Medical parasitology

Blood and tissue protozoa (*Plasmodium spp*.)

Malaria is a mosquito-borne infectious disease that affects humans and other animals. Malaria is caused by single-celled microorganisms of the *Plasmodium* group. The disease is most commonly spread by an infected female *Anopheles* mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood.

Malaria causes symptoms that typically include fever, tiredness, vomiting, and headaches. In severe cases, it can cause yellow skin, seizures, coma, or death. Symptoms usually begin ten to fifteen days after being bitten by an infected mosquito. If not properly treated, people may have recurrences of the disease months later. In those who have recently survived an infection, reinfection usually causes milder symptoms. This partial resistance disappears over months to years if the person has no continuing exposure to malaria.

Malaria parasites belong to the genus *Plasmodium* (phylum Apicomplexa). In Human, malaria is caused by *plasmodium falciparum, plasmodium malariae, plasmodium ovale, plasmodium vivax* and *plasmodium knowlesi*. Among those infected, *P. falciparum* is the most common species identified (~75%) followed by *P. vivax* (~20%). Although *P. falciparum* traditionally accounts for the majority of deaths, recent evidence suggests that *P. vivax* malaria is associated with potentially life-threatening conditions about as often as with a diagnosis of *P. falciparum* infection.

Plasmodium falciparum

Plasmodium falciparum is a unicellular protozoan parasite of humans, and the deadliest species of *Plasmodium* that causes malaria in humans. The parasite is transmitted through the bite of a female ***Anopheles*** **mosquito** and causes the disease's most dangerous form, falciparum malaria. It is responsible for around 50% of all malaria cases. *P. falciparum* is therefore regarded as the deadliest parasite in humans. It is also associated with the development of blood cancer (Burkitt's lymphoma) and is classified as Group 2A carcinogen.

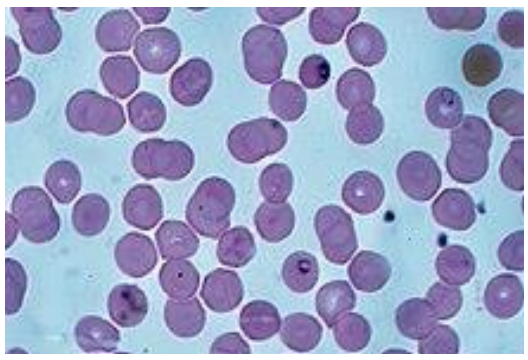
Morphology

P. falciparum does not have a fixed structure but undergoes continuous change during the course of its life cycle. A sporozoite is spindle-shaped and 10–15 μm long. In the liver it grows into an ovoid schizont of 30–70 μm in diameter. Each schizont produces merozoites, each of which is roughly 1.5 μm in length and 1 μm in diameter. In the erythrocyte the merozoite form a ring-like structure, becoming a trophozoite. A trophozoites feed on the haemoglobin and forms a granular pigment called haemozoin. Unlike those of other *Plasmodium* species, the gametocytes of *P. falciparum* are elongated and crescent-shaped, by which they are sometimes identified. A mature gametocyte is 8–12 μm long and 3–6 μm wide. The ookinete is also elongated measuring about 18–24 μm. An oocyst is rounded and can grow up to 80 μm in diameter.

Microscopic examination of a blood film reveals only early (ring-form) trophozoites and gametocytes that are in the peripheral blood. Mature trophozoites or schizonts in peripheral blood smears, as these are usually sequestered in the tissues. On occasion, faint, comma-shaped, red dots are seen on the erythrocyte surface. These dots are Maurer's cleft and are secretory organelles that produce proteins and enzymes essential for nutrient uptake and immune evasion processes.

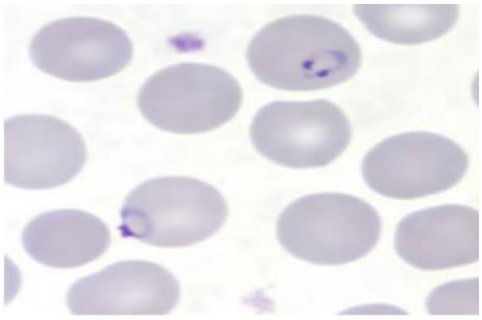
Blood smear from a *P. falciparum* culture (K1 strain - asexual forms) - several red blood cells have ring stages inside them. Close to the center is a schizont and on the left a trophozoite

Ring forms in red blood cells (Giemsa stain)

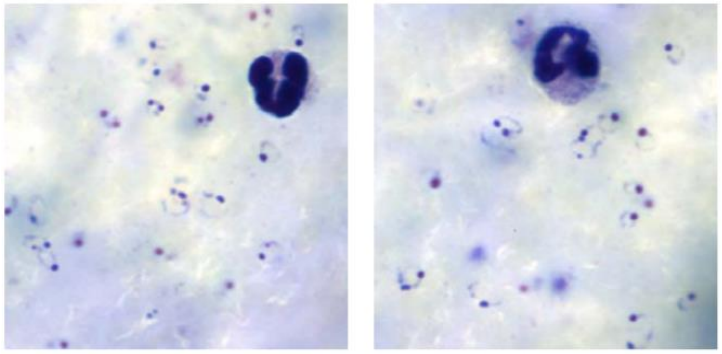


The infective stage called sporozoites released from the salivary glands through the proboscis of the mosquito enter the bloodstream during feeding.

1-Rings *P. falciparum* rings have delicate cytoplasm and one or two small chromatin dots. RBCs that are infected are not enlarged; multiple infection of RBCs is more common in *P. falciparum* than in other species. Occasional appliqué forms (rings appearing on the periphery of the RBC) can be present

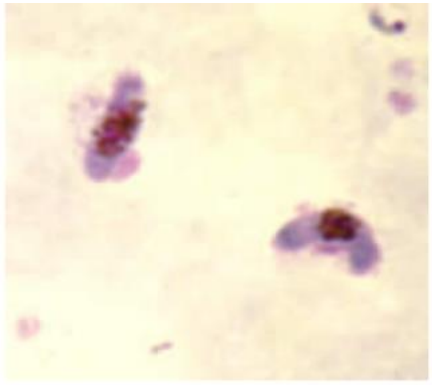


Thin, delicate rings in a thin blood smear. Note the double chromatin dot in the infected rbc at top, and the appliqué form in the infected rbc at bottom.

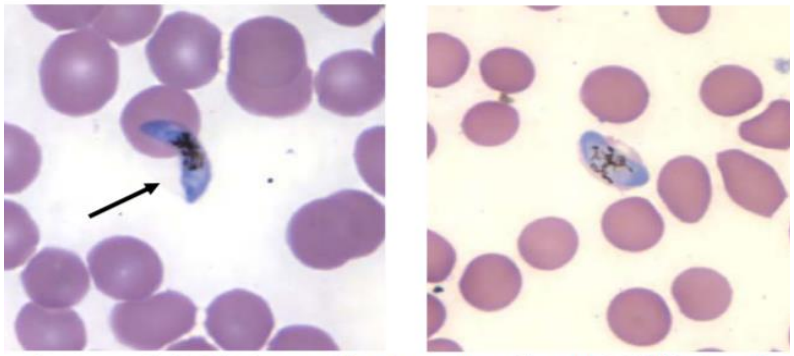


Images from a thick blood smear showing more rings. Note the classic "headphones" appearance of many of the rings.

2. Gametocytes *P. falciparum* gametocytes are crescent or sausage shaped. The chromatin is in a single mass (macrogamete) or diffuse (microgamete).

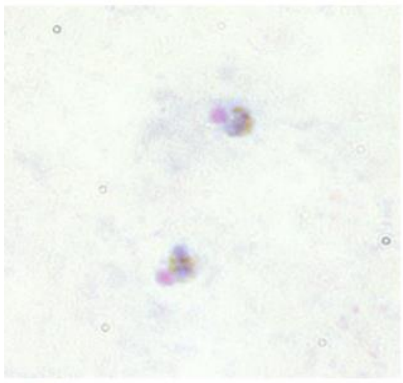


Gametocytes in a thick blood smear.

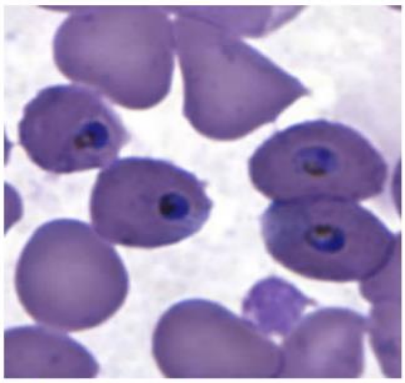


Gametocytes in thin blood smears. Note the presence of "Laveran's bib" (black arrow), which is not always visible.

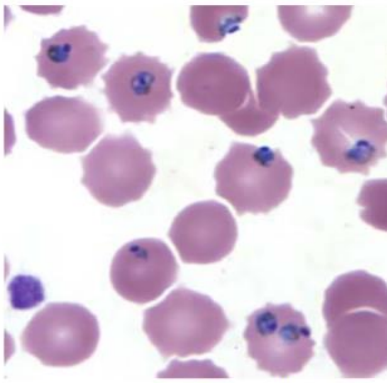
3. Trophozoites *P. falciparum* trophozoites are rarely seen in peripheral blood smears. Older, ring stage parasites are referred to as trophozoites. The cytoplasm of mature trophozoites tends to be more dense than in younger rings. As *P. falciparum* trophozoites grow and mature, they tend to retain their ring-like shape and sometimes trace amounts of yellow pigment can be seen within the cytoplasm. Growing trophozoites in *P. falciparum* can appear slightly amoeboid in shape.



Trophozoites in a thick blood smear.

