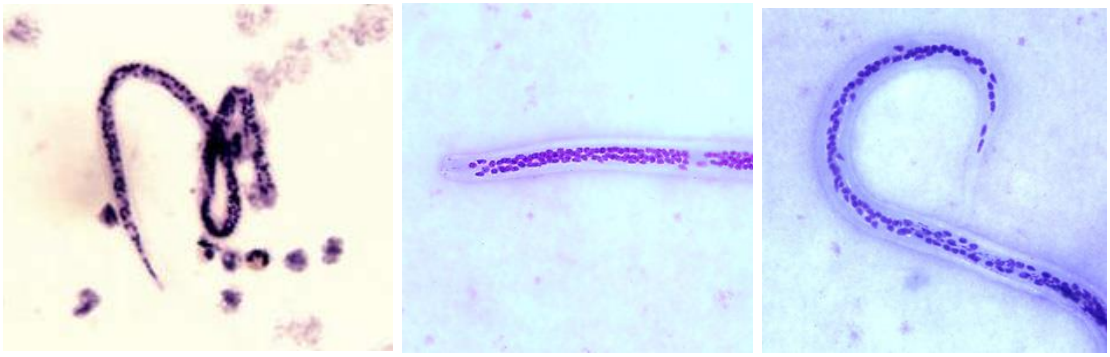
PARASITOLOGY CASE HISTORY #6 (BLOOD PARASITES)
(Lynne S. Garcia)

A 29-year old male from Haiti had blood drawn for complications associated with a congenital disorder. Thick blood films stained with Giemsa stain revealed the following image.



This image (left) was photographed from a stained thin blood film using the high dry objective 40X). The images on the middle and right were photographed using the 100X oil immersion objective. Note the distinct nuclei and pointed tail (nuclei stop before end of tail – right image).

Please identify the organism. Are there other tests that might confirm the diagnosis? If so, why does Giemsa present a problem in this case? Once the case was diagnosed as an incidental finding a much more extensive history was taken.

Discussion of Blood Parasite Quiz #6

The images presented in Diagnostic Blood Parasite Quiz #6 are the following:

This image shows a presumptive *Wuchereria bancrofti* microfilaria. There are eight filarial species in which the human is the definitive host, six of which are thought to be pathogenic. *W. bancrofti, B. malayi*, and *B. timori* all cause lymphatic filariasis. *Loa loa* causes Calabar swellings and allergic manifestations. *Mansonella streptocerca* causes skin disease. *O. volvulus* causes dermatitis and eye lesions. *Mansonella ozzardi* and *Mansonella perstans* are thought to be nonpathogenic.

Comment: Ancient Egyptian, Hindu, and Persian physicians (600 BC) were the first to note elephantiasis, which was probably due to W. bancrofti. Demarquay discovered microfilariae in hydrocele fluid in 1863, Wucherer

found organisms in chylous urine in 1868, Lewis found the microfilariae in blood in 1872, and the adult worm was found in a lymphatic abscess by Bancroft in 1872. By 1900, the entire life cycle of *W. bancrofti* had been elucidated; however, it was Patrick Manson's discoveries that showed for the first time that an arthropod was a vector for a parasitic disease. He had clarified the uptake of microfilariae by Culex mosquitoes and their subsequent maturation to infective forms. *W. bancrofti* infections are widely distributed throughout the tropics and subtropics. Humans are the only known reservoir hosts. At one time there was an endemic focus in the region of Charleston, S.C., which was probably related to the slave trade.

