IN THE NAME OF ALLAH, THE MOST GRACIOUS AND MOST MERCIFUL

BLOOD & TISSUE FLAGELLATES

1. LEISHMANIA SPP.

2. TRYPANOSOMA SPP.

LEISHMANIA

A. Old World Leishmaniasis

B. New World Leishmaniasis

CLASSIFICATION OF LEISHMANIASIS

A. Old World Leishmaniasis

Vector : Female Sandfly of genus Phlebotomus

- L. donovani,
- L. tropica,
- L. infantum,
- L. major
- L. aethiopica

CLASSIFICATION OF LEISHMANIASIS

B. New World Leishmaniasis

Vector : Sandflies of genus Lutzomyia & Psychodopygus

- L. peruviana,
- L. chagasi,
- L. mexicana complex
- L. braziliensis complex

PREVALENCE

Leishmaniasis is mainly a zoonotic disease,

but human-vector-human transmission is also found.

Endemic in India, Middle East, China, Turkey, Sudan,

Kenya, Somalia, Ethiopia , Morocco and Tunisia.

PREVALENCE

Continued

According to W.H.O. Latest estimates in 80 countries ,

- 0.5 million cases of Kala-azar occur yearly.
- 1.5 million cases of cutaneous leishmaniasis
- 12 million currently infected.
- 350 million people at risk.

OLD WORLD LEISHMANIASIS

LEISHMANIA DONOVANI

Sir William Leishman

in 1900 first discovered this parasite (Calcutta).

Donovan in 1903, reported (Madras).

OLD WORLD LEISHMANIASIS

LEISHMANIA DONOVANI

HABITAT

Obligate intracellular parasite

Reticulo-endothelial cells ,

Liver, Spleen, Bone marrow & Lymph nodes

of Man & other Vertebrate hosts (Dog)

where it occurs in Amastigote form.

MORPHOLOGY

Two morphological forms:

- Amastigote
- Promastigote.

MORPHOLOGY

a. AMASTIGOTE

The amastigotes reside in the cells of reticuloendothelial system i.e. (Macrophages, Monocytes, Neutrophils).

Round or oval body

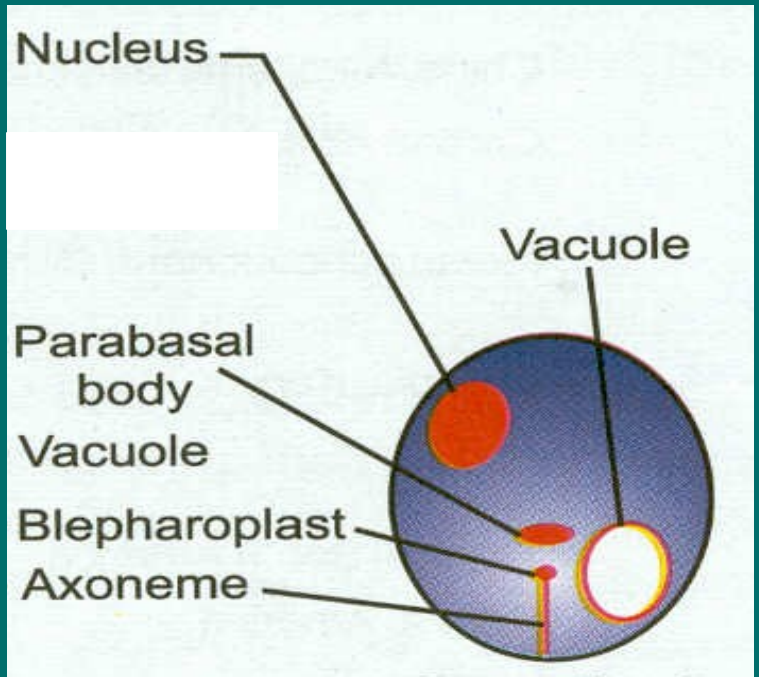
Non-motile (without flagellum)

Measuring 2- 4µm in diameter.

Nucleus is round or oval,

less than 1µm in diameter.

It is situated along the cell wall.



MORPHOLOGY (Continued)

AMASTIGOTE

Kinetoplast

consists of Parabasal body & Blepharoplast.