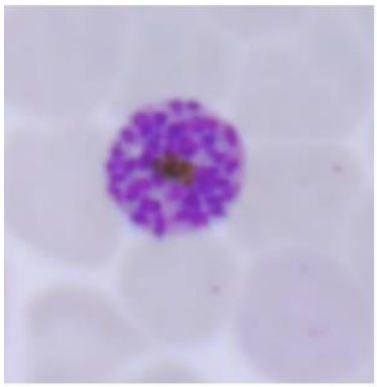


Mature, compact trophozoites in a thin blood smear.

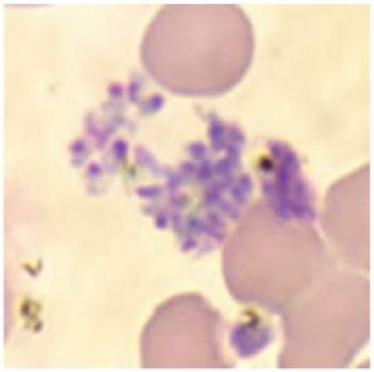


Compact trophozoites in a thin blood smear.

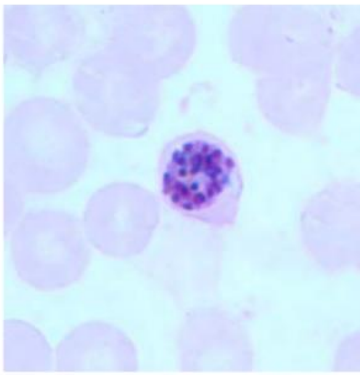
4. Schizonts *P. falciparum* schizonts are seldom seen in peripheral blood. Mature schizonts have 8 to 24 small merozoites; dark pigment, clumped in one mass.



Mature schizont in a thin blood smear.



Ruptured schizont in a thin blood smear.



Another schizont in a thin blood smear.

Plasmodium malariae

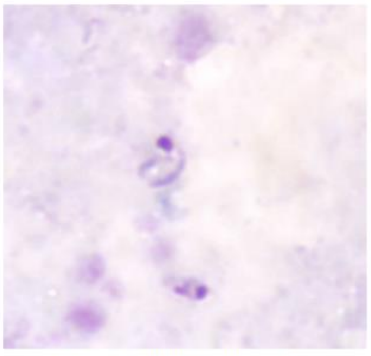
Plasmodium malariae is a parasitic protozoan that causes malaria in humans. it causes a so-called "benign malaria", not nearly as dangerous as that produced by *P. falciparum* or *P. vivax*. The vector of transmission of the parasite is the female Anopheles mosquito, but many different species have been shown to transmit the parasite at least experimentally. The mosquito serves as the definitive host and the human host is the intermediate. When the Anopheles mosquito takes a blood meal from an infected individual, gametocytes are ingested from the infected person.

Plasmodium malariae causes a chronic infection that in some cases can last a lifetime. Another defining feature of *P. malariae* is that the fever manifestations of the parasite are more moderate relative to those of *P. falciparum* and *P. vivax* and fevers show quartan periodicity.

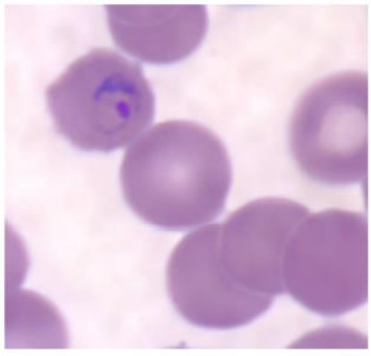
The ring stages that are formed by the invasion of merozoites released by rupturing liver stage schizonts are the first stages that appear in the blood. The ring stages grow slowly but soon fill one-fourth to one-third of the parasitized cell. Pigment increases rapidly and the half-grown parasite may have from 30 to 50 jet-black granules. The parasite changes various shapes as it grows and stretches across the host cell to form the band form.

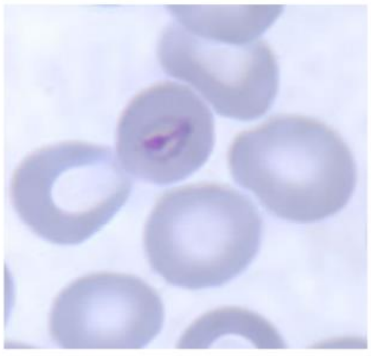
1. **Rings**

P. malariae rings have sturdy cytoplasm and a large chromatin dot.



Ring in a thick blood smear.

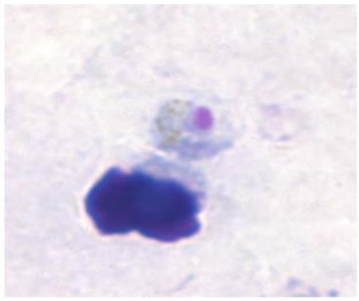




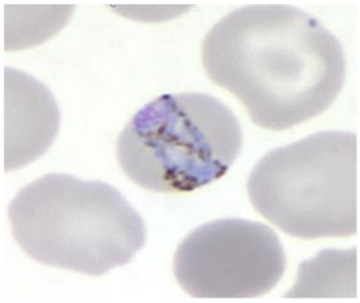
Rings in thin blood smears.

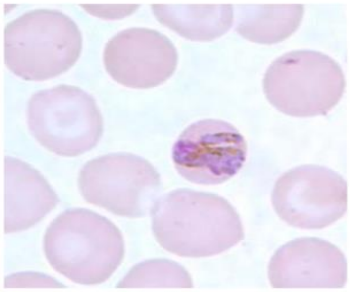
2. Trophozoites

P. malariae trophozoites have compact cytoplasm and a large chromatin dot. Occasional band forms and/or "basket" forms with coarse, dark-brown pigment can be seen.



Trophozoite in a thick blood smear.

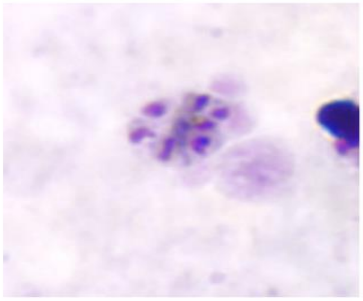




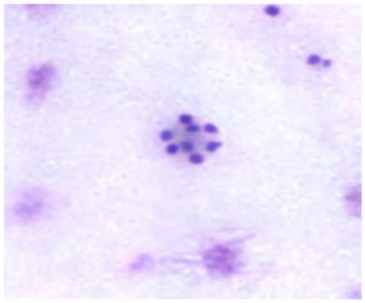
Band-form trophozoites in thin blood smears.

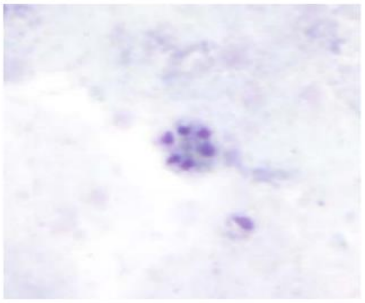
3. Schizonts

P. malariae schizonts have 6 to 12 merozoites with large nuclei, clustered around a mass of coarse, dark-brown pigment. Merozoites can occasionally be arranged as a rosette pattern.



Schizont in a thick blood smear.

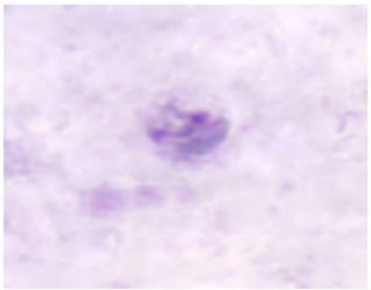


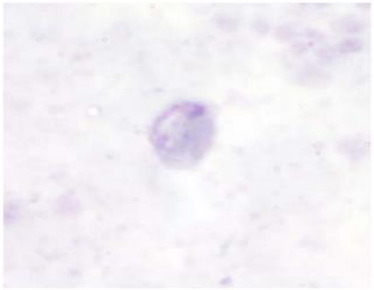


Schizont in thick blood smears. Note the classic "rosette" appearance of the merozoites.

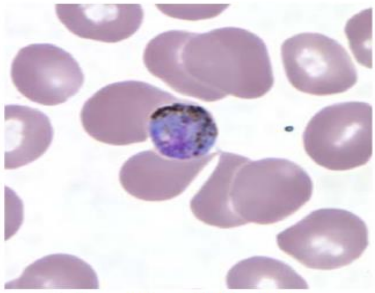
4. Gametocytes

P. malariae gametocytes are round to oval with scattered brown pigment; they may almost fill the infected red blood cell.





Gametocytes in thick blood smears.



Gametocyte in a thin blood smear.

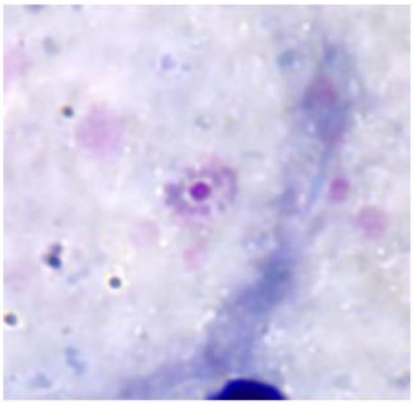
Plasmodium ovale

Plasmodium ovale is a species of parasitic protozoa that causes tertian malaria in humans. In humans, symptoms generally appear 12 to 20 days after the parasite has entered the blood. In the blood, the parasite's replication cycle lasts approximately 49 hours, causing tertian fever which spikes approximately every 49 hours as newly replicated parasites erupt out of red blood cells. In some cases, relapse may occur up to 4 years after infection.

