemia and death.

SOMNOLENCE

Diagnosis

- **Identification of the parasite in the blood**;
- Hematological examination - micro hematocrit;
- Inoculation of the parasite - a **biological experiment**;
- Serological tests - **ELISA**;

Therapy

- **isometamidium chlorid**
- **malarsomine**
- **diminazine**

} **resistence**

Prevention

- control of the vector
- permanent monitoring

EQUINE TRYPANOSOMIASIS/DURINE

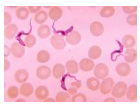
Dourine is a **chronic veneral trypanosomal disease** of horses that is transmitted predominantly **by coitus** and is characterized by **genital oedema, neurologic dysfunction** and **death.**

- ***Trypanosoma equiperdum***

- Durine is the only one trypanosomiasis that is **not vector-borne**;
- rarely, transmission can be carried by blood-sucking flies, even if only **mechanically.** Transmission by mechanical cleaning equipment used for genital washing, rubber gloves, etc. is also possible.
- Infection **from mare to foal** is transmitted via mucous membranes such as conjunctivae.
- Mare's milk is also infectious.
- Semen and vaginal excreta contain the highest numbers of parasites.

Pathogenesis, clinical manifestations

- Exacerbation, tolerance, relapse
- Pyrexia, swelling and local oedema of the genitals and mammary glands, oedematous skin eruptions, limb impingement, incoordination, facial paralysis, lesions on the eyes, anaemia and emaciation.
- Oedematous skin lesions - on the skin of the ribs, but also elsewhere on the body, persist for 3-7 days, then disappear, have no constant signs.
- The incubation period is 2-12 weeks, the disease has a chronic course of 6 months to 2 years.



Therapy and prevention

- In many countries, treatment is **not allowed**, it is forbidden and rigorous control is in place when transiting horses and crossing borders.
- Melarsomine (0.25-0.5 mg/kg b.w., i.m.)
- **Treatment is not recommended** because it can suppress clinical signs in animals, are transporters.
- EU - Strict control of breeding and **equine movement**, **quarantine** and **euthanasia of horses with clinical signs.**

HUMAN African Trypanosomosis

Protozoan hemoflagellates belonging to the complex ***Trypanosoma brucei brucei***

Two subspecies that are morphologically

indistinguishable cause distinct disease patterns in humans:

- ***T. b. gambiense*** causes **West African sleeping sickness (chronic form)**
- ***T. b. rhodesiense*** causes **East African sleeping sickness (acute form)**

Sleeping sickness – clinical symptoms
man – Africa

THREE PHASES OF DISEASE

- the primary lesion – **chancre**
  - **2 to 3 days after the bite**
- **parasitemia**
  - **trypanosomes in blood stream, irregular and intermitent fever in waves**
  - **cephalalgia, insomnia, generalised erythema and pruritus**
- invasion of **central nervous system**
  - **meningitis, encephalitis, cerebral edema, somnolence, lethargy, coma, death**

Sleeping sickness – **man –** Africa

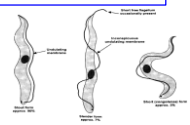
trypanosomal **chancre**
- the bite reaction

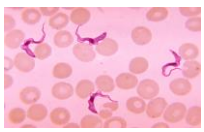
enlargement of cervical lymphatic glands
Winterbottoms's sign

trypanosomal rash/urticaria
- during peack of fever – skin eruption

Diagnosis of African trypanosomosis

- **Direct demonstration of the parasite – WET MOUNT, Geimsa stained thin or thick smears** (low sensitivity)
  - from chancre aspiration
  - the lymph glands
  - bone marrow
  - from blood during acute phase
  - from cerebrospinal fluid during acute phase
- **Concentration techniques**
  - HCT – haematocrit centrifugation technique
- Serological tests are of limited value e.g. **ELISA**
- **PCR**





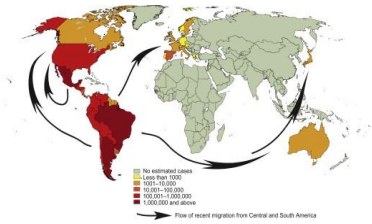
Control of African trypanosomosis

- **control of the principal reservoirs of infection**
  – livestock population (cattle) and wild (antelopes)
- **reduce of the vectors**
  – destruction of the flies habitats or use insecticides (can cause ecological changes)
  – fly traps
  – male flies sterilized in laboratory (Slovakia)

Therapy and control

- **Cattle, sheep, goats**
  - **Diminazene aceturate** – therapy
  - **Isometamidium** – prophylaxy
  - (long lasting efficacy: 2 – 6 months)
- **Camels, equids, dogs**
  - **Melarsormine**
  - (diminazene is toxic)

AMERICAN TRYPANOSOMOSIS
Chagas disease (*Trypanosoma cruzi*)



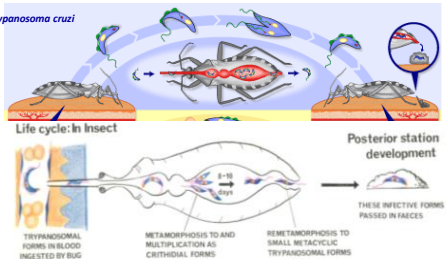
Trypanosoma species: **STERCORARIA GROUP**
American Trypanosomosis = **Chagas disease**