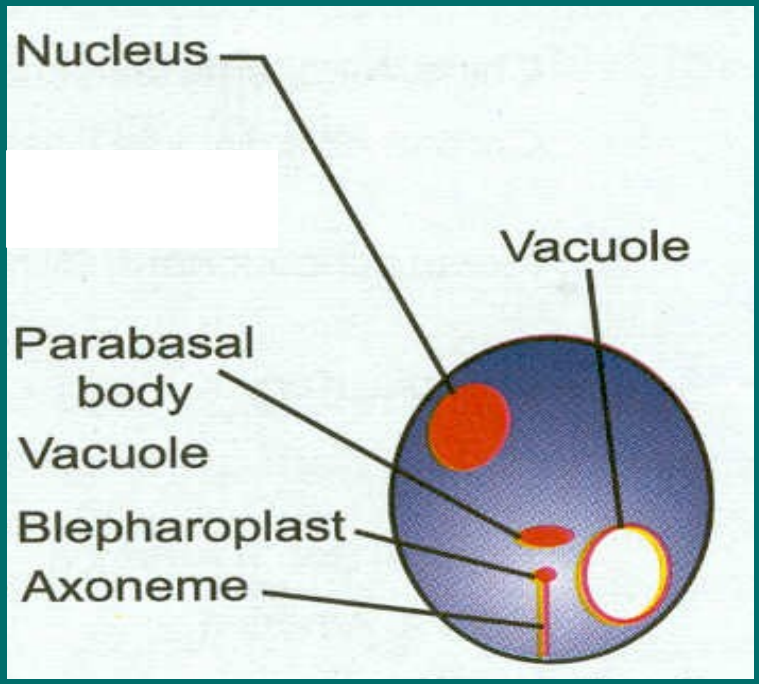
Axoneme

arises from the Blepharoplast represents the intracellular portion of the flagellum.

Vacuole , a clear unstained space lying alongside the axoneme.

MORPHOLOGY

Continued

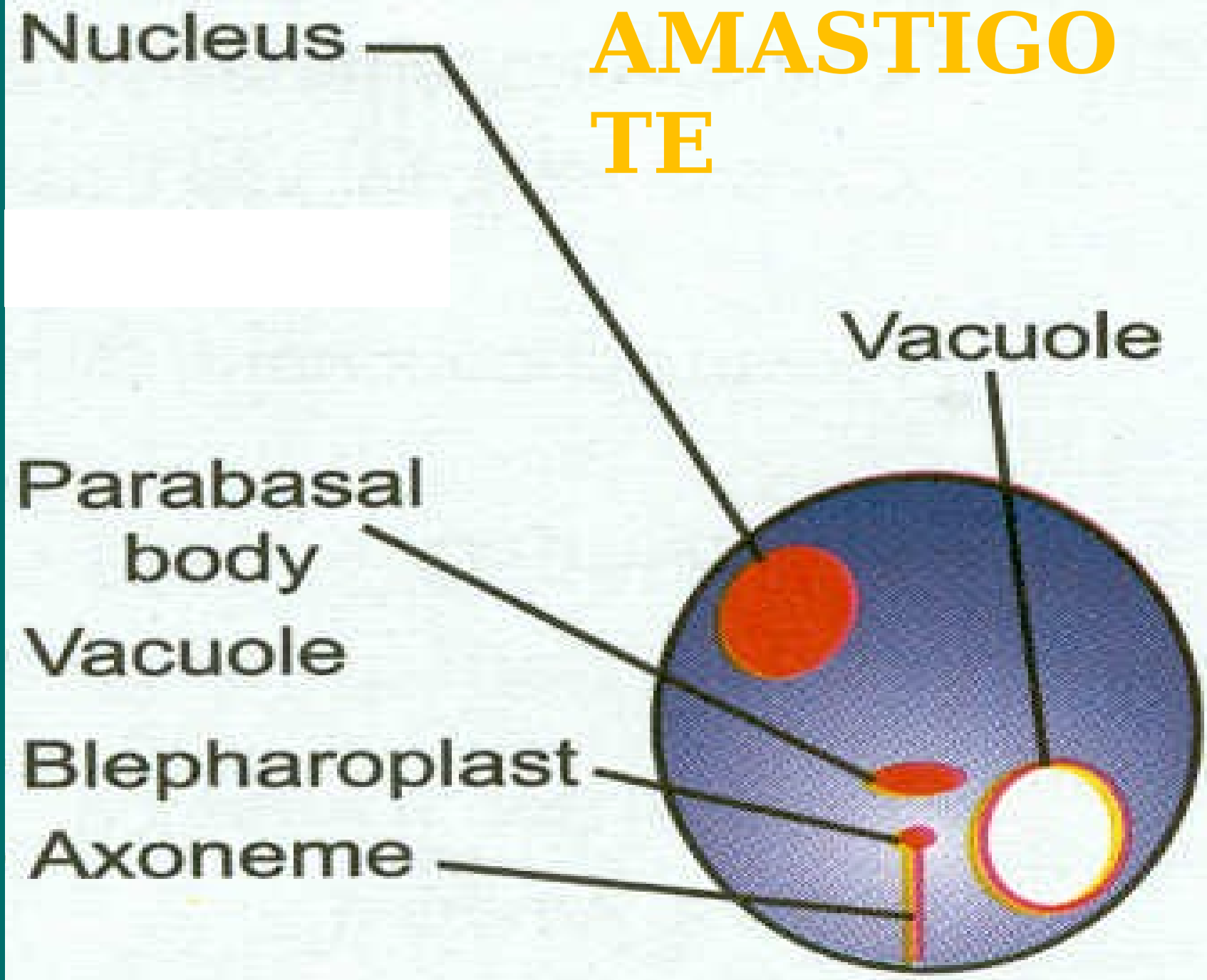


Miroscopically,

using Giemsa or Wright stain,

- **Cytoplasm appears pale blue,**
- **The inclusion bodies are red**
- **Nucleus is red,**
- **Kinetoplast is bright red.**

