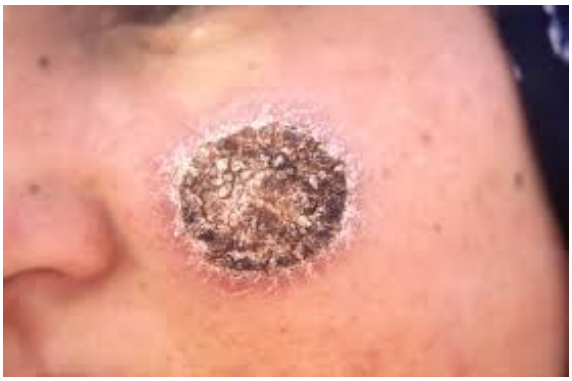
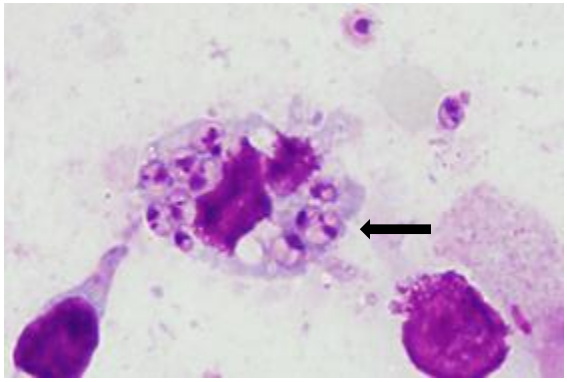
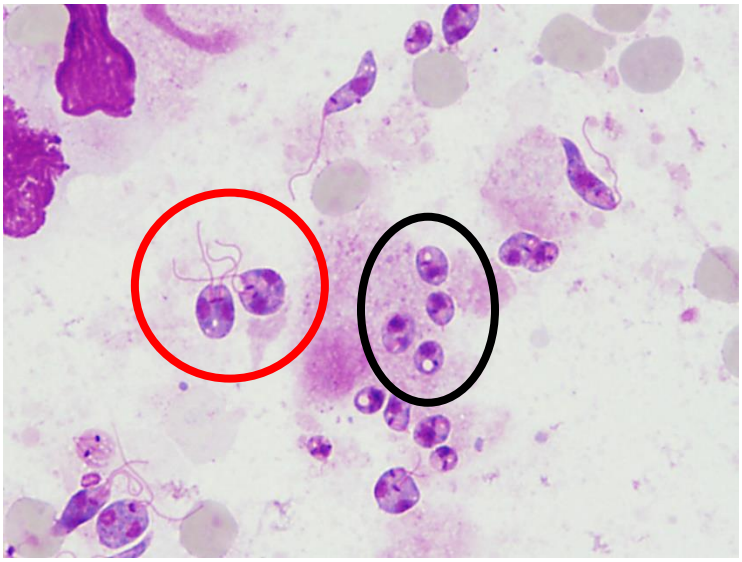
PARASITOLOGY CASE HISTORY 6 (HISTOLOGY)
(Lynne S. Garcia)

A 18 year-old female presented with a crusted nodule on her left cheek several months after traveling to Spain, Israel, Russia and other areas for an extended vacation with her family. Although they were in cities the majority of the time, many side trips were taken outdoors where they all recounted being bitten by insects numerous times. Antibiotic cream had been applied to her cheek with minimal changes; no other symptoms were seen. A biopsy was taken and submitted for histologic review. A Giemsa-stained touch preparation was also examined on site with the following results:





Courtesy of CDC, Washington, DC



Courtesy of CDC, Washington, DC.

- Based on these images, what is your diagnosis?

Scroll Down for Answer and Discussion

Answer and Discussion of Histology Quiz #6

This is a case cutaneous leishmaniasis (CL) caused by *Leishmania major*. Identification to the genus level was based on the presence of amastigotes (black oval and arrow) and promastigotes (red circle); molecular means are used to make the identification to the species since amastigotes all look the same. Amastigotes are small round to oval, approximately 1-5 µm long by 1-2 µm wide. They contain a round nucleus and a small bar-shaped kinetoplast (primitive flagella). Both have to be present for the identification of leishmaniasis. Occasionally, these amastigotes may transform to the promastigotes (culture/vector forms) (seen in the sand fly vectors (*Phlebotomus* spp. and *Lutzomyia* spp). The transformation may occur when the ambient temperature drops to that found in the vector and there is generally a delay in transport/processing. Promastigotes are larger, elongated or spindle-shaped and have a flagellum projecting from the anterior end.

The form introduced into the skin of the mammalian host by the sand fly is the promastigote. The organism is engulfed by reticuloendothelial cells (RE cells) of the mammalian host, where the parasite transforms into the intracellular amastigote form (Leishman-Donovan body, L-D body). Apparently, this process takes approximately 12 to 24 h. The large nucleus and small kinetoplast in the amastigotes can be seen in tissue after staining with Giemsa, Wright's, or other blood stains. The short intracytoplasmic portion of the flagellum (small bar) can also be seen within some of the amastigotes. The amastigotes multiply by binary fission within the macrophage until the cell

is destroyed, and the released parasites are phagocytized by other RE cells or ingested by sand flies. This phase is chronic and can last from months to years, even a lifetime, depending on the species involved. In CL, the amastigotes remain confined to the skin, usually as skin lesions such as raised papules or ulcers. Lesions usually occur on exposed parts of the body such as the face, hands, feet, arms, and legs; uncommon sites include the ears, tongue, and eyelids.