Life cycle: Humans and mosquitoes are necessary to complete the life cycle of *W. bancrofti*. The intermediate host, a mosquito, acquires the infection by ingestion of microfilariae in the blood meal. The major vectors in urban and semiurban areas are culicine mosquitoes, while anophelines are involved in the rural areas of Africa and Aedes is found in the Pacific Islands. An individual within an area of endemic infection is exposed to approximately 50 to 300 infectious larvae each year. Within hours after their arrival in the mosquito stomach, the microfilariae lose their sheaths. The larvae then penetrate the wall of the gut, migrate to the thoracic muscles, and develop into infective (filariform) larvae over a period of 7 to 21 days. The larvae migrate to the labella (distal end of the proboscis) of the mosquito and enter the skin of the definitive host through the puncture wound when a blood meal is taken. The infective larvae enter the peripheral lymphatic system and migrate to lymph vessels distal to the lymph nodes, where they grow to mature female and male adults and mate. Thousands of developing embryos can be found within the uteri of the female. Microfilariae are released from the gravid female and can be detected in the peripheral circulation in 8 to 12 months postinfection; however, filariasis without microfilaremia is not uncommon.

Adult worms are minute and threadlike, have a smooth cuticle, and are found in the lymph nodes and lymphatic channels. Adult males are about 40 mm long by 0.1 mm in diameter. Adult females are 80 to 100 mm long by 0.24 to 0.30 mm in diameter. To perpetuate their life cycle, sheathed microfilariae invade the blood; sometimes they can also be found in hydrocele fluid and chylous urine. The microfilariae range from 244 to 296 μm long and actively move about in the lymph or blood. The microfilaria has a sheath, and the body nuclei do not extend to the tip of the tail. Stains such as Giemsa, Wright's, or Delafield's hematoxylin have been used to help differentiate morphological features and thus identify the microfilariae to the species level. Stained microfilariae are about 245 to 300 μm long.

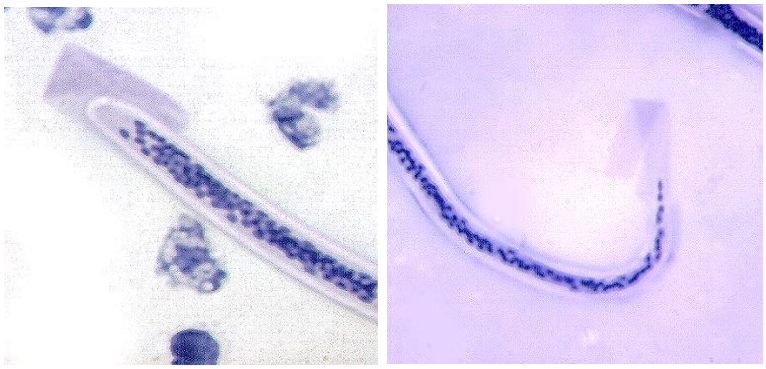
Clinical Disease: Lymphatic Disease. Estimates of 82 to 142 days for the prepatent period have been obtained from experimental human infections, and manifestations of filariasis can range from none to severe, depending on host factors and parasite strains. Early symptoms of filariasis include high fevers (filarial or elephantoid fever), lymphangitis, and lymphadenitis. Filarial fever usually begins with a high fever and chills that last 1 to 5 days before spontaneously subsiding, and in many cases, patients with filarial fevers do not have a microfilaremia. The lymphangitis extends in a distal direction from the affected nodes where the filarial worms reside.

Lymphadenitis and lymphangitis develop in the lower extremities more commonly than in the upper. However, in some areas of endemic infection such as northeastern Brazil, factors other than filarial worms can be the cause of subclinical pathology of the leg lymphatics and are not specific for bancroftian filariasis. In addition to limbs, there can be genital (almost exclusively a feature of *W. bancrofti* infection) and breast involvement. The lymph nodes most often affected are the epitrochlears and femorals. The nodes are firm, discrete, and tender and tend to remain enlarged, while the lymph vessel is indurated and inflamed. The overlying skin is tense, erythematous, and hot, and the surrounding area is edematous. Occasionally, abscesses form at the lymph node or along the lymphatic system and may take 2 to 3 months to heal.

Other potential sequelae include: obstructed lymphatics, acute filarial lymphangitis (death of adult worms leading to severe localized inflammation), tropical eosinophilia (pulmonary infiltrates, peripheral eosinophilia, cough, asthmatic attacks, and a history of prolonged residence in the tropics.