AMASTIGOTE


Nucleus
Vacuole
Parabasal body
Vacuole
Blepharoplast
Axoneme

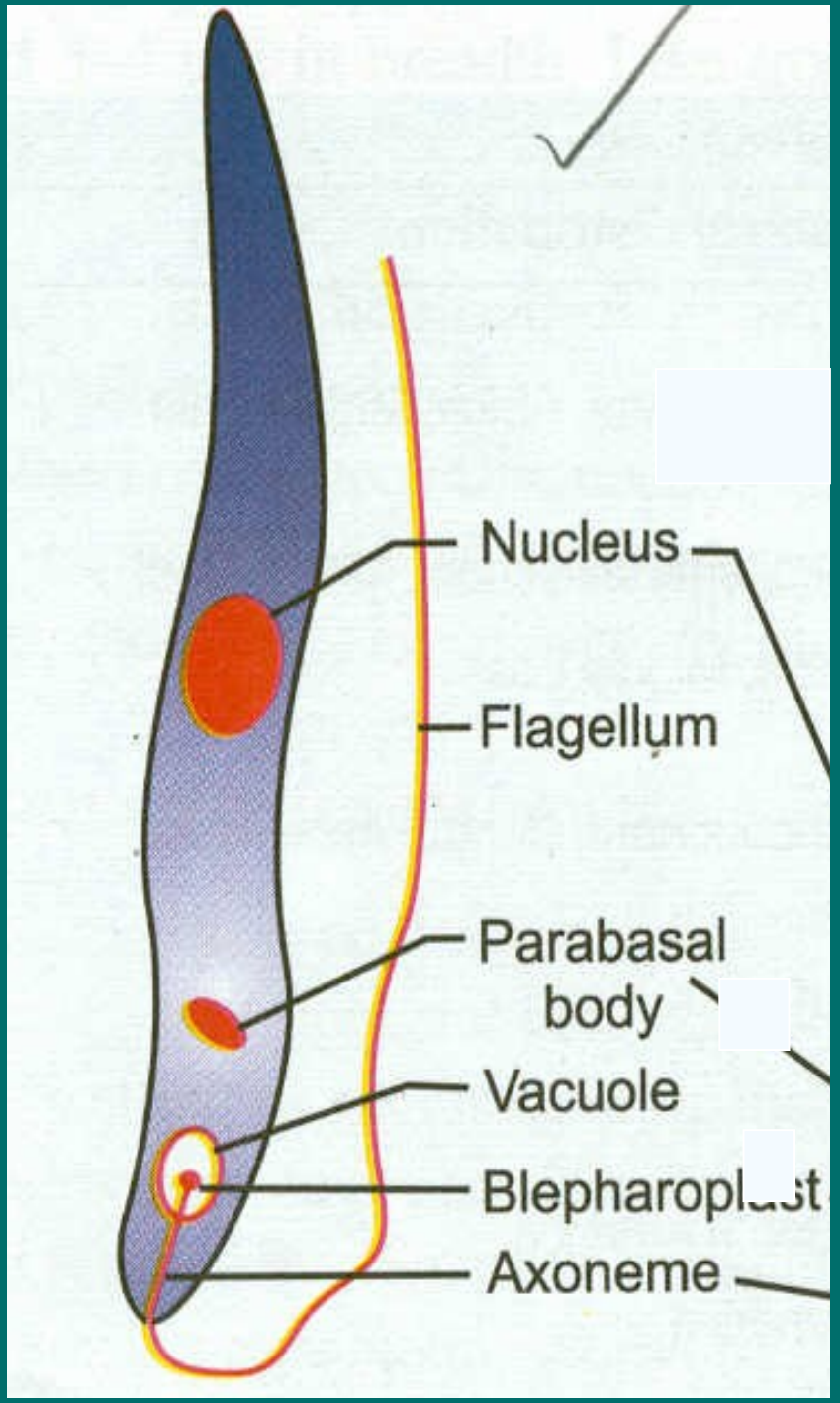
PROMASTIGOTE

Found in

GI Tract of Sandfly

&

Culture media.



