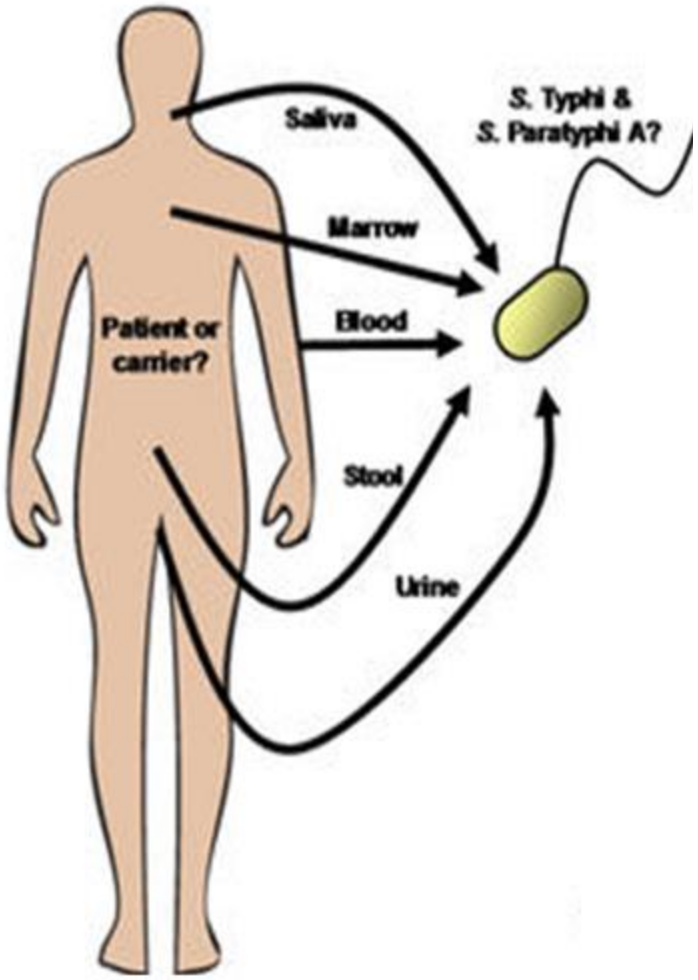
HLS
2024-2025
Practical

Dr. Mohammad Odaibat

Diagnosis of Salmonella

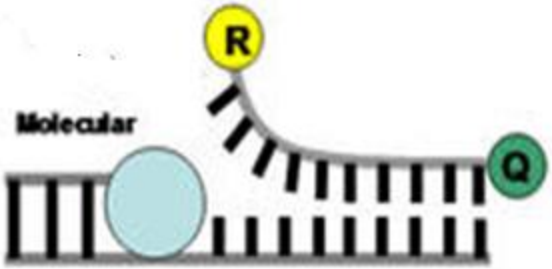
Which Sample?



Which Method?

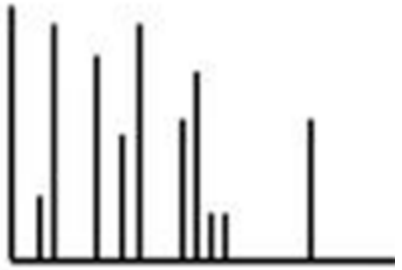
Microbiological

Serological



Molecular

lass Spectrometry



Diagnosis of salmonellosis

Cultural properties

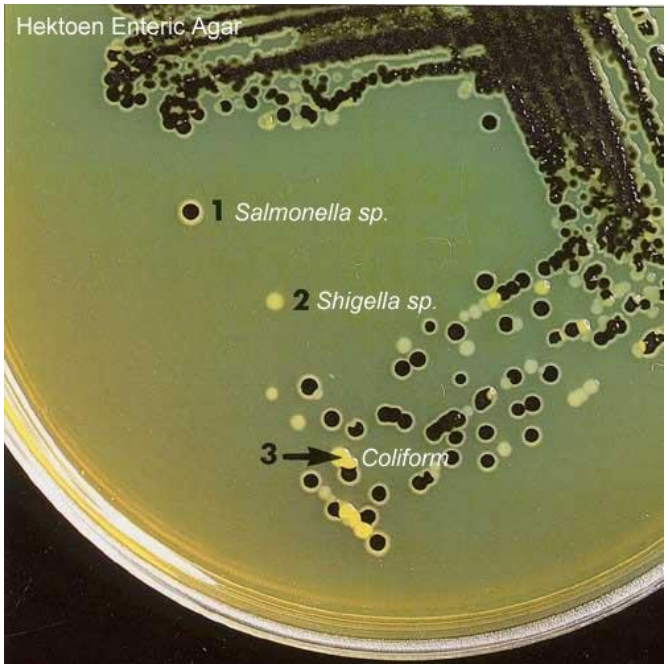
- Grow easily on simple culture media and on selective and differential media that contain biliary salts and lactose.
- Produce H2S, colonies have a “cat-eye” appearance.

Diagnosis of salmonellosis

Salmonella selective media:

Favor growth of *salmonellae* and *shigellae* over other *Enterobacteriaceae* including

1. Salmonella-Shigella (SS) agar
2. Hektoen enteric agar



Shigella: colorless colonies without black centers

Salmonella: colorless colonies with black centers

Lactose fermenter flora: pink to red colonies

Diagnosis of salmonellosis

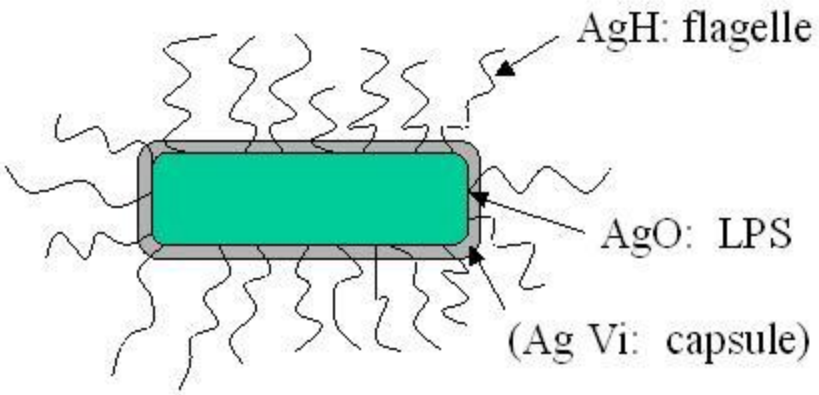
Suspected colonies from solid media are identified by biochemical reaction patterns

