

Order: Diplomonadorida

- Multiflagellates (1-4 pairs) with no undulating membrane.
- They are bilaterally symmetrical. Cyst form is often present.
- They are binucleated (trophozoite) or multinucleated (cyst).
- Axostyles and fibrillar filaments are present.
- Most of species are parasitic. Reproduction occurs by asexual binary fission.

Family: Hexamitidae

1) Genus *Giardia*

- A common intestinal and zoonotic parasite of mammals and birds worldwide, especially in warm climates.

Hosts and habitats:

It has a wide host range inhabiting the duodenum and upper small intestine and attaches to the brush border of epithelial cells. Common species are:

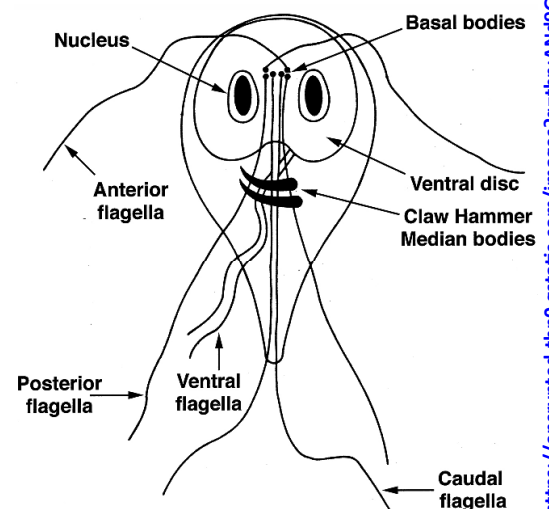
- *Giardia duodenalis* (*G. intestinalis*, *G. lamblia*) is the most important species in veterinary medicine. It infects a wide range of hosts including humans, monkeys, dogs, cats, cattle, sheep and birds.
- Other species as *G. cati* (cats), *G. canis* (dogs), *G. bovis* (cattle), *G. muris* (rodents), *G. caprae* (goats) and *G. gallinarum* (birds).

Morphology:

It has two life stages.

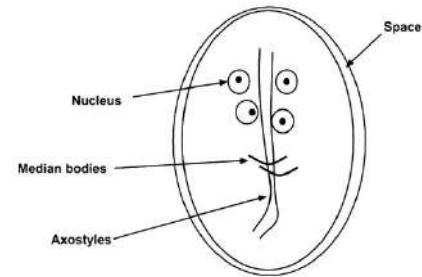
1) Trophozoite

- It is a motile feeding stage and non-infectious.
- Typically pear/pyriform to ellipsoidal-shaped (broadly rounded anterior end as well as narrower and tapered posterior end) (12-20 x 7-10 μ m). When seen from the front, trophozoite looks like a tennis racket with convex dorsal surface.
- The ventral surface has sucking (adhesive) disc containing 2 nuclei.
- Longitudinally, a ventral groove containing 2 rod-like axostyles run down the centre of the body ventrally with two median (parabasal) bodies.
- It has 4 pairs of flagella; anterior, posterior, caudal and ventral.



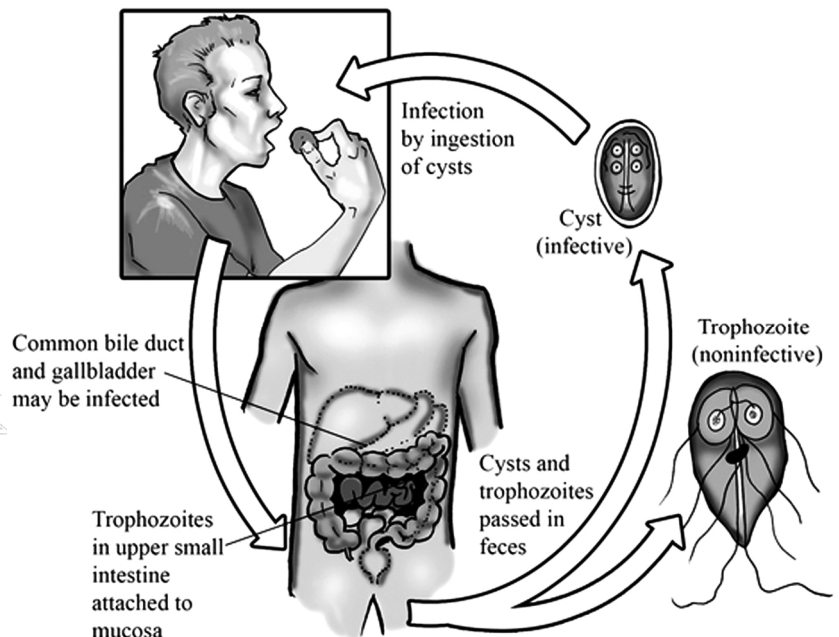
2) Cyst

- It is the infectious stage (immediately infectious to host) and environmentally resistant.
- It is oval-shaped (9-12 x 7-9 μm) with internal structures (remnants of axostyles and median bodies) are visible with a thick wall appears double-layers.
- It contains 2-16 nuclei. Mature cyst has at least 4 nuclei.



Life cycle:

- Simple and direct life cycle; cysts are ingested and excystation occurs releasing two trophozoites into the duodenum.
- Trophozoites reproduce by asexual binary fission & feed & colonize the small intestine (severity of disease is dependent on the number of trophozoites).
- Trophozoites encyst as they pass via the intestine towards rectum. Both cysts, rarely trophozoites, can pass in feces.
- Cysts are resistant, whereas trophozoites are fragile and die quickly.
- The parasite is transmitted when the cysts are swallowed along with feces-contaminated food and drinking water via improper hygienic conditions. Prepatent period is 7-10 days.



Pathogenesis:

Infection is often symptomless, except in children and young animals ingesting a large number of cysts. Diarrhea is a common sign associated with epigastric pain and flatulence stool is tinged with large amount of mucus and fat without blood.

Diagnosis: Detection of cysts/trophozoites in diarrheic feces.

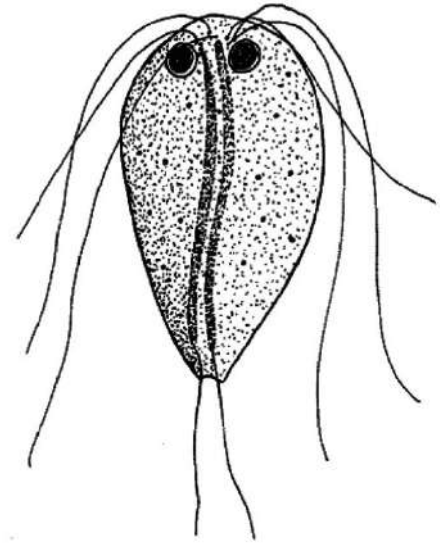
Control: Good hygiene and proper sanitation. Disinfectants on surfaces.

Treatment: Selective drugs e.g. fenbendazole and metronidazole.

2) Genus *Hexamita*

Morphology and general characters:

- Resembling *Giardia*, *Hexamita* has a narrow pear-shaped (pyriform) body with two nuclei anteriorly.
- It has 2 axostyles and 4 pairs of flagella; anterior 2 pairs, one lateral pair and one posterior pair. The later is closely associated with axostyles).
- The infection occurs via contaminated food/water with trophozoites/cysts. Multiplication is by longitudinal binary fission.