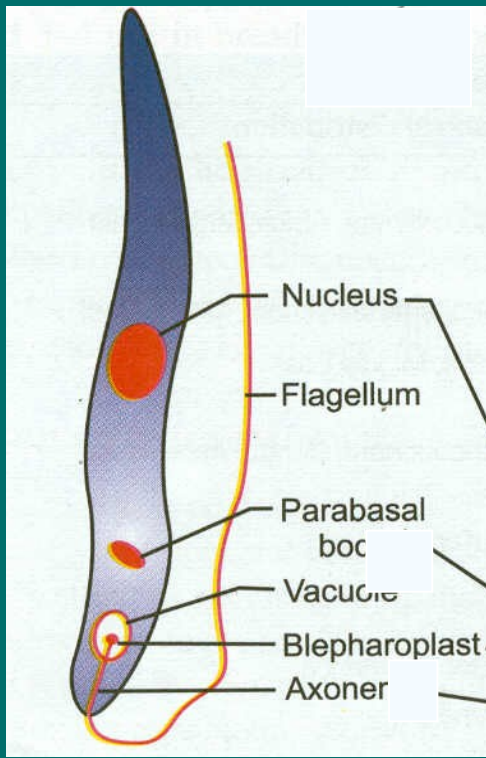
PROMASTIGOTE

Extracellular form

Elongated, motile,.

Mature promastigotes measure 15-25µm by 1.5-3.5µm.

Nucleus is situated centrally.



PROMASTIGOTE

Kinetoplast (Parabasal body & Blepharoplast) .

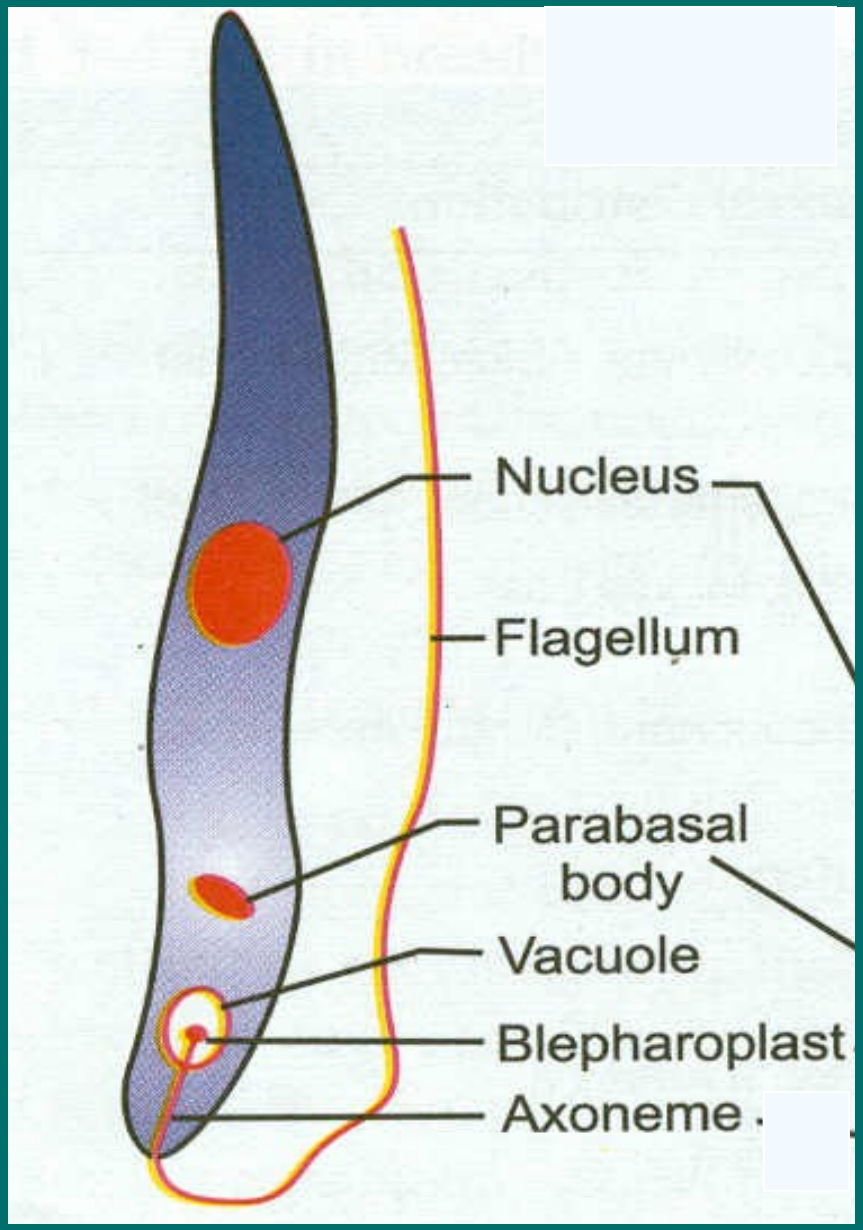
lies near the anterior end.

Vacuole In front of the kinetoplast lies a pale staining vacuole.

Axoneme arises from the **blepharoplast**

extending forward as a free flagellum.

Flagellum as long as the parasite body or longer.



PROMASTIGOTE

LIFE CYCLE

Passes its life cycle in two hosts:

1. Man / Dog are the vertebrate hosts.

2. Female sandfly is the invertebrate host.

LIFE CYCLE

Amastigotes : present in the blood stream of the patient,

(both free & intracellular).