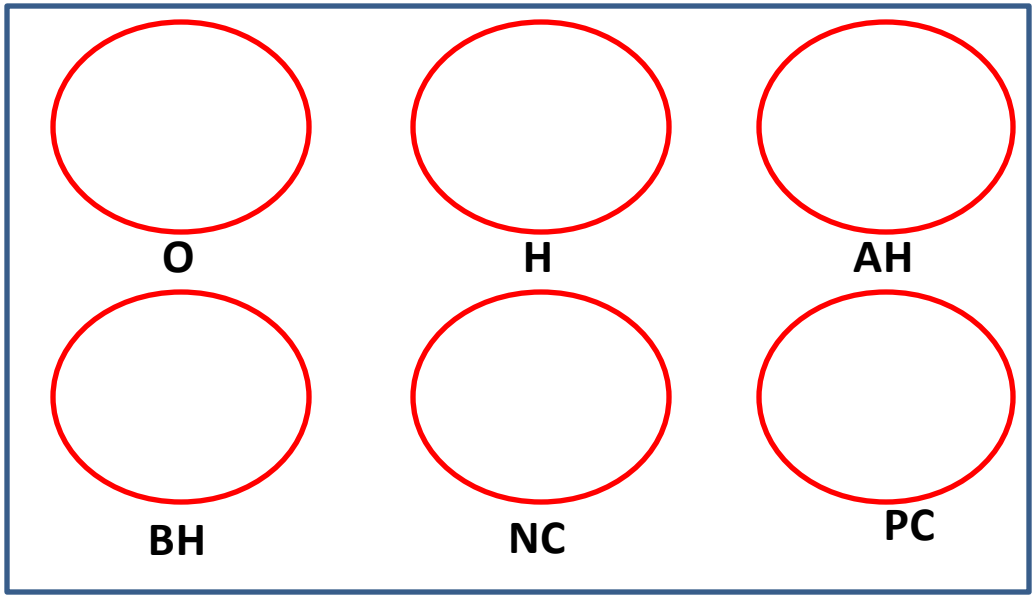
- **Motile**
- **Lactose negative**
- **acid and gas from glucose, mannitol, maltose, and sorbitol;**
- **Indole test negative**
- **Methyl red test positive**
- **Voges-Proskauer test negative**
- **Citrate positive (growth on Simmon's citrate agar)**
- **Urease negative**

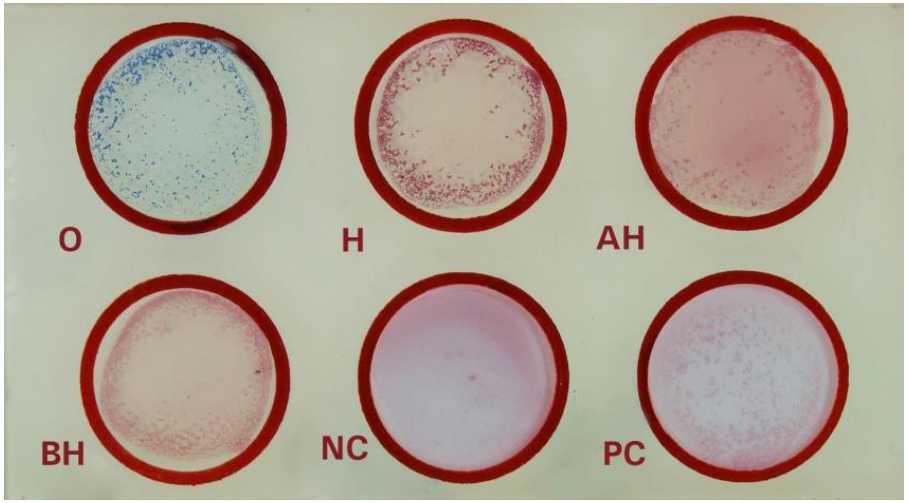
Slide agglutination tests with specific sera. Serologic techniques are used to identify unknown cultures with known sera **and may also be used to determine antibody titers in patients with unknown illness**

Diagnosis of salmonellosis

Serologic Methods (Widal test)







O: Somatic antigen (S. typhi)
H: flageller antigen (S. typhi)
S. pratyphi A , H antigen (AH)
S. pratyphi B, H antigen (BH)
NC: negative control
PC: positive control

Diagnosis of salmonellosis

Serologic Methods (Widal test)

- Principle: Patients' suffering from enteric fever would possess antibodies in their sera against *S. t*yphi O antigen, *S. t*yphi H antigen and *S. p*aratyphi AH antigen and *S. p*aratyphi BH antigen which can be detected by slide widal test.
- Procedure: One drop each of undiluted patients' serum samples for the four antigens are placed on the circled card and one drop of each of the four Salmonella antigens are added separately and gently rotated for one minute. Appearance of agglutination gives qualitative results

Yersinia pestis

Diagnosis

Acceptable Specimen Types .

- Bronchial wash/tracheal aspirate (≥ 1 ml) .
- Whole blood: 5-10 ml blood in EDTA, and/or Inoculated blood culture bottle .
- Aspirate or biopsy of liver, spleen, bone marrow, lung, or bubo

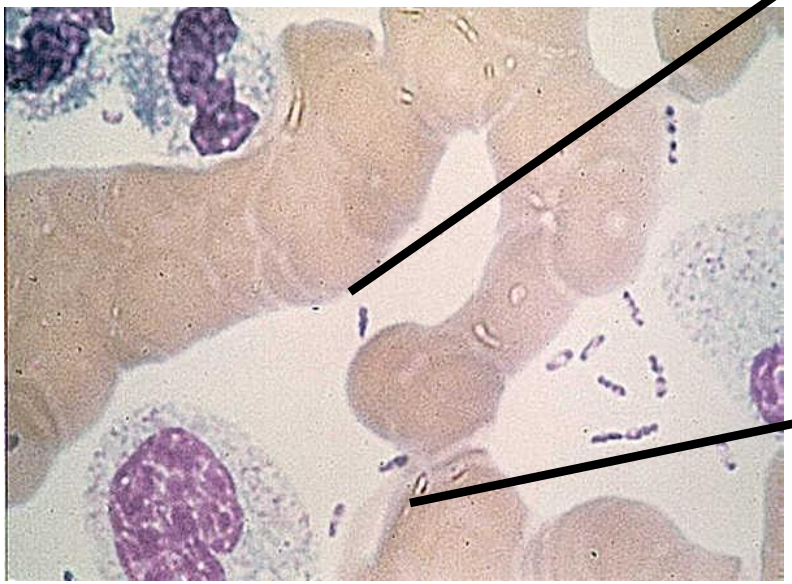
Diagnosis

- Giemsa stained Smears typically show the bacillus to have a **bipolar or "safety pin" appearance.**
- Send smears to a reference lab for fluorescent antibody microscopy.
- Most Gram-negative bacteria produce colonies within 24 h*; Y. pestis* do not. Because Cultures grow slower (1.25 hours/generation time) than other bacteria and thus require longer incubation times for optimal growth

