

Leishmaniasis in Veterans of Desert Storm & Iraqi Freedom



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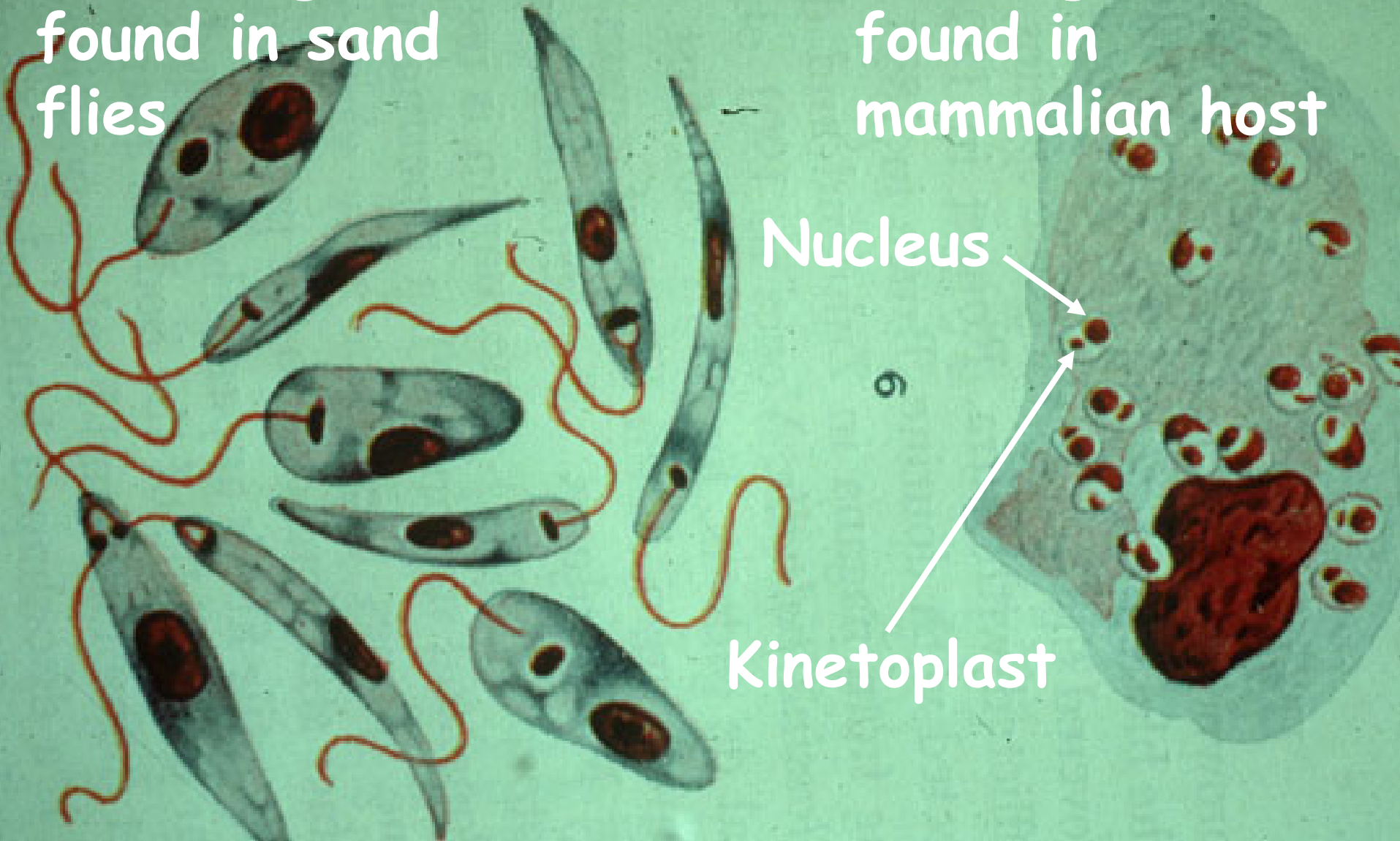
The Leishmaniases

- **A diverse group of protozoan parasites**
- **Intracellular pathogens of the macrophage**
- **Different clinical manifestations / syndromes**
- **Zoonosis**
 - Sand fly insect vector
 - Mammalian reservoir(s)
- **Anthroponotic**
 - Man is incidental host
 - Indian VL and *L. tropica* CL are exceptions



