42.1 INTRODUCTION
Sir William Leishman and Charles Donovan demonstrated the parasite in patients from Calcutta in year 1903. The Genus was so named by Sir Ronald Ross-Leishmania Donovani. It was first cultured by Rogers in 1904.

Geographic distribution-It is mainly seen in South and South–East Asia, China, Sudan tropical Africa South America. In India the regions mainly affected are – Bengal, Bihar, and Eastern Uttar Pradesh

OBJECTIVES
After reading this lesson, you will be able to:

- describe the morphology of Leishmaniasis
- explain the life cycle of Leishmaniasis
- discuss the pathogenecity of Leishmaniasis
- explain the laboratory diagnosis of Leishmaniasis

42.2 HABITAT
Amastigote forms are seen in human infections and are seen mainly in the cells of reticuloendothelial system located in liver, spleen, bone marrow, and peripheral blood

Promastigote forms are seen in the gut of sand fly Phlebotomus argentipes. It is also seen when grown in laboratory on artificial culture media.
42.3 MORPHOLOGY

(a) **Amastigote**: These are non motile and round to oval 2-4 µm long. The nucleus is round to oval. The nucleus is red and the kinetoplast is bright red on Leishman stain. A clear unstained space is present alongside the axoneme called the vacuole. The parasite has the blepharoplast and axoneme. Axoneme, arises from the blepharoplast and extends to the margin of the parasite. Amastigote form also has a parabasal body.

(b) **Promastigote**: It is a spindle shaped structure measuring 15-20 um by 1-2 um. It has a flagellum arising from the axoneme and coming out of the anterior end. There is a blepharoplast and a vacuole in the anterior end. The nucleus is round to oval and central in location. The nucleus is red and the kinetoplast is bright red on Leishman stain.
42.4 LIFE CYCLE

The parasite is transmitted amongst humans by the bite of infected Sand Fly-Phlebotomus and Lutzomiya. Transmission can also occur through infected blood transfusion.

After infected blood meal is taken by the vector sand fly Phlebotomus argentipes, the aflagellate develops into flagellate promastigote form in the gut of the insect in 8-20 days. The infective sand fly transmits the disease by biting man. Due to partial/complete blockage of mouth parts, parasites are lodged at the site of bite when it ingests blood.

The promastigotes penetrate the host macrophage and are converted to amastigote forms. They multiply and rupture the macrophages. There is ingestion of amastigote by other macrophages.

**Incubation period:** Usually 3-6 months, but can extend up to 1-2 years.

![Diagram of Leishmaniasis life cycle]

- a. Development in the sandfly
- b. Penetration of the promastigote into host macrophage
- c. Conversion of promastigote into amastigote
- d. Division of amastigote into many parasites
- e. Rupture of the infected macrophage
- f. Ingestion of the amastigote by another macrophage
- g. Multiplication and repetition of the cycle

Fig. 42.3
INTEXT QUESTIONS 42.1

1. Amastigote forms are seen in .......................
2. Promastigote forms are seen in .......................
3. Unstained space present alongside axoneme called .......................
4. Promastigote is transmitted by .......................
5. Kala azar is caused by .......................

42.5 PATHOGENECITY

Leishmania donovani causes a visceral disease called Kala azar. It is a parasite of the reticulo endothelial (RE) system and affects the organs containing the reticuloendothelial system like the bone marrow, liver and spleen. There is hepatosplenomegaly. The patient gets intermittent fever. There is associated anaemia, cachexia, loss of weight. There is dry skin, brittle hair and pigmentation of skin. There may also be diarrhoea, dysentery. Oedema is seen due to hypoalbuminemia.

Leishmania tropica causes Oriental sore (cutaneous leishmaniasis) On the skin which becomes dry there may appear solitary or multiple ulcerating papules. Healing occurs with scarification. Post kala azar dermal leishmaniasis

Leishmania braziliensis- Espundia (muco cutaneous leishmaniasis)

42.6 LABORATORY DIAGNOSIS

Demonstration of L-D bodies: The aetiological diagnosis is established by demonstrating the parasite in the patient specimen. The amastigote form of the parasite is seen in human infections. Parasite demonstration is done on the following specimen after staining the smears with Romanowsky stains, like Giemsa, Lishman stain.

- splenic aspirate
- bone marrow smears
- thick blood film
- enriched blood leucocyte fraction
Detection of specific antibodies: Antibodies can be detected in immunocompetant individuals in 99% of cases. ELISA, CIEP, DOT ELISA based kits are available for the same. In immunocompromised individuals like HIV patients with AIDS the 40-60% cases may be seronegative.

Culture on monophasic/biphasic media: The promastigote form of the parasite is seen on artificial culture media. The patient specimen can be cultured on the following media

- N N N media (Novy,MacNeal Nicolle)
- Brain heart infusion agar medium
- Schneider’s medium
Leishmaniasis

Detection of antigen: Antigen detection kits based on ELISA, IFA are available and can be used for establishing the diagnosis.

Non-specific tests
- progressive neutropenia
- relative lymphocytosis, monocytosis
- reversal of A:G ratio
- Napiers Aldehyde test
- Chopras Antimony test

Newer tests: PCR based detection can be done in laboratories having molecular biology facility.

Isoenzyme typing is one of the typing method which may be done in research laboratories.

INTEXT QUESTIONS 42.2

1. Kala azar (a) Leishmania Donovani
2. Oriental sore (b) Leishmania braziliensis
3. Espundia (c) Leishmania Donovani

WHAT HAVE YOU LEARNT

- Amastigote forms are seen in human infections and are seen mainly in the cells of reticuloendothelial system located in liver, spleen, bone marrow, and peripheral blood
- Promastigote forms are seen in the gut of sand fly Phlebotomus argentipes. It is also seen when grown in laboratory on artificial culture media.
- The parasite is transmitted amongst humans by the bite of infected Sand Fly-Phlebotomus and Lutzomiya. Transmission can also occur through infected blood transfusion.
- Incubation period is usually 3-6 months, but can extend up to 1-2 years
- Leishmania donovani causes a visceral disease called Kala azar, Leishmania tropica causes Oriental sore and Leishmania braziliensis causes Espundia
- The aetiological diagnosis is established by demonstrating the parasite in the patient specimen
- Parasite demonstration is done after staining the smears with Romanowsky stains like Giemsa, Lieszman stain
Antibodies can be detected in immunocompetent individuals, ELISA, CIEP, DOT ELISA based kits are available for the same.

The promastigote form of the parasite is seen on artificial culture media. The patient specimen can be cultured on the following media N N N media (Novy, MacNeal Nicolle), Brain heart infusion agar medium, Schneider’s medium,

Antigen detection kits based on ELISA, IFA are available and can be used for establishing the diagnosis.

PCR based detection can be done in laboratories having molecular biology facility and Isoenzyme typing is one of the typing method which may be done in research laboratories.

TERMINAL QUESTIONS

1. Describe the morphology of the amastigote and promastigote form of Leishmania donovani
2. Discuss the laboratory diagnosis of a case of leishmaniasis.

ANSWERS TO INTEXT QUESTIONS

42.1

1. Reticuloendothelial cells
2. Sand fly
3. Vacuole
4. Sand fly
5. Leishmania Donovani

42.2

1. (c)
2. (a)
3. (b)