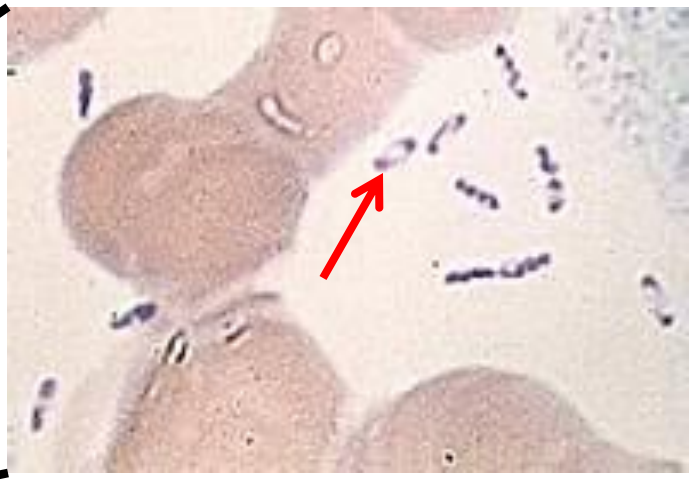
Diagnosis

Staining pattern

Gram-negative rods (0.5 - 0.8 x 1- 3 µm) Bipolar staining (resembling closed safety pin) may be evident with Gram stain but more apparent with Giemsa stain



Giemsa staining



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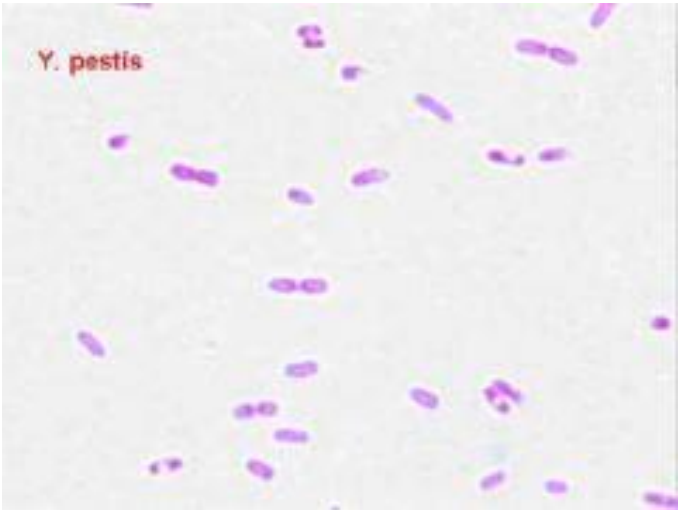


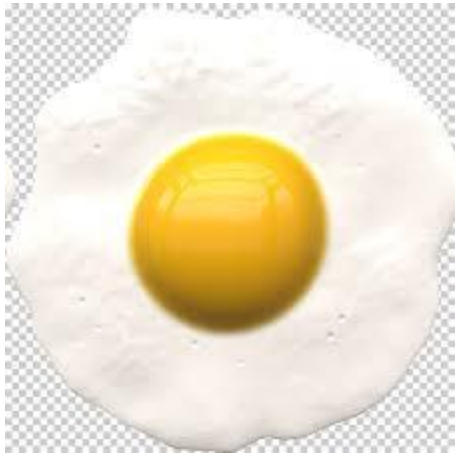
Gram staining

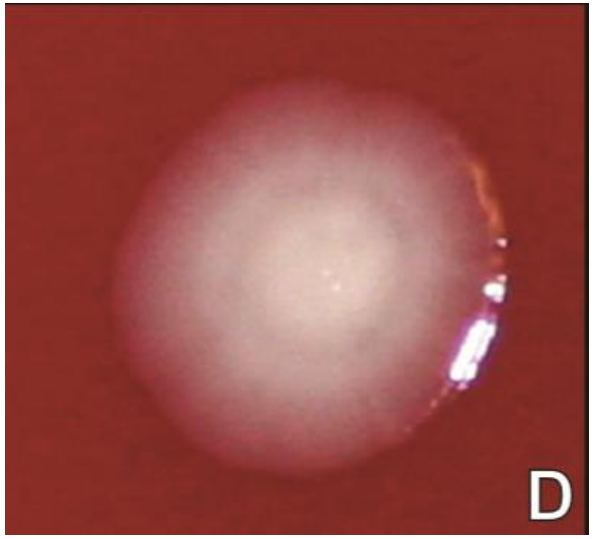
Diagnosis

Colony Morphology

- Grey-white translucent colonies on Blood Agar (BA) and Chocolate Agar (CA) at ambient and 35/37^{o}C (growth faster at 28^{o}C).
- "Fried egg" appearance on BA in older cultures

Yersinia pestis growth on BA at (A) 48 h, (B) 72 h, (C) 96 h, (D) 96 h "Fried egg"

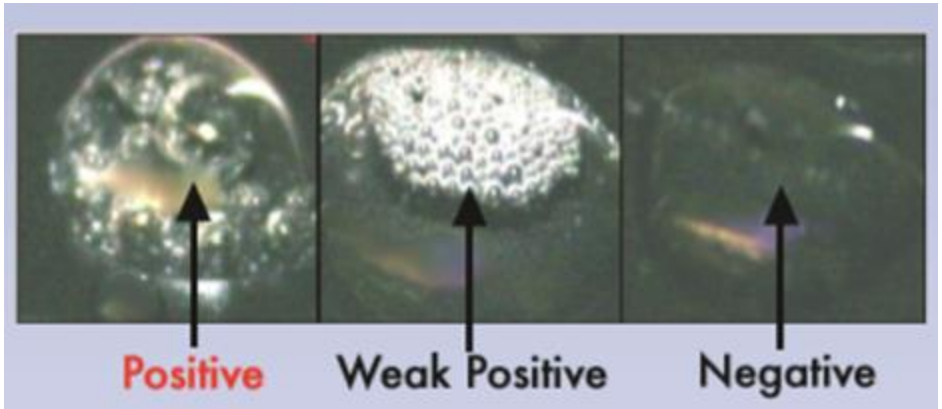




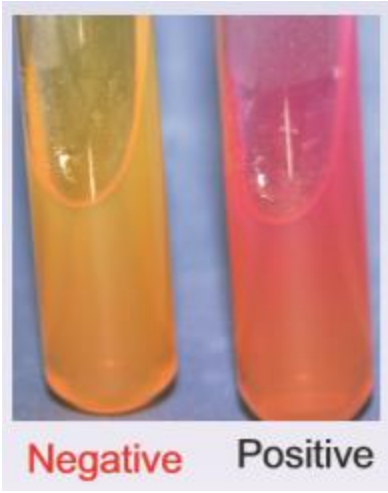
Diagnosis

Additional Lab Identification

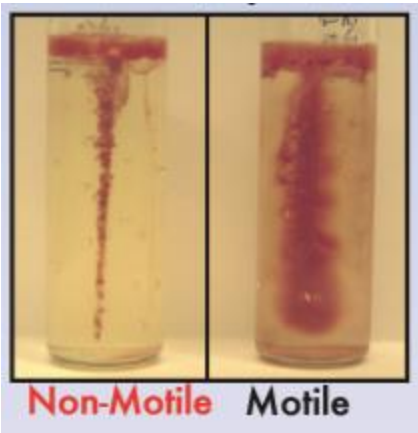
Catalase: positive



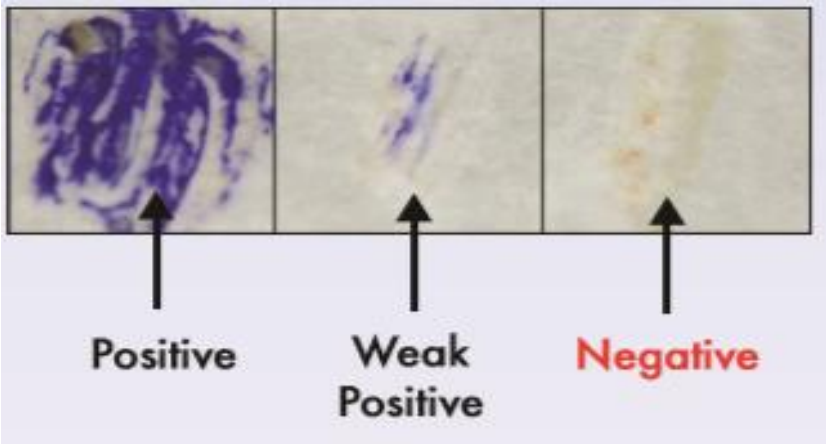
Urease: negative



Motility: nonmotile



Oxidase: negative Indole: negative



Diagnosis

Grey-white translucent, non-hemolytic colonies on BA or CA (24 h), Yellow and opaque (48 h).