Common species:

1. *Hexamita meleagridis*

It inhabits the small intestine of turkeys. Adult birds are symptomless and carriers, while poults suffer from severe catarrhal enteritis associated with high mortalities.

2. *Hexamita columbae*

It occurs in the small intestine of pigeons causing catarrhal enteritis.

3. *Hexamita muris*

It affects the posterior portion of small intestine and caecum of rat and mice.

Life cycle:

Direct life cycle. Trophozoites are the infective stage (cysts are rarely formed) and reproduce by binary fission. It has a fecal/oral route of transmission. Trophozoites colonize crypts of the duodenum and upper jejunum.

Pathogenesis:

It causes a disease called hexamitiasis or infectious catarrhal enteritis of birds (turkeys, pigeons, quail, pheasants, partridge, ducks & peafowl).

Order: Trichomonadordida

- Members of this order are motile with 4-6 flagella, one of which may be a recurrent flagellum associated with an undulating membrane (if present).
- No bilateral symmetry with a characteristic jerky and uneven movement (very diagnostic). They have one axostyle and one parabasal body.
- They may have one or two nuclei and reproduction is asexually by a longitudinal binary fission.

- Cysts are formed in some species.
- They present in the alimentary canal of animals and majority of them are non-pathogenic. The pathogenic forms are included in two families: Trichomonadidae and Monocercomonadidae.

i. Family: Trichomonadidae

- Parasites of the alimentary canal of animals, birds and humans. Few species parasitize the reproductive organs.
- Members are pyriform-shapes having a broad, rounded anterior end and somewhat pointed from posterior end. Anteriorly, there is a single nucleus, adjacent to which is the blepharoplast, which is associated with a number of basal granules. From the blepharoplast, a number of anterior flagella (according to species) arise together with one flagellum (posterior flagellum) which runs posteriorly, attached to the body by an undulating membrane and extend posteriorly beyond the posterior end. A rod-like axostyle runs along the body length, arising from the blepharoplast and emerges from the posterior end of the body.
- No cystic stage.

1) Genus *Tritrichomonas* Species *T. foetus*

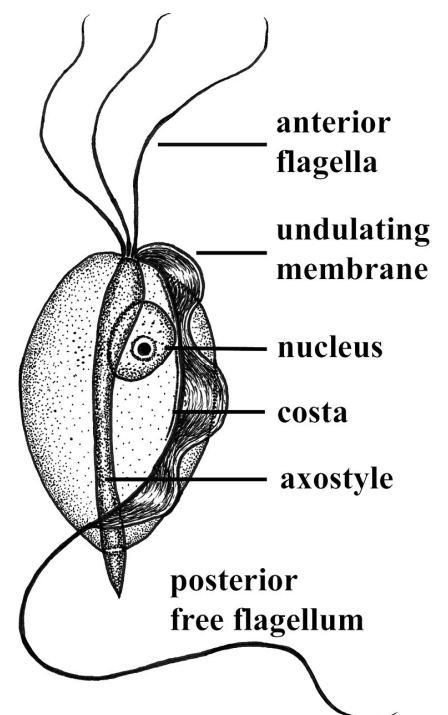
Host and habitats:

It is found in the superficial mucous membranes of male and female genital tracts of cattle and buffaloes.

Morphology:

The same characters of the family and:

- The parasite is 10-25 x 3-15 μm and pyriform-shaped with 3 whip-like anterior flagella.
- Well-developed undulating membrane and a prominent costa (deeply-stained structure extends along the base of the undulating membrane).
- A single anterior nucleus, thick and hyaline axostyle and sausage/ring-shaped parabasal body.
- There is **no** pelta.
- The axostyle is well-developed and emerges from the posterior end through a chromatic ring.



Life cycle:

Direct life cycle. Trophozoites (**infective stage**) are transmitted via copulation. In bulls, they reside in the prepuce, penis, epididymis and vas deferens (in cows/heifers, they reside in the vagina, cervix and uterus). Trophozoites reproduce by binary fission.

Transmission:

Bovine trichomoniasis is a **venereal** disease of cattle transmitted during coitus, artificial insemination, gynaecological examination, the contact between infected and uninfected cows (venereal) and by insects that feed on vaginal exudates from infected cows and transmit the flagellates to uninfected cows.

Pathogenesis:

It causes infertility as well as spontaneous abortion in the first trimester (invasion of the uterus leads to placentitis, which results in detachment and death of fetus).

A) Male animals:

Bulls do not show clinical signs and can infect females during coitus (only carrier). The infection does not affect the animal fertility or sperms viability. Infected bulls become permanently infected if untreated.

2) Female animals:

- First, trichomonads multiply in the vagina causing severe vaginitis. Then, they invade uterus and cervix with an early abortion (1-3 months) is a characteristic symptom.
- If placenta and fetal membranes are absolutely eliminated, infected cows spontaneously recovered, otherwise, chronic catarrhal/purulent endometritis develops leading to a permanent sterility. The uterus is filled with a large amount of odorless fluids change to offensive odour in pyogenic infections leading to pyometra.
- If no abortion, fetuses born dead/macerated.

Diagnosis:

Microscopic detection of trophozoites in preputial scrapings/washings, vaginal secretions, vaginal washings, or absorbed fetuses.

Other species: *T. equi* (horses), *T. suis* (pigs) and *T. eberthi* (chickens, turkeys, ducks).

Treatment:

- In females: It is unnecessary, as it is a self-limiting disease.
- In males: Preputial washing with acriflavin solution 1%.

2) Genus *Trichomonas*

Species *T. vaginalis*

Morphology:

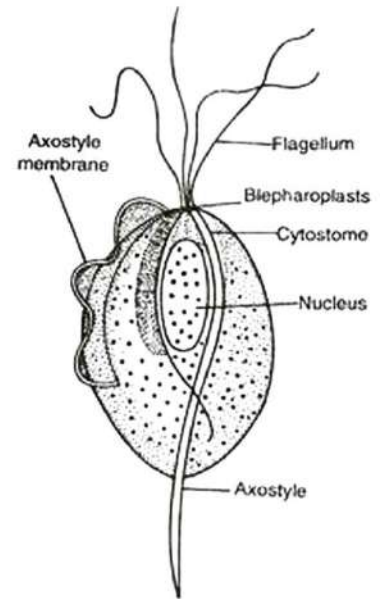
- Ovoid/pear-shaped organism with 4 anterior flagella and no posterior free flagellum.
- Large anterior nucleus.
- The undulating membrane extends from the base of flagella posteriorly to about half-length of the parasite.

Hosts and habitats: Vagina, prostate gland and urethra of humans.