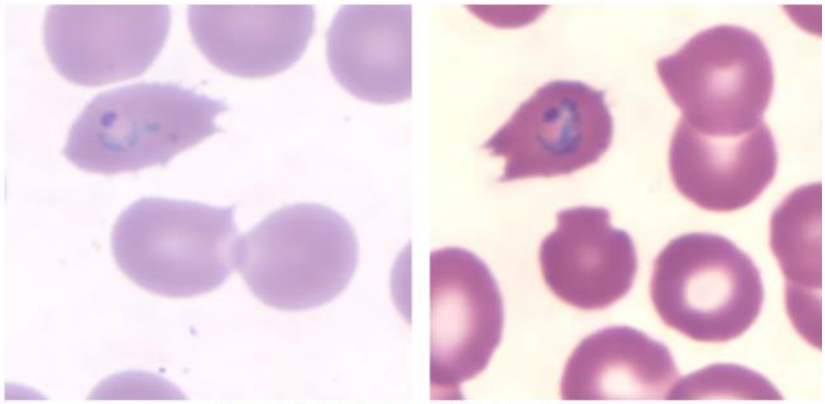
Anopheles gambiae and *Anopheles funestus* are likely the natural mosquito hosts of *P. ovale*. Experimentally, several other mosquito species have been shown to be capable of transmitting *P. ovale* to humans,

In *P. ovale* infections, red blood cells (RBCs) can be normal or slightly enlarged (up to 1 1/4×) in size, may be round to oval, and are sometimes fimbriated. Under optimal conditions, Schüffner's dots may be seen in Giemsa stained slides.

1. Rings *P. ovale* rings have sturdy cytoplasm and large chromatin dots.

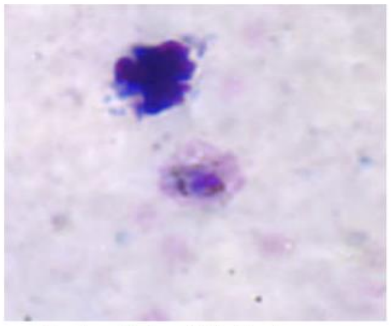


Ring in a thick blood smear.

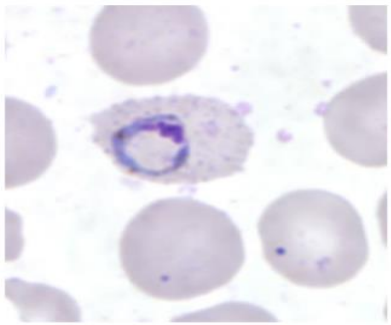


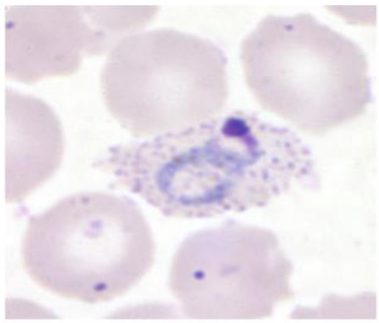
Rings in fimbriated rbcs in thin blood smears.

2. Trophozoites *P. ovale* trophozoites have sturdy cytoplasm, large chromatin dots, and can be compact to slightly irregular.



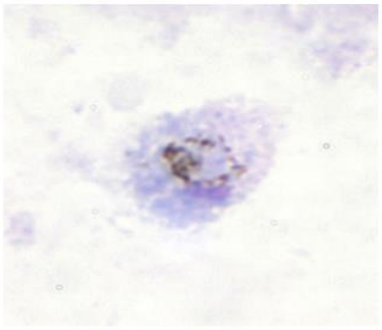
Trophozoite in a thick blood smear.



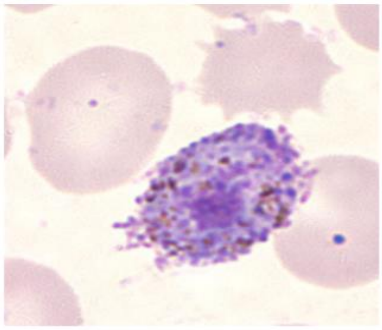


Compact trophozoites in fimbriated rbcs in thin blood smears. Schüffner's dots are also visible.

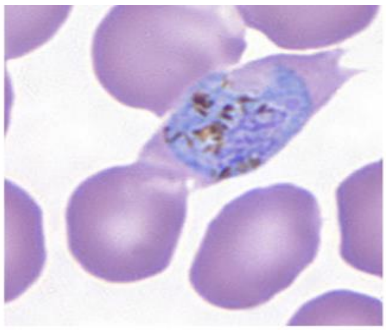
3. Gametocytes *P. ovale* gametocytes are round to oval and may almost fill the red blood cells. Pigment is brown and more coarse in comparison to *P. vivax*.



Gametocyte in a thick blood smear.

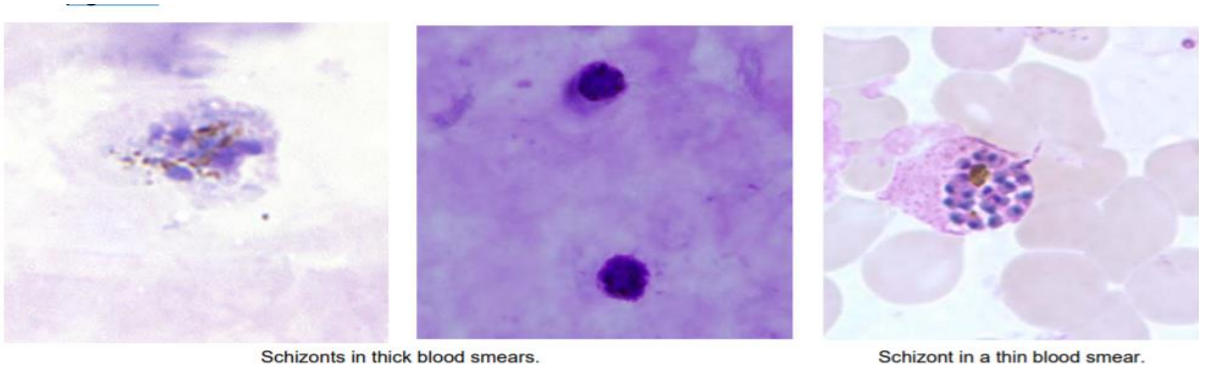


Gametocyte in a thin blood smear.



Gametocyte in a thin blood smear. The infected rbc shows some fimbriation

4. Schizonts *P. ovale* schizonts have 6 to 14 merozoites with large nuclei, clustered around a mass of dark-brown pigment.



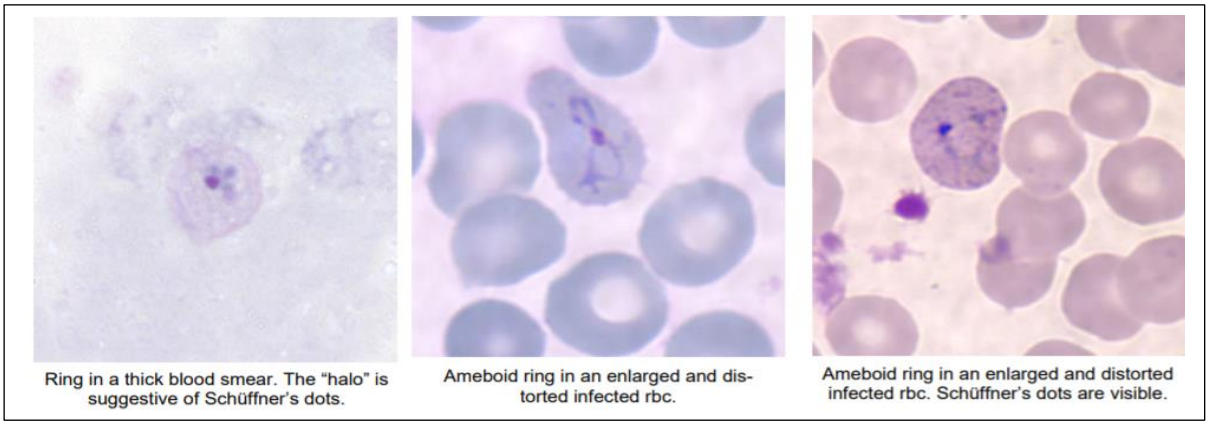
Schizonts in thick blood smears.

Schizont in a thin blood smear.

Plasmodium vivax

Plasmodium vivax is a protozoal parasite and a human pathogen. This parasite is the most frequent and widely distributed cause of recurring malaria. *P. vivax* malaria infections can lead to severe disease and death, often due to splenomegaly (a pathologically enlarged spleen).
P. vivax is carried by the female *Anopheles* mosquito; the males do not bite.

1. Rings *P. vivax* rings have large chromatin dots and cytoplasm can become ameboid as they develop.

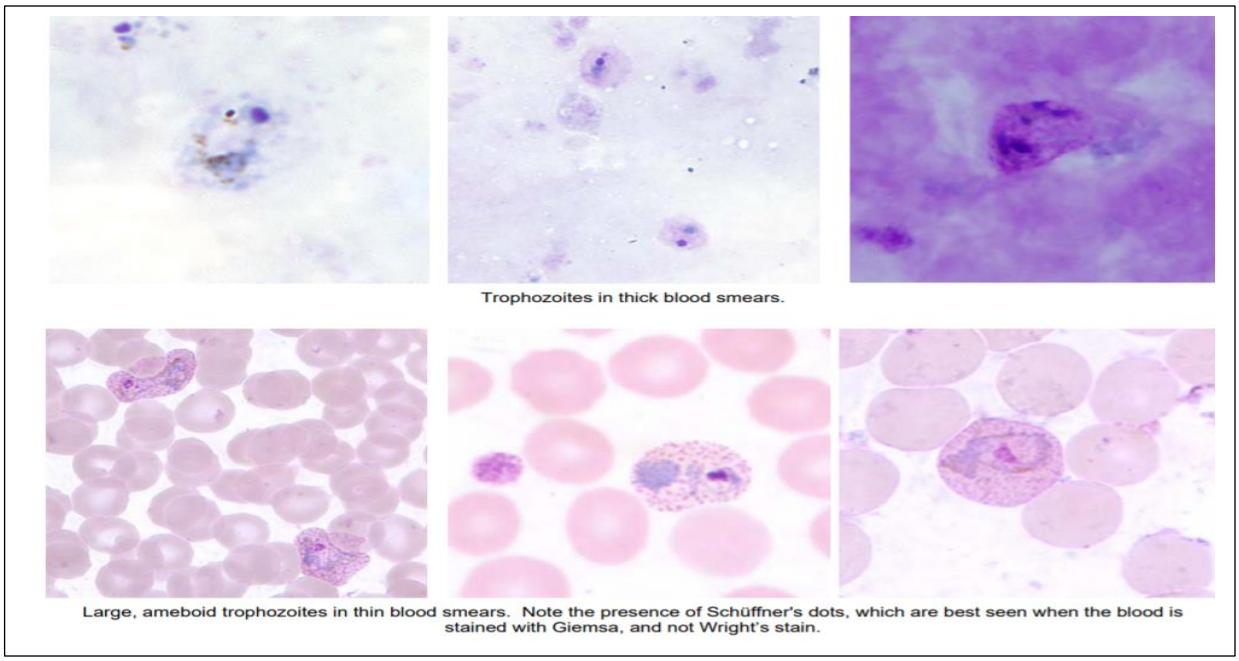


Ring in a thick blood smear. The "halo" is suggestive of Schüffner's dots.

