BLOOD PROTOZOA

Dr Sadia Ikram

Learning objectives

- By the end oleishmania ture students of third year MBBS should be able to remember the two medically important Protozoa.
- Plasmodium & leishmania.
- Their life cycles & cotellate the pathophysiology of the diseses caused by them with their clinical features.
- Diagnose the cases of Malaria and leishmania.

Blood Protozoa

Plasmodium & Leshmania

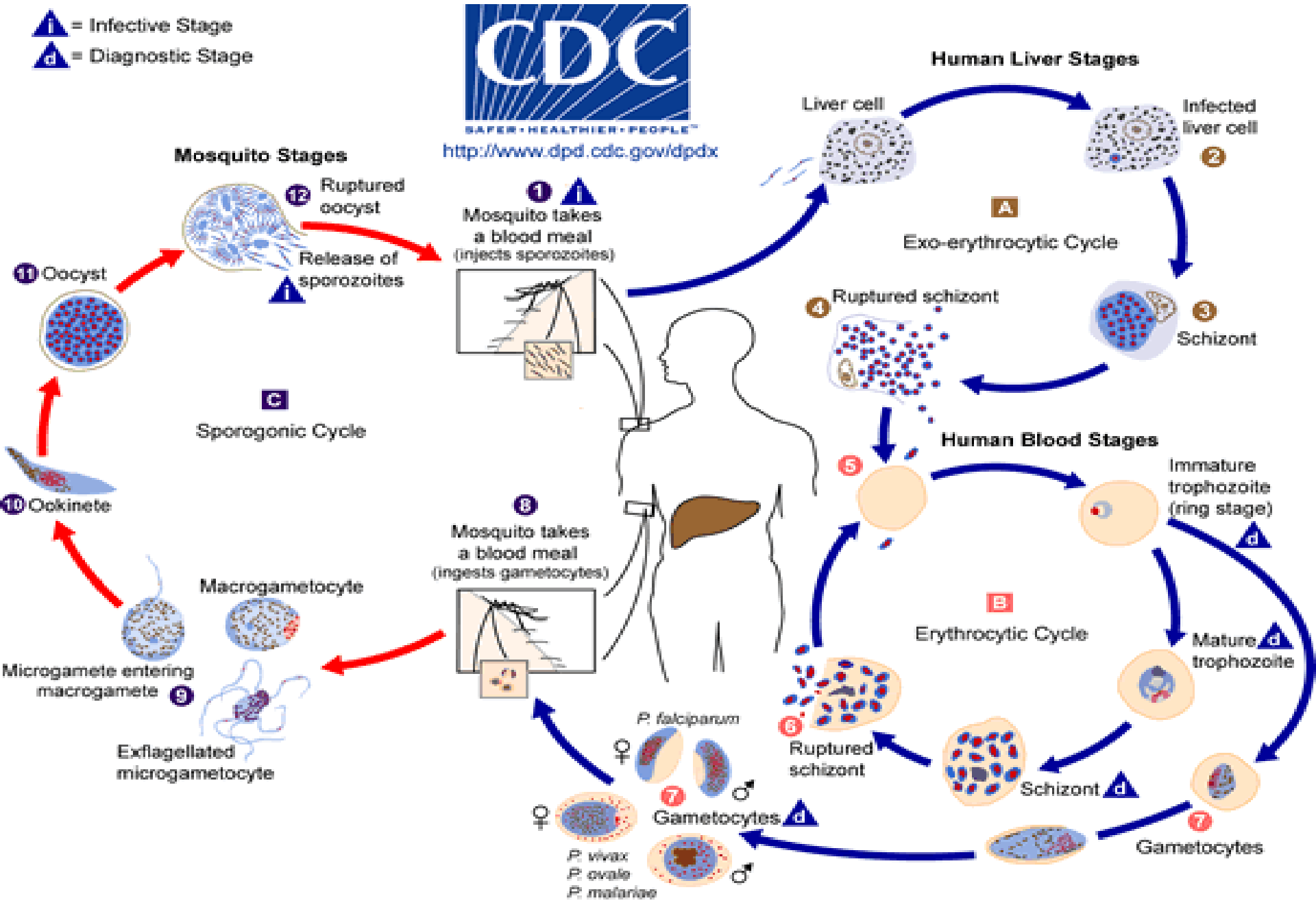
Plasmodium

- Malaria one of most common infectious diseases.
- Leading cause of death.
- **Vector & definitive host: female *Anopheles* mosquito.**
- **Four species of plasmodia:**
- *Plasmodium vivax*.
- *Plasmodium ovale*.
- *Plasmodium malariae*.
- *Plasmodium falciparum*.
- *P. vivax* & *P. falciparum* more common.

LIFE CYCLE: Indirect life cycle

- **Sexual cycle (sporogony): I**n mosquitoes.
- Mosquito: definitive host.
- **S**porozoites produced.

- **Asexual cycle (schizogony): I**n humans, liver cells.
- **H**umans: intermediate hosts.
- **S**chizonts made.


= Infective Stage
= Diagnostic Stage
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Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocytic Cycle
3
Schizont
4
Ruptured schizont
Human Blood Stages
5
Immature trophozoite (ring stage)
d
Mature trophozoite
d
B
Erythrocytic Cycle
6
Ruptured schizont
Schizont
d
7
Gametocytes
Gametocytes
d
P. falciparum
P. vivax
P. ovale
P. malariae
8
Mosquito takes a blood meal
(ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete
9
Exflagellated microgametocyte
10
Ookinete
11
Oocyst
C
Sporogonic Cycle
12
Ruptured oocyst
Release of sporozoites
Mosquito Stages
1
Mosquito takes a blood meal
(injects sporozoites)

- Introduction of sporozoites into blood from saliva of biting mosquito.
- Sporozoites taken up by hepatocytes within 30 minutes.

- "**Exoerythrocytic" phase**: Cell multiplication & differentiation into **merozoites.**
