Lecture 10: Emerging Parasitic protozoa part 2 (Hemoflagellates)

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HSC4933
Emerging Infectious Diseases

Readings-Lecture 10

- Ch. 7 (p. 144)
- Ch. 8 (p. 163 [table 8.2], pp. 167-172, special attention to tables)
- Ch. 11 (pp. 277-281, 281-82)
- Ch. 14 (p. 367 [table 14.1], 375



Monsters Inside Me

- **African sleeping sickness (Trypanosoma brucei rhodesiense/gambiense, hemoflagellate)**:

  Background: http://animal.discovery.com/invertebrates/monsters-inside-me/african-sleeping-sickness-trypanosomiasis/

  Videos: http://animal.discovery.com/videos/monsters-inside-me-african-sleeping-sickness.html

  http://animal.discovery.com/videos/monsters-inside-me-symptoms-of-sleeping-sickness.html

  Cutaneous Leishmaniasis (*Leishmania* spp., Hemoflagellate):

  Background: http://animal.discovery.com/invertebrates/monsters-inside-me/cutaneous-leishmaniasis-leishmania/

  Videos: http://animal.discovery.com/videos/monsters-inside-me-cutaneous-leishmaniasis.html

  http://animal.discovery.com/videos/monsters-inside-me-the-leishmaniasis-parasite.html

Learning objectives: Hemoflagellates

- Know basic life cycle and developmental stages
- Required hosts
  - Transmission strategy
  - Infective and diagnostic stages
- Know the common characteristics of each group
  - Be able to contrast and compare
- Diseases (differences?), high-risk groups
- Diagnostic methods, treatment
- Know important parasite survival strategies

Lecture 10: On the Menu

- Hemoflagellates
  - *Trypanosoma* spp.
  - *Leishmania* spp.

Protozoan flagellates in the family Trypanosomatidae are parasitic in the blood of many species of domestic and wild animals and birds

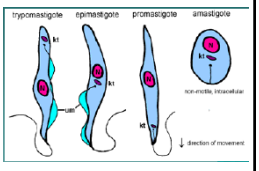
Table 77-1. Classification of Parasitic Protozoa and Associated Diseases

Phylum	Subphylum	Representative Genera	Major Diseases Produced in Human Beings	Chapter
Sarcomastigophora (with flagella, pseudopodia, or both)	Mastigophora (flagella)	*Leishmania*	Visceral, cutaneous and mucocutaneous infection	82
		Trypanosoma	Sleeping sickness; Chagas' disease	
		Giardia	Diarrhea	80
		Trichomonas	Vaginitis	
	Sarcodina (pseudopodia)	*Entamoeba*	Dysentery, liver abscess	79
		Dientamoeba	Colitis	
		Naegleria and *Acanthamoeba*	Central nervous system and corneal ulcers	81
		Babesia	Babesiosis	
Apicomplexa (apical complex)		*Plasmodium*	Malaria	83
		Isospora	Diarrhea	80
		Sarcocystis	Diarrhea	
		Cryptosporidium	Diarrhea	
		Toxoplasma	Toxoplasmosis	84
Microspora		*Enterocytozoon*	Diarrhea	—
Ciliophora (with cilia)		*Balantidium*	Dysentery	80
Unclassified		*Pneumocystis*	Pneumonia	85

Order Kinetoplastida

- Kinetoplast: spherical extranuclear DNA structure found in parasitic flagellates (family Trypanosomatidae)
- **Amastigote-intracellular form,** containing a nucleus and kinetoplast and the intracellular form of the flagellum; replicates within cells of reticuloendothelial system. **Can be infective stage for vector**
- Promastigote-possesses a flagellum anchored to the anteriorly located kinetoplast. **Can be infective stage for humans**
- Epimastigote-anterior flagellum is anchored to the kinetoplast; undulating membrane
- Trypomastigote: anterior flagellum and kinetoplast located at the posterior end of the body; undulating membrane.
  - **Can be infective stage for humans and vector**

Different forms appear in the life-cycles of trypanosomes, distinguished mainly by the position of the flagellum