Ameboid ring in an enlarged and distorted infected rbc.

Ameboid ring in an enlarged and distorted infected rbc. Schüffner's dots are visible.

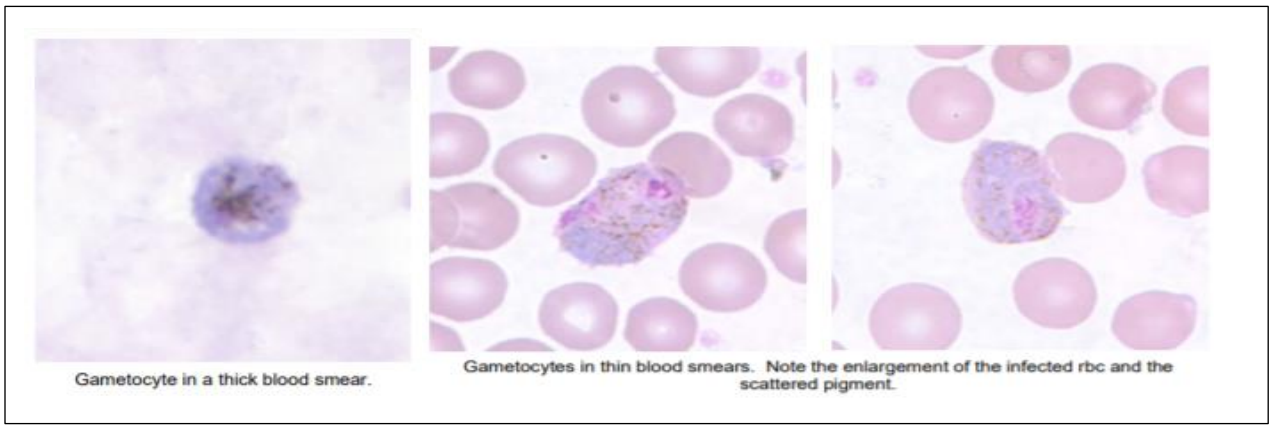
2. Trophozoites *P. vivax* trophozoites show amoeboid cytoplasm, large chromatin dots, and have fine, yellowish-brown pigment. Schüffner's dots may appear more fine in comparison to those seen in *P. ovale.*



Trophozoites in thick blood smears.

Large, ameboid trophozoites in thin blood smears. Note the presence of Schüffner's dots, which are best seen when the blood is stained with Giemsa, and not Wright's stain.

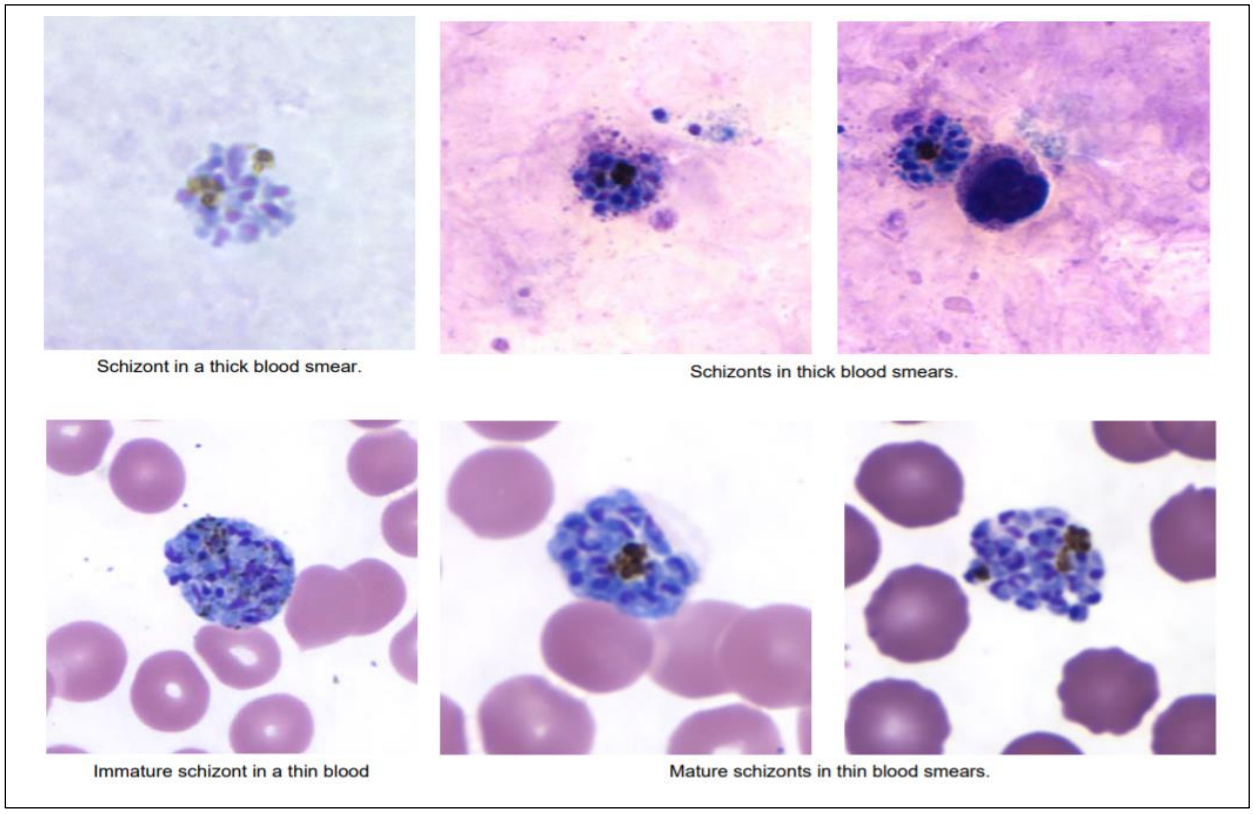
3. Gametocytes *P. vivax* gametocytes are round to oval with scattered brown pigment and may almost fill the RBC. Schüffner's dots may appear more fine in comparison to those seen in *P. ovale*



Gametocyte in a thick blood smear.

Gametocytes in thin blood smears. Note the enlargement of the infected rbc and the scattered pigment.

4. Schizonts *P. vivax* schizonts are large, have 12 to 24 merozoites, yellowish-brown, coalesced pigment, and may fill the RBC.



Diagnosis of malaria

The mainstay of malaria diagnosis has been the microscopic examination of blood, utilizing blood films. Although blood is the sample most frequently used to make a diagnosis, both saliva and urine have been investigated as alternative, less invasive specimens. More recently, modern techniques utilizing antigen tests or polymerase chain reaction have been discovered, though these are not widely implemented in malaria endemic regions. Areas that cannot afford laboratory diagnostic tests often use only a history of subjective fever as the indication to treat for malaria.

1- Blood film

The most economic, preferred, and reliable diagnosis of malaria is microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics. Two sorts of blood film are traditionally used. Thin films are similar to usual blood films and

allow species identification because the parasite's appearance is best preserved in this preparation. Thick films allow the microscopist to screen a larger volume of blood and are about eleven times more sensitive than the thin film, so picking up low levels of infection is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult. With the pros and cons of both thick and thin smears taken into consideration, it is imperative to utilize both smears while attempting to make a definitive diagnosis.

Diagnosis of species can be difficult because the early trophozoites ("ring form") of all four species look similar and it is never possible to diagnose species on the basis of a single ring form; species identification is always based on several trophozoites.

2- Antigen tests

For areas where microscopy is not available, or where laboratory staff are not experienced at malaria diagnosis, there are commercial antigen detection tests that require only a drop of blood. Immunochromatographic tests (also called: Malaria Rapid Diagnostic Tests, Antigen-Capture Assay or "Dipsticks") have been developed, distributed and field tested. These tests use finger-stick or venous blood, the completed test takes a total of 15–20 minutes, and the results are read visually as the presence or absence of colored stripes on the dipstick, so they are suitable for use in the field.

3- Molecular methods

Molecular methods are available in some clinical laboratories and rapid real-time assays (for example, QT-NASBA based on the polymerase chain reaction) are being developed with the hope of being able to deploy them in endemic areas.

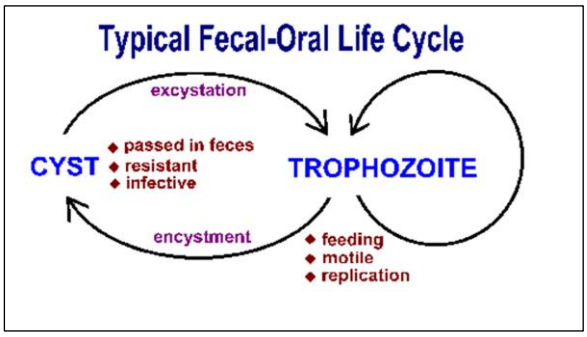
PCR (and other molecular methods) is more accurate than microscopy. However, it is expensive, and requires a specialized laboratory. Moreover, levels of parasitemia are not necessarily correlative with the progression of disease, particularly when the parasite is able to adhere to blood vessel walls. Therefore, more sensitive, low-tech diagnosis tools need to be developed in order to detect low levels of parasitemia in the field.

