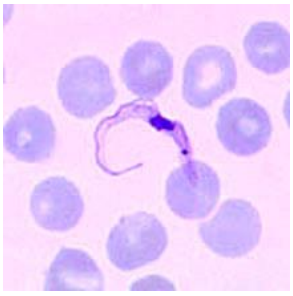
PARASITOLOGY CASE HISTORY #4 (BLOOD PARASITES)
(Lynne S. Garcia)

A 52-year old male presented after complaining of a week of fever and headaches. Other symptoms included malaise and lack of appetite, and he appeared to be somewhat confused. His initial symptoms of fever and headaches were sometimes accompanied by chills. Basically, he indicated he felt terrible and had absolutely no energy. His past medical history was unremarkable and he had no history of prior serious illness. His travel history revealed that he had recently returned from a lengthy trip to the western part of Africa; he had visited a number of rural areas where for several months he had lived in tents.

On physical examination, he had enlarged posterior cervical lymph nodes. His mental status was normal regarding orientation to time, location, and person; however, he had trouble responding to questions.

Complete stool examinations were performed (O&P exam: direct wet mount, concentration, and permanent stained slide), all of which were negative. Stained blood smears revealed the following:



This image was photographed from a stained thin blood film using the oil immersion objective (100X).

Please identify the organism.

Discussion of Blood Parasite Quiz #4

The images presented in Diagnostic Blood Parasite Quiz #4 are the following:

This image shows an African trypomastigote (trypanosome). The African trypanosomes belong to the subgenus *Trypanozoon* and as a group are referred to as the *T. brucei* complex. *T. brucei brucei* are parasites of domestic and wild animals and are not known to be infectious for humans.

Human infections are caused by *T. brucei gambiense* (West African Trypanosomiasis) and *T. brucei rhodesiense* (East African Trypanosomiasis). Infections caused by *T. brucei rhodesiense* are much more fulminant than those caused by *T. brucei gambiense*, and the parasitemia is much higher, with a much faster progression of disease. It is not possible to differentiate the three trypomastigotes of *T. brucei gambiense, T. brucei rhodesiense* and *T. brucei brucei* on the basis of morphology and all three organisms can be recovered in animal reservoir hosts. In the past, differentiation was based on clinical signs and geographic area. Differentiation is now based on isoenzyme characteristics and DNA and RNA methods in addition to the aforementioned factors. There are over 55 million people in 36 countries at risk and approximately 50,000 new cases per year of African trypanosomiasis.

Comment: Although the first descriptions of human sleeping sickness were made by European colonists in the late 19th and early 20th centuries, Atkins in 1742 described a "sleepy distemper" and in 1857 David Livingstone described a "fly disease". In 1901, *Trypanosoma brucei gambiense* was seen in the blood of a European steamboat captain working on the Gambia River. After Forde's discovery in 1901, Dutton in 1902 proposed the name *Trypanosoma gambiense*. By 1909, the mode of transmission by the tsetse fly, *Glossina palpalis*, was confirmed. The primary endemic area for *T. brucei gambiense* coincides with the tsetse fly belt through the heart of Africa. The disease remains, for the most part, unchecked due to the sophisticated biological defense mechanisms adopted by the parasite, tolerance to therapy, and the socioeconomic realities in a region where priorities may not include adequate surveillance and control.

Life cycle: Trypanosomal forms are ingested by the tsetse fly (*Glossina* sp.) when a blood meal is taken. Once the short, stumpy trypomastigote reaches the midgut of the tsetse fly, it transforms into a long slender procyclic stage. The organisms multiply in the lumen of the midgut and hindgut of the fly. After approximately 2 weeks, the organisms migrate back to salivary glands through the hypopharynx and salivary ducts, where the organisms attach to the epithelial cells of the salivary ducts and then transform to their epimastigote forms. In the epimastigote forms, the nucleus is posterior to the kinetoplast, in contrast to the trypomastigote, in which the nucleus is anterior to the kinetoplast. There is continued multiplication within the salivary gland, and metacyclic (infective) forms develop from the epimastigotes in 2 to 5 days. With development of the metacyclic forms, the tsetse fly is now infective. During the act of feeding, the fly introduces the metacyclic trypanosomal forms into the next victim in saliva injected into the puncture

wound. The entire developmental cycle in the fly takes about 3 weeks and once infected, the tsetse fly remains infected for life.

The trypanosomal (trypomastigote) forms can be found in the blood, cerebrospinal fluid (CSF), lymph node aspirates, and fluid aspirated from the trypanosomal chancre (if one forms at the site of the tsetse fly bite). The trypomastigote forms multiply by longitudinal binary fission. The organism is highly pleomorphic, having a variety of trypanosomal forms in the same blood smear. The forms range from long, slender-bodied organisms with a long flagellum (trypomastigote) attaining a length of 30 mm or more to short, fat, stumpy forms without a free flagellum, with a length of approximately 15 mm. The short, stumpy forms do not divide in the blood stream but are the infective stage for the tsetse fly.