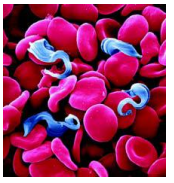


Hint: pay attention to bold writing

African trypanosomes

- African Sleeping Sickness
- *Trypanosoma brucei* complex with subspecies
  - *T. brucei brucei* infects game animals/livestock (causes nagana)
  - ***T. b. rhodesiense* causes East African trypanosomiasis**
  - ***T. b. gambiense causes* West and Central African sleeping sickness**
- Bloodborne disease transmitted by tsetse fly
- WHO estimates prevalence to be 50,000–70,000 cases worldwide (underestimated), based on a total number of 17,500 new cases per year

"Colorized" trypomastigotes in blood



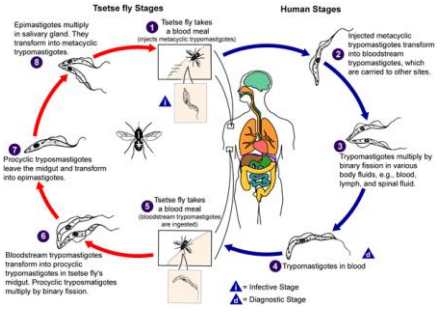
Testse fly



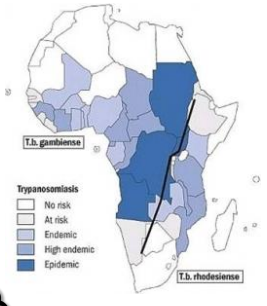
Comparison of two species causing human disease

- ***T. b. gambiense***
  - Habitat: riverine, rainforest, lakes
  - Reservoir: not common, domestic animals
  - Cycle: human-fly-human
  - Slower developing illness (>1 year)
  - Low parasitemia
- ***T. b. rhodesiense***
  - Savannah habitat: dry bush, woodland
  - Acute illness
  - Cycle: Ungulate-fly-human
  - Reservoir: wild animals
  - **Rapid onset of illness, often fatal**
  - High parasitemia

Trypanosomiasis: Life cycle



Disease Distribution



***T. b. gambiense* is found in foci in large areas of West and Central Africa.**

The distribution of *T. b. rhodesiense* is much more limited, found in East and Southeast Africa.

Which one is more serious?

Sleeping sickness



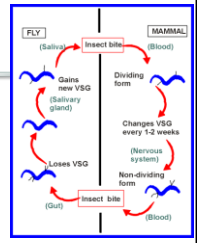
Clinical Features

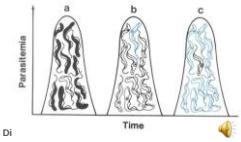
- Infection occurs in 3 stages:
  - A trypanosomal chancre can develop at the site of inoculation.
  - This is followed by a hemolymphatic stage with symptoms that include fever, lymphadenopathy, and pruritus.
  - **In the meningoencephalitic stage, invasion of the central nervous system can cause headaches, somnolence, abnormal behavior, and lead to loss of consciousness and coma.**
- **The course of infection is much more acute with *T. b. rhodesiense* than *T. b. gambiense*.**



VSG antigenic variation

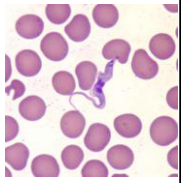
- Variable surface glycoprotein (VSG) coat
- **Hundreds of genes encode VSGs in each trypanosome's genome**
- Allows variants expressing a new VSG coat to escape the specific immune response raised against the previous coat.
- Helps to induce chronic infections in the hosts, which both ensures the transmission of the trypanosomes to other hosts and increases the severity of the pathology caused by trypanosome infection





Diagnosis and Treatment

- Demonstrating motile trypanosomes by microscopic examination of chancre fluid, lymph node aspirates, blood, bone marrow, or CSF
- Antibody detection has sensitivity and specificity that are too variable for clinical decisions.
- Treatment should be started as soon as possible
- The drug regimen depends on the infecting species and the stage of infection.
- Pentamidine isethionate and suramin are used for hemolymphatic stage of West and East African Trypanosomiasis, respectively.
- Melarsoprol for late disease with central nervous system involvement (infections by *T.b. gambiense* or *T. b. rhodiense*).

