

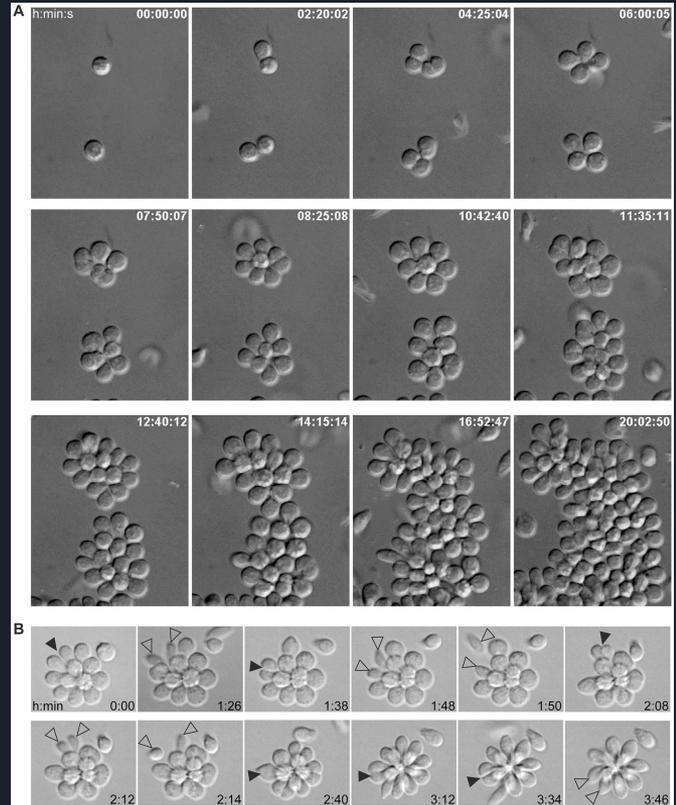


The growth curve of *Crithidia fasciculata*

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Introduction: 1.

Crithidia fasciculata



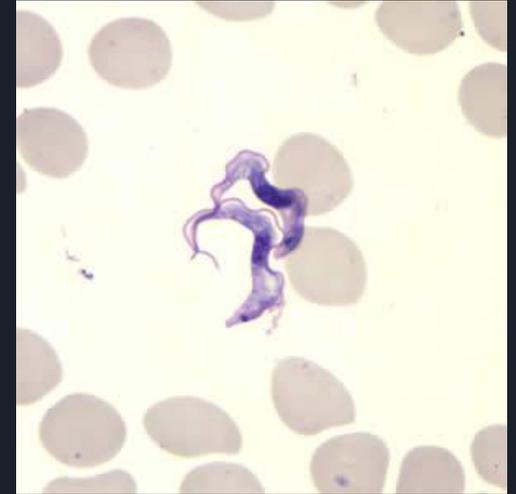
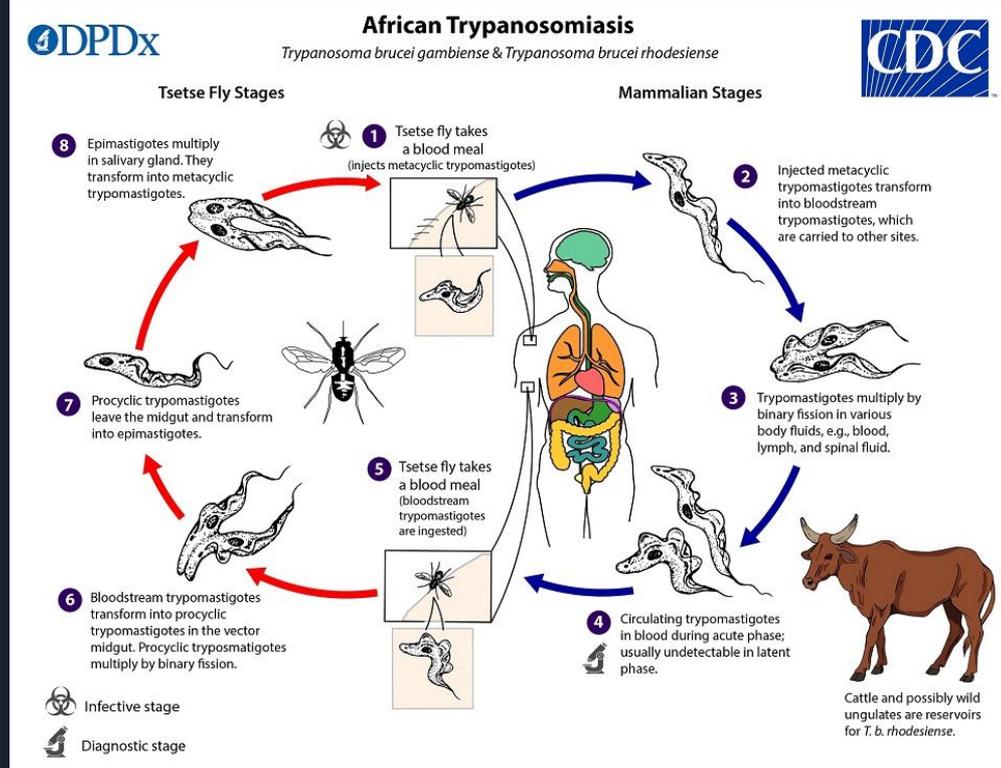
<https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0007570&rev=2>