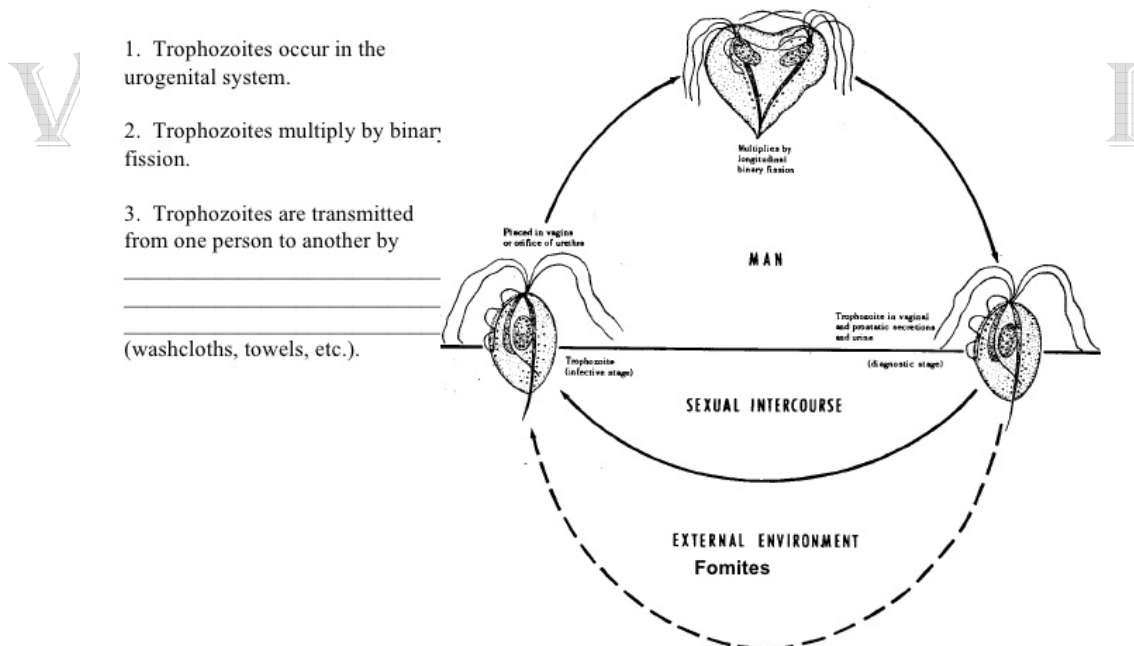
Pathogenesis:

Males are usually carriers (symptomless except urethritis in rare cases). Females suffer from burning micturition.

Treatment: Hygienic conditions, iodine solutions and antibiotics.



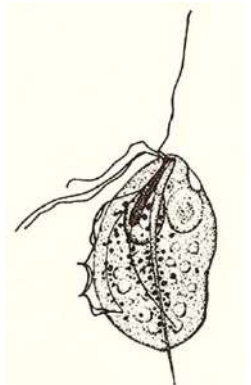
Life Cycle of *Trichomonas vaginalis*



Species *T. gallinae*

It resembles *T. vaginalis* but:

- A parasite of the upper digestive tract of pigeons and other birds.
- Undulating membrane extends along the two thirds of the body.
- Axostyle is narrow and extends a short distance posteriorly.



Pathogenesis:

- Young birds are mostly affected. Trophozoites invade the mucosa of the upper intestinal tract (buccal cavity, sinuses, pharynx, esophagus and crop) (greenish to yellow fluid appears in oral cavity).
- Raised yellow caseous lesions (**cankers**) first appear in mouth and spread to the upper digestive tract. Lesions enlarge and become confluent and caseous occluding the esophagus.

Diagnosis:

- Clinical signs and gross lesions.
- Microscopic detection of large numbers of *T. gallinae* in direct smears from oral fluids, crop or digestive tract.

Control:

- Elimination of infected birds and proper sanitation.
- Anti-flagellate drugs. e.g. dimetronidazole.

3) Genus *Tetratrichomonas*

Species *T. gallinarum*

A parasite of caecum and liver of turkeys, domestic fowls and guinea fowls.

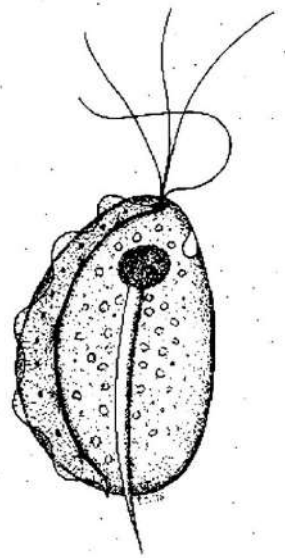
Morphology:

Pear-shaped with 4 anterior flagella and a posterior flagellum, which extends behind the body. Axostyle is long, slender and projects from the posterior end of the body with **no** chromatin ring.

Pathogenesis and clinical signs:

Liver lesions in turkeys (fatal enterohepatitis) are similar to that produced by *Histomonas meleagridis*. Differentiation could be done by detection of the parasite in a direct smear from the lesion.

Other species are *T. ovis* (sheep) and *T. anseris* (geese).

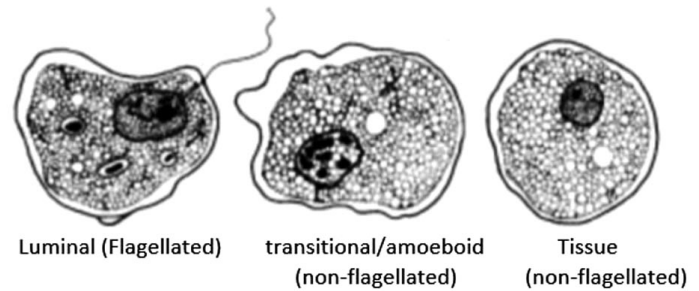


ii. Family: Monocercomonadidae

Genus *Histomonas*

Species *H. meleagridis*

• Actively amoeboid, rounded with a single nucleus (polymorphic). Within the tissue, it assume an amoeboid (8-15 μm) shape, while in the lumen (or free in the contents of caecum), it lives as an elongated flagellated form (up to 30 μm).



- A single fine flagellum arise from the blepharoplast close to the nucleus.
- It has a clear ectoplasm and granular endoplasm containing food vacuoles that include bacteria and RBCs.
- Vesicular nucleus.
- No cystic stage.
- Reproduction by binary fission.

Hosts and habitats:

Caecum and liver of turkeys (highly susceptible). It is a cosmopolitan in gallinaceous birds (turkeys, chickens, pheasant and quail).

Life cycle and transmission:

- Due to a relatively weak resistance of the parasite (maximum 2 hrs in feces), it is carried on **eggs** of the nematode *Heterakis gallinae*. When infected eggs hatch in caeca, both nematodal larvae and the protozoa are released.
- Moreover, histomonads may be ingested by worms, penetrate the intestinal wall and invade the reproductive system of both females and males. In the former, it invades ovaries, while in the later it affects testes and vas deferens. In such concern, female worms are infected via copulation.
- Within eggs, trophozoites may be viable for several years.

Pathogenesis:

- The disease in turkeys is called histomoniasis (blackhead disease, infectious enterohepatitis).
- In caecal walls of young turkeys, small raised pin-point ulcers containing the protozoa are present. Ulcers are enlarged and involve the whole mucosa with yellow-colored diarrhea.