During a blood meal taken by the sand fly, amastigotes are ingested and transform into the promastigote stage. Promastigotes multiply by longitudinal fission in the insect gut. Stages found in the sand fly vary from rounded or stumpy forms to elongated, highly motile metacyclic promastigotes. The metacyclic promastigotes migrate to the hypostome of the sand fly, where they are inoculated into humans when the sand fly takes its next blood meal. Various surface molecules on the promastigote, such as gp63 and lipophosphoglycans, help bind the parasite to the host macrophage receptors, thus allowing it to be phagocytized. Depending on the species, the duration of the life cycle in the sand fly varies from 4 to 18 days.

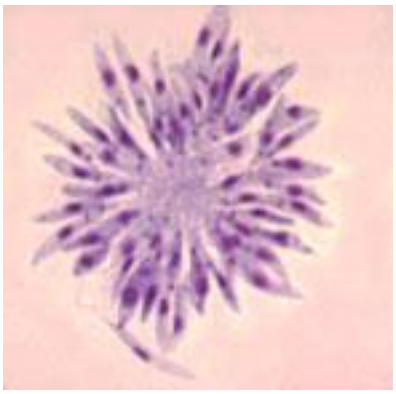
Sand flies are pool feeders and possess cutting mouthparts that slice into the skin, allowing a small pool of blood to form. Thus, although they are small flies (2 to 3 mm), the bite can be painful. In most endemic foci, the vast majority of sand flies are uninfected; however, those that are infected are very efficient vectors. Infected flies probably remain so for life, although this is just a matter of weeks. Under optimal conditions, transformation of the amastigotes to promastigotes generally takes 5 to 7 days; by that time, the female fly is ready to take her next blood meal.

The incubation period of CL is usually 2 to 8 weeks but can be as long as several years. The first sign of cutaneous disease is a lesion (generally a firm, painless papule) at the bite site. Although a single lesion may appear insignificant, multiple lesions of *L. major* or disfiguring facial lesions of *L. tropica* may be psychologically or physically devastating. In general, all lesions on a patient have a similar appearance and progress at the same speed.

The original lesion may remain as a flattened plaque or may progress, with the surface becoming covered with fine, papery scales. These scales are dry at first but later become moist and adherent, covering a shallow ulcer. As the ulcer enlarges, it produces exudate and may develop a crust. The edge of the lesion is usually raised. Depending on the species, satellite lesions are common and merge with the original lesion.

L. major causes rural disease, a variety of CL. Multiple lesions are present and are accompanied by marked inflammation and crusting. The center of the lesion tends to be necrotic and exudative, forming a loose crust. The lesions tend to mature and may heal relatively quickly, often lasting only a few months. Lymphatic spread may occur in *L. major* infections, with subcutaneous nodules in a linear distribution and regional lymphadenopathy; if the initial lesion is on the hand, this clinical presentation may resemble sporotrichosis. *L. major* infections may result in severe scarring.

In areas of the world where physicians are very familiar with leishmaniasis, the diagnosis may be made on clinical grounds. However, in other areas of the world, where the disease is rare, the condition may not be recognized. Definitive diagnosis depends on demonstrating the amastigotes in tissue specimens or the promastigotes in culture. CL may have to be differentiated from a number of other lesions and diseases, including basal cell carcinoma, tuberculosis, various mycoses, cheloid, and lepromatous leprosy.



Promastigotes from culture.

All smears to be stained with Giemsa should be air dried and fixed with 100% methanol prior to staining. Any of the other blood stains can also be used. The amastigotes are found within the macrophages or close to disrupted cells. Although this intracellular form may mimic other intracellular parasites (*Histoplasma capsulatum* and some of the microsporidia), the presence of the intracytoplasmic kinetoplast (primitive flagellum/small bar) confirms the identification. After staining, the cytoplasm appears light blue and the nucleus and kinetoplast appear red to purple. Regardless of the blood stain used, the parasites will stain like the WBCs and/or tissue cells. In very early and older lesions, very few organisms may be present, so that prolonged examination of the slide may be required.

From August 2002 to February 2004, over 500 cases of parasitologically confirmed cases of CL were found in U.S. military personnel serving in

Afghanistan, Iraq, and Kuwait. The majority of these cases were probably acquired in Iraq, with *L. major* being confirmed for most of the cases through isoenzyme electrophoresis of cultured parasites.

References:

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

2. **Magill, AJ, WM Meyers, RC Neafie, MK Klassen-Fischer.** 2011. *Topics on the pathology of protozoan and invasive arthropod diseases, Cutaneous leishmaniasis.* Uniformed Services University of the Health Sciences, Bethesda, MD.

3. **Willard, R. J., A. M. Jeffcoat, P. M. Benson, and D. S. Walsh.** 2005. Cutaneous leishmaniasis in soldiers from Fort Campbell, Kentucky, returning from Operation Iraqi Freedom highlights diagnostic and therapeutic options. *J. Am. Acad. Dermatol.* **52**:977–987.