Promastigotes
found in sand
flies

Amastigotes
found in
mammalian host



Leishmania infection and 1990-91 Gulf War

- **What did we expect to see?**
 - NEJM article, 21 Mar 1991. 324; 859
- **Typical Cutaneous Leishmaniasis**
 - *L. major* parasites
 - Desert rodent reservoir
 - *Ph. papatasi* sand fly vector
- **N = 20 cases**
- **Visceral Leishmaniasis not described**

Leishmania infection and 1990-91 Gulf War

- **What else did we see?**
- **Atypical “viscerotropic leishmaniasis”**
 - *L. tropica* parasites
 - **Desert rodent or human reservoir??**
 - **Sand fly vector?**
- **N = 12 cases, parasitologically confirmed**
- **N = ?? cases total**

What was unusual?

- **Did not expect to see VL in Saudi Arabia**
- **Atypical, non-specific clinical syndrome**
 - **Not typical Visceral Leishmaniasis**
 - **Smear negative, culture positive = low parasite burden**
- **Isolation of *Leishmania* from bone marrow**
- **Characterization of isolates as *L. tropica***
- **Difficult diagnosis, insensitive tests**

NEJM. May 13, 1993

Table 1. Clinical Presentation of Eight Male Patients with Visceral Leishmaniasis, at the Time of Confirmatory Culture.

PATIENT NO.	INCUBATION PERIOD (Mo)	SIGNS AND SYMPTOMS AT PRESENTATION	FEVER	ABDOMINAL PAIN*	MALAISE*	FATIGUE*	PHYSICAL EXAMINATION
1	2	Adenopathy	Yes	++	+	++	Hepatomegaly, splenomegaly, adenopathy
2	1-4	Fever	Yes	+	++	+	Normal findings
3	2-8	Gastroenteritis	No	+++	+++	+	Splenomegaly
4	2-6	None	No	No	No	No	Normal findings
5	4-12	Chronic fatigue with hepatosplenomegaly	Yes	+	+	+++	Hepatomegaly, splenomegaly
6	7-14	Chronic fatigue with adenopathy	No	+	+	+++	Hepatomegaly, adenopathy
7	1-6	Mononucleosis	Yes	+/-	+++	+	Normal findings
8	3-12	Fever of unknown origin	Yes	+	++	++	Hepatomegaly, splenomegaly

*One plus sign indicates that the patient reported the symptom when questioned by the examiner; two plus signs, that the patient himself reported the symptom without questioning; and three plus signs, that the symptom was the primary one. Patient 7, represented by the plus-minus sign, reported abdominal pain of brief duration associated with diarrhea.

Leishmania in 1st Gulf War



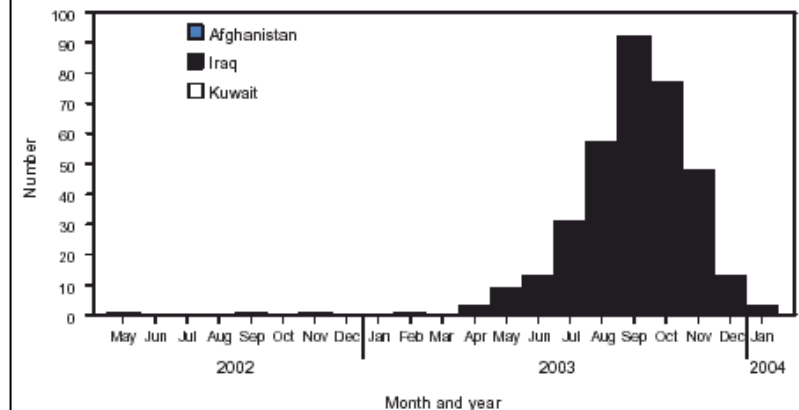
FIGURE 1. Map of the possible sites for infection of the Desert Storm participants. Open symbols = *Leishmania major*; solid symbols = *L. tropica*; ☆ = capitals; X = major cities. The numbers are Desert Storm identification numbers (as in Tables 1 and 2).