- *P. vivax* and *P. ovale* produce a latent form (**hypnozoite**) in liver, (relapses with vivax & ovale malaria).
- Merozoites released from liver cells & infect red blood cells.
- **Erythrocytic phase:** Organism differentiates in to ring-shaped trophozoite.
- Ring form grows into ameboid form & differentiates into schizont filled with merozoites.
- Merozoites infect other erythrocytes.
- Cycle in red blood cell repeats at regular intervals typical for each species.
- Periodic release of merozoites causes typical recurrent symptoms of chills, fever, and sweats seen in malaria.

Epidemiology & Transmission:

- **Transmitted primarily by mosquito bites.**
- **Transmission across placenta, in blood transfusions, & by intravenous drug abuse also occurs.**

More than 200 million people infected worldwide.

Occurs primarily in tropical and subtropical areas, especially Asia, Africa, and Central and South America.

Mortality: 1 million/ year.

lethal infectious disease.

Seen in United States seen in Americans who travel to areas of endemic infection & in immigrants from areas of endemic infection.

Certain regions in Southeast Asia, South America, and east Africa affected by chloroquine-resistant strains of *P. falciparum*.

Pathogenesis

Pathological findings of malaria result from:

- destruction of red blood cells.
- Red cells destroyed both by release of merozoites & by action of spleen, which sequester infected red cells & lyse them.
- Enlarged spleen characteristic of malaria due to congestion of sinusoids with erythrocytes, hyperplasia of lymphocyte & macrophages.

- *P. falciparum* more severe.
- Occlusion of the capillaries with aggregates of parasitized red cells.
- Life-threatening hemorrhage and necrosis, brain (cerebral malaria).

- *P. falciparum*
- Extensive hemolysis and kidney damage, with resulting hemoglobinuria.
- Dark color of urine ("blackwater fever“).
- Hemoglobinuria lead to acute renal failure.