Crithidia fasciculata are a species of parasitic excavates. They have a single host life cycle within the mosquito. Their species specificity is low which enables them to infect as many different species of mosquito. *C. fasciculata* are found in two different morphological life cycle stages; the immotile, attached, amastigote form which is found in the gut of the mosquito and the free swimming choanomastigote form which uses an external flagellum that is long for motility. Transmission of *Crithidia fasciculata* occurs when amastigotes are ingested by mosquito larvae after they are washed into standing water and they are found typically in the larva's rectum. The larva's molt concludes in loss of infection but the infection is re-acquired quickly by the larva from the surrounding by the ingestion of more amastigotes. The infection of the amastigote will go through metamorphosis which will give rise to an adult infected mosquito when the amastigote is maintained in the gut by the fourth instar pupates of the larva.

C. fasciculata is an example of a non-human infective trypanosomatid and is related to several human parasites, including *Trypanosoma brucei* (which causes African trypanosomiasis) and *Leishmania* spp. (which cause Leishmaniasis). *C. fasciculata* parasitizes several species of insects and has been widely used to test new therapeutic strategies against parasitic infections. It is often used as a model organism in research into trypanosomatid biology that may then be applied to understanding the biology of the human infective species.

2. *Trypanosoma brucei*





Trypanosoma brucei belongs to the species kinetoplastid which belongs to the genus *Trypanosoma* which happens to be parasitic. They are carried by the genera of tsetse fly in sub-Saharan Africa. *T. brucei* causes sleeping sickness or African trypanosomiasis in humans while it causes nagana also called animal trypanosomiasis in horses and cattle. There are three subspecies: *T. b. brucei* (non-human parasite), *T. b. Gambiense* (known human parasite) and *T. b. rhodesiense* (known human parasite).

Trypanosoma brucei brucei Causes animal trypanosomiasis, along with several other species of *Trypanosoma*. *T. b. brucei* is not infective to humans due to its susceptibility to lysis by trypanosome lytic factor-1 (TLF-1). However, it is closely related to, and shares fundamental features with the human-infective subspecies.

Trypanosoma brucei gambiense is found in 24 countries in west and central Africa. This form currently accounts for 98% of reported cases of sleeping sickness and causes a chronic infection.