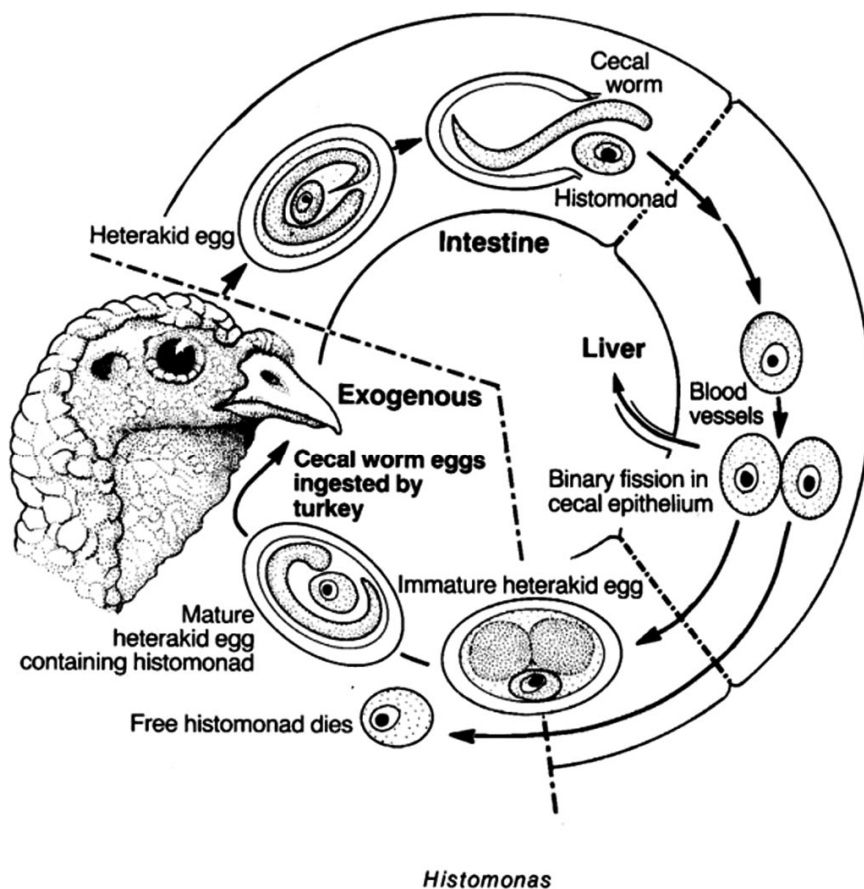
- Greatly thickened and necrotic mucosa, and caeca are hemorrhagic containing a hard caseous core.
- Histomonads deeply penetrate mucosa to blood vessels and reach the liver via the portal circulation.
- In the liver, yellowish green areas of focal necrosis of about 1 cm in diameter may develop and extend to include all the hepatic surface.
- A darkening of the head may develop, so the disease name is derived.

Diagnosis:

Brilliant yellow (sulfur) feces associated with the cecal and liver lesions.

Control:

- Good sanitation and control of *Heterakis gallinae* in birds and limit access to nematode eggs.
- Prophylactic and therapeutic treatment with nitroimidazoles (e.g. dimetronidazole).



B) Subphylum: Sarcodina

- Organs of locomotion are pseudopodia and most of them are free living while few are parasitic in the digestive tract of their hosts.
- Reproduction is asexually by simple binary fission.
- Most of them are holozoic.
- The cytoplasm is divided into ectoplasm and endoplasm. The later contains nucleus, food vacuoles, contractile vacuoles (in some species) ...etc
- They are usually in two forms, vegetative (trophozoite) form and the encysted form. Vegetative form is responsible for reproduction and the pathologic effects caused by the parasite. The encysted form (**infective stage**) is diagnostic for species identification.
- It includes family Endamoebidae that contains the following genera, *Entamoeba*, *Dientamoeba*, *Iodamoeba* and *Endolimax*.

1) Genus *Entamoeba*

General characters:

The same characters of the subphylum. The nucleus is vesicular with the endosome (karyosome) is central/eccentric and the nuclear membrane is lined with varying numbers and sizes of periendosomal chromatin granules. The cyst contains 1-8 nuclei according to the species. So, this genus includes 4 groups:

- 1) 8-nucleated group (Coli group): Non-pathogenic. e.g. *E. coli*, *E. muris*.
- 2) 4-nucleated group (Histolytica group): Pathogenic. e.g. *E. histolytica*, *E. equi*.
- 3) One-nucleated group (Bovis group): Non-pathogenic. e.g. *E. bovis*, *E. ovis*.
- 4) No cyst group (Gingivalis group): Non-pathogenic. e.g. *E. gingivalis*.

Life cycle:

- Direct life cycle. The infective stage (**cysts**) are transmitted via contaminated food/water and a broad range of insects is incriminated as being act as mechanical intermediate hosts (by hairs, legs,).
- Trophozoites multiply by simple binary fission. The produced amoebae round up with expulsion of all vacuoles, then cease feeding and change into cysts, **encystment**, in the lumen of small intestine and nuclei divide according to species. Each nuclear fragment is surrounded by a portion of the cytoplasm to form new forms (cysts) which disseminate in feces.

- Some individuals undergo excystation in the large intestine (by proteolytic enzymes) with nuclear and cytoplasmic divisions to form new colonies of trophozoites which either stay in the lumen or invade another tissues.

Pathogenesis:

- Members cause amoebic dysentery which is characterized by violent diarrhea (usually bloody and/or mucoid in *E. histolytica* infection), severe colitis. Foul-smelling stool, frequent flatulence, moderate to severe anemia and dehydration.
- Some species gain access into the lymphatics and then to the liver forming amoebic abscess (hepatic amoebiasis). Abscesses may be formed in other organs as brain and lung.
- The condition is aggravated by a secondary bacterial infection.

Diagnosis:

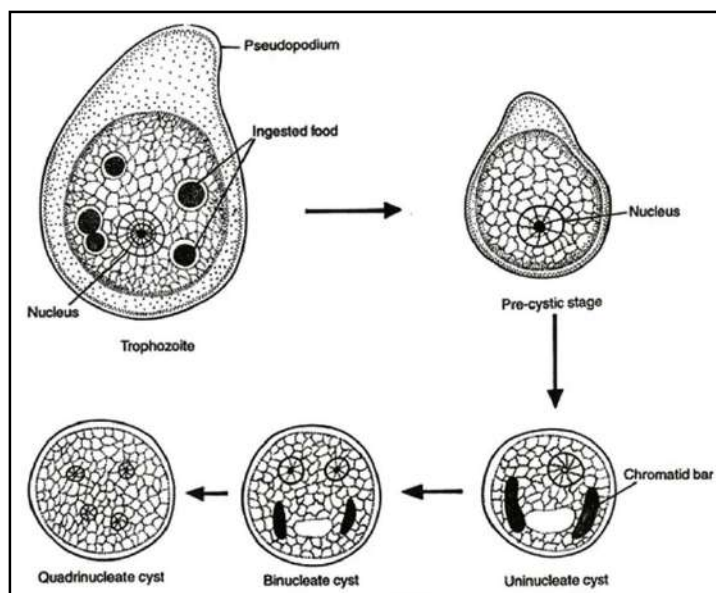
- Direct fecal examination and zinc sulphate floatation technique are used to detect the parasite forms.
- Iodine solution 1% is helpful to detect nuclei and glycogen vacuoles.
- Normally passed stool contains only cysts, while diarrheic one contains both forms.