Clinical forms of malaria

Quartan malaria: Fever recurs every fourth day

Tertian malaria: Fever recurs every third day

- **Benign Quartan malaria:** *P. malariae (72 hours)*
- **Malignant tertian malaria:** P. *falciparum. (48h)*
- **Benign tertian malaria:** *P. vivax* and *P. ovale. (48h)*

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Clinical Findings

- Incubation Period: 2 weeks.
- Abrupt onset of fever, chills, headache, myalgias & arthralgia.
- Fever continuous or periodic.
- Fever spike reaching up to 41°C with shaking chills, nausea, vomiting & abdominal pain.
- Fever followed by drenching sweats.
- Splenomegaly in most patients.
- Hepatomegaly in one-third.
- Anemia prominent.

- Untreated malaria by *P. falciparum* life-threatening due to extensive brain (cerebral malaria) & kidney (blackwater fever) damage.

- Malaria by other plasmodia self-limiting.

- Relapses of *P. vivax* and *P. ovale* malaria up to several years after initial illness due to hypnozoites latent in liver.

Complications

- **Malaria caused by P. falciparum:**
- Cerebral malaria
- Blackwater fever
- Anaemia
- Hypoglycemia

- **Malaria caused by P. vivax and ovale:**
- Relapses due to hypnozoite stage.

- **Malaria caused by P. malariae:**
- Nephrotic syndrome leading to renal failure, due to deposition of Ag-Ab complexes on basement membrane of kidney. (Oedema, proteinuria, low serum albumin levels).