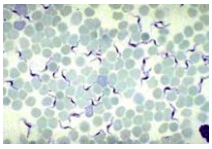


Prevention and Control

- Avoid tsetse fly habitats
- Wear neutral-colored clothing.
- Insect Repellent
- Surveillance: Regular screening of blood helps prevent the spread of infection which could occur through transfusion of blood.
- Control of vector population- trapping, insecticides
  - Cows used as bait to collect tsetse flies for surveillance
- Domestic animals e.g. cattle, pigs, may act as reservoirs

American trypanosomiasis (Chagas Disease)

- ***Trypanosoma cruzi* causes a** zoonotic disease that can be transmitted to humans by blood-sucking triatomine bugs.
  - 12 species of bug occur in U.S.
- Chagas disease is endemic throughout Mexico and Central and South America
- ~7.7 million persons infected, 108.6 million persons considered at risk, 3–3.3 million symptomatic cases, an annual incidence of 42,500 cases (through vector transmission), and 21,000 deaths every year
- Chagas disease can also be acquired by humans through blood transfusions and organ transplantation , congenitally (from a pregnant woman to her baby), and through foodborne transmission

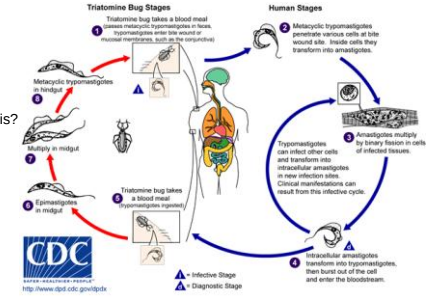


Reduvid bug pooping



Chagas Disease: Life cycle

How is this different from African trypanosomiasis?



Geographic Distribution





IMAGES

Chagas Disease in endemic areas

- Millions of people in South and Central America are infected and many are unaware they are infected
- Chagas' disease was endemic in Uruguay and kissing bugs were present in 80% of households
- An intensive program of vector reduction by spraying and replacing thatched roofs with metal, reduced incidence of the disease
- Uruguay was declared free of Chagas' disease in 1997

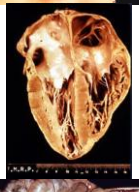


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Disease features

- The acute phase is usually asymptomatic, but can cause fever, anorexia, lymphadenopathy, mild hepatosplenomegaly, and myocarditis.
  - **Romaña's sign** may appear as a result of conjunctival contamination with the vector's feces.
  - A nodular lesion or furuncle, usually called **chagoma**, can appear at the site of inoculation.
  - Most acute cases resolve over a period of a few weeks or months into an asymptomatic chronic form
- The **symptomatic chronic form** may not occur for years or even decades after initial infection.
  - **Cardiomyopathy (the most serious manifestation);** pathologies of the digestive tract such as megaesophagus and megacolon
  - Acute infection can be lethal, and cardiomyopathy develops in 25%–30% of infected persons





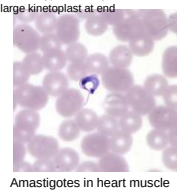


IMAGES

Diagnosis and Treatment

- Microscopic examination of blood smears, tissue sections
- Isolation of the agent
  - a) inoculation in culture with specialized media
  - b) inoculation into mice
  - c) Xenodiagnosis, where uninfected triatomine bugs are fed on the patient's blood, and their gut contents examined for parasites 4 weeks later.
- Molecular diagnostic tools
- Treatment
  - Medication for Chagas disease is usually effective when given during the acute phase of infection
  - Antiparasitic treatment with Benznidazole or Nifurtimox
  - Management of symptoms and signs of infection.

Different morphology compared to African tryps- "C shaped" and large kinetoplast at end



Amastigotes in heart muscle

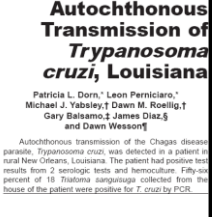


Prevention

- Spraying infested dwellings with residual-action insecticide
- Using bed nets treated with long-lasting insecticides
- Wearing protective clothing and applying insect repellent to exposed skin.
- In addition, travelers should be aware of other possible routes of transmission, including bloodborne and foodborne.
- No vaccine exists

Outbreaks and spread

- Foodborne in Brazil: sugar cane juice (2005), acai fruit (2006)
- Organ transplantation
  - Los Angeles: two cases of acute Chagas disease in heart transplant recipients reported by two hospitals in February 2006.
- Blood Transfusion
  - American Red Cross Study in CA, AZ- identified 32 donations (approximately one in 4,655) as confirmed positive for *T. cruzi* antibodies
- New Orleans: elderly woman infected by *T. cruzi* in 2006 (significance?)
- Risk of bloodborne disease from various animals: identified directly or by serologic analysis in ≥18 species of mammals



Autochthonous Transmission of *Trypanosoma cruzi*, Louisiana

Patricia L. Dorn,* Leon Perniciaro,* Michael J. Yabsley,† Dawn M. Roellig,† Gary Balsamo,‡ James Diaz,§ and Dawn Wesson¶

Autochthonous transmission of the Chagas disease parasite, *Trypanosoma cruzi*, was detected in a patient in rural New Orleans, Louisiana. The patient had positive test results from 2 serologic tests and hemoculture. Fifty-six percent of 18 *Triatoma sanguisuga* collected from the house of the patient were positive for *T. cruzi* by PCR.