Treatment:

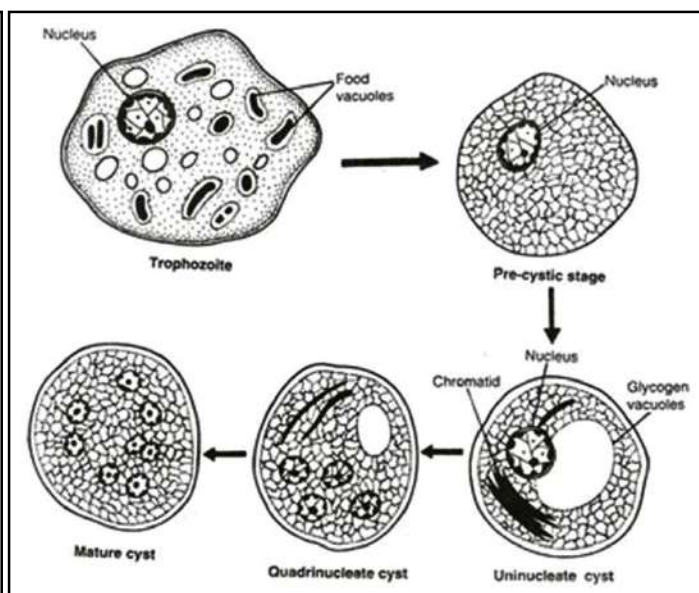
- Treatment with antiprotozoal drugs e.g. Flagyl, Flagentyl, Amerizole and streptoquin.
- The use of some antibiotics to prevent the secondary bacterial infection.
- Good sanitation, personal hygiene and eradication of flies.

Item	<i>E. histolytica</i> (Histolytica group)	<i>E. coli</i> (Coli group)
Host & habitats	Inside tissues of caecum and colon of humans and dogs	In the lumen of caecum and colon of humans and dogs
Pathoogenecity	Pathogenic forming amoebic dysentery	Non-pathogenic
Morphology		
Trophozoite		
Size	Small-sized (10-60 µm diameter)	Large-sized (15-50 µm diameter)
Cytoplasm	Small mass, well-differentiated into outer hyaline ectoplasm and inner fine granular endoplasm	Not well-differentiated into ectoplasm and endoplasm except at the region of pseudopodia

	It usually has one pseudopodium	It usually has more than one pseudopodium
	Endoplasm contain both food vacuoles (with bacteria, tissue debris and RBCs) and contractile vacuoles (for removal of waste products)	The same, but food vacuoles contain bacteria and fecal debris only.
Nucleus	Spherical in shape and vesicular	
	Small-sized and central endosome	Large-sized and eccentric endosome
	Nuclear membrane is thin and lined with peripheral fine chromatin granules which are regular in shape, size and distribution. They are arranged in a circle giving the appearance of ring with small beads and a clear zone (halo)	Thick nuclear membrane lined with coarser chromatin granules which are irregular in shape, size and distribution. They are also scattered in the space between the endosome and nuclear membrane
Cyst	Spherical-shaped (20 µm)	Globular-shaped (10-35 µm)
	Small-sized with a thick cyst wall	Large-sized with a thin cyst wall
	It contains 1-4 nuclei. Fully mature cyst (infective stage) contains 4 nuclei. Each nucleus has the same characters of the trophozoite.	It contains more than 4 nuclei. Fully mature cyst (infective stage) contains 8 nuclei. Each nucleus has the same characters of the trophozoite.
	Chromatoid bodies are cigar -shaped or rod-shaped with rounded ends. They persist for a long time, but they disappear in fully mature cysts.	Chromatoid bodies are splinter -like with sharp ends. They disappear by aging.



Stages of life cycle of *E. histolytica*



Stages of life cycle of *E. coli*

Image at: <http://www.yourarticlelibrary.com/zoology/parasite-entamoeba-histolytica-life-cycle-mode-of-infection-and-treatment/24274>

Image at: https://en.wikipedia.org/wiki/Entamoeba_coli

Bovis group

They are similar to *E. histolytica* but often in cattle, non-pathogenic, smaller endosome, chromatin granules vary in size and irregular in distribution, fully mature cyst has one nucleus, chromatoid bodies are cigar-shaped or splinter-like and glycogen vacuoles are well-developed.

Gingivalis group

They are similar to *E. histolytica* but they habit the buccal cavity (between teeth), non-pathogenic (except producing bad odour of mouth due to the presence of waste products in contractile vacuoles), smaller endosome and chromatin granules which form a continuous ring and no cystic stage.

2) Genus *Dientamoeba* (*D. fragalis*)

Small amoebae in caecum and colon of humans and monkeys, usually binucleated and with no cystic stage.

3) Genus *Iodamoeba* (*I. butschlii*)

Members habit caecum and colon of humans, monkeys and pigs, with a large endosome and uninucleated cysts (no chromatoid bodies and a large compact mass of glycogen).

4) Genus *Endolimax* (*E. pana*)

Non-pathogenic small amoebae in caecum and colon of humans, monkeys and pigs, trophozoite with no nuclear peripheral granules and with tetranucleated cysts.

Phylum: Apicomplexa (syn. Sporozoa)

General characters:

- All are entirely parasitic species and produce spores. Most of them are intracellular.
- Members are so called due to the presence of a group of organelles (complex structures) at the apical end of the parasitic stages, particularly **sporozoites** (under electron microscopy, EM). They play a role in interaction of the parasite with the host cell and the subsequent invasion of the host cell. These structures are:

1- Electron dense polar ring: at the anterior end.

2- Conoid: Cone-shaped hollow structure of one or more spirally coiled microtubules and has a penetrating function.

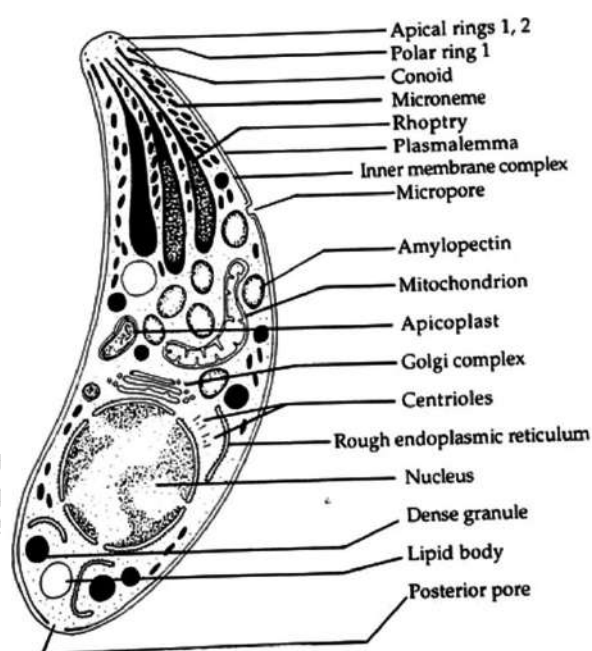
3-Rhoptries: Tubular structures run via the conoid to the anterior end and expand into a bag-like structure at the other end. It secretes proteolytic enzymes.

4- Micronemes: Rod-like structures, more or less great in number and may be well-attached to rhoptries.

5- Subpellicular microtubules: A number of subpellicular microtubules run back words from the polar ring and are involved in locomotion.

6- Micropores: One or more and may found along the cell wall probably for ingestion of food.

- Organs of locomotion are absent except species having microgametes which are flagellated. Locomotion in apicomplexa is by body flexion (gliding).
- The nucleus is vesicular and cystic stage is present.
- The reproduction occurs in two forms; asexual form [simple or multiple binary fission (**schizogony**)] and sexual form [fusion of male and female gametes (**gametogony**)]. Following this process, zygote is formed and then sporozoites, infective stages, are formed (**sporogony**) (See page 95).
- All stage of the life cycle may occur in one (monoxenous) or more (heteroxenous) hosts. Moreover, in some species schizogong and gametogony occur **inside** the host, while sporogony is **outside**. In other, all stages occur **inside** the host.
- It includes 3 classes of veterinary medical importance, Coccidia, Piroplasmidia and Haemosporidia.