Diagnosis: When a patient is suspected of having filariasis, the clinical history helps determine the most appropriate specimen and collection time. The optimal time for drawing blood to detect nocturnal periodic *W. bancrofti* infections is between 10 p.m. and 4 a.m. Blood used to detect subperiodic *W. bancrofti* may be drawn any time, although peak microfilaremias occur in the late afternoon. Finger prick, earlobe, or venous blood (using EDTA anticoagulant [purple-top tube]) may be taken for direct wet, thin, and thick blood smears. Blood films may be stained with Giemsa or Delafield's hematoxylin stain; however, Giemsa stain does not stain the microfilarial sheath adequately, although hematoxylin stains do so (see images below). Examination of a thin blood film for microfilariae should include low-power review of the entire film, not just the feathered edge. Sheathed microfilariae often lose their sheath when drying on thick films. All thin and thick blood films submitted/prepared for examination for parasites should first be

examined using the 10x objective; immediate examination at higher magnifications may miss microfilariae, particularly if in low numbers.



Note the visibility of the sheath beyond the head (left) and beyond the tail (right). Blood films stained with hematoxylin stain.

Key Points - Laboratory Diagnosis:

1. A travel and geographic history should be obtained to maximize the best type of specimen and optimal collection time for the filarial infection suspected.

2. In addition to multiple thin and thick blood films, Knott or membrane concentration techniques should be used to detect microfilariae normally found in the peripheral blood.

3. It is important to examine every portion of the thin and thick blood films; microfilariae are often found at the outside edges or in the original drop from which the thin film was “pulled.” All thin or thick blood films should first be examined using the 10x objective (low power). If immediate examination is undertaken at a higher magnification, the microfilariae may be missed, particularly if the parasite numbers are low.

4. Giemsa stain does not stain the W. bancrofti sheath as well as a hematoxylin-based stain (Delafield’s hematoxylin).

5. Serologic tests are more meaningful in patients who have not resided in the areas of endemicity for extended periods.

6. Antigen detection tests are commercially available and may be very helpful in the detection of circulating filarial antigens.

7. PCR may prove to be valuable in the diagnosis of lymphatic filariasis; however, these procedures are often limited to research facilities. Some testing is now available commercially.

8. Ultrasonography has proven to be very valuable in assessing lymphatic filariasis in both adults and children; this approach can be much more sensitive than a physical examination alone**.**

Epidemiology and Control: *W. bancrofti* infections are widely distributed throughout the tropical and subtropical regions of Africa, Asia, Central and South America, the Caribbean islands, and the Pacific Islands. This species infects an estimated 115 million individuals; however, based on diagnostic test limitations, the actual number may be twice as great. *Anopheles* and *Culex* mosquitoes are night-biting vectors for the nocturnally periodic W. bancrofti, while the subperiodic strain is transmitted by day-biting Aedes mosquitoes. In areas of endemicity, exposure begins early in childhood, with microfilaria rates increasing with age, although the infection may not be clinically apparent.

Research on lymphatic filariasis, has mainly focused on rural environments, where it also has its major impact. However, there is also a potential for urban transmission, which has been identified as one of the major future challenges. *W. bancrofti* has have a significant potential for urban transmission; one of its vectors, *Culex quinquefasciatus*, thrives and reproduces in crowded city areas with poor sanitary, sewerage and drainage facilities. There don't seem to be any major differences in the pattern of infection and disease prevalence between the surveyed urban sites and that seen in rural areas. Individual protection involves the use of insect repellents and bed netting treated with long-lasting insecticide; however, long-term protection has been sought through vector control and the use of chemotherapy.

Treatment: It is recognized that some patients with lymphatic filariasis may be asymptomatic; however, if they have microfilaria in the blood, they probably have subclinical disease (like the patient). Early treatment of these patients is recommended to prevent additional disease manifestations such as lymphatic damage. For patients who appear to have no circulating microfilariae but have a confirmed presence of adult worms, treatment with DEC and albendazole is recommended

References:

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