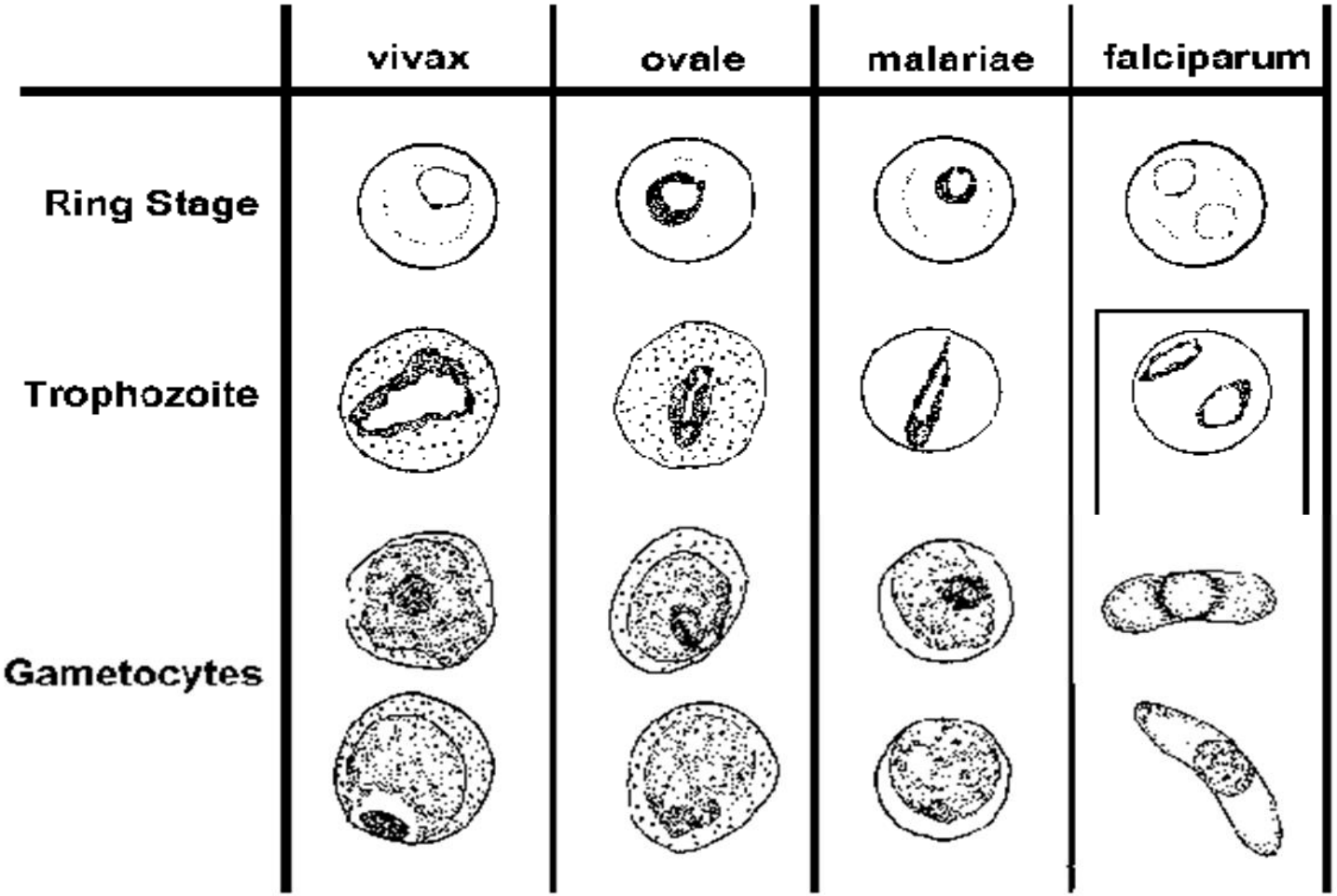
Diagnosis

1. **Clinical Diagnosis:** Based on patient's symptoms & on physical findings. Fever, chills, sweats, headaches, muscle pains, nausea and vomiting.

- In *Plasmodium falciparum malaria*: confusion, coma, neurologic focal signs, severe anemia, respiratory difficulties.

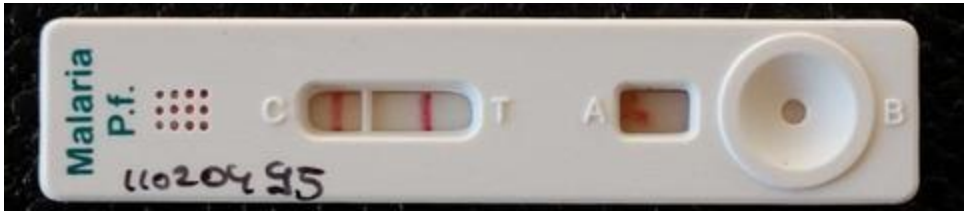
2. Microscopic Diagnosis

- **Malaria parasites** identified by examining Giemsa stained blood smear under the microscope. Gold standard for laboratory confirmation of malaria.
- Thick smear: To screen for organisms.
- Thin smear: For species identification.
- Ring-shaped trophozoites within infected red blood cells.
- Gametocytes of *P. falciparum* **crescent-shaped** ("banana-shaped").
- Gametocytes of other plasmodia spherical.


vivax
ovale
malariae
falciparum
Ring Stage
Trophozoite
Gametocytes

3. Antigen Detection (Rapid Diagnostic Test)

- Immunochromatographic test, provide results in 2-15 minutes.



4. Molecular Diagnosis: PCR-based test for *Plasmodium* nucleic acids.

- Technique slightly more sensitive than smear microscopy. Result not available quickly. useful for confirming species of malarial parasite.

5. Serology: Detects antibodies against malaria parasites, by either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Does not detect current infection, measures past exposure.

Treatment

- Chloroquine drug for acute malaria (kills merozoites, reducing parasitemia).
- Hypnozoites of *P. vivax* & *P. ovale* in liver killed by primaquine.
- Mefloquine for chloroquine-resistant strains of *P. falciparum*.
- Intravenous administration of either quinidine (or quinine) plus doxycycline or clindamycin for strains of *P. falciparum* resistant to both chloroquine and mefloquine.

Prevention

- Mefloquine or doxycycline for **chemoprophylaxis** of malaria for travelers to areas with endemic chloroquine-resistant *P. falciparum*.
- Chloroquine for sensitive strains of *P. falciparum*.
- Mosquitoe nets and repellents.

Other Preventive Measures

- Mosquito netting, window screens, protective clothing &insect repellents.
- Protection important during night.
- Drainage of stagnant water in swamps & ditches reduces breeding areas.
- No vaccine available.