Order: Eucoccidiorida

- Members are intracellular parasites of the epithelial cells of invertebrates and vertebrates.
- Sporozoites are either naked or in oocysts when ingested and enter tissue cells, where they multiply by repeated schizogony for some generations. The last generation of merozoites develops into gametocytes. These forms are dissimilar gametes, which undergo syngamy, forming zygotes and further sporozoites are produced. Intermediate host may or not be involved.

Family: Eimeriidae

- Intracellular parasites of the intestinal epithelium of vertebrate hosts causing a disease, **coccidiosis**. Few species occur in other organs such as liver and kidneys.
- Schizogony and gametogony occur **within** the host, while sporogony (maturation of the fertilized zygote and production of infective spores) usually takes place **outside** the host.
- All of them are monoxenous.
- Infective stage is the sporulated oocyst which contains up to 4 sporocysts, each containing one or more sporozoites according to species. The infection is done by eating the sporulated oocysts via contaminated food/water.
- Microgametes are often biflagellated.
- Genera of veterinary medical importance are *Eimeria*, *Isospora*, *Tyzzeria* and *Cryptosporidium*.

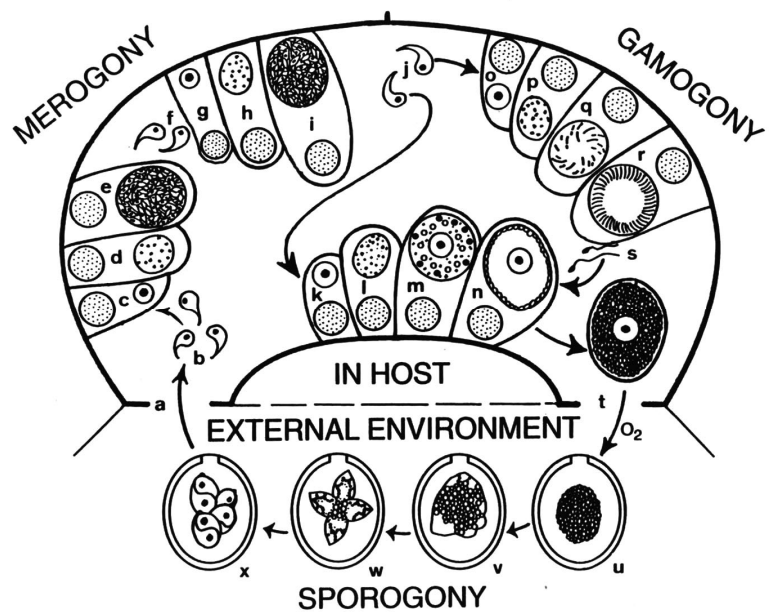
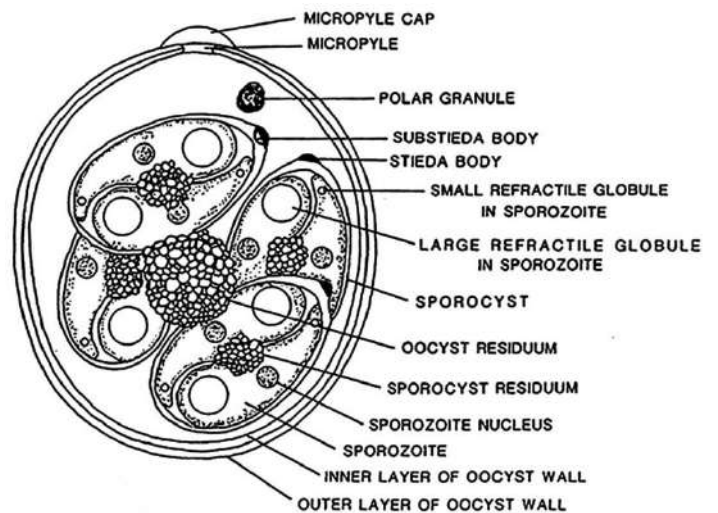
1) Genus *Eimeria*

- Species of this genus cause **coccidiosis** among poultry, rabbits and other animals.
- Most are intracellular parasites of the intestinal tract, but few species occur in other organs as the liver (*E. stiedae* in rabbits) and kidney (*E. truncata* in geese).
- It is host specific tissue specific. Each species is often found in a specific location within the intestinal tract, some are found in the caecum, others in the duodenum. Some species are found above the host cell nucleus, while others are found beneath it.
- Species of *Eimeria* in different animals:
 - In chicken: *E. tenella*, *E. necatrix*, *E. maxima*, *E. mivati*, *E. mitis*, *E. brunette*, *E. acervulina* and *E. praecox*.
 - In ducks and geese: *E. anatis*, *E. anseris* in the intestine and *E. truncata* in kidneys.
 - In rabbits: *E. intestinalis*, *E. magna* and *E. perforans* in the intestine and *E. stiedae* in liver.

- In cattle: *E. bovis* and *E. ellipsoidalis*.
- In sheep: *E. faurei* and *E. arloingi*.
- In equine: *E. leukarti* and *E. solipedum*.
- In pigs: *E. deblicki* and *E. spinosa*.

Life cycle:

- Direct life cycle including 3 successive phases; the schizogony following the infection; gametogony (sexual reproduction) and sporogony (formation of spores) which is the unique stage outside the host.
- The host ingests the **infective stage** i.e. sporulated oocyst (4 sporocysts, each containing 2 sporozoites, **tetrasporocystic dizoic oocyst**). It is spherical or ellipsoidal-shaped with double-layered wall and variable sizes according to species. Some species has a micropyle at one pole of the oocyst and may cover by a micropyle cap.



Stages of life cycle:

1. Shizogony (asexual reproduction): Following the infection by ingestion of the infective sporulated oocysts, excystation occurs in the digestive tract and 4 sporocysts are liberated. Then, sporozoites (activated by trypsin and bile) leave the sporocysts. Each sporozoite penetrates an intestinal epithelial cell, rounded up and then known as **trophozoite**.

- Within few days, each trophozoite divides by multiple fission forming a **schizont**. The latter contains a large number of elongated, nucleated organisms, **merozoites**. This development is known as **schizogony** or **merogony**. This is the 1st generation schizont. The later becomes mature and both the host cell and the schizont rupture, and merozoites liberate into the intestinal lumen, escape to invade new cells forming the 2nd generation schizont.

- This stage of schizogony may be repeated and the number of schizont generations is species dependent (e.g. 1st generation of *E. tenella* infection contains 900 merozoites, while in *E. bovis* infection, it is 100000).

2. Gametogony (sexual reproduction): Following the schizogony, the stage of sexual reproduction (gametogony or gamogony) begins when the last generation of merozoites (telomerozoites) invades the intestinal epithelium and differentiate into male and female gametocytes.

- The female gametocytes (macrogametocytes) remain unicellular but increase in size to fill the parasitized cell. It can be differentiated from the trophozoite and the developing schizonts by having a single large nucleus with a clear central karyosome and peripheral granules arranged on the inner surface of its wall. These granules will later form the inner wall of the oocyst after fertilization.

