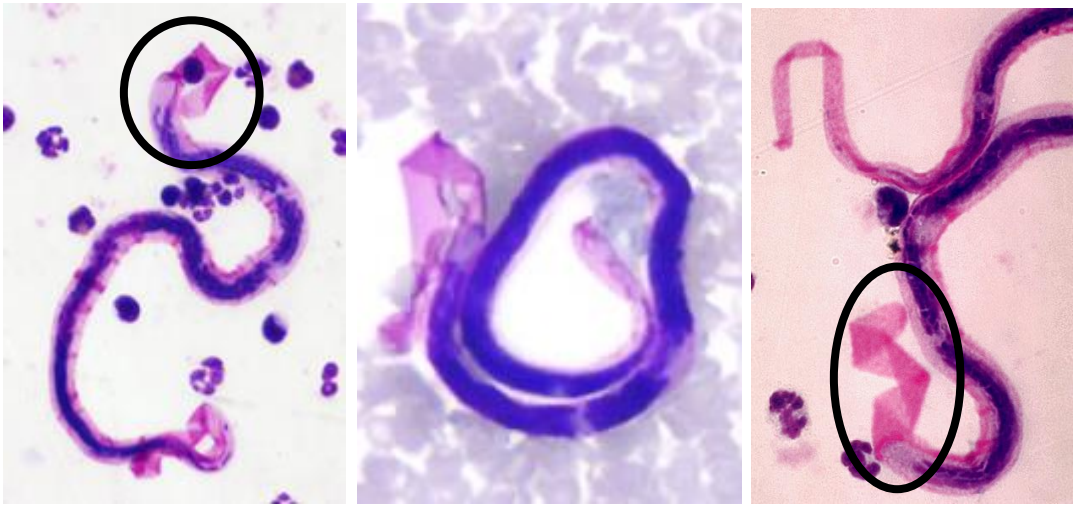
PARASITOLOGY CASE HISTORY #13 (BLOOD PARASITES)
(Lynne S. Garcia)

An epidemiologic survey was undertaken in a small town in Myanmar (Burma) endemic for lymphatic filariasis. Blood specimens were collected at night and stained using Giemsa stain. Approximately one-third of the cases were symptomatic with fevers and various degrees of lymphedema. Microscopic review of the stained blood smears revealed the following:



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

Answer and Discussion of Blood Parasite Quiz #13

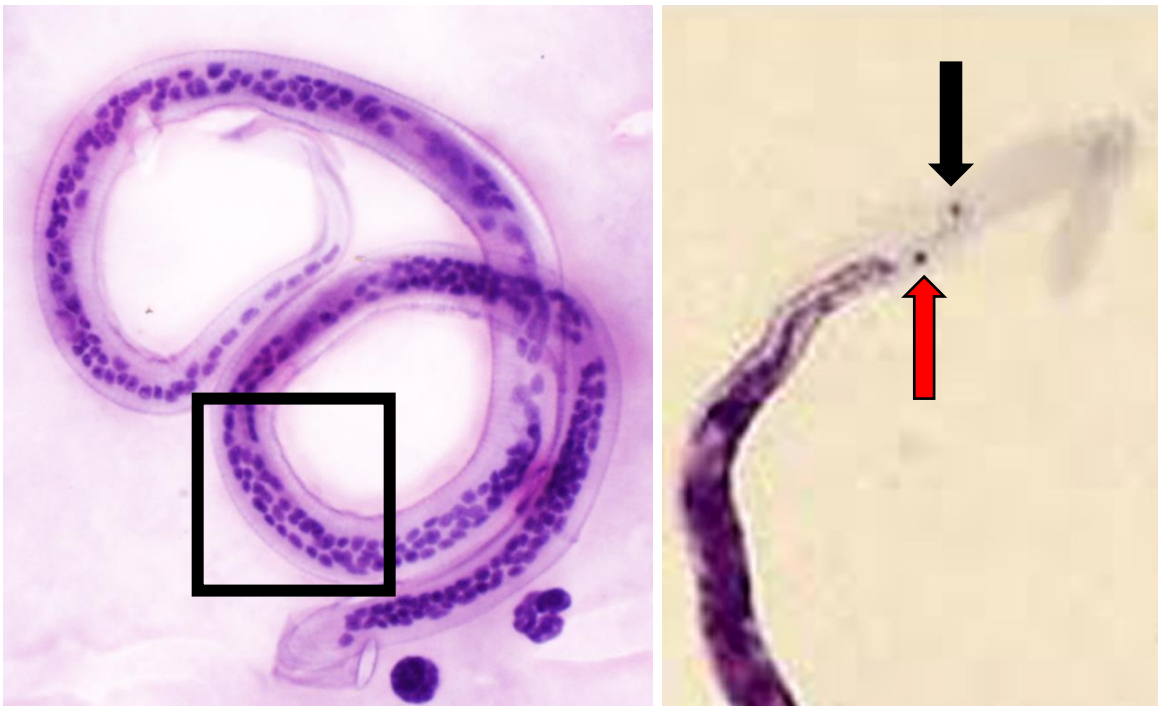
The images presented in this quiz are the following: This was a case of lymphatic filariasis caused by *Brugia malayi*. Diagnostic morphologic features included the following:

1. Microfilarial sheaths that stain pink with Giemsa stain (circle and oval).

2. Overall size range of 175 – 230 microns.

3. Dense column of nuclei, which are clearly defined (square).

4. Microfilariae with a relatively long head space and a tail with terminal (black arrow) and subterminal (red arow) nuclei (see below)



Life Cycle and Morphology

Although the two species can be differentiated morphologically, the life cycle of *B. malayi* is similar to that of *W. bancrofti*. Brugia has a shorter development time in the mosquito vector (3 to 4 months). The adult worms inhabit the lymphatics, and the females give birth to sheathed microfilariae. The microfilariae differ from those of *W. bancrofti* by having **two terminal nuclei that are distinctly separated from the other nuclei in the tail.** The last terminal nucleus is quite small and is found at the tip of the tail. The microfilariae range from 175 to 230 µm in length. The intermediate host is a mosquito that may be infected with a periodic or subperiodic strain, depending on the geographic area.

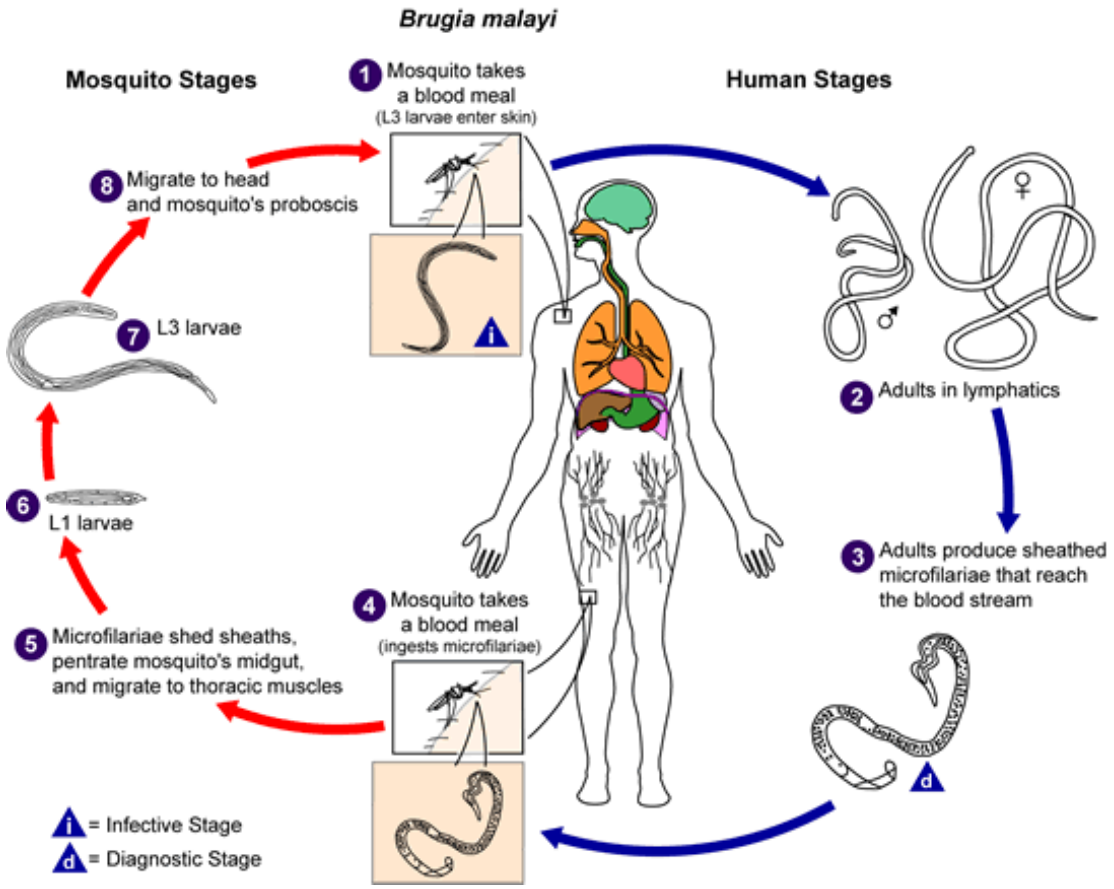
The Endosymbiont

Infection with *Wuchereria bancrofti, Brugia malayi,* or *Brugia timori* causes lymphatic filariasis, while *Onchocerca volvulus* causes “river blindness,” which can lead to severe ocular involvement and blindness. These parasites contain an endosymbiotic alpha-proteobacterium of the genus *Wolbachia;* these bacteria are *Rickettsia*-like, matrilineally inherited, obligate intracellular bacteria. They are required for larval worm development and adult worm viability and fertility. *Wolbachia* spp. have also been implicated in the pathogenesis of filariasis, including inflammatory responses related to filarial

chemotherapy and death of the parasites. These bacteria are linked to the onset of lymphedema and blindness.