↓

Gram-negative rods bipolar staining (closed safety pin)

↓

*Catalase: positive *Motility: nonmotile
* Urease: negative *Oxidase: negative * Indole: negative

No
Continue laboratory identification procedure

Yes
Immediatelly notify the physician to treat and to take the the proper isolation precautions

Brucellosis

Brucellosis

Specimen collection, transport, and processing

- A definitive diagnosis of brucellosis requires isolation of the organisms in cultures of blood, bone marrow, CSF, pleural and synovial fluids, urine, abscesses, or other tissues.
- If processing will be delayed, the specimen may be held in the refrigerator.

Brucellosis

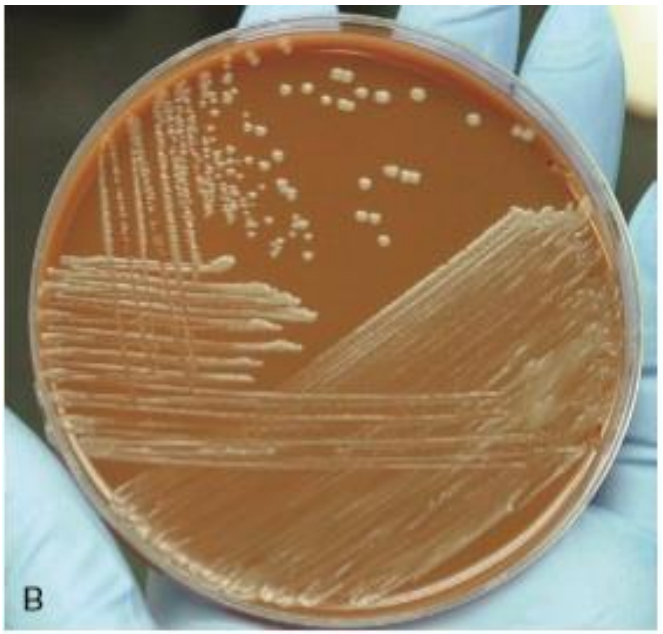
Direct detection methods

- Conventional and real-time polymerase chain reaction (PCR) assays are reliable and specific means of directly detecting Brucella organisms in clinical specimens.

Brucellosis

Cultivation

- Brucella can grow on blood and chocolate agars
- More enriched agars including Brucella agar or infusion base agar are used to isolate *Brucella*
- All subculture plates should be held for a minimum of 7 days.
- On culture, colonies appear small, convex, smooth, translucent, nonhemolytic, and slightly yellow and opalescent after at least 48 hours of incubation
- Brucella spp. are catalase and urease positive, and most strains are oxidase positive



Brucellosis

Serologic test

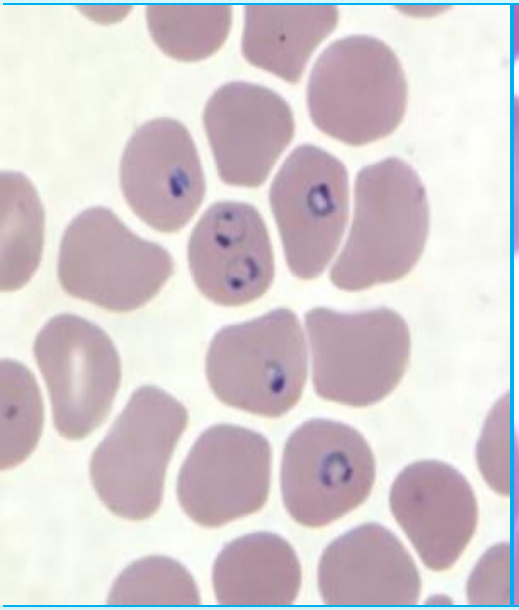
- Is widely used (e.g., serum agglutination test [SAT] or microplate agglutination [MAT]) because isolating brucellae is difficult
- A titer of 1 : 160 or greater in the SAT is considered diagnostic if this result fits the clinical and epidemiologic findings.

Diagnosis Q fever (Coxiella burnetii)

- Serology (rise in titer)
  - IFA, CF, ELISA, microagglutination
- DNA detection methods
  - PCR
- Isolation of organism
  - Risk to laboratory personnel
  - Rarely done

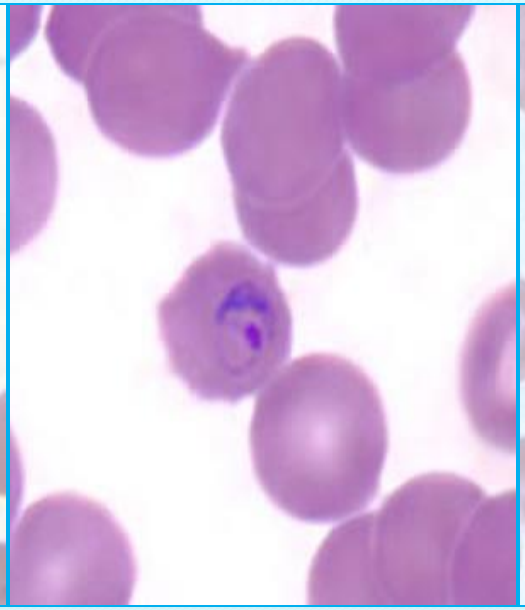
Parasitology

Ring stage of malaria species



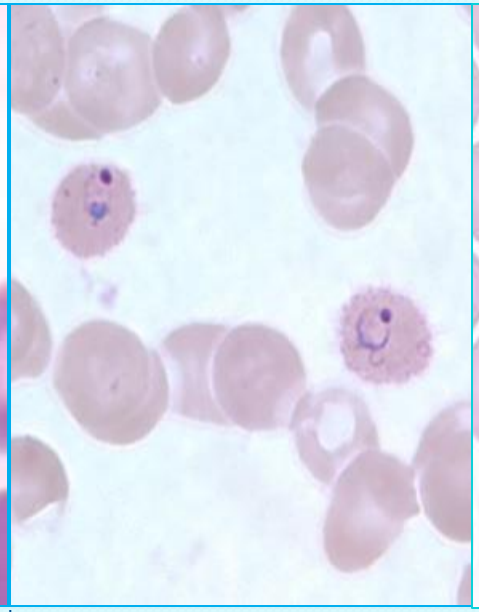
P. falciparum

- Infected RBCs are normal in size.
- Scanty cytoplasmic ring fills 1/6 RBCs surrounds a small vacuole.
- One or 2 chromatin dots (headphone).
- multiple rings are common.
- Seen in periph.blood