- Characterizations of *L. tropica* based on CAE of 21 enzymes
- 3 clusters of *L. tropica*
- *Am J Trop Med Hyg.* 1993. 49:357

OIF / OEF Event Timeline

- **1996 - 2000:** Army kills Leish research program
- **October 2001:** OEF begins
- **March 2003:** OIF begins
- **April 2003:** Infected sandflies found in Iraq
- **Sept 2003:** Epidemic of CL caused by *L. major* begins
- **May 2004:** Army begins Leish R&D
- **May 2005:**
 - >1000 cases of CL
 - All *L. major* (MON26) from Iraq
 - 3 *L. tropica* from Afghanistan
 - 2 cases of VL from Afghanistan
 - 2 cases of VL from Iraq

FIGURE. Number* of cases of cutaneous leishmaniasis in U.S. military personnel, by self-reported onset of skin lesions — Afghanistan, Iraq, and Kuwait, May 2002–January 2004



* N = 350 (Iraq 346, Afghanistan two, and Kuwait two); onset data were not available for 11 cases.

***L. major* and persistent infection**

- ***L. major* infections not persistent**
 - **No case of viscerotropic *L. major* ever identified**
 - **No case of transfusion or congenital transmission**
 - **No presentation as an opportunistic infection in late stage AIDS**
 - **No increase in risk or cases with HIV co-infection**
 - **Infection = disease = no asymptomatic infections**

Visceral Leishmaniasis



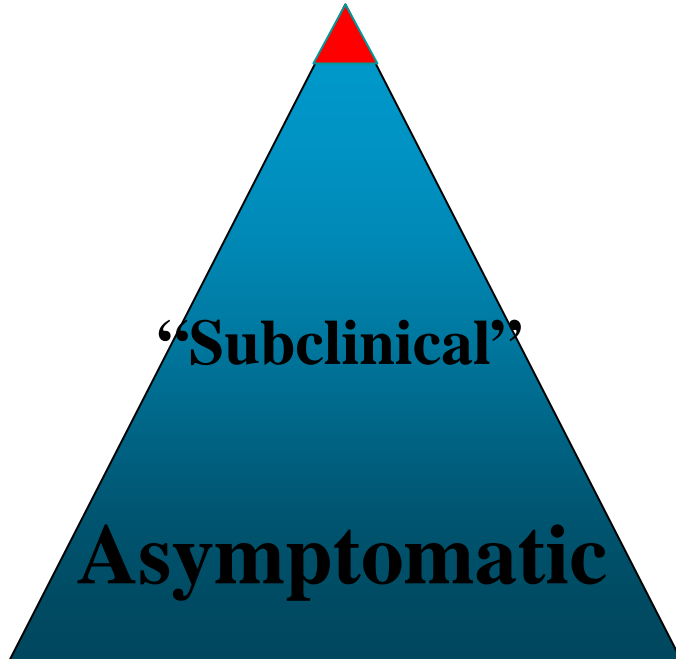
- *Classic “pentad”*
 - Fever
 - Cachexia
 - Splenomegaly
 - Pancytopenia
 - Hypergamma-globulinemia
- *L. donovani,*
L. infantum/chagasi

Clinical Presentation of VL in the Immunocompetent, Well Nourished Adult

- *Most and Levietes. 1947. Medicine. 26:221*
 - N=30, US military in WWII
 - Incubation period: 3 weeks to 33 months
 - Symptom onset to definitive diagnosis, mean 10 weeks (range 2 -26 weeks)
 - Abrupt onset of fever and chills - 96%
 - Splenomegaly in 90% and hepatomegaly in 73% when Dx confirmed
 - Parasitologic diagnosis
 - 21 of 49 (43%) smears from bone marrow aspirations in 29 patients were (+)
 - 8 of 29 (28%) were not confirmed by bone marrow
 - 18 of 18 (100%) splenic aspirates were both smear and culture positive

Visceral Leishmaniasis Disease Spectrum

1-3% with overt VL



- **“Subclinical” Syndromes**
 - Chronic systemic illness
 - Acute febrile illness
- **Risk factors for progression**
 - Malnutrition
 - Immunosuppression (AIDS)
 - Genetic?
- **Cause of death**
 - Measles
 - Pneumonia
 - TB
 - dysentery

“Subclinical Disease”: Brazil

- **Prospective pediatric cohort study, Bahia**
- **N = 86 seroconversion, 5 year follow-up**
- **28 of 86 (33%) progressed to VL between 2 weeks and 15 months**
- **20 of 86 (23%) remained asymptomatic**
- **38 of 86 (44%) had a prolonged “subclinical” illness, resolved 35 mo on average**
 - **Intermittent hepatomegaly, diarrhea, failure to thrive, fatigue, malaise**