Taken up by the sandfly in a blood meal

Reach midgut of the insect.

Transformation of Amastigotes into Promastigotes & multiply.

Migration to the pharynx and buccal cavity on 6^{th}-9^{th} day of meal.

Life Cycle in Human

(Continued)

- Prick

This sandfly pricks the skin and punctures with its **proboscis ,**

Regurgitates the promastigotes into the wound thus produced.

- Phagocytosis of the promastigotes by nearby Macrophages

Life Cycle in Human

(Continued)

- Transformation into Amastigotes within the cytoplasm of macrophages.

- Here the amastigotes multiply slowly remain dormant for weeks – months.

- Migration of infected cells to Viscera :

  Parasitized macrophages migrate from the skin to spleen, liver,

- Interaction of Fixed Macrophages

Amastigotes are taken up by fixed macrophages

(e.g. Kupffer's cells in the liver),

which become packed with the parasites (50-200 or more).

- Rupture of cells & release of more Amastigotes.

  On rupture of host cells , the released amastigotes are taken up by new reticuloendothelial cells followed by multiplication and rupture of these cells.

- Ultimately the entire reticuloendothelial system becomes progressively infected.

Life Cycle (Continued)

- Some of these free amastigotes are phagocytosed by Polymorphonuclear leucocytes and Monocytes.

- A blood-sucking insect draws both types of amastigotes during blood meal

- Cycle is repeated.

PATHOGENICITY

Visceral leishmaniasis or **Kala-azar.**

Incubation period:

Varies from 3-6 months, (but it may be 10 days to 2 years).

PATHOGENESIS OF VISCERAL LEISHMANIASIS or KALA-AZAR.

- The parasite spreads from the site of inoculation to reticuloendothelial cells of various organs.

- Marked proliferation of macrophages.

- Leading to progressive enlargement of the viscera (Spleen & Lymph nodes).

Visceral Leishmaniasis or **Kala-azar.**

Disturbance of Haemopoiesis

- These macrophages occupy a large part of the bone marrow,
- Compromising both the erythropoietic & granulocytic activity,
- Pan-cytopenia

Blood Picture in Kala-azar

Occupation of bone marrow by Macrophages results in

- Anaemia (usually normocytic)
- Leucopenia
- Thrombocytopenia
- Hyper-globulinemia
- Reversal of A:G ratio

- Hypersplenism also helps in aggravation of anaemia.
- Erythrocytes adsorb immune complexes
- Become prone to enhanced phagocytosis by the macrophages of the liver & spleen.
- Production of globulin is greatly increased.
- This leads to reversal of the A:G ratio.

Immunity in Kala azar

In contrast to cutaneous leishmaniasis,

Cell-mediated immunity is impaired in active kala-azar patients

who consequently lack a Delayed Type Hypersensitivity response,

but re-appears after cure.

Clinical Presentation of Kala azar

- Fever,
- Malaise,
- Headache,
- Weight Loss,
- Dry, Rough, Pigmented skin
- Brittle hair.
- Enlarged spleen, liver and lymph nodes.

Clinical Presentation of Kala azar

If left untreated , 75-95% patients die within 2 years.

Death in kala-azar is due to Secondary infections.

HIV infection activates subclinical Leishmaniasis
or increases suscetibility of patients to a new infection.

The presentation of visceral leishmaniasis in HIV patients is very atypical and

serological tests may be negative.

LABORATORY DIAGNOSIS

A. NON-SPECIFIC LABORATORY TESTS

1. Haemoglobin:

Decrease in erythrocyte count leading to anaemia.

2 . Blood count:

Pancytopenia, (mainly Neutropenia).

The count may fall to 1,000/µl of blood or even below. (Normal= 4000-11000)

3. Estimation of serum proteins:

Raised serum proteins with reversal of the **A:G ratio** (albumin : globulin ratio)

due to greatly raised IgG levels.

DIAGNOSIS OF LEISHMANIASIS

B. SPECIFIC LABORATORY TESTS

Diagnosis of leishmaniasis can be confirmed by:

1. Peripheral blood film:

Thick film method:

Reveals Amastigote form of the parasite inside circulating Monocytes & Neutrophils, in the stained thick blood film.

Thin film method: Often negative.

(Continued)

DIAGNOSIS OF KALA -AZAR

2. Needle biopsy / aspiration :

Deeper tissues e.g. lymph node, bone marrow, liver & spleen.

Spleen aspirate being the most reliable material.

NOTE :

Bleeding from the puncture wound in the spleen, may be fatal.

Special care in patients with haemorrhagic diathesis and leukaemia.