Chagas Disease- emerging problem in the U.S. ?

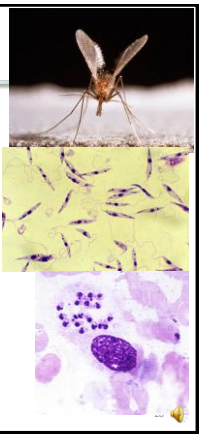
- The disease is becoming a serious health issue in the U.S. because blood donors becoming seropositive for *T. cruzi*.
  - A small number of seropositive blood donors have never left the United States. Only 7 cases of this disease have been acquired in the U.S. - all in the southern half of the country.
- In Arizona, humans may be at a greater risk for getting the disease than previously thought because humans are **expanding into habitats where infected bugs and wild mammalian reservoirs exist.**
- Reports of dogs infected in Texas (high percentage of infected bugs), bugs in California, Arizona, and New Mexico were also infected with *T. cruzi*
- A recent study found that 41.5% of collected bugs in southern Arizona were infected with *T. cruzi*, and that 63% of the collection sites yielded ≥1 infected specimens.
- Many cases of Chagas disease in the United States may be overlooked because the early phase of the infection is often asymptomatic, and health professionals are largely unaware of this disease, or it is misdiagnosed.
- Vector transmission reduced?

WHO elimination program

- WHO 2007 effort for elimination by 2010
  - Strengthen surveillance, drug programs, case management
- Bayer manufactures Nifurtimox, a drug used to treat Chagas Disease.
  - Funding to expand WHO's Chagas disease elimination efforts along with 2.5 million tablets free of charge, allowing the treatment of an estimated 30,000 patients over a period of five years.
- http://www.who.int/mediacentre/news/releases/2007/pr36/en/index.html
  http://www.who.int/mediacentre/news/notes/2007/np16/en/index.html



Leishmania spp.

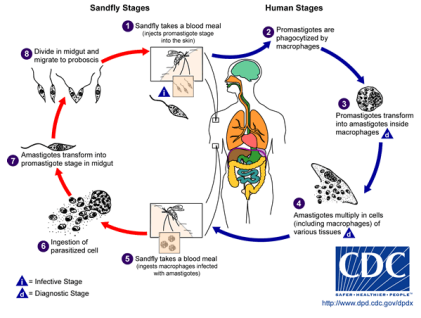


- Obligate intracellular parasites transmitted by sandfiles
  - Old World forms of *Leishmania* are transmitted by *Phlebotomus* sandflies, while *Lutzomyia* sandflies transmit leishmaniasis in Western Hemisphere
- **Zoonotic parasites, infecting a wide range of vertebrates in tropical/subtropical areas**
- affect 15 million people, mainly children and young adults, in 88 countries worldwide. It is estimated that 350 million people are at risk of leishmaniasis.
- **50-60,000 deaths/yr.**
- Several forms of disease in humans
  - Visceral (Kala-azar)
  - Cutaneous
  - Mucocutaneous

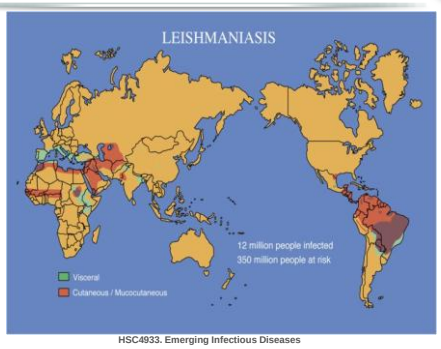
Leishmania spp.