Badaro R, Jones TC, Lorenzo R, et al: A prospective study of visceral leishmaniasis in an endemic area of Brazil. J Infect Dis 154:639, 1986

“Subclinical Disease”

- **Perception of ill health and disease is culturally and resource dependent**
- **“subclinical” in the favelas of Brazil or rural Bihar, India = overt disease in suburban North America**
- **Cannot extrapolate reported experience in endemic areas to non-immune, immunocompetent adults**

Spectrum of Disease : Diagnosis

Oligoparasitic

Polyparasitic

Antigen Detection

PCR

In vivo culture

In vitro culture

Giemsa Stain

Parasite Burden

Antibody

DTH

Choice of Diagnostic Test?

- Sensitivity of diagnostic test depends on parasite burden and thus clinical syndrome



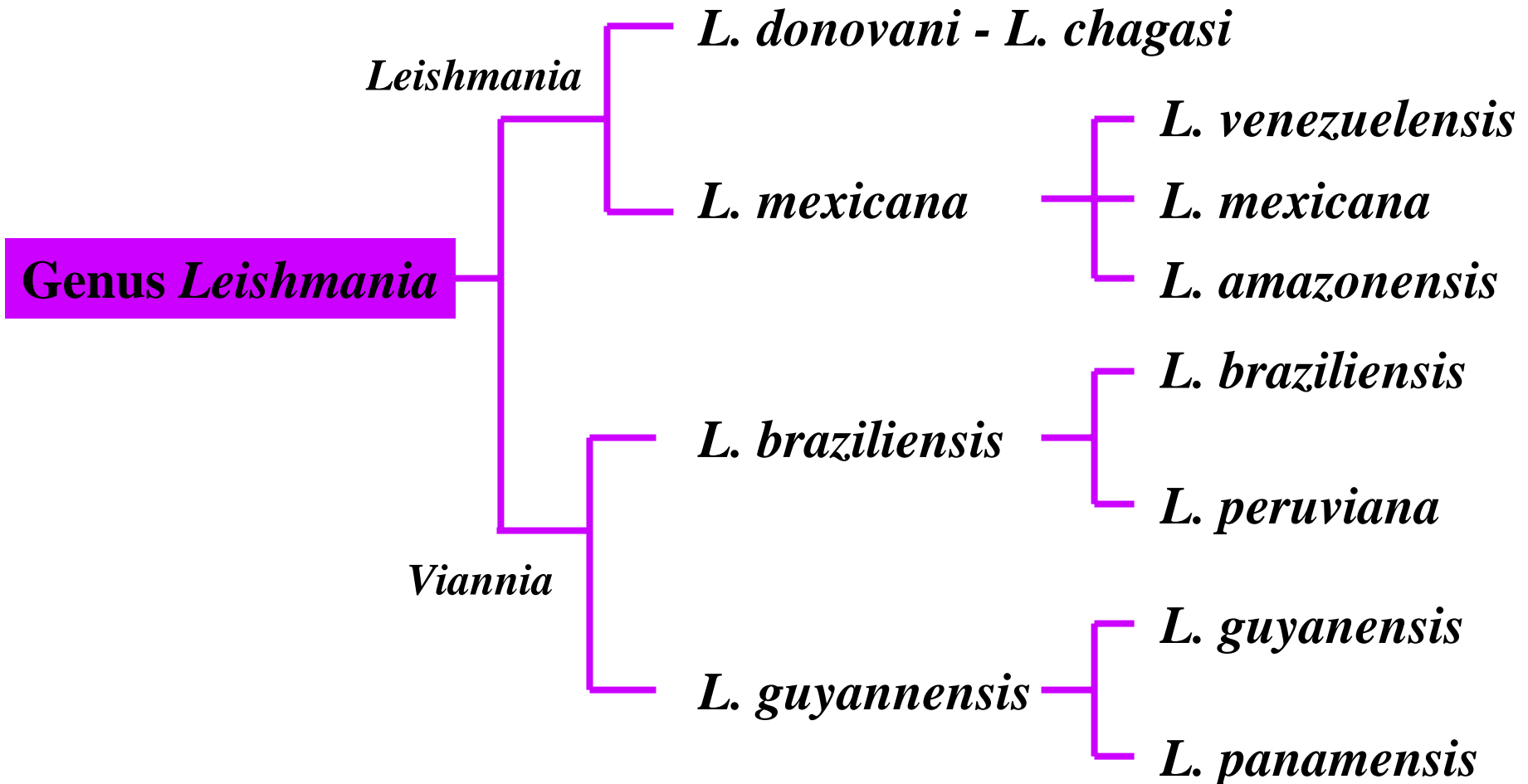
Other Systemic Syndromes: “Viscerotropic”