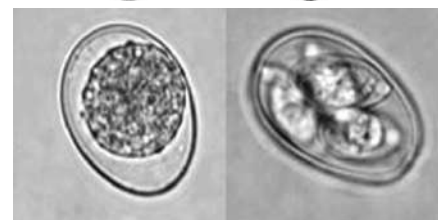
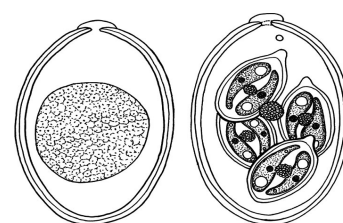
- The male gametocyte (microgametocyte) undergoes a repeated division to form a large number of flagellated, nucleated organisms, the microgametes, which later liberated by the rupture of the host cell.

- Fertilization occurs when a microgamete penetrates a macrogamete, fusion of their nuclei takes place and the zygote is formed (**unsporulated oocyst**). There is no further development usually takes place until the unsporulated oocyst is liberated from the host's body in feces.

3. Sporogony (formation of sporocysts): It begins on the ground and under suitable conditions of oxygenation, high humidity and optimal temperature of about 27°C.

- The nucleus divides twice followed by the protoplasmic mass resulting in 4 rounded sporoblasts and in some species, the remaining protoplasm forms the **oocystic residual body**. Each sporoblast secretes a wall of retractile material and known as **sporocyst**, while its protoplasm divides into 2 banana-shaped sporozoites. In some species, the remaining protoplasm within the sporocyst forms a **sporocystic residual body**.

- The oocyst becomes consisting of outer double-layered wall enclosing four sporocysts, each containing 2 sporozoites and referred to as a sporulated oocyst (**tetrasporocystic dizoic, the infective stage**).



Pathogenesis and clinical signs of *Eimeria* species infection:

- *Eimeria* species are the cause of a disease among poultry and other farm animals, known as **coccidiosis**.

- Pathogenicity of coccidia depends on number of the infective dose of ingested oocysts, number of the host cells destroyed, host cell infected, age and general condition of the animal and degree of acquired and natural immunity.
- Coccidiosis is characterized by high mortality rates due to massive destruction of the epithelial cells of intestine either due to massive invasion of the infective sporozoites or rupture of cells containing schizonts or male gamonts.
- Clinically, there is an unthriftiness and usually **bloody** diarrhea and/or allergic reactions due to the rupture of intestinal cells. In *E. tenella* infection, lesions are basically in caeca which are filled with destructed cells and clotted blood forming a characteristic lesion, **caecal core**. Most pathological lesions are due to the 2nd generation schizont. In chicken, *E. tenella* is highly pathogenic, and in cattle *E. zurnii* causes severe lesions.
- Coccidial infections are self-limiting where the asexual reproduction does not continue in the absence of reinfection and the host can get rid of the infection after it has expelled the formed oocysts. Also, reinfection usually occurs with development of immunity against the concerned species with formation of few oocysts.

Diagnosis:

- Clinical signs.
- Detection of sporulated/unsporulated in feces. Failure to find oocysts in diarrheic feces does not necessarily exclude the disease.
- Post-mortem findings are confirmatory. In the later, all stages including schizonts, merozoites and gametes must be recognized.

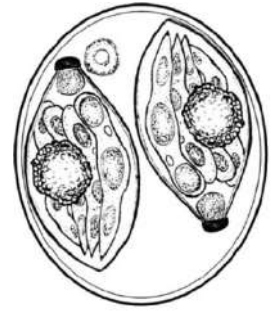
Control:

- Treatment with sulphonamides e.g. trimethoprim, sulphadimidine, sulphaguanidine, sulphaquinoxaline. The use of drugs including derivatives of diphenylmethane, diphenylsulphide, nitrofurazone, imidazole, benzamide and amprolium as prophylactic is more effective.
- Sanitation and isolation are effective in preventing coccidiosis.
- Overstocking and crowding should be avoided.
- Feed and water containers should be high enough to prevent fecal contamination.
- The buildings must be regularly cleaned, disinfected, kept dry and well-drained.
- Young and old animals should be separated, since the old animals are parasitic carriers and serve as a source of infection.

2) Genus *Isospora*

It is similar to genus *Eimeria* but:

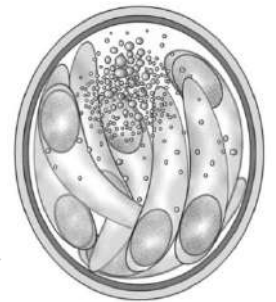
- The infective stage is the sporulated oocyst that contains 2 sporocysts, each containing 4 sporozoites (**disporocystic tetrazoic oocyst**).
- In species infecting pigs, spleen, liver and lymph nodes may be involved.
- Rodents may take the infection and may act as reservoirs for dog species.
- Common species are *I. canis* and *I. bigemina* (dogs), *I. felis* (cats), *I. suis* (pigs), *I. natalensis* (humans), and *I. gallinae* (chicken).
- Most species are only mildly pathogenic but can cause transient diarrhea, colic, weight loss and fever.



3) Genus *Tyzzeria*

It is similar to genus *Eimeria* but:

- The sporulated oocyst contains **8** naked sporozoites (no sporocysts).
- Common species are *T. perniciosa* (ducks) and *T. anseris* (geese).
- It is highly pathogenic for ducklings which are crying continuously. On post mortem, thick and hemorrhagic intestinal wall with blood, white spots of cheesy materials are present.



Family: Cryptosporidiidae

Genus *Cryptosporidium*

During the 1980's, *Cryptosporidium* was recognized as a major cause of diarrhea in AIDS-patients and resulted in death. Initially, it was believed to be an exotic disease.

It is similar to genus *Eimeria* but:

- Under, EM polar ring and conoid are absent.
- Unlike *Eimeria* spp., it is not host specific or tissue specific i.e. a transverse infection is possible and the organism is found in different locations (in the small and large intestines, stomach, respiratory tract, bursa of Fabricius, gall bladder and bile duct).
- All developmental stages are found enclosed in a parasitophorus vacuole (its outer covering is derived from the enterocyte cell membrane) adhered to the brush border of enterocytes in both small and large intestine. They are present at the tip, sides or in crypts of villi of the brush border, **not** in the cell proper.
- **All** stages occur **within** the host (sporulation occurs **inside** the host) and the shed oocysts are sporulated. Oocysts can survive for several months under favourable conditions.

- The infective stage is the oocyst containing 4-naked sporozoites.

- In schizogony stage, up to 2 generations of schizonts are produced, type I schizont (8 merozoites) and type

II schizont (4 merozoites). Microgametes are non-flagellated.

- The prepatent period is 4-14 days.

- Two types of sporulated oocysts are produced:

- Thick-walled oocyst: It contains 4-naked sporozoites (infective stage).

- Thin-walled oocyst: It easily undergoes rupture in the intestine with the release of free sporozoites resulting in an **autoinfection**.

- Common species are *C. parvum* (sheep and humans), *C. hominis* (humans), *C. andersoni* (cattle), *C. muris* (rats) and *C. meleagridis* (turkeys).