- Human infection is caused by about 21 of 30 species that infect mammals.
- The Subgenus *Leishmania* exists in Old, New world; Subgenus *Viannia* only in New World
- Western Hemisphere (South America mainly), includes (not all):
  - *L. donovani* complex with 3 species (*L. donovani*, *L. infantum*, and *L. chagasi*); the *L. mexicana* complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*); *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana*).
- Eastern Hemisphere (Africa, Asia, Europe)
  - *L. donovani*, *L. infantum*, *L. aethiopica, L. major, L. tropica*
- **The factors determining the form of disease include leishmania species, geographic location, and immune response of the host**

Leishmania spp. General Life cycle



Overall distribtion of Leishmaniasis



Epidemiology

- Overall, leishmaniasis is found in focal areas of about 88 countries, where 350 million people live.
- Over 90 percent of the cases of **cutaneous leishmaniasis** occur in parts of Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, and Syria (in the Old World) and in Brazil and Peru (in the New World);
- Over 90 percent of the cases of **visceral leishmaniasis** occur in parts of India, Bangladesh, Nepal, Sudan, and Brazil.
- Leishmaniasis is found in Mexico, Central America, and South America—from northern Argentina to Texas
- Cases of leishmaniasis evaluated in the United States reflect travel and immigration patterns.
  - Cases in U.S. civilian travelers typically are cases of cutaneous leishmaniasis acquired in common tourist destinations in Latin America (rather than in places in the Old World).

Visceral Leishmaniasis (Kala-azar)

- Approximately 500,000 new cases annually, worldwide.
- Incubation period = 3 to 8 months
- **Most severe form of the disease, may be fatal if left untreated**
- Parasites migrate to internal organs (spleen, bone marrow, liver)
- Usually associated with **fever,** chills, weight loss, and an **enlarged spleen and liver**
- Anemia (low RBC), leukopenia (low WBC), and thrombocytopenia (low platelets) are common
- Lymphadenopathy may be present
- Visceral disease from the Middle East is usually milder than visceral leishmaniasis from other areas of the world





Visceral Leishmaniasis-species

- *L. donovani*
  - Indian subcontinent, north/eastern China, Pakistan, Nepal, east Africa
- *L. infantum*
  - Middle East, Mediterranean, Balkans, central and southwestern Asia, northern and western China China, North and sub-Saharan Africa, U.S.
- *L. chagasi*
  - Latin America
- *L. amazonensis*
  - Brazil (Bahia state)
- *L. tropica* (viscerotropic, rare-usually cutaneous)
  - Middle East, Saudi Arabia (U.S. troops), India, North Africa, Pakistan, Mediterranean, central and western Asia

Visceral Leishmaniasis: Treatment

- Liposomal amphotericin-B (AmBisome) is the drug of choice
- Pentostam is an alternative therapy
  - 28 days of therapy is required, very expensive
- Miltefosine (oral formulation)

Post kala-azar dermal leishmaniasis (PKDL)

- A condition that develops after treatment of visceral leishmaniasis
- About 20% of treated patients develop, 6 months to years later
- Lesions are filled with histocytes containing promastigotes-source of infection to sandflies



Study of Canine Visceral Leishmaniasis, United States and Canada, 2000-2003



Distribution of hunt clubs with confirmed cases of visceral leishmaniasis, United States and Canada. States in which hunt clubs or kennels had ≥1 dog infected with *Leishmania infantum* are shaded. *Leishmania*-positive foxhounds were also found in Nova Scotia and Ontario.

Importance for humans

- In 2000, public health authorities in the United States found hunting dogs in 21 U.S. states and Ontario were infected with *Leishmania infantum* (can cause visceral leishmaniasis)
  - The infection appears to be widespread in foxhounds, but so far transmission appears to be limited to dog-to-dog mechanisms.
- The local form of *L. infantum* can become adapted for transmission by indigenous sandflies, greatly increasing the chances of human infection

Cutaneous Leishmaniasis

- The number of new cases each year in the world is thought to be about 1.5 million.
- 20 species of *Leishmania* may cause cutaneous leishmaniasis.
- **Most common form of disease**
- Reservoirs can maintain infections (dogs, rodents)
- PCR for dx to distinguish from other dermatological conditions