- **Acute febrile illness**
 - Self limited?
 - Progressive to VL
 - Weeks to months
- **Adenopathy**
 - Localized, generalized, transient
- **Chronic gastrointestinal syndromes**
- **Failure to thrive**

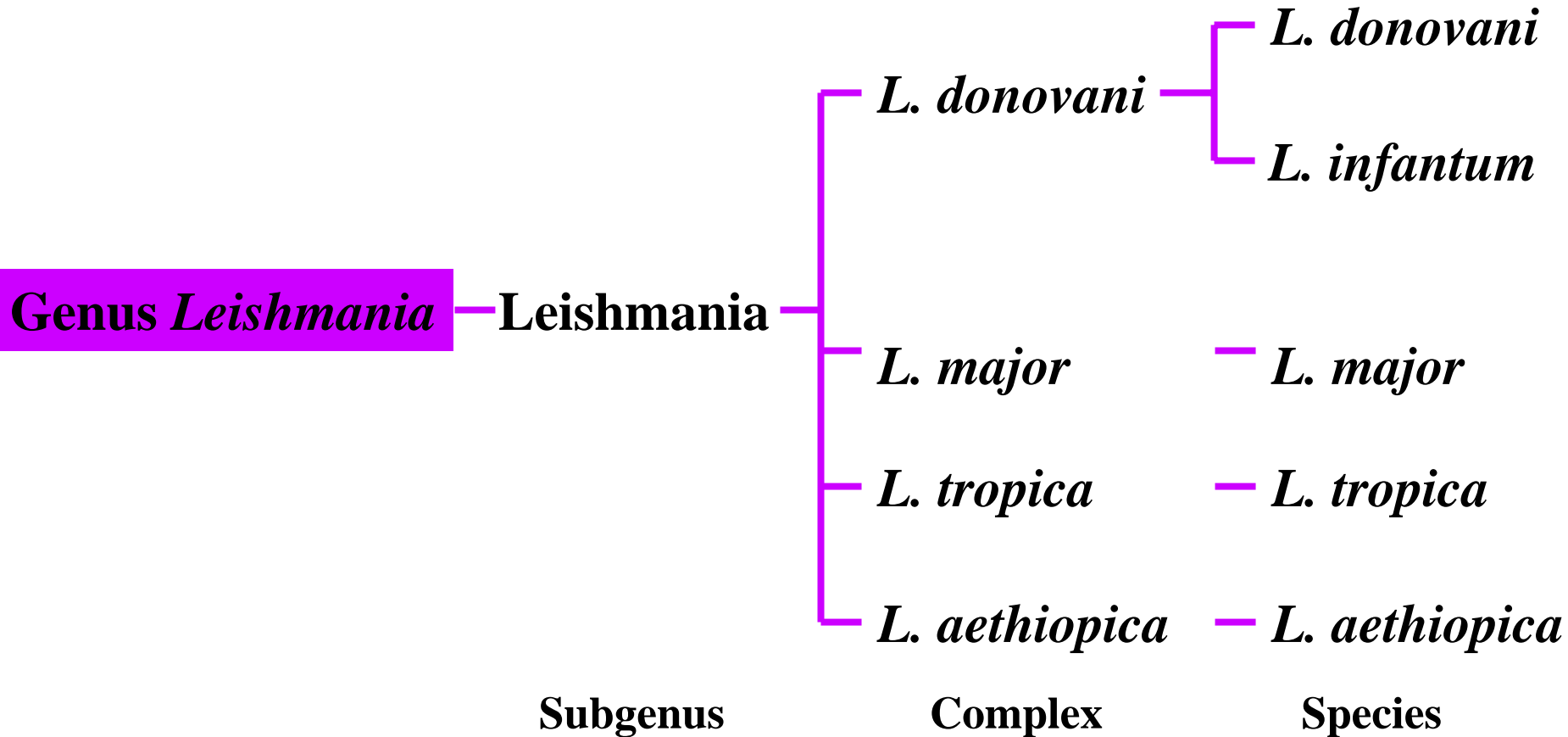
Diagnosis

- **Parasitologic diagnosis = confirmed diagnosis**
 - Smear, Culture, Antigen detection, PCR
- **Availability**
 - Routine clinical versus specialty labs
- **Clinical recognition**
 - Classic syndromes versus Gulf War *L. tropica*
- **Walter Reed Army Medical Center referral bias**

Taxonomic Classification of the Leishmaniases: New World



Taxonomic Classification of the Leishmaniases: Old World



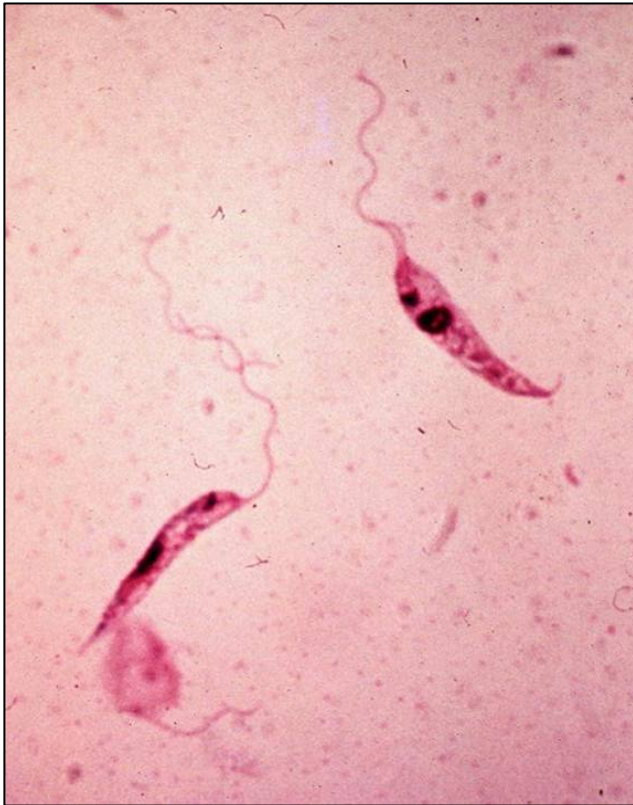
What is a *Leishmania* species?

- **Classic definition**
- **Type organism isolated from a location and clinical syndrome (reference strain)**
- **Characterized by zymodeme analysis**

L. tropica Reference Strains

	Where isolated	Source	International Ref No.	Clinical Manifestation