4- **Another approach** is to detect the iron crystal byproduct of hemoglobin that is found in malaria parasites feasting on red blood cells, but not found in normal blood cells. It can be faster, simpler and precise than any other method.

Intestinal flagellates

Numerous protozoa inhabit the gastro-intestinal tract of humans. The majority of these protozoa are non-pathogenic commensals, or only result in mild disease. Some of these organisms can cause severe disease under certain circumstances. For example, *Giardia lamblia* can cause severe acute diarrhea which may lead to a chronic diarrhea and nutritional disorders. *Trichomonas vaginalis* does not reside within the gastro-intestinal tract, but is often discussed with the intestinal flagellates. It infects the urogenital tract and causes a sexually-transmitted disease.

Intestinal protozoa are transmitted by the fecal-oral route and tend to exhibit similar life cycles consisting of a cyst stage and a trophozoite stage (not all intestinal parasites have cyst stage).



Giardia lamblia

Giardia lamblia is a protozoan parasite that colonizes the upper portions of the small intestine. It is the most common flagellate of the intestinal tract, causing Giardiasis. Human are the only important reservoir of infection. Typically *Giardia* is non-invasive and often results in asymptomatic infections. Symptomatic giardiasis is characterized by acute or chronic diarrhea and/or other gastro-intestinal manifestations.

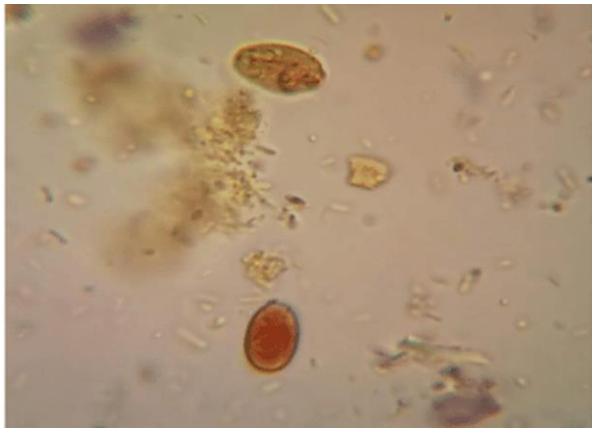
Giardia lamblia has two morphological stages: the trophozoite and cyst.

1- **trophozoite** of *G lamblia* is a heart-shaped, symmetric organism 10-20 m in length. There are four pairs of flagella, two nuclei with prominent central karyosomes, and two

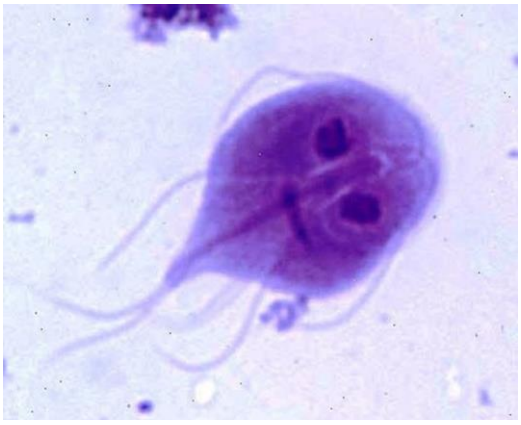
axostyles (rod-like supporting organelles). A large concave **sucking disk** in the anterior portion occupies much of the ventral surface. The swaying or dancing motion of giardia trophozoites in fresh preparations is unmistakable. As the parasites pass into the colon, they typically encyst.

2- **Cysts** are found in the stool-often in enormous numbers. They are thick-walled, highly resistant, 8-14 m in length, and ellipsoid and contain two nuclei as immature, four as mature cysts.

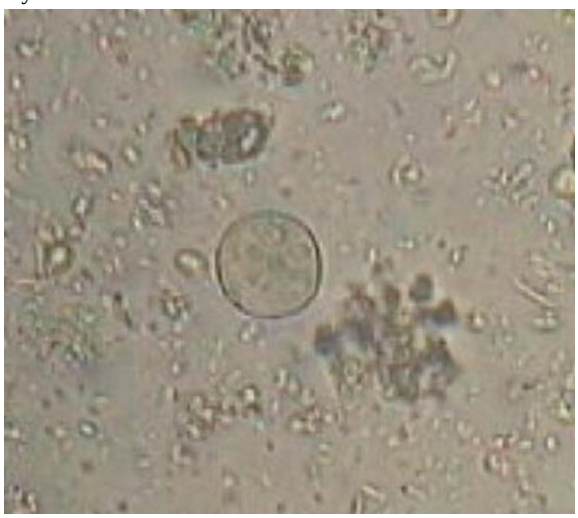
Trophozoite of the parasites *Giardia lamblia*



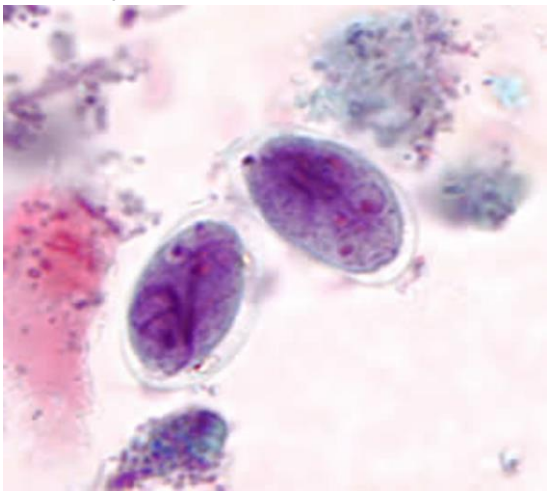
Giardia lamblia trophozoite, as visualized by Trichrome staining



Cyst of *Giardia lamblia*



Intestinal cysts stained with trichrome.



DIAGNOSIS

Parasite Detection
Stool Examination • 3 non-consecutive days • wet mount or stained • IFA, copro-antigens **Duodenal Aspirate or Biopsy** • Enterotest

Diagnosis is confirmed by finding cysts or trophozoites in feces or in duodenojejunal aspirates or biopsies. Detection of the parasites can be difficult since *Giardia* does not appear consistently in the stools of all patients. Some patients will express high levels of cysts in nearly all the stools, whereas others will only exhibit low parasite counts in some of the stools. In addition, parasites are easier to find during acute infections than chronic infections.

Stool examination is the preferred method for *Giardia* diagnosis. Watery or loose stools may contain motile trophozoites which are detectable by the immediate examination of wet smears. The hardier cysts are relatively easy to recognize in either direct or stained smears. In addition, diagnostic kits based on immunofluorescence or the detection of copro-antigens are also available.

Diagnosis can also be made by examining duodenal fluid for trophozoites. A small intestinal biopsy, preferably from multiple duodenal and jejunal sites, may also reveal trophozoites attached to the intestinal epithelium.

Chilomastix mesnili

Chilomastix mesnili is a nonpathogenic flagellate that is often described as a commensal organism in the human gastrointestinal tract. The cyst stage is resistant to environmental pressures and is responsible for transmission of *Chilomastix*. Both cysts and trophozoites can be found in the feces (diagnostic stages) . Infection occurs by the ingestion of cysts in contaminated water, food, or by the fecal-oral route (hands or fomites) . In the large (and possibly small) intestine, excystation releases trophozoites. *Chilomastix* resides in the cecum and/or colon; it is generally considered a commensal whose contribution to pathogenesis is uncertain.

Morphology:

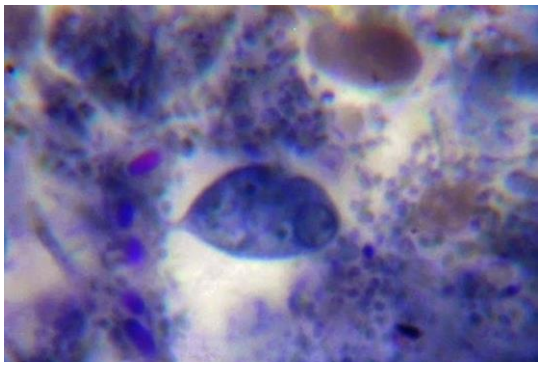
Trophozoite: pear form.

Size: 7-10μm in length.

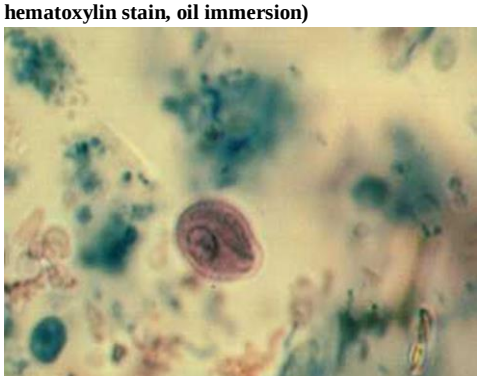
4 flagella, 3 forewords, 4th in the cleft.

Cyst: pear form, 8μm, single nucleus.

Chilomastix mesnili trophozoite (iron hematoxylin stain 1000x)



***Chilomastix mesnili*. Cyst in fecal smear (iron hematoxylin stain, oil immersion)**



Laboratory diagnosis

Stool examination: examination of freshly passed liquid stool from patients infected with *chilomastix mesnili* typically reveal only trophozoites, while examination of formed stool samples from such patients usually reveal only cyst. Sample of semi formed consistency may contain both trophozoites and cysts.