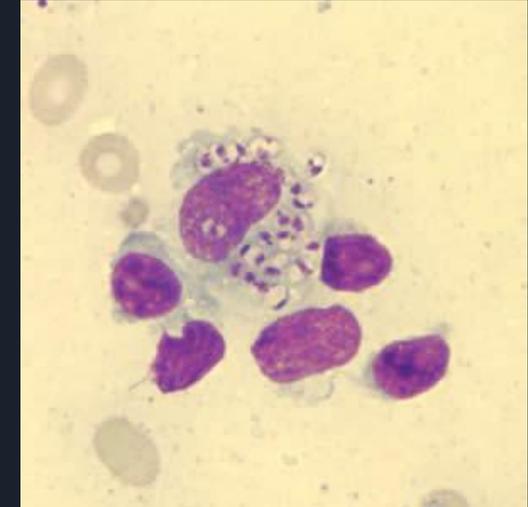
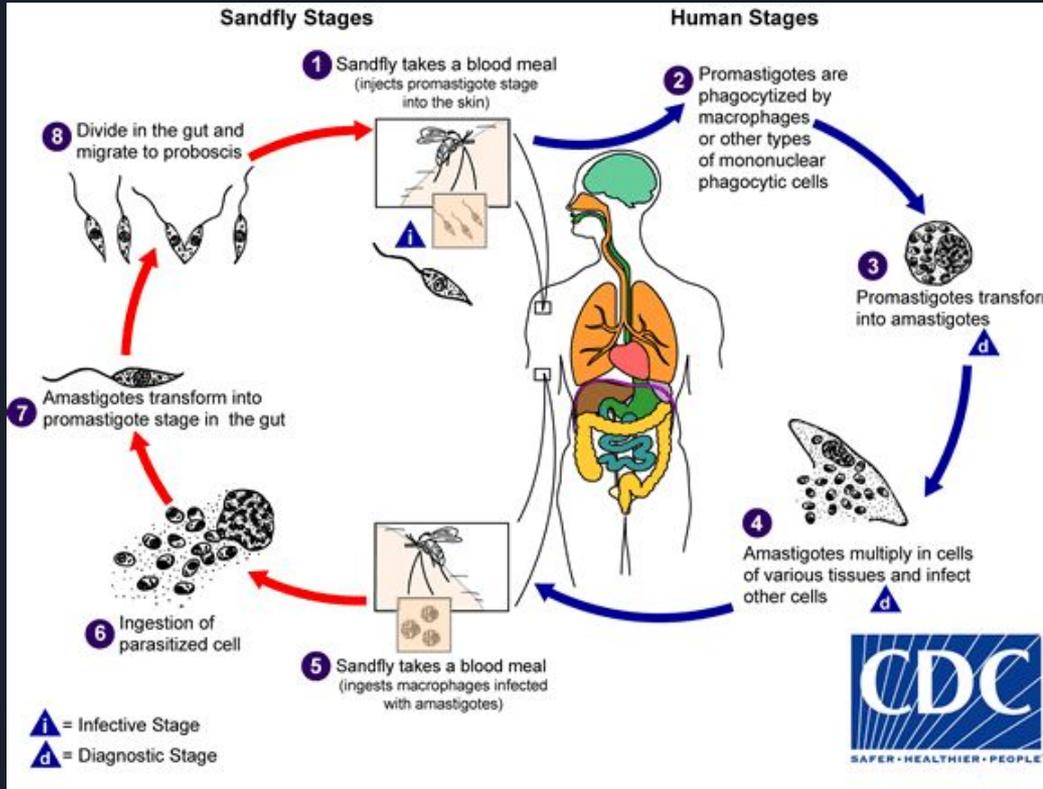
Trypanosoma brucei rhodesiense is found in 13 countries in eastern and southern Africa. Nowadays, this form represents under 2% of reported cases and causes an acute infection.

In 2009, the number reported dropped below 10, 000 for the first time in 50 years, and in 2018 there were 977 cases recorded due to sustained controlled efforts.

T. brucei is transmitted through biting during the insect's blood meal. Mammalian bloodstream provides a conducive environment for the parasite to grow which leads to chronic infection.

T. brucei is known to pass the blood brain barrier which happens to be one of the few pathogens with the ability. New development of therapeutic drugs are in demand because the ones available now may have side effects which may be fatal for patients.

3. *Leishmania* spp





Leishmania belongs to a genus of trypanosomes which are the cause of leishmaniasis. They are spread by at least 93 sandflies worldwide. *Leishmania* commonly affects vertebrates; humans, rodents, canids and hyraxes. They are unicellular eukaryotes that have a nucleus, kinetoplasts and flagella among other cell organelles.

Leishmania exist in two structural variants depending on the lifecycle stage they are in. Their amastigote form is found in the circulatory system and in the phagocytes which are mononuclear in humans. Its amastigote doesn't have an external flagellum, and it is in a nonmotile and intracellular form. Their promastigote form is found in sandflies' alimentary tracts. It is in a motile and extracellular form. There are 53 species recognised in this genus but at least 20 species infect humans. Some of them are evolving.



Purpose of the research

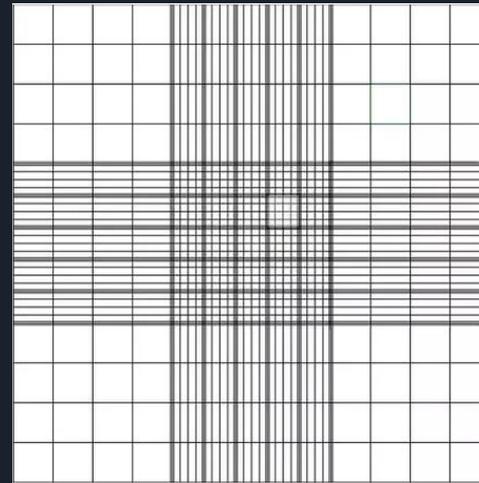
- Find out how fast *Crithidia fasciculata* grows.
- Find out how fast they divide.
- Find the approximate time it takes for them to double.
- Count the cells using a hemocytometer and an OD600.