<i>L. tropica</i>	USSR Turkestan	Man	MHOM/SU/60/LRC-L39	CL
<i>L. tropica</i>	Iraq	Man	MHOM/IQ/00/Avraham	LR
<i>L. tropica</i>	Iraq	Man	MHOM/IQ/66/L75	CL

What is a zymodeme?



- **Electrophoresis of parasite pellet**
- **3 vs. 7 vs. 11 vs. 21 isoenzymes**
- **Cellulose acetate vs. starch gel**
- **How different is different enough?**
- **Importance of minor enzyme allomorphs?**

Genotypic classification

- **Targets**
 - **kDNA**
 - **rRNA**
 - **Repetitive nuclear sequences**
- **Methods**
 - **PCR based methods**
 - **RLFP, RAPD**
 - **sequencing**

They may look the same...



- **Different parasites**
- **Different diseases**
- **Different epidemiology**
- **Different vectors**
- **Different reservoirs**

Examples of Lt causing VL

- **Kenya**
 - Am J Trop Med Hyg 41:289
- **Israel**
 - Personal communication
- **India**
 - Ann Trop Med Parasitol 75:131
 - Lancet. 1995 Apr 15;345(8955):959-61
- **Morocco**
 - Ann Trop Med Parasitol. 2002 Sep;96(6):637-8.