Culture: in laboratory media.

Trichomonas vaginalis, trichomonas hominis, T. tenax, Enteromonas hominis, and Retortamonas intestinalis

*** T. hominis (intestinal).**

***T. vaginalis (genital organs).**

***T. tenax (human mouth)**

Trichomonas hominis

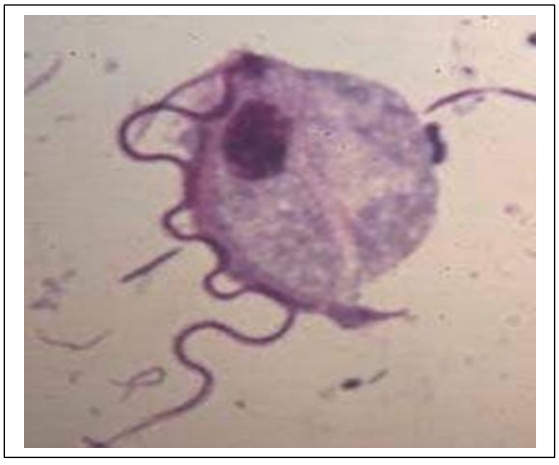
Trichomonas hominis is a protozan flagellate and therefore motile organism. It is thought to be non-pathogenic although it has been associated with diarrheic stools. It is the most commonly found flagellate next to *Giardia lamblia* and *Dientamoeba fragilis*.

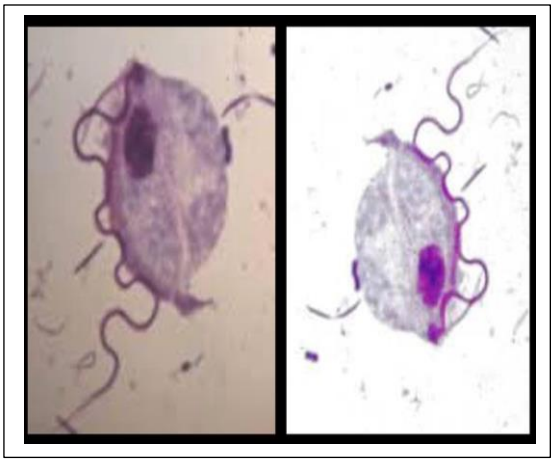
Morphology of *Trichomonas hominis*

It has no cystic stage. The trophozoite measures from 5-15µm in length by 7-10µm in width. The shape is pyriform and has an axostyle which runs from the nucleus down the center of the body and extends from the end of the body and undulating membrane which extends the entire length of the body and projects from the body like a free flagellum. It has 4 free flagella and a single nucleus at the anterior end. Trophozits characterized by Jerky movement.

Laboratory Diagnosis

In a fresh stool, the flagellates move very rapidly in a jerky, non-directional manner. The axostyle and undulating membrane are diagnostic. The flagellates are difficult to stain, however, the axostyle can be seen on a stained preparation and is diagnostic.





Trichomonas vaginalis

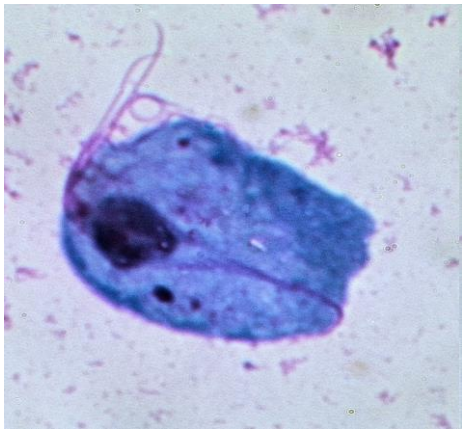
Trichomonas vaginalis is an anaerobic, flagellated protozoan parasite and the causative agent of trichomoniasis and is a sexually transmitted infection. Transmission usually occurs via direct, skin-to-skin contact with an infected individual, most often through vaginal intercourse.

Morphology

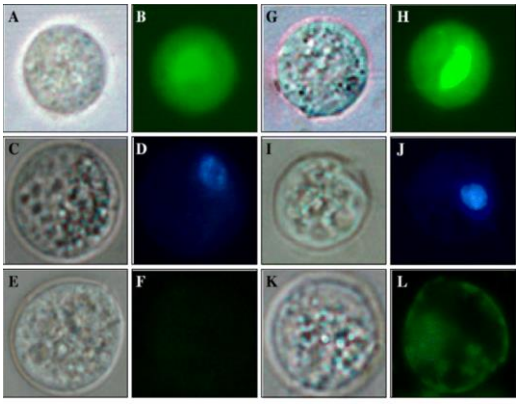
Trichomonas vaginalis exists in only one morphological stage, a trophozoite, and cannot encyst. The *T. vaginalis* trophozoite is oval as well as flagellated, or "pear" shaped as seen on a wet-mount, measuring 9×7 μm. Five flagella arise near the cytostome; four of these immediately extend outside the cell together, while the fifth flagellum wraps backwards along the surface of the organism. In addition, a conspicuous barb-like axostyle projects opposite the four-flagella bundle. The axostyle may be used for attachment to surfaces and may also cause the tissue damage seen in trichomoniasis infections. The nucleus is usually elongated, and the cytoplasm contains many hydrogenosomes.

While *T. vaginalis* does not have a cyst form, organisms can survive for up to 24 hours in urine, semen, or even water samples. A nonmotile, round, **pseudocystic form** with internalized flagella has been observed under unfavorable conditions. This form is generally regarded as a degenerate stage as opposed to a resistant form, although viability of pseudocystic cells has been occasionally reported. The ability to revert to trophozoite form, to reproduce and sustain infection has been described, along with a microscopic cell staining technique to visually discern this elusive form.

Trophozoite *Trichomonas vaginalis*



Pseudocyst forms of *Trichomonas vaginalis*



Laboratory diagnosis

1- Vaginal or urethral secretions or discharge should be examined microscopically in a drop of saline for characteristic motile trichomonads. Dried smears may be stained with hematoxylin or other stains for later study.

2- Culture of vaginal or urethral discharge, of prostatic secretion, or of a semen specimen may reveal organisms when direct examination is negative.

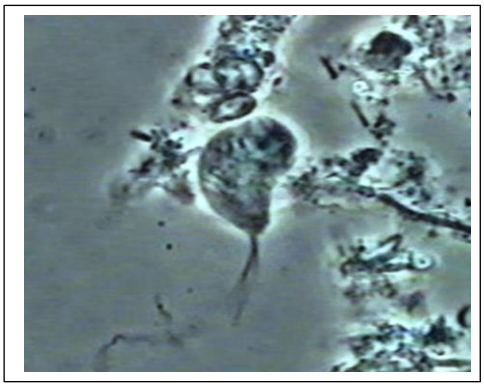
3- Nucleic acid detection Recombinant DNA technology has been adapted over the past decade as a diagnostic tool.

Trichomonas tenax

Trichomonas tenax, or oral trichomonas, is a species of *Trichomonas* commonly found in the oral cavity of humans. Routine hygiene is generally not sufficient to eliminate the parasite. *T. tenax* is generally not found on the gums of healthy patients. It is known to play a pathogenic role in necrotizing ulcerative gingivitis and necrotizing ulcerative periodontitis, worsening preexisting periodontal disease. This parasite is also implicated in some chronic lung diseases.

morphology

Of the three species in the genus Trichomonas, T. tenax is the smallest, measuring only 5-14 µm long and 6-9 µm wide; specimens can be identified by their long axostyles and tails, 4 anterior flagella, and by the recurrent flagellum that raises an undulating membrane which is two thirds the length of the body. This undulating membrane may appear like small legs. It may occasionally appear larger, allowing it to be confused with Trichomonas vaginalis due to similar morphology. In such cases, the presence of an oral or vaginal parasite should be confirmed, due to the ease with which the parasite can be transmitted through direct contact of mucous membranes.



Diagnosis

The specimen of choice for diagnosing *Trichomonas tenax* trophozoite is mouth scrapings. Microscopic examination of tonsillar crypts and pyorrhea pockets of patients suffering from *T. tenax* infections often yields the typical trophozoites. Tartar between the teeth and the gingival margin of the gums are the primary areas of the mouth that may also potentially harbor this organism. *T. tenax* may also be cultured onto appropriate media

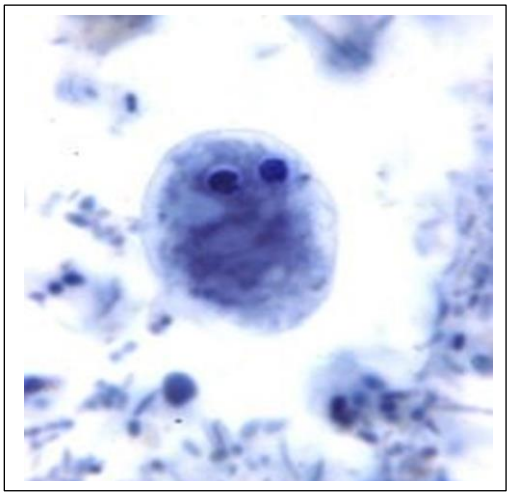
Other Intestinal Flagellates

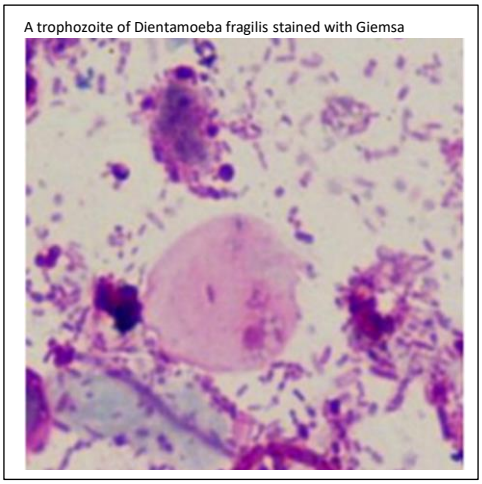
Dientamoeba Fragilis

Dientamoeba fragilis causes diarrhea and other gastrointestinal symptoms. Its reservoir seems to be restricted to humans and other primates. Like the other members of Class Trichomonadida, it is found only in the trophozoite form. The organism lives in the large intestine.