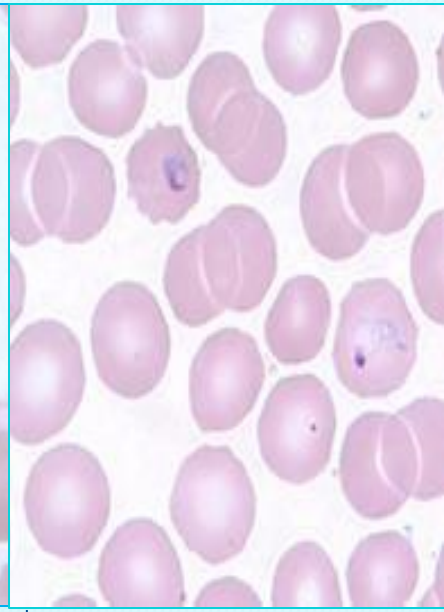
P. malariae

- Infected RBCs are normal in size.
- Cytoplasmic ring fills 1/3 RBCs.
- One chromatin dot inside the ring.



P. ovale

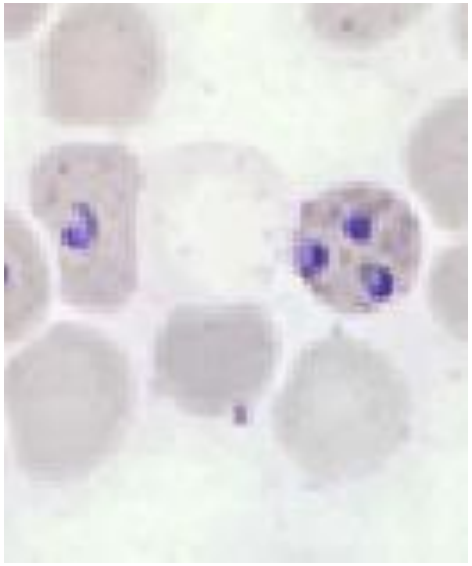
- Infected RBCs are oval, larger than non infected ones with irregular surface.
- Dense cytopl. ring larger than *P. vivax* fills 1/3 of RBCs
- Dense one chromatin mass.



P. vivax

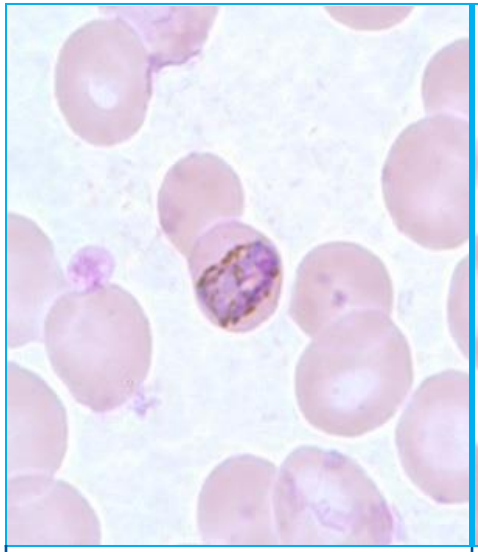
- Infected RBCs are larger than non infected ones.
- Delicate cytoplasmic ring fills 1/3 of RBCs.
- One chromatin dot.
- Ring surrounds a vacuole.

Trophozoite stage of malaria species



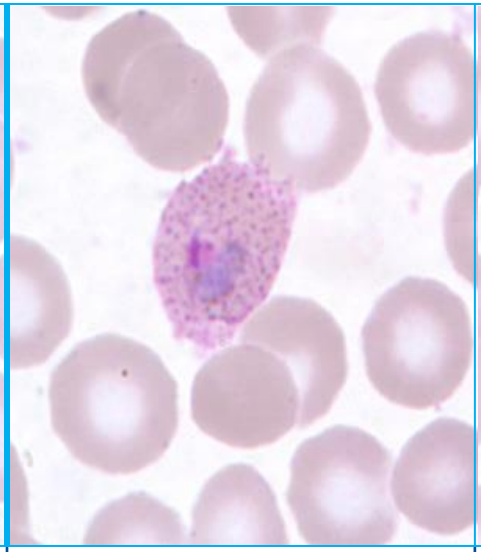
P. falciparum

- Thin and delicate, measuring on average 1/5 the diameter of the red blood cell



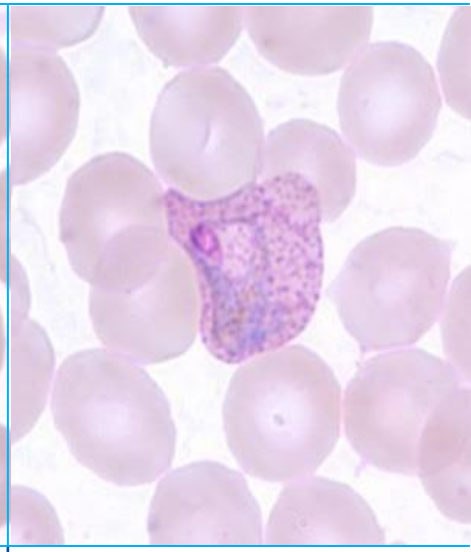
P. malariae

- Band shaped & less vacuolated



P. ovale

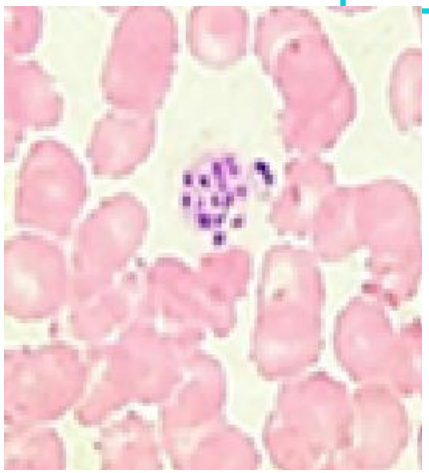
- Small, compact, oval
- Less vacuolated.
- Fimbrial end.



P. vivax

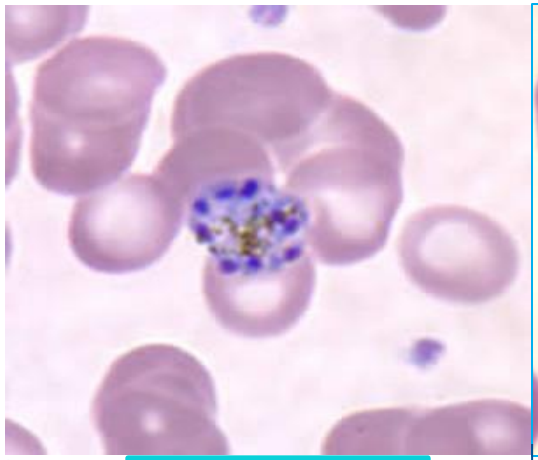
- Large amoeboid. & highly vacuolated.