(Continued)

DIAGNOSIS OF KALA -AZAR

3. Culture:

NNN medium *(Novy , Nicolle , Mc Neal)*

Hockmeyer's medium

IMMUNOLOGICAL TESTS

Non-specific and More specific tests :

1. Non-specific tests

- Aldehyde test
- Antimony test
- Complement fixation test with W.K.K. antigen

IMMUNOLOGICAL TESTS

2. More specific tests

More specific tests which become positive earlier in kala-azar include

1. Direct agglutination test (DAT),
2. Indirect haemagglutination (IHA) test
3. Indirect fluorescent antibody test (IFAT).
4. Enzyme-linked immunosorbent assay (ELISA)

(Continued)

IMMUNOLOGICAL TESTS

5. Detection of Leishmania Antigen

6. Species-specific Monoclonal antibodies

7. DNA probes.

IMMUNOLOGICAL TESTS (Continued)

Leishmanin or Montenegro test

Delayed hypersensitivity reaction to

intradermal injection of crude Leishmania antigen.

Procedure :

0.2 ml of a crude suspension of killed Promastigotes of L. tropica

(containing 6-10 Million promastigotes per ml of 0.5% phenol in saline)

is injected intradermally and read after 48-72 Hrs.

IMMUNOLOGICAL TESTS (Continued)

Leishmanin or Montenegro test

Leishmanin Test is Negative in active kala-azar cases,

Because CMI is impaired in active kala-azar patients.

The test becomes positive 6-8 weeks after cure from kala-azar.

DIFFERENTIAL DIAGNOSIS OF KALA-AZAR

1. Malaria,
2. Liver abscess,
3. Brucellosis,
4. Tuberculosis,
5. Chronic Myeloid leukaemia,
6. Lymphoma,
7. Cirrhosis of liver,
8. Thalassaemia.
9. Trypanosomiasis,
11. Schistosomiasis,

TREATMENT OF THE OLD WORLD LEISHMANIASIS

Pentavalent Antimonials:

- Sodium stibogluconate
- Meglumine antimoniate

Aromatic Diamidines:

- Pentamidine

Others:

- Monomycin
- Paromomycin
- Aminosidine
- Amphotericin B
- Allupurinol

PROPHYLAXIS

No vaccine available.

Preventive Measures.

1. Detection of Active cases & treatting them.
2. Elimination of sandflies by spraying of insecticides.
3. Insect repellents such as dimethyl-phthalate.
4. Use of fine mesh bed nets (45 holes per square inch)
5. Insecticide-impregnated bed-nets & curtains.

Prophylaxis

(Continued)

6. Sleeping on the roof or second floor

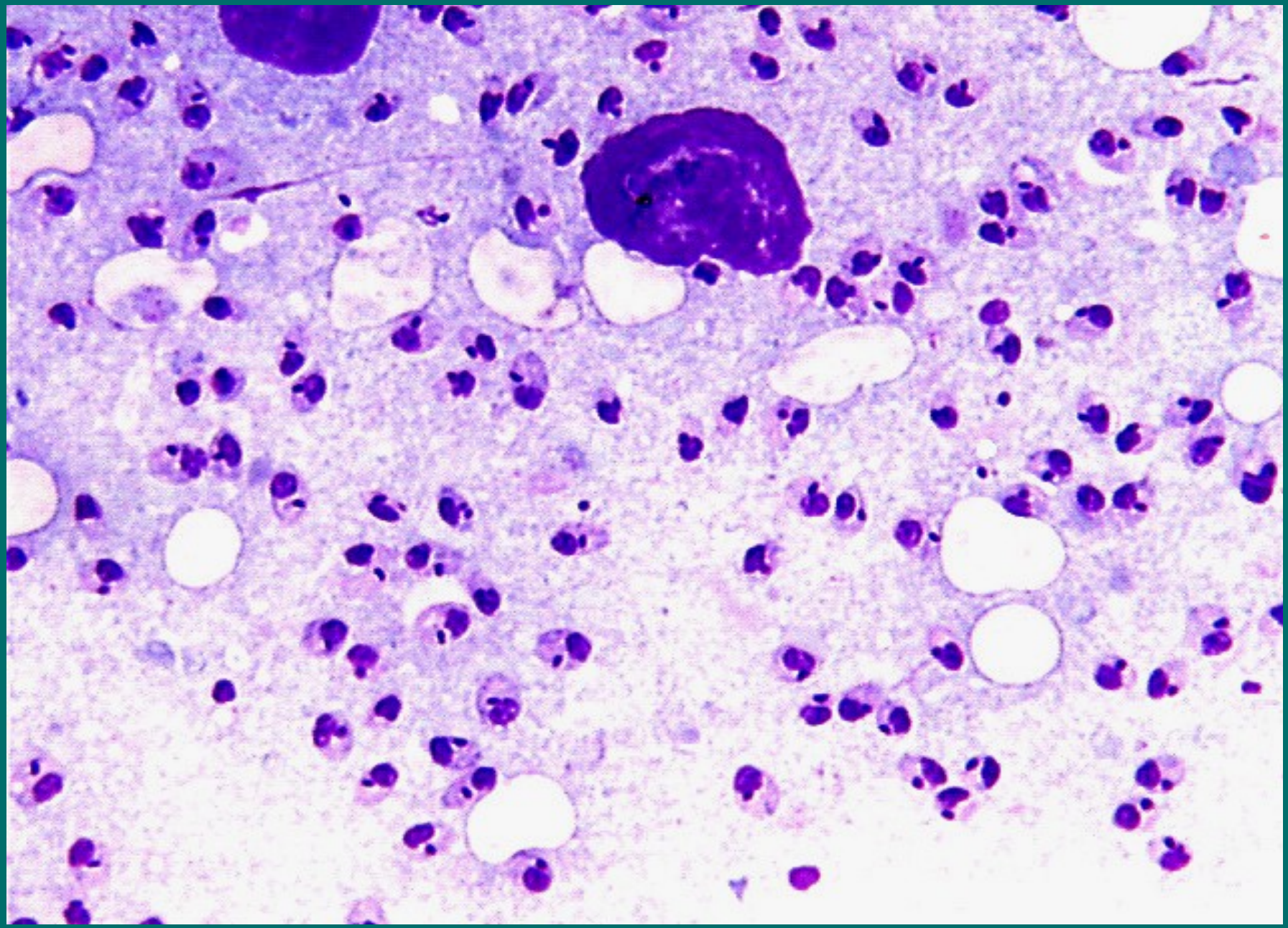
   (Phlebotomus is nocturnal and can't fly high above ground level).