BLOOD PROTOZOA: *LEISHMANIA*

Blood Protozoa:
Leishmania

Cutaneous Leishmania	Mucocutaneous Leishmania	Kalazar/Visceral Leishmania
• Leishmania Tropica (**oriental sore**) • Leshmania Mexicana (**bay sore**)	• Leishmania Brazilliensis **(espundia)**	• Leshmania Donovani **(kalazar)**

Important Properties: Indirect life cycle

Life cycle involves the female sand fly as the vector

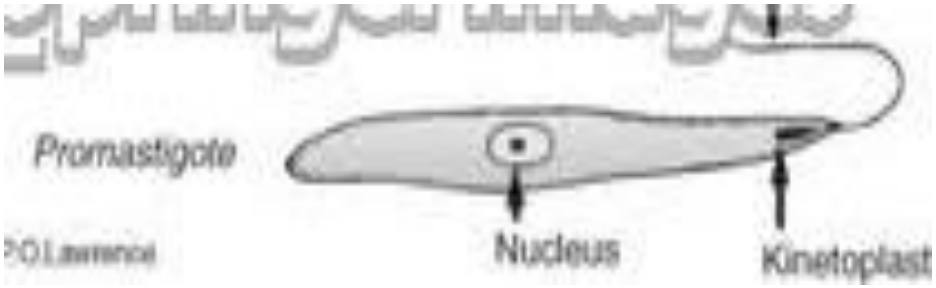
Reservoirs are dogs, foxes and rodents

Female sand fly takes the blood meal

Morphological types

- **Amastigote form:**

- Oval in shape
- 2-4um
- Have nucleus and rod shaped kinetoplast
- Resides in Reticulo-endothelial system of host
- Called as LT or LD bodies