Cutaneous Leishmaniasis: species

- Old World
  - *L. major*: Middle East, India, Pakistan, Africa, central and western Asia, northern and western China
  - *L. tropica*: Mediterranean littoral, Middle East, North Africa, India, Pakistan, central and western Asia
  - *L. aethiopica*: Ethiopian highlands, Kenya, Yemen (Diffuse cutaneous leishmaniasis)
  - *L. infantum* (rare): Middle East, Mediterranean littoral, central Asia, northern and western China, North and sub-Saharan Africa
- New World
  - *L. mexicana* (chicle ulcer): Central and South America, Texas
  - *L. amazonensis*: Amazon Basin, neighboring areas Bahia and other
  - *L. (V.) guyanensis*: (forest yaws)-Guyana, Surinam, northern Amazon Basin
  - *L. (V.) peruviana* (uta) areas, Peru (western Andes) states of Brazil
  - *L. pifanoi* - Venezuela
  - *L. garnhami*- Venezuela
  - *L. venezuelensis*- Venezuela
  - *L. (Viannia) braziliensis*-Central and South America Argentinian highlands
  - *L. (V.) panamensis*-Panama, Costa Rica, Colombia
  - *L. (V.) colombiensis*-Colombia and Panama
  - *L. chagasi*-Central and South America

Do not memorize, but know extent of different species and locations for this type of disease

Cutaneous Leishmaniasis

- Characterized by one or more cutaneous lesions on areas where sandflies have fed.
- Most sores develop within a few weeks of the sandfly bite, however they can appear up to months later
- Skin sores of cutaneous leishmaniasis can heal on their own, but this can take months or even years
  - Significant scars and be disfiguring if they occur on the face
  - Usually painless but can become painful if secondarily infected
- Swollen lymph nodes may be present near the sores





Diffuse Cutaneous Leishmaniasis

- Produces disseminated and chronic skin lesions resembling those of lepromatous leprosy.
- Characterized by one or more sores, papules or nodules on the skin
- Non-ulcerative nodules that spread over body
- *L. amazonensis*-Amazon Basin, neighboring areas, Bahia and other states of Brazil
- *L. pifanoi*-Venezuela
- *L. mexicana*-Central and South America, Texas
- *L. spp.*-Dominican Republic

- As of 2008, 30 indigenous cases of cutaneous leishmaniasis (CL) have been reported in south-central Texas since 1903.
  - Reports of spreading (9 cases in North Tex 2007) -*Leishmania mexicana*





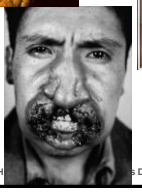


Mucocutaneous Leishmaniasis

- Occurs with *Leishmania* species from Central and South America (*L. braziliensis*)
- This type occurs if a cutaneous lesion on the face spreads to involve the nose or mouth, and there is no treatment administered
- May occur months to years after original skin lesion
- Lesions can partially or totally destroy the mucous membranes of the nose, mouth and throat cavities and surrounding tissues.
  - Chronic inflammatory process involving the nasal, pharyngeal, and laryngeal mucosa, which can lead to extensive tissue destruction
- Cases are focused in South America, especially in Brazil, Paraguay, Ecuador, Bolivia, Peru, Colombia, and Venezuela. Ninety percent of the cases occur in Brazil, Bolivia, and Peru.

Mucocutaneous Disease





Cutaneous and Mucocutaneous Leishmania: Treatment

- **Antimony (Pentostam, Sodium stibogluconate) is the drug of choice**
  - 20 days of intravenous therapy
- Fluconazole may decrease healing time in *L. major* infection
  - Biopsy and culture to determine species is required
  - Six weeks of therapy is needed
- Cutaneous: liquid nitrogen freezing of lesions

Laboratory Diagnosis

- Examination of Giemsa stained slides of the relevant tissue biopsies
- Antibody detection can prove useful in visceral leishmaniasis, but is of limited value in cutaneous disease, where most patients do not develop a significant antibody response.
- **The different species are morphologically indistinguishable**, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies

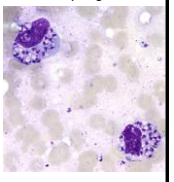
Visceral disease

- Presentation is usually very non-specific and should be considered in febrile patients returning from endemic areas
- Diagnosis requires finding *Leishmania* on biopsy of bone marrow, liver, enlarged lymph node, or spleen

Mucocutaneous disease

- Early diagnosis and treatment is critical to avoid disfigurement
- Because few parasites are present, PCR may be particularly useful

Amastigotes in macrophages



Prevention

- Protection from sand fly bites.
- Avoid outdoor activities, especially from dusk to dawn, when sand flies generally are the most active.
- Insect repellent, bed nets
- Insecticides in houses to kill sandflies