Antigenic Variation: A unique feature of African trypanosomes is their ability to change the surface coat of the outer membrane of the trypomastigote, helping to evade the host's immune response. The trypomastigote surface is covered with a dense coat of approximately 10 million molecules of the membrane-form variant surface glycoprotein (VSG). There are approximately 100 to 1,000 genes in the genome responsible for encoding for as many as 1,000 different VSGs. More than 100 serotypes have been detected in a single infection. It is postulated that the trypomastigote changes its coat about every 5 to 7 days (antigenic variation). This change is responsible for successive waves of parasitemia every 7 to 14 days and allows the parasite to evade the host's humoral immune response. There is no evidence the host's immune system induces the VSG switches.

Antigenic variation, however, is probably only one of several mechanisms enabling these parasites to thrive in spite of the host's immune defenses. The ability to grow in high levels of interferon-gamma, to avoid complement-mediated destruction and to vary their susceptibility to a subclass of human high density lipoproteins may also facilitate organism survival.

Clinical Disease: In general, African trypanosomiasis caused by *T. b. gambiense* (West African Sleeping Sickness) has a long, mild chronic course that ends fatally with CNS involvement after several years' duration. This is unlike the disease produced with *T. b. rhodesiense* (East African Sleeping Sickness) that has a short course and ends fatally within a year.

Symptoms may occur within months to years after infection. The first distinct symptoms appear upon invasion of the lymph nodes, which is followed by the onset of remittent, irregular fevers with night sweats. Headaches, malaise,

and anorexia frequently accompany the fevers. The febrile period of up to a week is followed by an afebrile time frame of variable duration and then another febrile period. Many trypomastigotes may be found in the circulating blood during fevers, but few are seen during afebrile periods. Lymphadenopathy is a consistent feature of Gambian trypanosomiasis. Enlarged lymph nodes are soft, painless, and nontender. Although any lymph node may be affected, posterior cervical regions are most frequently involved (Winterbottom's sign). Trypomastigotes can be aspirated from the enlarged lymph nodes. In addition to lymph node involvement, the spleen and liver become enlarged. With Gambian trypanosomiasis, the blood-lymphatic stage may last for years before the sleeping sickness syndrome occurs.

Diagnosis: Physical findings and clinical history are very important in establishing the diagnosis. Diagnostic symptoms include irregular fever, enlargement of the lymph nodes (particularly those of the posterior triangle of the neck, which is known as Winterbottom's sign), delayed sensation to pain (Kerandel's sign), and erythematous skin rashes. Definitive diagnosis depends upon demonstration of trypomastigotes in blood, lymph node aspirates, sternum bone marrow, and CSF. Due to the greater frequency of higher parasitemia, there is a better chance of detecting organisms in body fluids in infections caused by *T. brucei rhodesiense* (East Africa) than *T. brucei gambiense* (West Africa). Because of periodicity, parasite numbers in the blood may vary; therefore, multiple specimens should be collected and a number of techniques used to detect the trypomastigotes.

Key Points - Laboratory Diagnosis:

1. Trypomastigotes are highly infectious, and health care workers must use bloodborne pathogen precautions.

2. Trypomastigotes may be detected in aspirates of the chancre and enlarged lymph nodes in addition to blood and CSF.

3. Concentration techniques, such as centrifugation of CSF and blood, should be used in addition to thin and thick smears (any of the blood stains).

4. Trypomastigotes are in highest numbers in the blood during febrile periods.

5. Multiple daily blood examinations may be necessary to detect the parasite.

6. Blood and CSF specimens should be examined during therapy and 1 to 2 months posttherapy.

Epidemiology and Control: *T. brucei gambiense* is transmitted from person to person by the bite of the tsetse fly (*Glossina palpalis* and *G. tachinoides*) after infectivity develops within the insect. The development cycle in the fly, depending on temperature and moisture, varies from 12 to 30 days and averages 20 days. Less than 10% of the tsetse flies become infective after obtaining blood from infected patients, and vertical transmission from infected fly to offspring is not known to occur. *G. palpalis* and *G. tachinoides* can be found in areas of thick shrubbery and trees near the banks of rivers, streams, or water holes; therefore, transmission can readily occur when people frequent these areas. Both female and male tsetse flies can transmit the infection. In addition to biological transmission, the tsetse fly may mechanically transmit the infection with its proboscis if it bites an uninfected person within a few hours of biting an infected person. Congenital transmission in humans has also been documented.

Although *T. brucei gambiense* can be transmitted to animals, no animal reservoirs for West African sleeping sickness have been documented. Trypanosomal strains isolated from hartebeest, kob, chickens, dogs, cows, and domestic pigs in West Africa are identical to those isolated from humans in the same area. These findings suggest that animals may serve as reservoirs; however, there is no indication that these animal trypanosomal forms may be directly transmitted to humans and that a patent infection will develop. Epidemiologic evidence suggests that in endemic areas, transmission may be entirely human to human. Asymptomatic individuals are thought to be the residual reservoir of the disease.