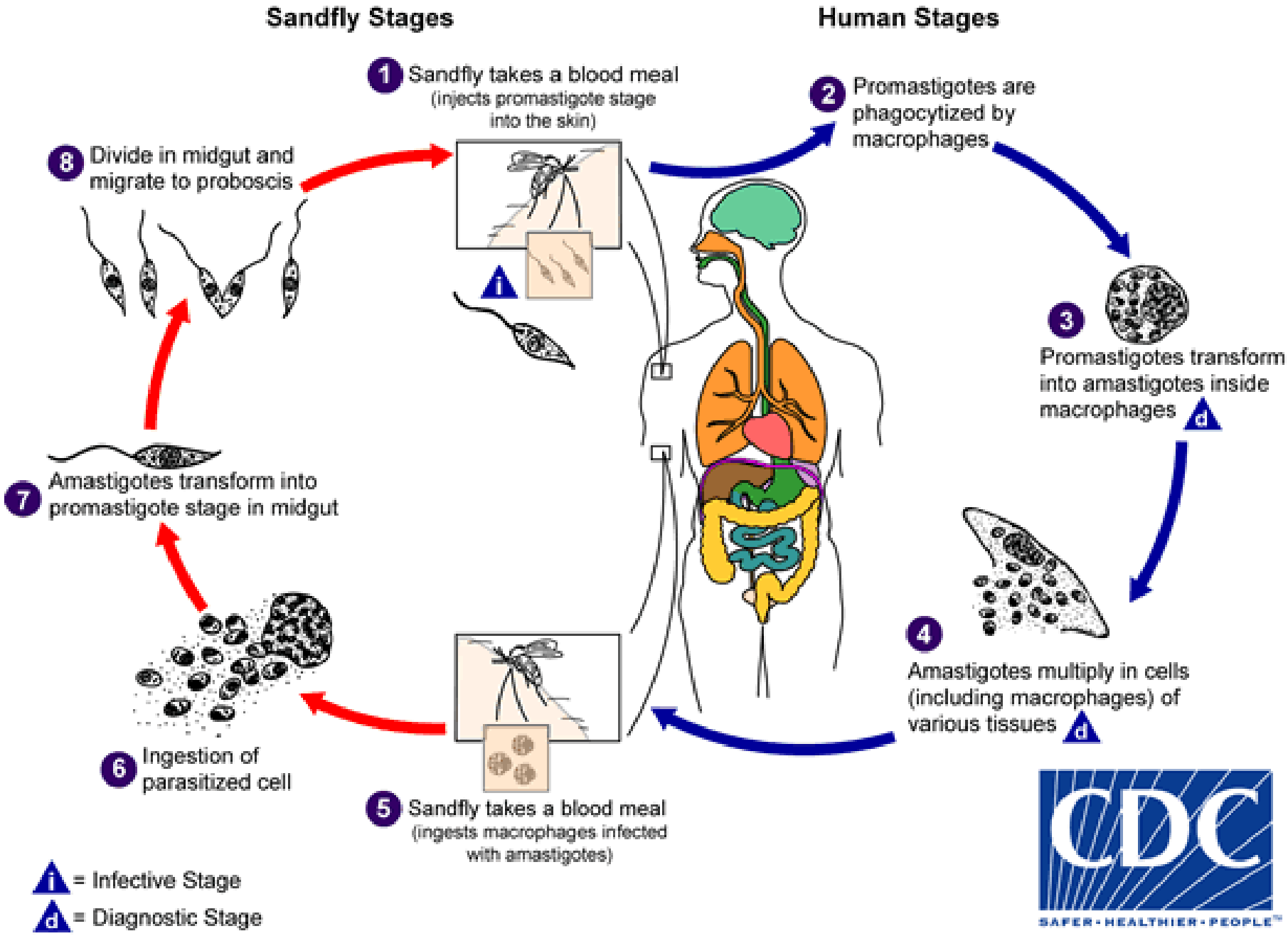


- **Promastigote form:**
- Large, elongated.
- Has nucleus, kinetoplast, polar flagellum
- Seen in sand fly and in cultures

Epidemiology

- **Cutaneous Leishmaniasis:** Eastern Baluchistan, Southern Punjab, NWFP and Kashmir.

- **Visceral Leishmaniasis:** Common in Azad Kashmir and Baltistan.


Sandfly Stages
Human Stages
1 Sandfly takes a blood meal (injects promastigote stage into the skin)
2 Promastigotes are phagocytized by macrophages
3 Promastigotes transform into amastigotes inside macrophages
4 Amastigotes multiply in cells (including macrophages) of various tissues
5 Sandfly takes a blood meal (ingests macrophages infected with amastigotes)
6 Ingestion of parasitized cell
7 Amastigotes transform into promastigote stage in midgut
8 Divide in midgut and migrate to proboscis
i = Infective Stage
d = Diagnostic Stage
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Pathogenesis

- **Cutaneous Leishmaniasis:** Lesions confined to skin.
- **Mucocutaneous Leishmaniasis:** Lesions confined to mucous membrane, cartilage & skin.
- **Visceral Leishmaniasis:** Organs of RE system affected (liver, spleen, bone marrow).
- Reduced bone marrow activity.
- Cellular destruction in spleen.
- Anemia, Leukopenia, Thrombocytopenia.
- Enlarged spleen.
- Marked increase in IgG

Clinical Findings

- **Cutaneous Leishmaniasis:**
  - Initial lesion red papule at site of bite.
  - Enlarges slowly to form multiple satellite nodules that coalesce and ulcerate.
  - Lesions heal spontaneously.
  - In immuno-compromised patients, it spread to involve large areas of skin & contain numerous organisms.

- **Mucocutaneous Leishmaniasis:**
  - Papule at site of bite.
  - Metastatic lesions at mucocutaneous junction of nose & mouth.
  - Granulomatous, ulcerating lesions destroy nasal cartilage.
  - Lesions heal slowly.
  - Death may occur from secondary infection.

Visceral Leishmaniasis:

- Intermittent fever, weakness and weight loss.
- Massive enlargement of spleen.
- Hyper-pigmentation of skin.
- Gastrointestinal bleeding in advanced stages.
- Untreated cases always fatal.

Complications

- **Post-kala-azar dermal leishmaniasis (PKDL):** characterized by hypopigmented macular, maculopapular, and nodular rash usually in patients who have recovered from Visceral leishmania .