7. Destruction of desert rodents (Natural Reservoirs).

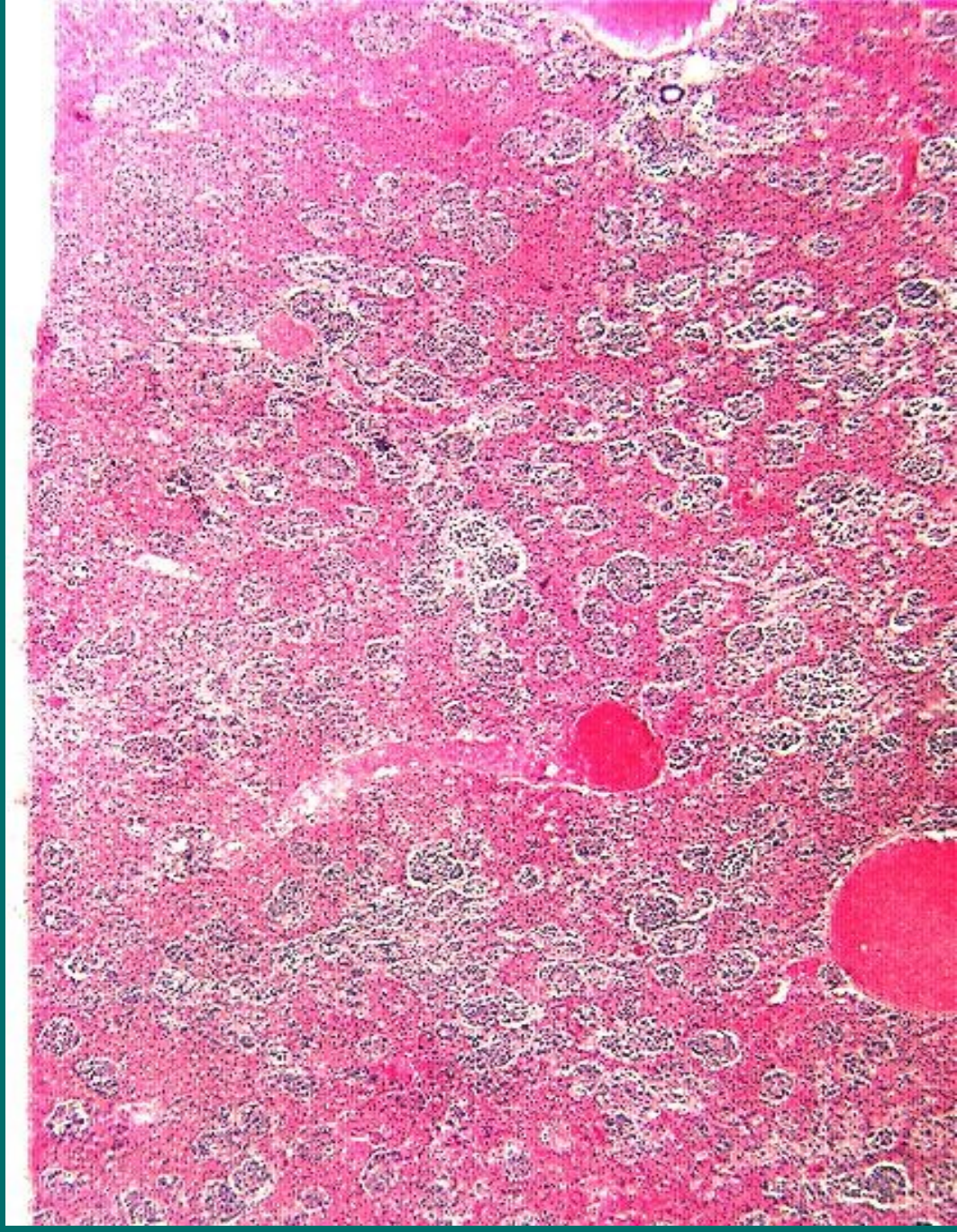
8. Elimination of dogs, (reservoir hosts, as in China).