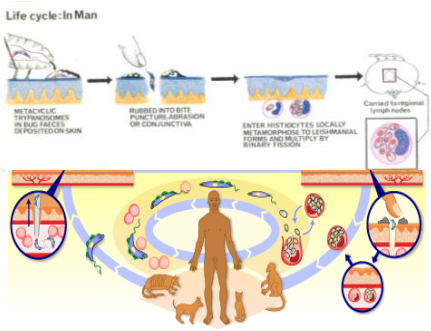
Trypanosoma spp. Distribution (D)	Disease; hosts; pathogenicity	Vectors; mode of transmissiion
T. cruzi D: Central & South America, USA	**Chagas disease** humans***, dog, cat**, opossum, armadillos, many other mammals	***Triatoma*** (kissing bugs): cyclic
T. theileri D: worldwide	apathogenic or facultative pathogenic in cattle*	Tabanidae: cyclic & Ixodidae: cyclic
T. melophagium D: worldwide	apathogenic sheep*	*Melophsgus ovinus* (sheep ked): cyclic
T. cervi D: worldwide	apathogenic red deer*	Tabanidae: cyclic
T. lewisi D: worldwide	apathogenic	fleas: **cyclic**

ROUTE OF INFECTION

- Vector - *Triathoma*, *Rhodnius* and *Panstrongylus* genus;
- Ingestion of food contaminated with parasites;
- Blood transfusion;
- Fetal transmission (13% deaths / year in Brazil);

Life cycle *Trypanosoma cruzi*





American trypanosomosis

MAMMALIA
- Extracellular trypomastigotes – blood
- Intracellular amastigotes – tissue

VECTORS – kissing bugs (*Triatoma*)
Epimastigotes – intestine
Trypomastigotes (metacyclic f.) – rectum

Chagas disease – reservoir hosts

Armadillo

Opossum

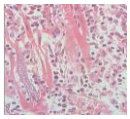
Naturally infected up to 150 species of domestic and wild mammals
IMPORTANT: rodents, opossum, armadrilos, dogs, cats, HD, pigs, etc.

DOGS – pathogenesis and clinical signs

DOGS up to 1 year
- **acute infection**: hard failure organs = **DEATH**
- **In surviving dogs**: hepato-splenomegaly, anaemia, oedema, ascites cachexia respiratory symptoms, fever
- **Acute phase** is followed by a **transitional period (latent)** (8 – 36 months) – no clinical signs
- Progressive **chronic form** – myocardia and destruction of ganglia cells

Chagas disease – clinical symptoms in humans

- largely **inapparent during acute phase**
- **incubation period**: **10 – 20 years**
- **serious pathological changes** can occure
  - damage of the tissue cells
  - damage of the ganglia of the autonomic nervous system



AMASTIGOTES in cardiac muscle

Chagas disease – clinical symptoms

3 phases of the disease:

- **Acute Phase**
  - **active infection**
  - **1-4 months duration**
  - **most are asymptomatic (children most likely to be symptomatic)**
- **Indeterminate Phase**
  - **10-30 years of latency**
  - **relatively asymptomatic with no detectable parasitemia**
  - **seropositive**
- **Chronic Phase**
  - **10-30% of infected exhibit cardio-myopathy or megasyndromes**

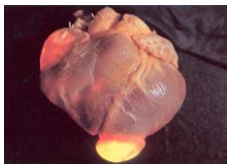
Chagas disease – clinical symptoms in humans

Romagna sign
– oedema of the eyelids
Chagoma
– oedema of the satelit lymph node – local reproduction of the parasite

Chagas disease – pathology: **visceromegaly**

DENERVING OF THE VEGETATED NERVOUS SYSTEM - IMUNOPATHOLOGY

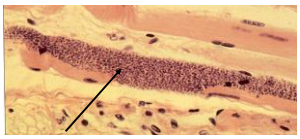
cardiomegaly



apical aneurysma – heart

Chagas disease – pathology: visceromegaly

megaoesophagus, RTG



AMASTIGOTES in muscle cells of oesophagus
„nests" or „pseudocysts"

Chagas disease – pathology: visceromegaly

megacolon

Diagnosis of Chagas disease – dog

- **Acute stage** – blood
  thin and thick blood smear, concentration techniques
- **Chronic stage** – trypomastigotes are missing or rarely present

- **Alternative methods** – serological – ELISA
  antigen from epimastigotes *Trypanosoma crusi* cultivated *in vitro*
  !!! cross reaction with *Leishmania* in dogs

- **PCR methods**

Treatment and prevention

Chemotherapy in dogs is difficult

- **Acute cases**
  – benzidazole 5-7 mg/kg b.w. daily for about 2 months (mitigate the clinical course, but not prevent subsequent chronic cardiac disease)
- **Vector control – insecticides**
- **Blood transfusion**

Humans

- **acute stage**
  - **nifurtimox (8-16 mg/kg/day, 60-90 days)**
  - **benzidazole (5-7 mg/kg/day, 30-120 days)**
  - **allopurinol (experimental)**
  - **azole antifungal agents (experimental)**
- **chronic stage**
  treat symptoms