The Kinetoplastids

*Leishmania* spp.

*Trypanosoma* spp.

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**African Trypanosomiasis**

*Trypanosoma brucei gambiense*  
(Dutton 1902)

*Trypanosoma brucei rhodesiense*  
(Stephens & Fantham 1910)

African Sleeping Sickness
Terms from the documentary - Sleeping Sickness

Democratic republic of the Congo (DRC; aka Zaire)
3rd largest African country (area)
>68 million people
Second Congo War began in 1998:
7 foreign armies
fighting continues in east
deadliest conflict since World War II
~5.4 million people killed
Citizens of the DRC are among the poorest in the world,
lowest per capita GDP ($210./year in 2011)
~50% of deaths are children under age 5.
Lumbar puncture = spinal tap (cerebrospinal fluid)
Melarsoprol- arsenic containing drug
Eflornithine, Nifurtimox: other anti-trypanosome drugs
Combination therapy: giving two drugs together

Kill or Cure:
Sleeping Sickness
Season 4 Episode 6
Experts thought Sleeping Sickness had been destroyed decades ago. But it's back with a vengeance. Kill or Cure journeys to the Democratic Republic of Congo to see the devastation Sleeping Sickness causes. Without treatment sufferers face almost certain death. Whole communities have been crippled by the disease. Mobile teams are in the field carrying out rapid blood tests and lumber punctures to identify and beat the disease.

http://www.rockhopper.tv/programmes/3/
African Trypanosomiasis

only in sub-Saharan Africa
lives in bloodstream of mammals
cattle disease: “nagana” (Trypanosoma brucei brucei)
something (perhaps HDL) in human serum lyses this parasite so it does not cause human infection
human disease: “Africans sleeping sickness”
Trypanosoma brucei gambiense (1902)
Trypanosoma brucei rhodesiense (1910)
2005: 450,000 cases throughout sub-Saharan Africa
60 million people are at risk
tsetse fly vector
recently classified as a re-emerging infection:
extensive forced migration caused by civil turmoil
breakdown of control measures against vector

Gambiense versus Rhodesiense

• infection through a broad region of equitorial Africa
(restricted by boundaries of Sahara desert to north & dryer temperate regions south of equator)

• T. gambiense mainly found in western & central Africa
(Cameroon, Benin, Central African Republic, Gabon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria, Senegal, Gambia, Uganda & Democratic Republic of Congo)
• the domestic pig is only important reservoir for gambiense

• T. rhodesiense mainly found in east Africa
(Burundi, Botswana, Congo, Ethiopia, Kenya, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zambia & Zimbabwe)
• many wild animal species & domestic cattle of East Africa are reservoirs for rhodesiense
Kinetoplastid Morphological Forms:
African trypanosome = *Trypanosoma brucei*

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**metacyclic trypomastigotes**: located in tsetse fly salivary glands; injected into host skin by bite \(\rightarrow\) become bloodstream forms

**bloodstream trypomastigotes**: in human host bloodstream.
- “long slender form” = proliferative, *minimal* mitochondrion, not infectious to tsetse fly
- “short stumpy form” = *pre-adapted* for infection of tsetse fly; non-dividing, *developed* mitochondrion \(\rightarrow\) infects vector

**procyclic trypomastigotes**: attaches to insect tsetse midgut & replicates, develops *fully functional* mitochondrion. Migration to salivary glands and differentiation to epimastigotes.

Epimastigote: replication in fly salivary gland, transformation to metacyclic trypomastigotes \(\rightarrow\) infection of human host

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**Trypanosomes & Energy**

- In all eukaryotes, ATP produced by oxidation of carbohydrates
  - *glycolysis* (glucose \(\rightarrow\) pyruvate) = 2 ATP
  - tricarboxylic acid (TCA) cycle = \(~28\) ATP
- *Vertebrate bloodstream is rich in glucose and oxygen*
- *In the vertebrate host, trypanosomes*: shut off mitochondrial TCA cycle depend entirely *glycolysis for energy* \(\rightarrow\) parasite uses 10X more fuel for energy than in the insect
  - First 9 glycolysis steps are organelle-associated (*glycosome*)
- *Insect gut is NOT rich in glucose and oxygen*
- *In the insect host, trypanosomes*:
  - develop a conventional cytochrome chain
  - mitochondria develop a TCA cycle

*The loss/gain of TCA happens in all kinetoplastid parasites, not just African trypanosomes*
Kinetoplastid Morphological Forms: African trypanosome = *Trypanosoma brucei*

- **metacyclic trypanomastigotes**
  - insect
- **long slender bloodstream trypomastigotes**
  - mammal
- **procyclic trypanomastigotes**
  - insect
- **short stumpy bloodstream trypomastigotes**
  - mammal

**African Trypanosomes**

- Live *extracellularly* in mammalian & insect hosts
- Infection by infective **metacyclic trypanomastigotes** injected by infected tsetse fly during blood meal
- Transform into bloodstream form **trypomastigotes** (long slender form & short stumpy form)
- Replication at bite site
- Repeated replication cycles → buildup of metabolic wastes
- Extensive necrosis & formation of a soft painless chancre
- Replication continues in blood → millions of trypomastigotes

