

PARASITOLOGY CASE HISTORY #9 (BLOOD PARASITES) (Lynne S. Garcia)

A 26 year old male graduate student returned from a trip with colleagues to observe the lowland gorillas in the Congo. He and his companions reported many insect bites; many within the group had not taken malarial prophylaxis. He developed a fever after returning; about a month later he began to complain of headaches, malaise, anorexia, and swollen lymph nodes. He was also complaining of itchy skin and a rash. Routine blood work was ordered, including thick and thin blood films that were stained with Wright-Giemsa stain. The following images were seen on routine microscopic examination of the thin blood films. Some information courtesy of CDC.



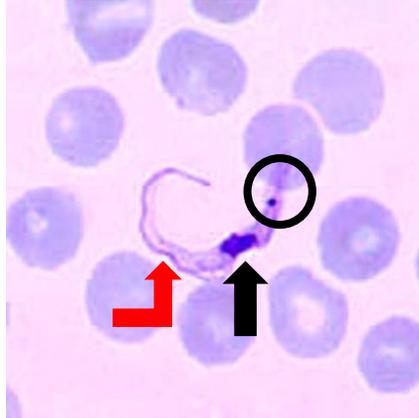
What infection most likely matches these images? What key characteristics support your diagnosis?

Answer and Discussion of Blood Parasite Quiz #9

The images presented in this quiz are the following: *Trypanosoma brucei* ssp. Note very small kinetoplast, nucleus, and undulating membrane, all typical for one of the African trypanosome subspecies (second row below).



Left: swollen lymph node (circle); right: sleeping sickness



Circle: small, compact kinetoplast; black arrow: nucleus; red arrow: undulating membrane.

Comments on the Patient:

After the host has been bitten by an infected tsetse fly, metacyclic trypomastigote stages are introduced into the skin, where they multiply and set up a local inflammatory reaction. A nodule or chancre at the site (3 to 4 cm) may develop after a few days. However, this primary lesion will resolve spontaneously within 1 to 2 weeks. The chancre is seen frequently in white Europeans but rarely in patients indigenous to an area where the disease is endemic. Trypomastigotes may be detected in fluid aspirated from the ulcer. The trypomastigotes enter the bloodstream, causing a symptom-free low-grade parasitemia that may continue for months. This is considered stage I disease, where the patient can have systemic trypanosomiasis without CNS involvement. During this time, the parasites may be difficult to detect, even by thick blood film examinations. The infection may self-cure during this period without development of symptoms or lymph node invasion. The patient presents with typical symptoms with stage I African trypanosomiasis.

Clinical Disease:

West African trypanosomiasis (*Trypanosoma brucei gambiense*): more chronic leading to actual sleeping sickness.

East African trypanosomiasis (*Trypanosoma brucei rhodesiense*): much more acute, leading to death (often prior to the development of actual sleeping sickness syndrome).

Morphology cannot differentiate between the two subspecies.

Once trypomastigotes invade the CNS, the sleeping sickness stage of the infection is initiated (stage II disease) (see images above). The trypomastigotes are found primarily in the frontal lobes, pons, and medulla. Behavioral and personality changes are seen during CNS invasion. Gambian trypanosomiasis is characterized by steady progressive meningoencephalitis, apathy, confusion, fatigue, coordination loss, and somnolence. In the terminal phase of the disease, the patient becomes emaciated and progresses to profound coma and death, usually from secondary infection. Although this disease has always been considered as invariably fatal, it is now well documented that this outcome may not be the case. Information is found in a long-term follow-up (15 years) of a cohort of 50 human African trypanosomiasis patients from the Ivory Coast among whom 11 refused treatment after their initial diagnosis (12). In 10 out of 11 subjects who continued to refuse treatment despite repeated visits, parasite clearance was observed using both microscopy and PCR. Most of these subjects (7/10) also displayed decreasing serological responses, becoming progressively negative to trypanosome variable antigens.

The West African form (*Trypanosoma brucei gambiense*) is characterized by a slow progression to actual sleeping sickness, while the East African form (*Trypanosoma brucei rhodesiense*) is characterized by a more progressive disease that can lead to death prior to the actual sleeping sickness syndrome.