Morphology

Dientamoeba fragilis is relatively small, varying from 3 - 22 microns in diameter and there can be considerable variation in size among organisms in the same specimen. The organism has only a trophozoite stage and in a permanently stained preparation, one, two or rarely three nuclei can be seen, two being the most common. The nuclear chromatin is usually fragmented into three to five granules but these have not been visualised by Giemsa Stain, and there is normally no peripheral chromatin on the nuclear membrane. The cytoplasm is usually vacuolated and may contain ingested debris as well as some large uniform granules. The cytoplasm can also appear uniform and clean with a few inclusions. *Dientamoeba fragilis* lives in the lumen of the caecum and upper colon.





Laboratory Diagnosis

Diagnosis is dependent on examination of the fresh direct wet preparation or permanently stained smears prepared from unformed or formed stools with mucus. It is particularly important that permanent stained smears of stool preparations should be examined, because survival times of the organism in terms of morphology, is very limited and specimens must be examined immediately or preserved in a suitable fixative as soon as possible after defaecation. The recommended stains are Fields' and Giemsa. The trophozoite is destroyed in a formol-ether concentrate.

Balantidium coli

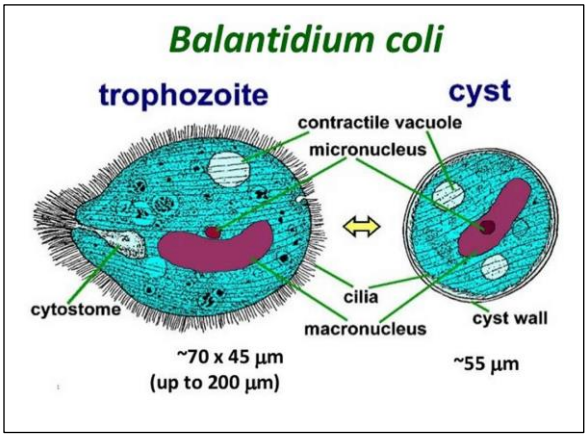
Balantidium coli is a parasitic species of ciliate alveolates that causes the disease balantidiasis. It is the only member of the ciliate phylum known to be pathogenic to humans.

Morphology

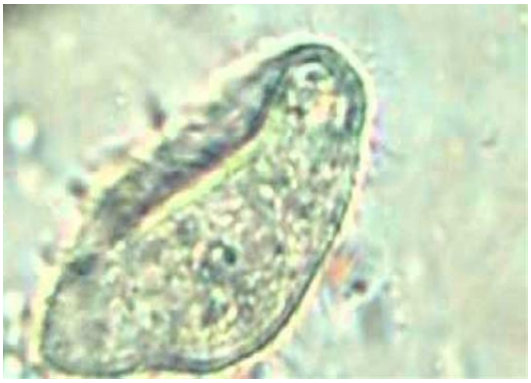
Balantidium coli has two developmental stages, a trophozoite stage and a cyst stage. In trophozoites, the two nuclei are visible. The macronucleus is long and sausage-shaped, and the spherical micronucleus is nested next to it, often hidden by the macronucleus. The opening, known as the peristome, at the pointed anterior end leads to the cytostome, or the mouth.

Cysts are smaller than trophozoites and are round and have a tough, heavy cyst wall made of one or two layers. Usually only the macronucleus and sometimes cilia and contractile vacuoles are visible in the cyst, however, both nuclei are present because nuclear multiplication does not occur when the organism is a cyst. Living trophozoites and cysts are yellowish or greenish in color

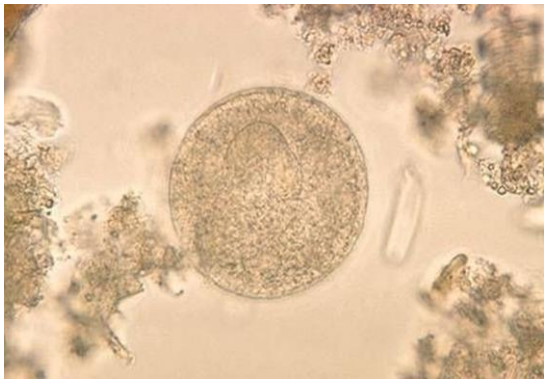
Balantidium coli trophozoite



Balantidium coli trophozoite



Balantidium coli cyst



Laboratory Diagnosis:

Stool microscopy

Trophozoites- detected in acute disease (dysenteric stool)-easy to identify by its rotatory motility, large kidney shaped macronucleus and presence of cilia.

Cysts- seen in chronic cases or carriers-round, 40-60μm in size, surrounded by a cyst wall and presence of two nuclei**.**

Histopathology:

Scrapings of colonic and ceacal mucosa can be stained with H&E.

Histopathological staining of biopsy tissue or scrapping of the ulcers taken by sigmoidoscopy-reveals clusters of trophozoites, cysts and lymphocytic infiltration found in sub mucosa.

Culture:

Media used: Boeck and Drbohlav egg serum media and Balamuth's media.

Culture rarely necessary as parasites are easily detected by stool microscopy or histopathology

Medical Protozoa

(intestinal protozoa)

Human parasitology is the study of those organisms which parasitize humans .Human parasitology, an important part of parasitology, study the medical parasites including their morphology , life cycle, the relationship with host and environment. So it is considered that parasitic infection /or parasitic diseases are still one of the important problems in public health.

Parasites of medical importance come under the kingdom called protista and animalia . The microscopic single-celled eukaryotes (having true nuclear membrane) known as protozoa. In contrast, helminthes are macroscopic, multicellular worms possessing well differentiated tissues and complex organs belonging to the kingdom animalia.

Classification schemes differed, but throughout much of the 20th century the major groups of Protozoa included:

- Flagellates, or Mastigophora (motile cells equipped with whiplike organelles of locomotion, e.g., *Giardia lamblia*)
- Amoebae or Sarcodina (cells that move by extending pseudopodia or lamellipodia, e.g., *Entamoeba histolytica*)
- Sporozoans, or Sporozoa (parasitic, spore-producing cells, whose adult form lacks organs of motility, e.g., *Plasmodium knowlesi*)
- Ciliates, or Ciliophora (cells equipped with large numbers of short hairlike organs of locomotion, e.g. *Balantidium coli*)

Amoeba (Sarcodina)

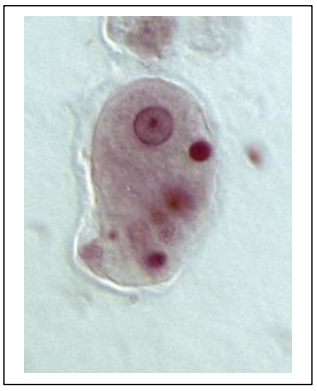
Amoeba any protozoan of the superclass (sometimes class or subphylum) Sarcodina. These organisms have streaming cytoplasm and use temporary cytoplasmic extensions called pseudopodia in locomotion (called amoeboid movement) and feeding.

Sarcodines include the genus *Amoeba* and pathogenic species, e.g., dysentery causing *Entamoeba histolytica.* These protozoans' cells may be spherical or irregular in shape;

the pellicle (or envelope) is usually thin and flexible. Sometimes there is an external shell or skeleton. The cytoplasm, composed of ectoplasm and endoplasm, may contain more than one nucleus. Food, which adheres to the body surface or is trapped by pseudopodia, is digested in food vacuoles.

Entamoeba histolytica

A single-celled protozoan parasite is an anaerobic parasitic amoebozoan, part of the genus *Entamoeba*. Predominantly infecting humans and other primates causing amoebiasis,

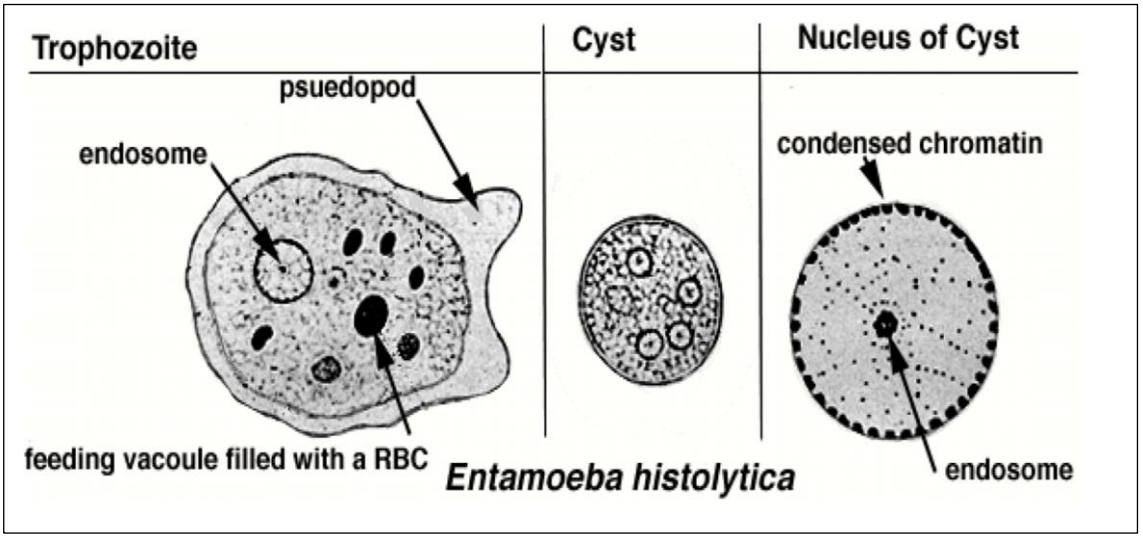


The active (trophozoite) stage exists only in the host and in fresh loose feces; cysts survive outside the host in water, in soils, and on foods, especially under moist conditions on the latter.