- Appears 6 months to 1 or more years after apparent cure of of disease but may occur earlier or even concurrently with visceral leishmaniasis.

Diagnosis

1. Microscopy

- In human host, amastigotes stage is seen upon microscopic examination of tissue specimens. Stained by Giemsa and hematoxylin and eosin (H&E) stains.

2. Isoenzyme analysis

- Isolation done by using biphasic medium which includes a solid phase composed of blood agar base (e.g., NNN medium), with defribinated rabbit blood.
- Species identification by isoenzyme analysis.

3. Serology

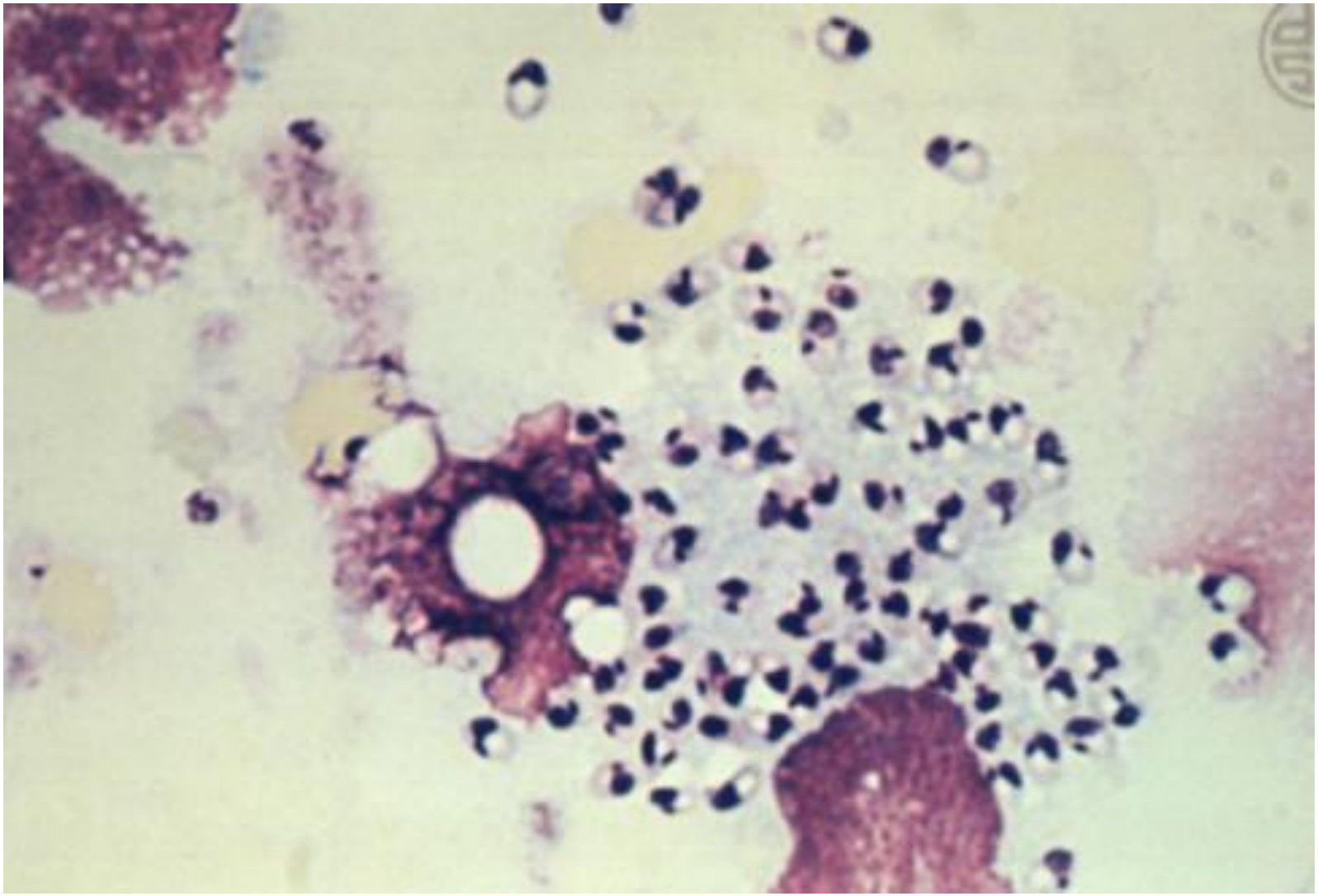
- Antibody detection in visceral leishmaniasis.