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 immune response. The trypomastigote surface is covered with a dense coat of approximately 10⁷ molecules of the membrane-form variant surface glycoprotein (VSG). There are approximately 100 to 1,000 genes in the genome, responsible for encoding 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 humoral immune response. There is no evidence that the host immune system induces the VSG switches. During antigenic switching, VSG is both internalized by the trypomastigote and released or shed into the blood. This shed VSG is most probably responsible for the immune dysfunctions noticed during infections. There is a release of gamma interferon (which stimulates parasite growth), suppression of interleukin-2, and hypergammaglobulinemia. Based on studies using *T. brucei*, bloodstream forms when aggregated in the presence of

antibodies can subsequently disaggregate, and this mechanism may function to aid survival of the trypomastigotes in the presence of antibody in the host prior to the occurrence of a VSG switching event.

VSG is a 58-kDa glycoposphatidylinositol (GPI)-anchored glycoprotein, having predominantly an α -helical secondary structure, with highly variable sequences embedded at the N terminus and monoallelically expressed from a repertoire of hundreds of genes. The disaggregation appears to be strictly energy dependent, and the organisms remain motile, metabolically active, and infective following this process. Antigenic variation, however, is probably only one of several mechanisms enabling these parasites to thrive in spite of the host immune defenses.

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 [Winterbottom's sign]), delayed sensation to pain (Kerandel's sign), and erythematous skin rashes. However, diagnosing imported human African trypanosomiasis outside endemic areas is difficult and diagnosis is often delayed. A recent case was reported with *T. gambiense* with an unusually long incubation period of at least 7 years. Definitive diagnosis depends on demonstration of trypomastigotes in blood, lymph node aspirates, sternum bone marrow, and CSF. Due to the greater frequency of high parasitemia, there is a better chance of detecting organisms in body fluids in infections caused by *T. brucei rhodesiense* than in those caused by *T. brucei gambiense*. Because of periodicity, parasite numbers in the blood may vary; therefore, multiple specimens should be collected and a number of techniques should be used to detect the trypomastigotes. Health care personnel must adhere to standard precautions when handling specimens from patients with suspected cases of African trypanosomiasis because the trypomastigotes are highly infectious.

The following specimens can be examined: chancre aspirate, lymph node aspirate, blood, CSF, antigen (serum/CSF) and/or antibody (blood, plasma, serum) detection, and molecular methods. Although animal inoculation and culture are also performed, these methods are not practical for the majority of diagnostic laboratories.

Key Points - Laboratory Diagnosis:

1. Trypomastigotes are highly infectious, and health care workers must use blood-borne-pathogen precautions.
2. Trypomastigotes may be detected in aspirates of the chancre and enlarged lymph nodes in addition to blood and CSF; trypomastigotes tend to be more numerous in the blood in Rhodesian trypanosomiasis, and diagnosis is normally confirmed from blood examination.
3. Concentration techniques, such as centrifugation of CSF and blood, should be used in addition to thin and thick smears (Giemsa, Wright's, or other blood stains). Due to heavier parasitemias, concentration may not be necessary for Rhodesian trypanosomiasis.
4. Trypomastigotes are present in largest numbers in the blood during febrile periods.
5. Examinations of multiple daily blood samples may be necessary to detect the parasite.
6. Blood and CSF specimens should be examined during therapy and again 1 to 2 months posttherapy.

Epidemiology and Prevention:

The incidence of *T. brucei rhodesiense* infections is considerably lower than that of *T. brucei gambiense* infections, and the extent of distribution is smaller. East African trypanosomiasis is usually found in woodland and savannah areas away from human habitation. The tsetse fly vectors of Rhodesian trypanosomiasis are game feeders that may incidentally transmit the disease from human to human or animal to human. Because the infection results in acute rather than chronic disease, asymptomatic carriers are not a source of transmission as they are in Gambian trypanosomiasis. The disease is an occupational hazard for persons working in game reserves and a threat to visitors to game parks. *T. brucei rhodesiense* has been isolated from a variety of game animals (bushbuck, hartebeest, and lion) and domestic animals (cattle and sheep), a group that may prove to be the more important group of reservoir animals.

References:

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