Entamoeba histolytica is a common protozoan parasite found in the large intestine of human. The parasite is responsible for amoebiasis and liver absceses. It is the third leading parasite cause of death in the developing countries.

Morphology:

Entamoeba histolytica occurs in three stages; trophozoite, precyst and cyst.



1. Trophozoite:

•It is the growing and feeding stage of parasite

•**Shape;** not fixed because of constantly changing position

•**Size:** ranging from 18-40 μm; average being 20-30 μm

•**Cytoplasm:** cytoplasm is divided into two portion; a clear

transparent ectoplasm and a granular endoplasm. Ingested RBCs, tissue granules and food materials are also found in endoplasm

•**Nucleus:** It is single, spherical shape and size ranging from 4-6μ Nucleus contains central karyosome and fine peripheral chromatin.

•Trophozoites are actively motile with the help of pseudopodia.

•Trophozoites are anaerobic parasite, (present in large intestine)

2. Pre cyst:

It is the intermediate stage between trophozoite and cyst
It is smaller in size; 10-20μ
It is round or slightly ovoid with blunt pseudopodium projecting from periphery
No RBC or food materials are found on its endoplasm.

3. Cyst:

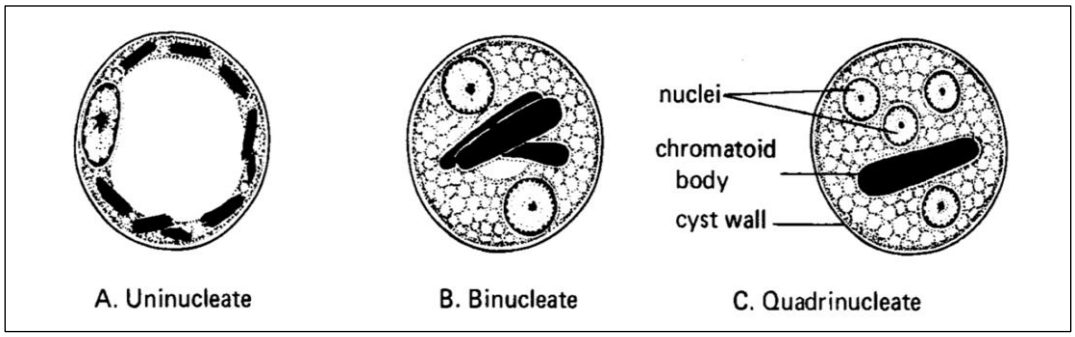
•It is the infective form of parasite.

•**Shape:** It is round or round or oval in shape

•**Size:** 12-15 μm in diameter

•It is surrounded by a highly refractile membrane called cyst wall. The cyst wall is resistant to digestion by gastric juice in human stomach

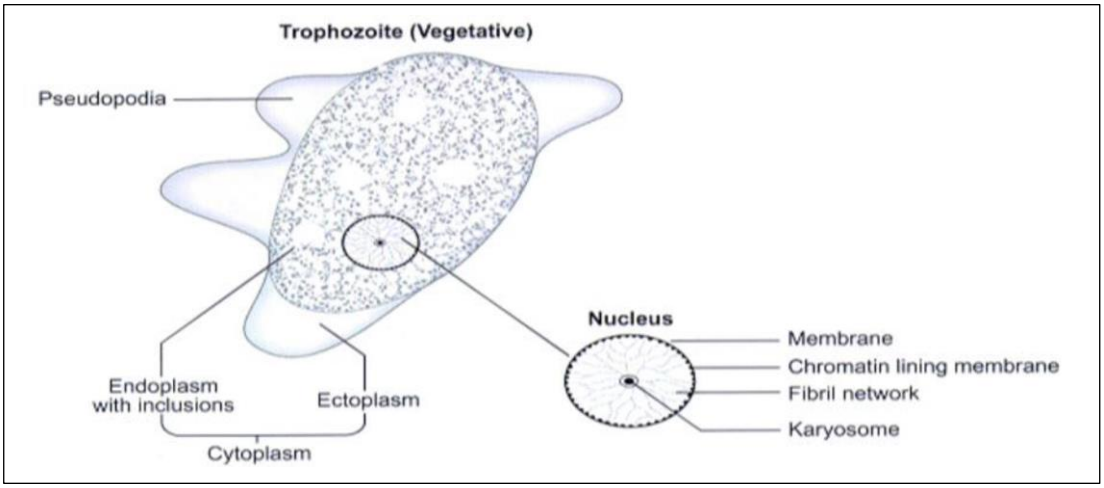
The cystic stage of E. histolytica is either mature or immature , the maturity of cyst depend on the number of nuclei found in the cyst.The immature cyst includes uni and bi –nucleated cyst while the mature cyst is quadrinucleated cyst

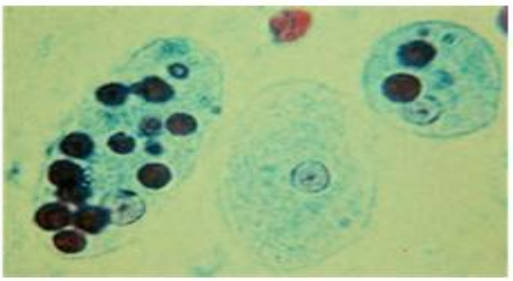


•**Nucleus:** A mature cyst is quadrinucleated.

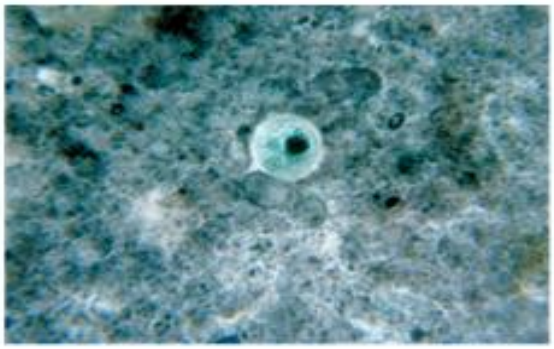
•**Cytoplasm:** Cytoplasm shows chromatid bars and glycogen masses but no RBCs or food particles.

•Mature cyst passed out in stool from infected patient and remained without further development in soil for few days.





Trophozoites of *E. histolytica* with ingested erythrocytes



E. histolytica cyst

Lab Diagnosis:

•**Specimen:** stool, pus or liver abscesses, sputum and biopsy samples

•**i. Stool macroscopy:** in amoebic dysentery stool is offensive, semi-solid, dark brown color and acidic in nature, mixed with blood, mucus and faecal materials.

•**ii. Microscopy**: Normal saline preparation of fresh faecal material revels trophozoites with RBCs in its cytoplasm and its amoebic motility.

•**iii. Stool Ag detection:** ELISA to detect 170KD lectin of E. histolytica

•**iv. Stool culture:** Robinson's medium and NH polyxenic culture medium are used to culture E. histolytica

•**v. Serology:** many serological tests are used to detect antibody in serum against E. histolytica such as ELISA

•**vi. PCR:** It is sensitive test , used to differentiate E. histolytica from other Entamoeba species.

•**vii. Radiological finding:** X-rays, MRI, CT scan, ultrasonography etc for extra intestinal amoebiasis..

Entamoeba coli

 Non pathogenic amoeba that very closely resembles Entamoeba histolytica

 does not invade tissues

 common inhabitant of the lumen of the cecum and colon of man and other animals

 Has the typical Entamoeba nucleus

Morphology of Trophozoite:-

 Usually 15-25 μm in diameter (range 10-50 μm) ,perhaps a little larger than the trophozoite of Entamoeba histolytica

 Cytoplasm: More vacuolated or granular endoplasm with bacteria and debris but no RBCs

Pseudopodia: broad short pseudopodia and little locomotion. sluggish, non-directional motility.

function more to ingest food

Nucleus :1 n–Thicker, irregular, coarsely granular peripheral chromatin with a large eccentric karyosomeucleus

Morphology of cyst:-

-size: 10-35 μm

-Nucleus

 Usually spherical

 mature cyst: 8 nuclei

 Immature cyst: 2 or more nuclei

 Karyosome is large, may/may not be compact and/or eccentric

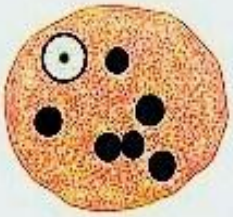
-Cytoplasm: coarsely granular

-chromatoidal bodies: Splinter-shaped or broom-shaped with rough, pointed end.

Laboratory diagnosis:-

Routine microscopic examination of stool is sufficient for diagnosis.

Stained by iron haematoxylin

E. histolytica		Trophozoite	E. coli
	Purplish brown Faintly granular	Cytoplasm	Greyish blue Coarsely granular
	RBC black	Inclusions	Vacuoles black, as are bacteria etc.
	Lined with minute black granules	Nucleus: Membrane	Thick with plaques of black chromatin
	Small black central dot	Karyosome	Eccentric black dot or plaque
	Trace only seen	Fibril network	More conspicuous; may have chromatin plaques
		Precyst	
	Round	Shape	Round
	As trophozoite	Cytoplasm Nucleus	As trophozoite
	Black chromidial bodies or bars	Inclusions	May have slender black chromidial bars
	Glycogen (dissolved) replaced by vacuoles		Glycogen (dissolved) replaced by vacuoles
		Cyst	
	Grey-blue	Cytoplasm	Greyish-blue, granular
	As precyst; less conspicuous or absent	Inclusions	As precyst, less conspicuous or absent In 2 nuclei stage glycogen vacuoles may be dumb-bell-shaped
	Unstained, hyaline	Wall	Unstained, hyaline
	As trophozoite 1–4	Nuclei	As trophozoite 1–8