UV/VIS lambda (spectrophotometer) OD600



<https://pr.vwr.com/store/product/4832209/lambdatm-25-35-series-uv-vis-spectrophotometers-perkinelmer>

Hemocytometer



<https://www.scienceabc.com/pure-sciences/what-is-a-hemocytometer-calculation-counting-how-to-use.html>

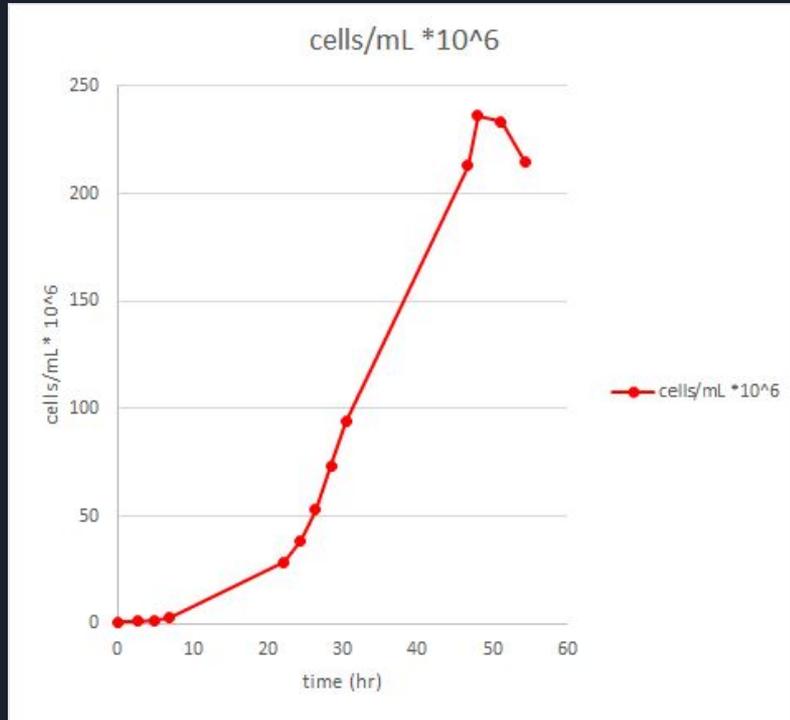


Instruments used.

Hemocytometer - Used to approximate the number of cells by using small squares.

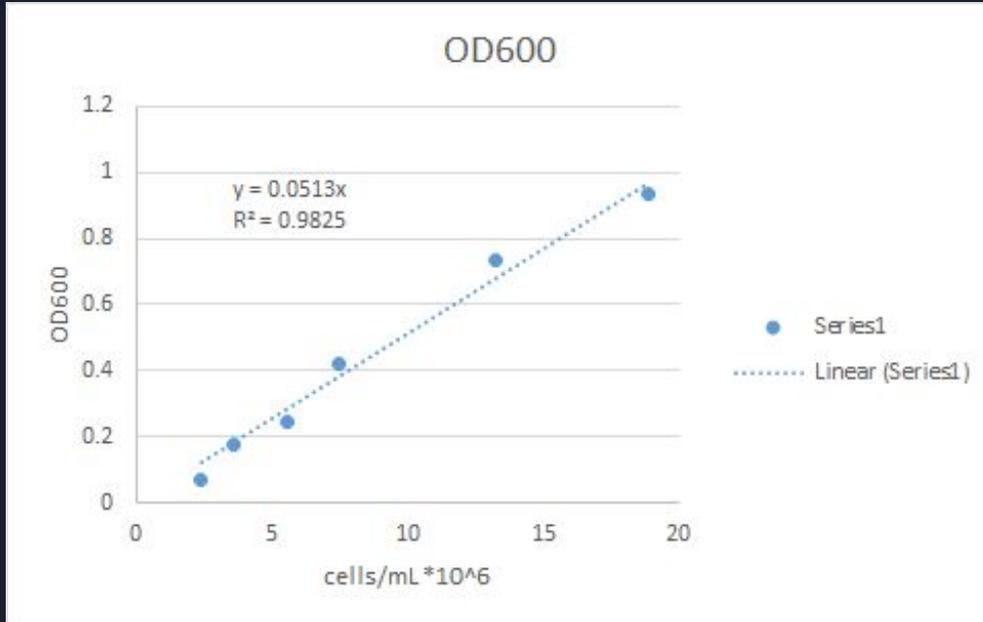
UV/VIS spectrophotometer - Used to measure the OD600 of samples by the amount of light that goes through a solution in a cuvette.

Graphs: Cell count by hemocytometer



The graph shows the peak of the growth of the cells.

Cell count by OD 600



The graph shows the the trendline that was used to find the formula to be used to find the cell count faster.



Discoveries from the data collected

- From the data that was collected, it took the *Crithidia fasciculata* 4.3 hours to double.
- A formula was found that could be used to predict the number of cells without using a hemocytometer which was $OD600 = 0.0513(\text{cells} * 10^6)$.
- The results from this data may help in some ways to better understand the biology and chemistry to some extent of *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* parasites that are fatal to humans.



Future of research

- This data might be used to help understand oxidative stress and other ways to develop drugs to kill the cells in the future.



Reference

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