Filarial and *Wolbachia* antigens activate the release of proinflammatory and chemotactic cytokines, which induce cellular infiltration and amplification of the inflammatory process. Toll-like receptors (TLRs), especially TLR2 and TLR6 (not TLR4 as originally thought), appear to play a role in this process. The increased levels of vascular endothelial growth factor (VEGF) VEGF-C and sVEGF-A seen in lymphedema patients were reduced following doxycycline treatment, which eliminates *Wolbachia*. In patients with hydrocele, doxycycline therapy also reduced the size of the hydrocele with reduced circulating levels of VEGF-A. Repeated and extensive exposure to filarial and *Wolbachia* antigens can lead to scarring and fibrosis in lymphatic filariasis and skin thickening and corneal scarring in onchocerciasis.

Like other bacteria in the family Rickettsiaceae, *Wolbachia* is sensitive to the tetracyclines (tetracycline and doxycycline) and to azithromycin and rifampin. The use of anti-*Wolbachia* antibiotics is promising as a means to improve the success of other mass treatment programs.



Identification of different *B. malayi* strains

In 1957, two subspecies of human infecting *Brugia malayi* were discovered by Turner and Edeson in Malaysia based on the observation of different patterns of microfilaria periodicity. Periodicity refers to a pronounced peak in microfilariae count during a 24 hour interval when microfilariae are present and detectable in the circulating blood. The basis for this phenomenon remains largely unknown. The specimens in this study were collected at night.

Nocturnal periodicity: microfilariae are not detectable in the blood for the majority of the day, but the microfilarial density peaks between midnight and 2 AM nightly.

Nocturnal subperiodicity: microfilariae are present in the blood at all times, but appear at greatest density between noon and 8 PM.

Clinical Disease

The clinical pathology of *B. malayi* infections in humans is similar to that of *W. bancrofti*. Clinical manifestations usually develop months or years after infection, and many of the patients are asymptomatic even when they have microfilaremia. Lymphangitis and filarial abscesses occur with a greater degree of frequency than in *W. bancrofti* infections. If elephantiasis occurs, the swelling is normally restricted to the lower extremities below the knee. Sclerotic cordlike lymphatics and enlarged nodes in the arms and legs are common; urogenital involvement with chyluria does not occur. In disease caused by *B. malayi*, episodes of prolonged fever, adenolymphangitis, abscesses of affected lymph nodes, and local residual scarring occur quite frequently. Chronic lymphedema or elephantiasis, as seen in bancroftian filariasis, does not occur frequently.

The accumulation of many infective mosquito bites – several hundreds to thousands - is required to establish infection. This is due to the fact that a competent mosquito usually transmits only a few infective L3 larvae, and less than 10% of those larvae progress through all the necessary molting steps and develop into adult worms. Thus those at greatest risk for infection are individuals living in endemic areas – <u>short term tourists are unlikely to develop lymphatic filariasis.</u>

Diagnosis

The diagnostic methods are similar to those for *W. bancrofti* and include thin and thick blood films, wet preparations and concentrations, PCR, ultrasonography, and antigen and antibody detection. There are nocturnally periodic and nocturnally subperiodic strains, so travel history can be helpful in determining optimal specimen collection times. Giemsa stains the sheath of *B. malayi* (will stain pink) but does not stain the sheath of *B. timori*, a species found in the islands near Indonesia. Apparently, the sheath is absent in 50% of periodic microfilariae but in only 5% of subperiodic microfilariae. A multiplex, TaqMan-based, real-time PCR assay capable of simultaneously detecting *W. bancrofti* and *B. malayi* DNA extracted from human bloodspots or vector mosquito pools has recently been developed, with significant cost and labor savings.

KEY POINTS — LABORATORY DIAGNOSIS

Filariasis

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). The sheath of *B. malayi* stains pink with Giemsa stain.

References:

1. **Garcia, LS**, 2016. *Diagnostic Medical Parasitology*, 6th Ed., ASM Press, Washington, DC.

2. **Taylor, M. J., C. Bandi, and A. Hoerauf.** 2005. *Wolbachia* bacterial endosymbionts of filarial nematodes. Adv. Parasitol. 60:245–284.

3. **Babu S, TB Nutman.** 2012. Immunopathogenesis of lymphatic filarial disease. Semin Immunopathol 34:847-861.

4. **Scott AL, Ghedin E.** The genome of *Brugia malayi* - all worms are not created equal. Parasitol Int. 2009 Mar;58(1):6-11. doi: 10.1016/j.parint.2008.09.003. Epub 2008 Sep 24.