**Chancre due to early infection with T. b. gambiense.**
African Trypanosome Life Cycle

short stumpy form (non-dividing)
  some long slender forms transform into short stumpy form
  biochemistry similar to form in insect vector
  short stumpy forms are pre-adapted to vector
well-developed mitochondrion & partial TCA cycle
tsetse fly infected by ingestingssf in blood from infected host
develops into procyclic trypomastigotes in fly mid-gut
divides for ~10 days here
fully functional TCA cycle
parasites migrate to salivary glands
transform into epimastigotes
  epimastigotes divide in salivary gland
differentiate into metacyclic trypanosomes
  (infectious stage for humans & reservoir hosts)

African Trypanosome Life Cycle

insect cycle takes 25-50 days
depending upon species of fly
strain of trypanosome
ambient temperatures

vectors remain infected for life (2-3 months)
tsetse flies inject >40,000 metacyclic trypanosomes when feeding
minimum infectious dose for most hosts is 300-500 organisms

infection also acquired by eating raw meat from an infected animal
  this maintains cycle in some reservoir hosts
  (lions, cheetahs, leopards, hyenas & dogs)
Winterbottom’s sign: swelling of lymph nodes along the back of neck, as trypanosomes travel in the lymphatic fluid & cause inflammation.

African Trypanosomes – Immune Evasion

trypanosome membrane associated antigen → (variant surface glycoprotein, VSG)

antibodies destroy organisms with first VSG

few trypanosomes produce 2nd completely different VSG

these parasites escape lysis & replace destroyed parasites cycle continues until infected individual is overcome by exhaustion due to glucose depletion & build up of metabolic wastes from parasites

• from a 1910 report of T. gambiense infection
• number of bloodstream trypanosomes (parasitemia) has periodicity
• due to antigenic variation:
  patient makes antibodies
  5-7 days → antibodies destroy most trypanosomes parasitemia decreases
  new trypanosomes unaffected by existing antibodies immune system starts again (new antibodies)
  immune system exhausted → death of the host
**African Trypanosomes – Immune Evasion**

surface coat formed by 10,000,000 copies of single VSG protein antigenic variation depends on genomic reorganization large repertoire of antigenic variants (~1,000 VSG genes) trypanosome selects a gene from large VSG gene family moves VSG gene to telomeric location chromosome end gene at this site $\rightarrow$ protein (other VSG genes silent) frequent switching of surface VSG $\rightarrow$ antigenic variation antigenic variation is one reason why vaccines do not protect!

Why would antigenic variation be more important to African than South American trypanosomes?

**African Trypanosomes – Neuropathy**

parasites in bloodstream & lymph nodes throughout infection invasion of CNS induces a lethargic condition $\rightarrow$ coma & death patients with trypanosomes in CNS: severe headache, stiff neck, sleeplessness & depression focal seizures, tremors & palsies coma develops patient usually dies of associated causes (pneumonia or sepsis) parasite replication in CSF results in meningitis cerebral edema encephalopathy Parasites induce astrocytes to release prostaglandin D2 (sleep regulating molecule)
African Trypanosomes – Clinical Disease

both species of trypanosomes cause same clinical disease
timescale of disease evolution differs

*rhodesiense*
- infection rapidly progresses to disease
- incubation period of 2-3 weeks
- CNS involvement at 3-4 weeks after infection

*gambiense*
- incubation period of weeks to months
- CNS involvement months to years after infection

**Diagnosis:**
organisms in blood smears or CSF fluid
PCR laboratory method of future (rapid diagnosis)

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African Trypanosomes – Treatment

**Suramin:** developed in 1916
only used for early stages of infection
*doesn’t cross blood-brain barrier*
unknown mode of action
associated with possibly severe kidney dysfunction

**Pentamidine:**
used for early phase of infection with *gambiense*
unknown mechanism of action
kidney & bone marrow damage
### African Trypanosomes – Treatment

**Difuoromethylornithine (DMFO):**
- recommended drug – “wake up from the dead” drug
- relatively non-toxic
- inhibits single point in *polyamine biosynthesis* pathway
  - resistance is likely to develop
- resistance seen in some people under treatment

**Melarsoprol:**
- contains arsenic
- side effects: convulsions & fever
- *only effective drug for treatment of disease with CNS involvement*
  - ~3% of cases: drug associated with encephalopathy
  - ~10% failure to cure

### African Trypanosomes – Prevention & Control

*political upheavals in Africa → dramatic increase in sleeping sickness*
- prior to 1995: <70,000 cases/year
- 450,000 new cases in 2005 alone

*military action & civil unrest:*
- Sudan & Ethiopia, Sierra Leone, Congo & Liberia
- large influxes of refugees

*forced migration → high risk from opportunistic parasitic infections*

*limited resources cannot keep up with vector control & caring for refugees (food, water & medicine)*