9. Protection of skin lesions from insects with gauze bandage.

Thank You



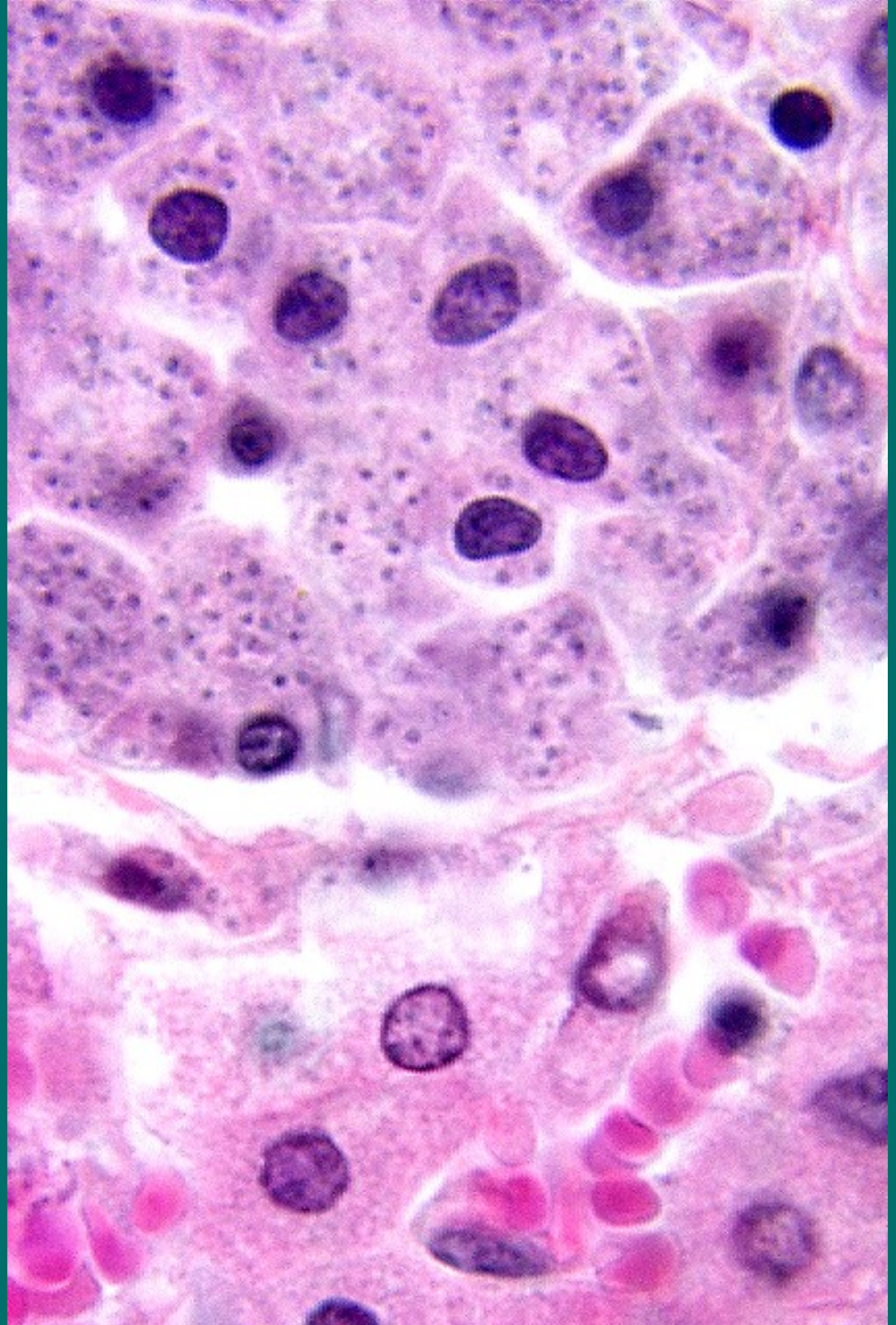
a. Low magnification view,
showing extensive infection of
ver macrophages (Kupffer cells)
by amastigotes,
apparent as dark, mottled areas.



. Higher magnification,

showing infected host cells

adjacent to uninfected hepatocytes.



Promastigotes of *Leishmania donovani*, culture smear.

This is the stage found in the gut of the sand fly.

Note the absence of an undulating membrane,

and the anterior location of the kinetoplast (K) relative to the nucleus (N).