PHE National Parasitology Reference Laboratory, Hospital for Tropical Diseases, 3rd Floor Mortimer Market, Centre, Capper Street, London WC1E 6JB, TEL: +44 (0) 207 383 0482, FAX +44 (0) 207 388 8985

Visceral Leishmaniasis

Introduction

Human visceral leishmaniasis (VL), sometimes known as Kala-azar, is caused by *Leishmania donovani* complex; *L. donovani* and *L. donovani infantum* in the old world and *L. donovani chagasi* in the new world. The clinical features -azar caused by these species are similar, but they have different epidemiological features. The parent species *L. donovani* occurs in Asia (Northeastern China, India and Iran) and Africa (primarily Sudan, Kenya and Ethiopia) and can affect people of all ages. The parasite (*L. d. infantum*) which causes VL in countries bordering the Mediterranean, (Southern Europe as well as North Africa), affects young children as well as infants. It is now being seen in the immuno-compromised. In the New World also, VL is mainly a disease of young children, with the causative organism *L. d. chagasi* being closely related to, but slightly different from, *L. donovani*. The main geographical foci of VL in Latin America are in northern and northeastern Brazil. Small foci are found in northern Argentina, Columbia and Venezuela. Sporadic cases are found in central American countries, including Mexico.

Life cycle

Infection is initiated by the bite of the sandfly ie the *Plebotomus* species. When a sandfly takes a blood meal from an infected host, minute amounts of blood, lymph and infected macrophages are ingested. The number of **amastigotes** ingested is thought to be extremely low. Once ingested in the sandfly the **amastigotes** transform to **promastigotes**, There is sequential division and development of the **promastigotes** from a non infective to an infective stage. The infective forms are called **metacyclic promastigotes**. These are formed in the midgut of the sandfly and migrate to the proboscis. The persistence of extracellular inoculated promastigotes at the site of the bite is dependent on the rate of recruitment of macrophages to the bite area (thought to be a matter of hours). After phagocytosis, transformation to dividing **amastigotes** occurs within 24 hours. Reproduction at all stages of the lifecycle is believed to occur by binary fission. No sexual stage has been identified.

Clinical Disease - Visceral leishmaniasis

The incubation period of VL may vary between 2 weeks and 18 months. The onset of VL is usually insidious with fever, sweating, weakness and weight loss. The most prominent findings are fever, hepatosplenomegaly and anaemia. The sites mainly affected are the liver, spleen and bone marrow. Enlargement of the liver is due to hyperplasia of Kupffer cells which are packed with amastigotes.

The bone marrow is infiltrated with parasitised macrophages. Some organs, notably the kidneys, may show pathological changes secondary to deposition of immune complexes. In advanced cases, ascites and oedema can develop. Deaths are usually due to secondary bacterial infections such as pneumonia, tuberculosis or dysentery.

Laboratory Diagnosis of visceral leishmaniasis

1. **Microscopy**

   Parasites may be found in a splenic aspirate, liver biopsy or bone marrow biopsy. These techniques, especially splenic aspirate and liver biopsy, can be hazardous and require previous expertise in the procedure.

   a) Air dry smears.
   b) Fix in methanol for 1 minute
   c) Stain with Giemsa 1 in 10 in buffered distilled water pH 6.8 for 30 minutes (or use the rapid Field's stain)
   d) Wash the slide in buffered water and drain dry

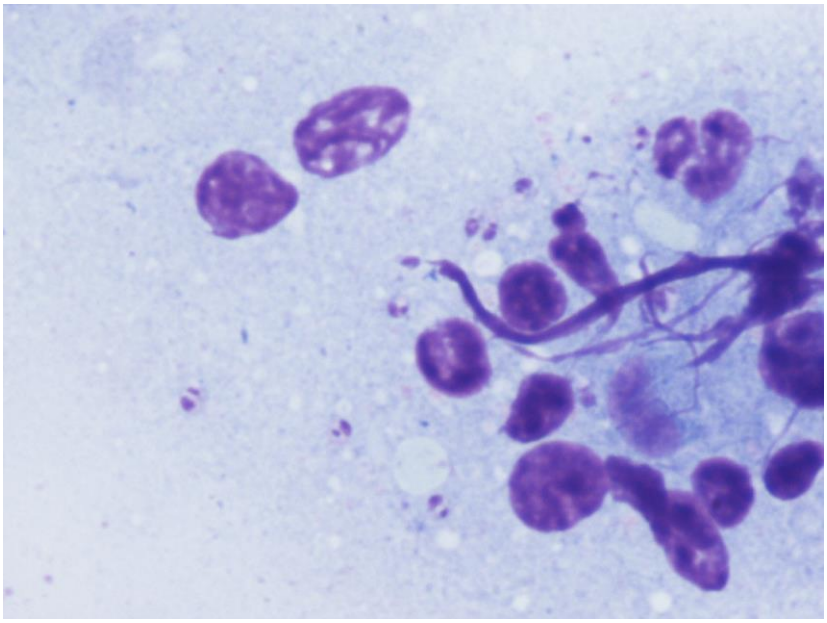
   Amastigotes of leishmania should be seen in positive smears. They are approximately 2-4 µm in size, oval and are frequently seen within the cytoplasm of the macrophage. The amastigotes possess a nucleus and a rod - shaped kinetoplast within the cytoplasm. In many samples a very small number of parasites are present. Extensive searching of the film is necessary.

2. **Culture**

   The aspirates can be cultured in Novy-Nicolle-MacNeal (NNN) or Schneider's Drosophila medium. In culture the **amastigote** stage converts to the **promastigote** stage. However, this is not a rapid technique, as the parasites may take anything from 10 - 21 days to grow.

3. **Serodiagnosis**

   VL produces large amounts of specific IgG which can be used for diagnosis. Currently the most used sero diagnostic tests are Indirect-immuno Fluorescent Antibody Test (IFAT), Enzyme Linked Immunosorbent Assay (ELISA) and Direct Agglutination Test (DAT).



Amastigotes in a splenic dab