***L. tropica* Genetic Heterogeneity**

- Isoenzyme profiles of 27 stocks of *Leishmania tropica* from widely separated geographical areas were compared with those of reference strains of *L. tropica* and *L. major* using starch-gel electrophoresis of 13 enzymes (GPI, GD, ES, PGM, PEPD, NH, ASAT, ALAT, PK, MPI, 6PGD, SOD, MDH).
- 18 zymodemes were seen.
- *L. tropica* showed considerable intraspecific variation which did not correlate with its epidemiological uniformity.
- Isolates from cases of cutaneous and visceral leishmaniasis and leishmaniasis recidivans were identified as *L. tropica*.
- Only one isoenzyme band was held in common with the enzyme profile of the *L. major* reference strain thus supporting the status of *L. tropica* as a separate species.
- *Trans R Soc Trop Med Hyg.* 1986;80(1):113-9

Clinical heterogeneity: the Host

- **Near identical *L. tropica* isolates**
 - Nested PCR of kDNA
 - Restriction digests of amplicons
 - Shared fingerprint = schizodeme
- **Epidemic outbreak in a refugee camp**
- **21 isolates**
 - Nodular, 4-21 lesions, 1-12 mo duration
 - Outcome dependent on host response
- **J Clin Microbiol. 1998. 36:2877**

***L. infantum* and persistent infection**

- **Infection rate is 10-100 X higher than disease rate**
- **Disease in infants, rarely in immunocompetent adults**
- **Opportunistic infection in late stage AIDS**

Can Cytokines Cause Disease?

- **Acute disease**
 - **Fever, malaise, myalgias, arthralgias, fatigue, anorexia, nausea**
 - **Influenza, dengue, malaria, tuberculosis, etc.**
 - **TNF α , INF γ , IL-2, IL-12, etc.**

Can Cytokines Cause Disease?

- **Chronic disease**
 - **Fever, malaise, myalgias, arthralgias, fatigue, anorexia, nausea**
 - **Inflammatory bowel disease, rheumatoid arthritis,**
 - **TNF α , INF γ , IL-2, IL-12, etc.**

Side Effects of IFN γ as Therapy

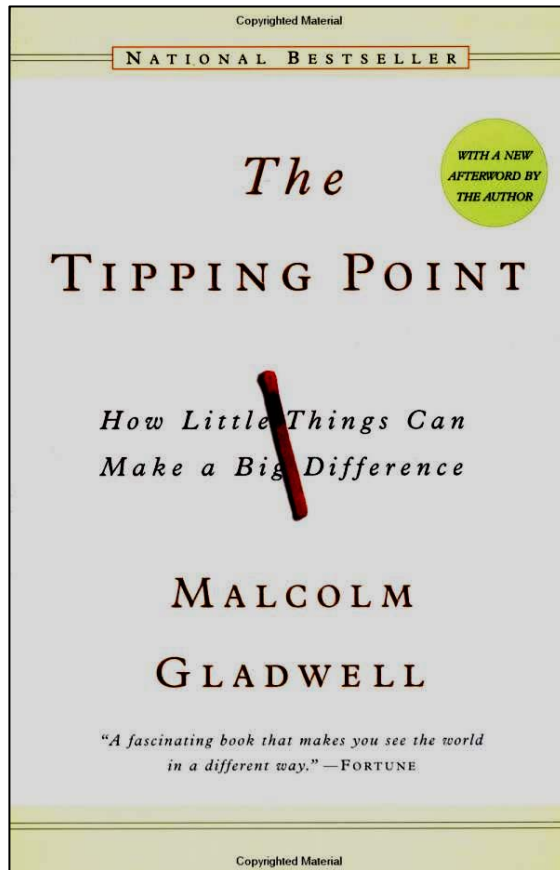
- **Constitutional: flu-like illness, fever, rigors, arthralgia, myalgia, fatigue**
- **Neuropsychiatric**
 - **depression**
 - **insomnia**
 - **irritability**

Persistent *Leishmania* Infection

- Intracellular pathogen of the macrophage
- Lifelong, persistent infection
- Treat disease, never eradicate parasites
- Mycobacteria: TB, leprosy
- Bacteria: *Brucella*
- Fungal: *Histoplasma*
- Viral: *HIV*

The Tipping Point

Malcolm Gladwell



- “Ideas and products and messages and behaviors spread just like viruses do”
- “...small numbers of people start behaving differently, that behavior can ripple outward until a critical mass or "tipping point" is reached...”
- **Role of media**