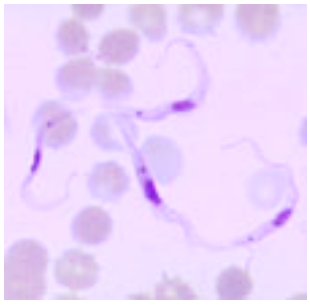
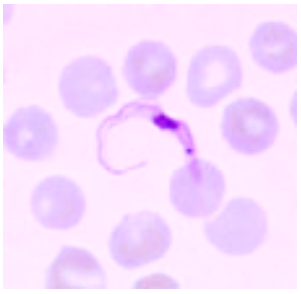
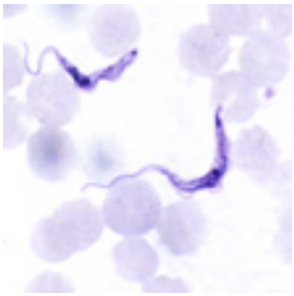
In areas where West African sleeping sickness is endemic, *T. brucei gambiense* has been controlled by eliminating the parasite through regular population-screening programs. This approach effectively reduces the prevalence of the disease to low levels; however, with interruptions in surveillance, resurgence of the disease will occur. An example of reassurance of West African trypanosomiasis was well documented in southern Sudan where the disease prevalence found was among the highest every documented.

In regions where the disease is endemic, natives appear to be more resistant to infection than are new arrivals to the area, even though there is no evidence of acquired immunity. West African sleeping sickness affects primarily rural populations and tourists are rarely infected. Chemo-prophylaxis

is not recommended because of drug toxicity and vaccines are unavailable. It may be possible to develop a vaccine because immunity to reinfection occurs with *T. brucei gambiense*.

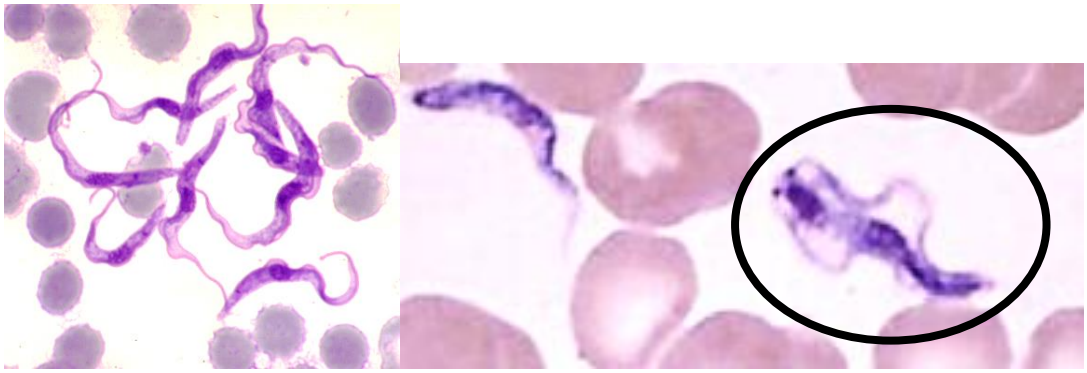
Tourists are usually not at great risk unless they are traveling and spending long periods of time in rural areas of Western and Central Africa. Persons visiting areas where the disease is endemic should avoid tsetse fly bites by wearing protective clothing (long-sleeved shirts and long pants). Wear khaki or olive colored clothing; the tsetse fly is attracted to bright colors and very dark colors. Because heavy clothing is not always practical because of heat and humidity, other measures include use of insect repellents, bed netting, and screen

Treatment: Treatment should be started as soon as possible and is based on the patient's symptoms and laboratory findings. The choice of antiparasitic drug will depend upon whether the CNS is infected. Suramin or pentamidine isethionate can be used when the CNS is not infected. Pentamidine isethionate does not cross the blood-brain barrier and is administered intramuscularly; side effects include an immediate hypotensive reaction, nausea, vomiting, and Herxheimer-type (inflammatory) reactions. Suramin (Bayer 205), a diamidine is effective in treating the hemolymphatic stage and CNS disease of *T. brucei gambiense*. When using suramin, a test dose should be given to the patient to ensure the drug can be tolerated. It should not be given to patients with renal disease, and if proteinuria, red blood cells or casts are detected in the urine, treatment should be stopped. Suramin is given intravenously; its side effects include nausea, vomiting, loss of consciousness, seizures, pruritus, edema, and hepatitis. Although rare, fatalities have been reported during suramin therapy. It is recommended that an infectious disease consultation be obtained prior to the start of therapy.

These images are representative of African trypomastigotes (trypanosomes). The centrally located nucleus stains reddish to purple (depending on the stain). At the organism's posterior end are the kinetoplast (very small) and the

remaining intracytoplasmic flagellum (axoneme), which may not be noticeable. The flagellum arises from the kinetoplast, as does the undulating membrane. The flagellum runs along the edge of the undulating membrane until the undulating membrane merges with the trypanosome body at the organism's anterior end. At this point, the flagellum becomes free to extend beyond the body. It is important to remember that dividing trypomastigotes can be seen on the blood films, while those of American Trypanosomiasis (*Trypanosoma cruzi*) do not divide within the peripheral blood (see images below). Note the dividing organisms in both images, especially the one on the right – circle.



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