Schizont stage of malaria species



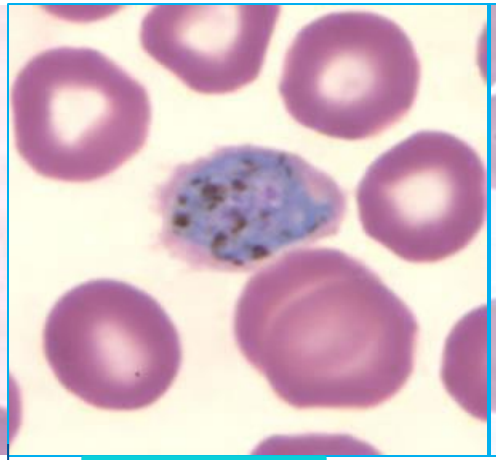
P. falciparum

- When seen, schizonts contain anywhere from 8-24 merozoites..



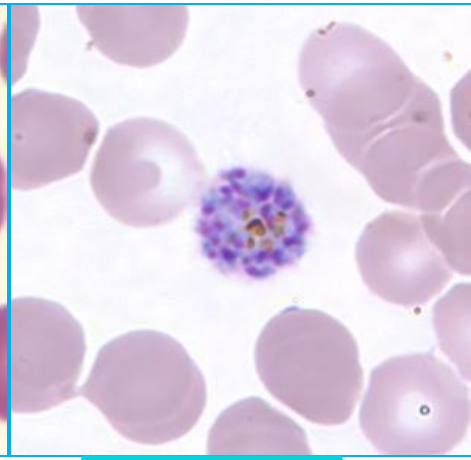
P. malariae

- Fills RBCs.
- Contain 6-12 merozoites (8) arranged symmetrically around central mass of malarial pigment (rosette-shaped)



P. ovale

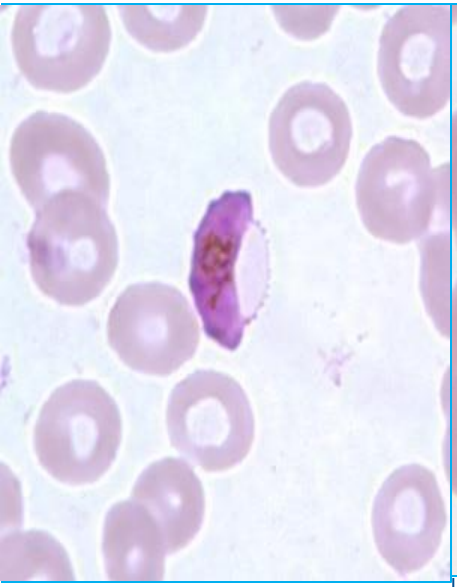
- Fills ¾ of RBCs with fimbrial end.
- Contain 6-12 merozoites (8) arranged irregularly around central mass of malarial pigment



P. vivax

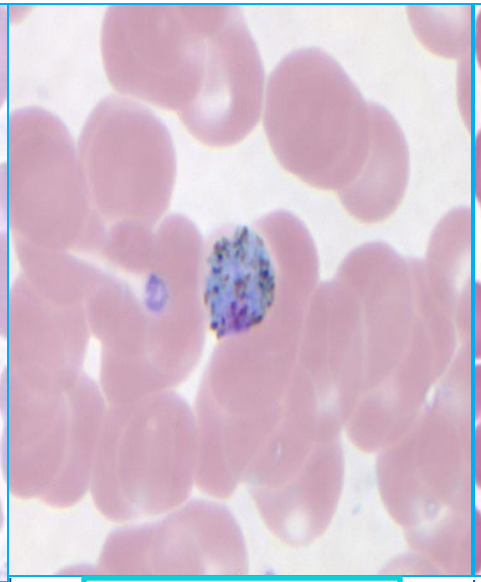
- Fills RBCs.
- Contain 12-24 merozoites (18) arranged irregularly around central mass of malarial pigment

Gametocytes (male & female) of malaria species



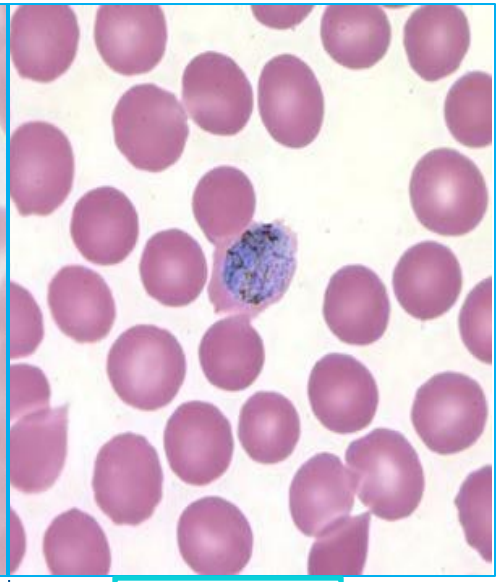
P. falciparum

- Crescent or banna-shaped.
- Seen in peripheral blood



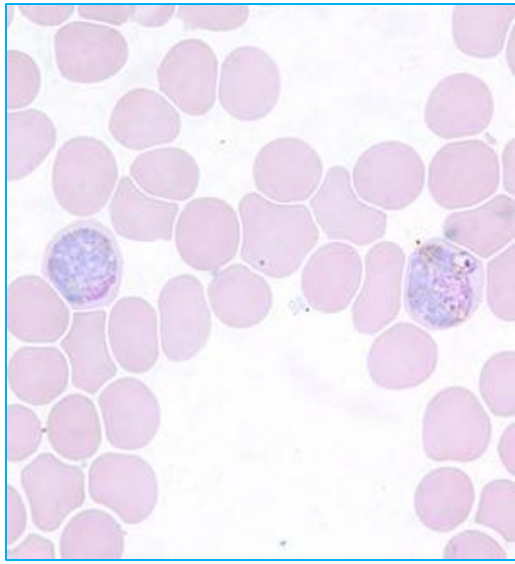
P. malariae

- Fills RBCs.
- Spherical & compact.