4. Molecular Diagnosis

- Molecular approaches e.g. PCR, more sensitive and rapid; e.g. results available within days versus weeks.

Laboratory Diagnosis

- **Cutaneous & Mucocutaneous Leishmaniasis:**
- **Microscopy:** Demonstration of LD bodies in smear taken from skin lesions.
- **Leismanin test: Po**sitive when skin ulcer appears.
- **Visceral Leishmaniasis:**
- **Microscopy:** Detection of LD bodies in bone marrow, spleen, lymph node biopsy.
- **Culture:** Of ogansims on NNN (Novy McNeal & Nicole) medium.
- **Serology:** Indirect immunoflourescence test positive in most patients. Raised IgG levels.
- **Antigen Detection:** Leishmanin test:



Treatment & Prevention

Treatment

- Sodium stibogluconate.
- Antimony compound.

Prevention

- Protection from sandfly bites & insecticide spray

MCQ # 1

- A woman, recently returned from Africa, complains of having paroxysmal attacks of chills, fever, and sweating; these attacks last a day or two at a time and recur every 36 to 48 h. Examination of a stained blood specimen reveals ringlike and crescent-like forms within red blood cells. The infecting organism most likely is
- a. Plasmodium falciparum
- b. Plasmodium vivax
- c. Trypanosoma gambiense
- d. Wuchereria bancrofti
- e. Schistosoma mansoni

MCQ # 2

- **Patient came to the emergency department with history of fever, shivering and body aches. Plasmodium vivax was diagnosed as a cause of malaria. This plasmodium is responsible for which type of malaria?**
- Benign tertian malaria
- Quartan malaria
- Malignant tertian malaria
- Benign quartan malaria
- Malignant quartan malaria

MCQ # 3

- **A young boy had history of intermittent fever for last week, associated with dark colored urine. Which specie of *Plasmodium* is responsible for his disease?**
- *P. falciparum*
- *P.vivax*
- *P.ovale*
- *P.malariae*
- None of the above

MCQ # 4

- **A 35 year old adult had an official trip to India. After 3-6 months of returning he developed dry, rough and pigmented lesions on skin along with fever. On physical examination he had hepatosplenomegaly. Skin biopsy revealed LD bodies. What is the most likely diagnosis?**
- Lesishmaniasis
- Black water fever
- Infantile Kala-azar
- Malaria
- Amoebiasis

MCQ # 5

- A 35 year old adult had an official trip to India. After 3-6 months of returning he developed dry, rough and pigmented lesions on skin along with fever. On physical examination he had hepatosplenomegaly. He had Intradermal test positive. What is the most likely diagnosis?
- Visceral lesion
- Muco-cutaneous leishmaniasis
- Infantile Kala-azar
- Cutaneous leishmaniasis
- None of the above

SEQ # 1

- A 20 years old farmer develops periodic bouts of fever with chills and rigors occurring every 36-48 hours. He is anemic on appearance and has splenomegaly. His peripheral smear shows cresenteric structures.
- What is the most likely diagnosis?
- How will u diagnose this case in laboratory ?
- What are its complications?

SEQ # 2

- a)Name hemoflagellates infecting humans.

- b) Describe pathogenesis of Plasmodium Falciparum infection.

SEQ # 3

- A lesion appeared on face and extremities weeks to months after bite of the fly in the resident of a tropical country resident adult male. The blood picture shows ingested bodies (LD bodies) in the leucocytes.
- What is the name of this parasite?
- Name the vector of the parasite.
- Discuss the laboratory diagnosis of this parasite.
- Draw the life cycle of this parasite.
- Which form of parasite is present in sandfly?