P. ovale

- Fills ¾ of RBCs.
- Spherical & compact & smaller than *P. vivax.*

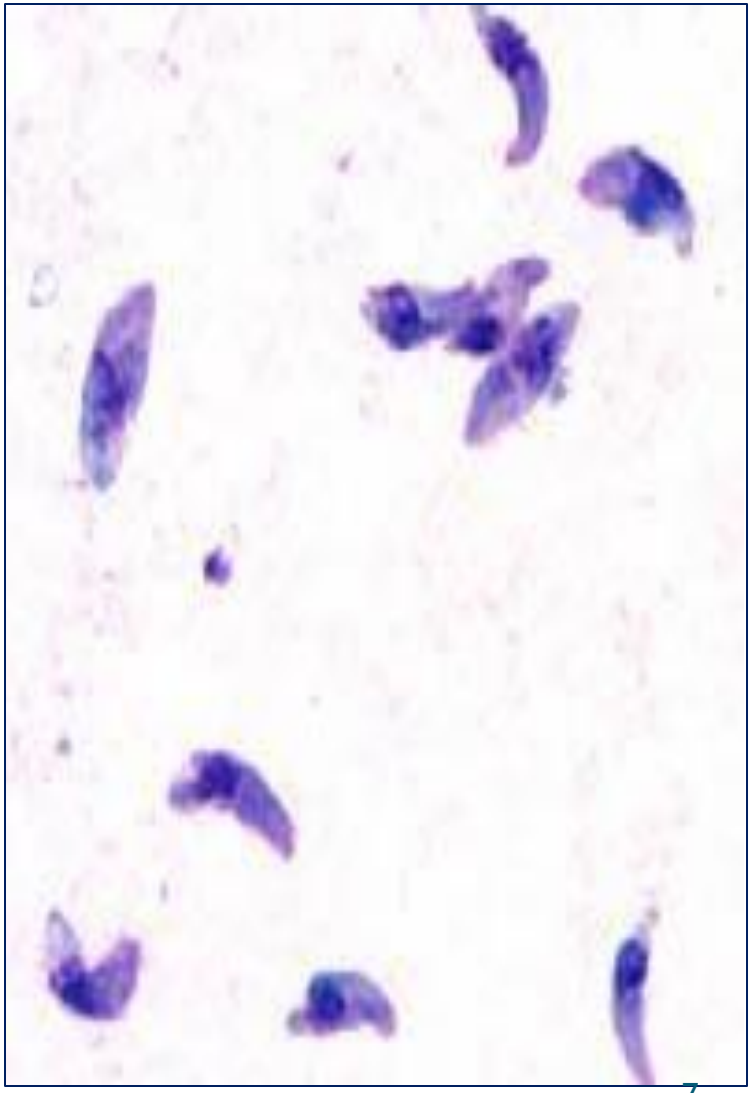


P. vivax

- Fills RBCs.
- Spherical & compact.

Toxoplasma gondii Trophozoite

- **Obligate intracellular parasite.**
- **6 x 2 um.**
- **Crescentic in shape with one pole more pointed than the other.**
- **Vesicular nucleus nearer to one end.**
- **Multiply by longitudinal binary fission.**



Toxoplasma gondii oocyst

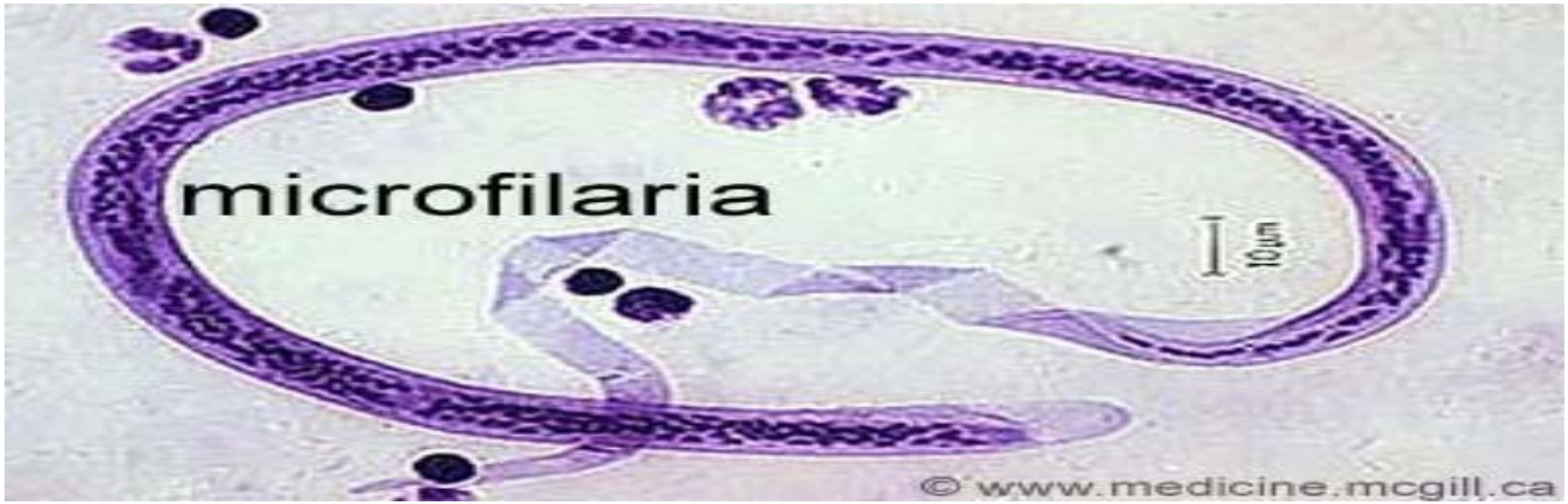
- 10 x 12 µm.

- Oval in shape.

-Contents: 2 sporocysts each with 4 sporozoites (disporocystic tetrazoic).

-Excreted in faeces of infected cat & remains infective in soil for long time.

Microfilaria of *Wuchereria bancrofti*



250 µmx8 µm, body with smooth curves, loose sheath with deeply stained nuclei with empty ant. and post. ends & have nocturnal periodicity (10 p.m. to 2 a.m.).

protozoa

- The tissue and blood protozoa include the apicomplexa (Plasmodium spp., Babesia spp., and Toxoplasma gondii),
- The flagellates (Leishmania spp., Trypanosoma spp., and Trichomonas vaginalis),
- The free-living amebae (Naegleria fowleri, Acanthamoeba spp., and Balamuthia mandrillaris.

Amastigote (D.S) of visceral Leishmaniasis

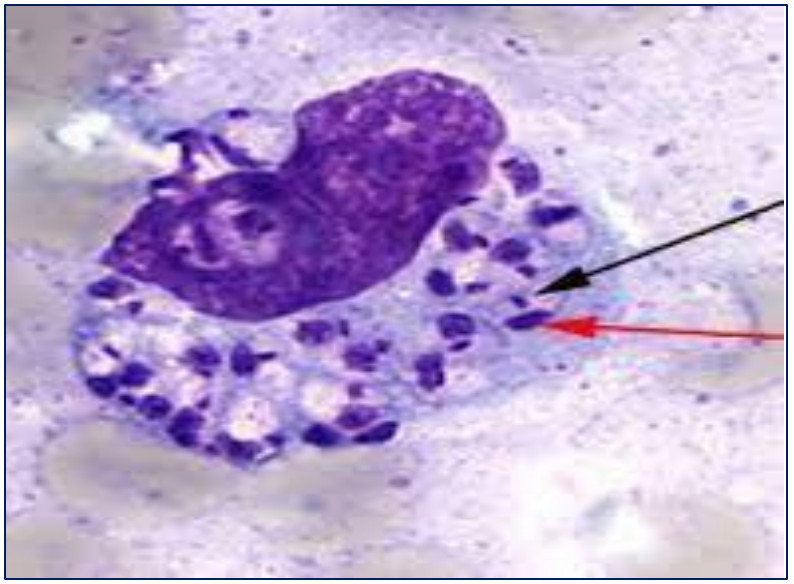
Oval in shape

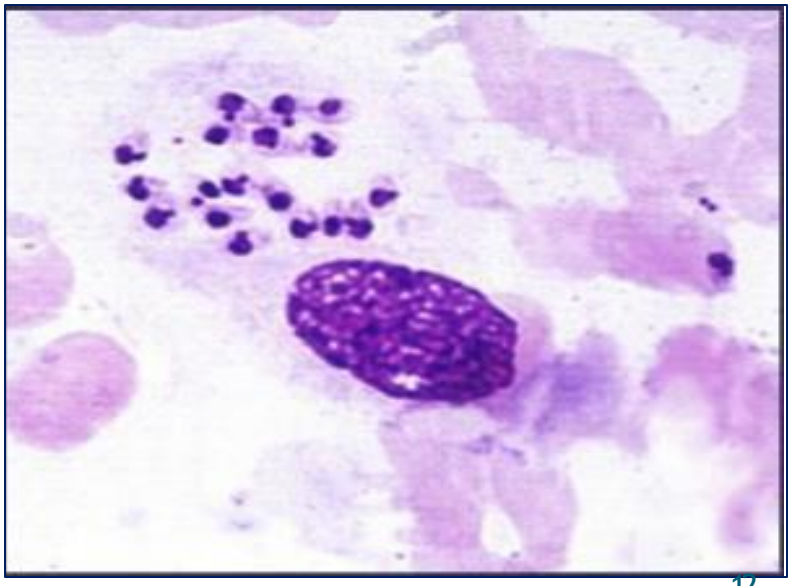
Nucleus: -Eccentric with central Karyosome .

Flagellum: Absent

Kinetoplast: Beside the nucleus.

Habitat: -Intracellular (macrophage) &Tissue culture





Promastigotes in culture

Shape: Fusiform or spindle.

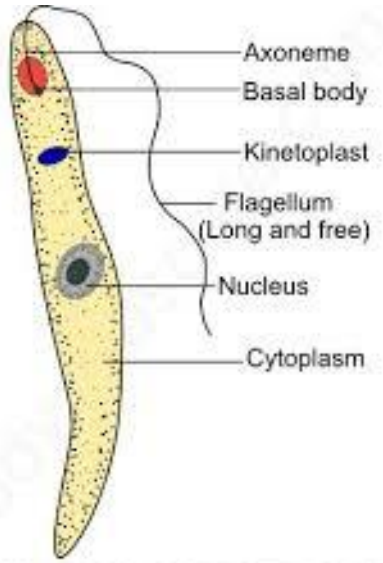
Kinetoplast: At the anterior end

Flagellum: Present.

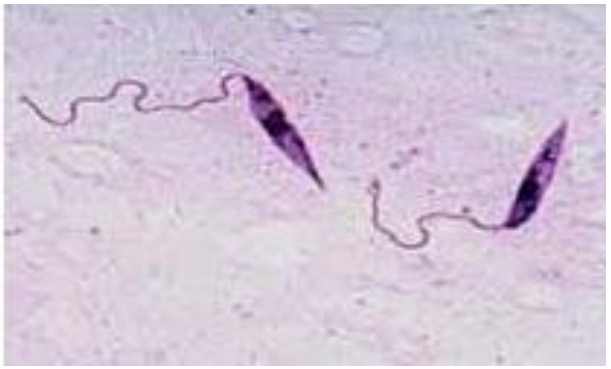
Nucleus: Central with

central karyosome

Habitat: Midgut of the insect



LEISHMANIA: PROMASTIGOTE FORM



Polymorphic trypanosomes

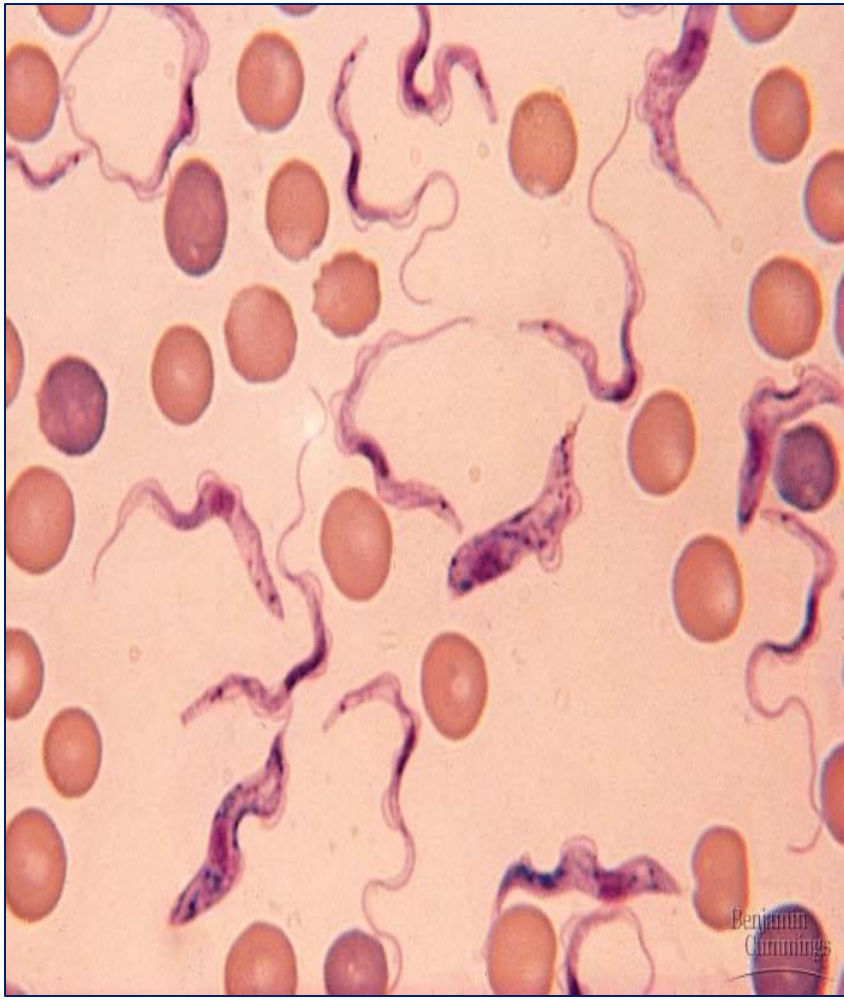
T. gambiense & T. rhodesiense

In the blood film,trypomastigote (*Trypanosoma*) has different shapes:

1 Long slender form (30 µm), active and with long free flagellum.

2 Short stumpy form (15µm), sluggish in motility and without free flagellum.

3 Intermediate form (20µm), with a short free flagellum.



Epimastigotes in culture medium

- Shape: Fusiform or
- spindle
- Kinetoplast: Anterior to the nucleus.
- Flagellum: Present
- Nucleus: - Slightly moved
- posterior.

Undulent membrane -Short

- Habitat: - In the salivary glands of vector & NNN culture medium.