Pathogenesis:

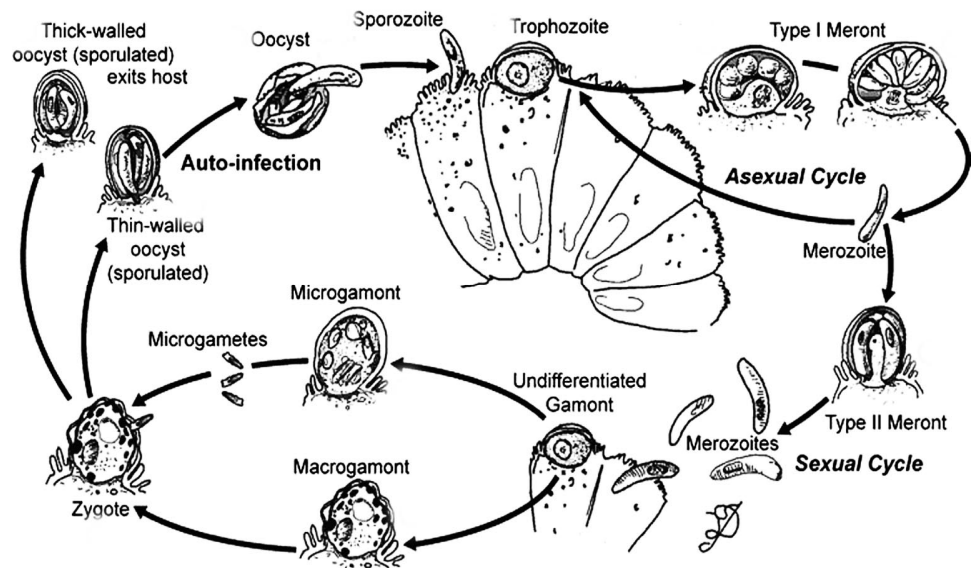
- Profuse watery diarrhea is a common sign due to villous atrophy, reduction in the intestinal mucosal surface, decreased absorbing capacity of the small intestine and decrease in the digestive enzymes of the intestinal mucosal.

- Other signs as off food, gastrointestinal discomfort, abdominal tension, nausea and a mild fever develop.

- The onset of clinical signs coincides with the shedding of oocysts. Severe infections may lead to dehydration. Oocysts shedding often continue after the clinical signs have disappeared. Mortality rates are usually low.

Diagnosis: Clinical signs. In the laboratory, a film is prepared from the fecal sample then stained with acid-fast for the detection of *Cryptosporidium* oocysts (Modified Ziehl-Neelsen technique) where oocysts appear deep red with blue granules against pale green background.

Treatment: Halocur (Halofuginone lactate) is used to decrease the symptoms.



Family: Sarcocystidae

- Parasites-forming cysts in the intermediate hosts (**tissue cyst-forming coccidia**).
- The life cycle needs an intermediate host where the asexual stages occur, while the sexual stages occur in the final host.
- It includes the genera *Toxoplasma*, *Sarcocystis*, *Neospora* and *Besnoitia*.

1) Genus *Toxoplasma*

(Toxon= arc, plasma= form)

- It is a cosmopolitan parasite commonly in warm-blooded hosts. It was first described in gondii rats (*Ctenodactylus gundi*) in Tunisia in 1908.
- It is a facultative/obligatory heteroxenous parasite. *Toxoplasma* is a parasite of nucleated cells, but those of CNS and muscles are predilection sites.
- It forms pseudocysts i.e. the thin cyst wall is formed by the host and **not** by the parasite.

Species *T. gondii*

General characters:

- Domestic cats and other felids are final hosts and the intermediate hosts are mammals including humans as well as rodents and birds.
- Toxoplasmosis in animals is an acute fatal infection, a subclinical, or most importantly, a disease of pregnancy in women, sheep, goats and pigs. It is one of the most common opportunistic infections in patients with AIDS.
- The infective stage is the **disporocystic tetrazoic oocyst** (2 sporocysts, each with 4 sporozoites).

Life cycle

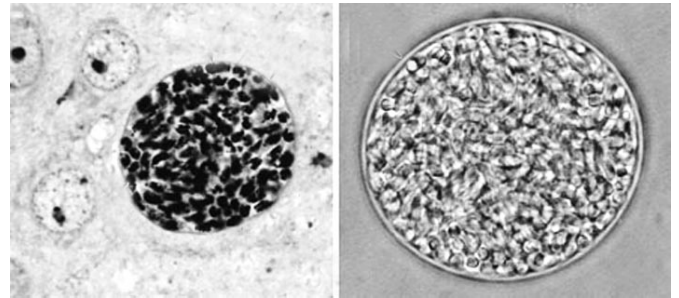
- *T. gondii* primarily exists in 3 forms: oocysts, tachyzoites and bradyzoites.
- **Unsporulated oocysts** (spherical with double clear wall) pass in feces of cats (definitive host) and sporulate within 1-5 days on the ground and then ingested. Sporulated oocysts can infect humans and other intermediate hosts.

A) Extra-intestinal phase:

- In the later, rupture of the cyst wall and release of sporozoites which penetrate the intestinal wall to blood, lymph to be engulfed by macrophages found in the peritoneal cavity, liver, lung and other locations where they develop into **tachyzoites (rapidly multiplying zoites)**. They divide rapidly in cells, causing tissue destruction and spreading the infection. Multiplication takes place by **endodyogony**.

- The process is repeated several times invading many other cells and this condition is called **acute toxoplasmosis**. During this stage, tachyzoites may penetrate barriers and appear in urine, milk and other secretions, so, the occurrence of abortion in pregnant women, particularly in the 1st trimester is highly possible, and infected hosts suffer from signs similar to those of the common flu (fever, headache, anemia, lymphadenitis,etc).

- Due to increased number of tachyzoites, they liberate (after cells rupture) to the blood and circulate to certain organs, particularly brain, heart, skeletal muscles and other viscera and multiply by **endopolygony** forming large numbers of similar merozoites, **bradyzoites**



Pseudocyst of *Toxoplasma gondii*

(slowly multiplying zoites) which are collected together and surrounded by a fibrous connective tissue derived from the host cell forming what is called pseudocyst (*Toxoplasma* cyst, zoitocyst or tissue cyst). This condition is referred as **chronic toxoplasmosis**. In the intermediate hosts, the tissue cyst may persist for long periods without causing harms, otherwise, it undergoes a rupture on immunodeficiency releasing bradyzoites which change to tachyzoites (autoinfection) or may be calcified if not ingested by another host.

B) Entero-epithelial (intestinal) phase: (schizogony and gamogony)

- It occurs only in cats and other felids.
- Upon ingestion of infective tissue cysts, bradyzoites liberate in the intestine and attach the villi of the intestinal epithelium and multiply giving 5 generations of schizonts. From the last generation, often in ileum, micro- and macrogametes are produced and the fertilization occurs forming zygote. Then, shedding of unsporulated oocysts takes place.

Note:

- Cats shed oocysts after ingesting any of *T. gondii* stages; tachyzoites, bradyzoites or sporozoites (sporulated oocysts).
- Prepatent periods are 3-10 days after ingesting tissue cysts, ≥ 18 days after ingesting oocysts, and ≥ 13 days after ingesting tachyzoites.
- In cats, some ingested sporozoites invade enterocytes, to mesenteric L.Ns., the blood then to internal organs forming tissue cysts (**autoinfection**).

- The infective stages for humans are sporulated oocysts, tachyzoites (easily destroyed by pepsin) and tissue cysts (bradyzoites). In the later, partial life cycle may occur i.e. a human can get the infection from another infected one.

Mode of infection (often for humans):

1. Oral route via ingestion of:

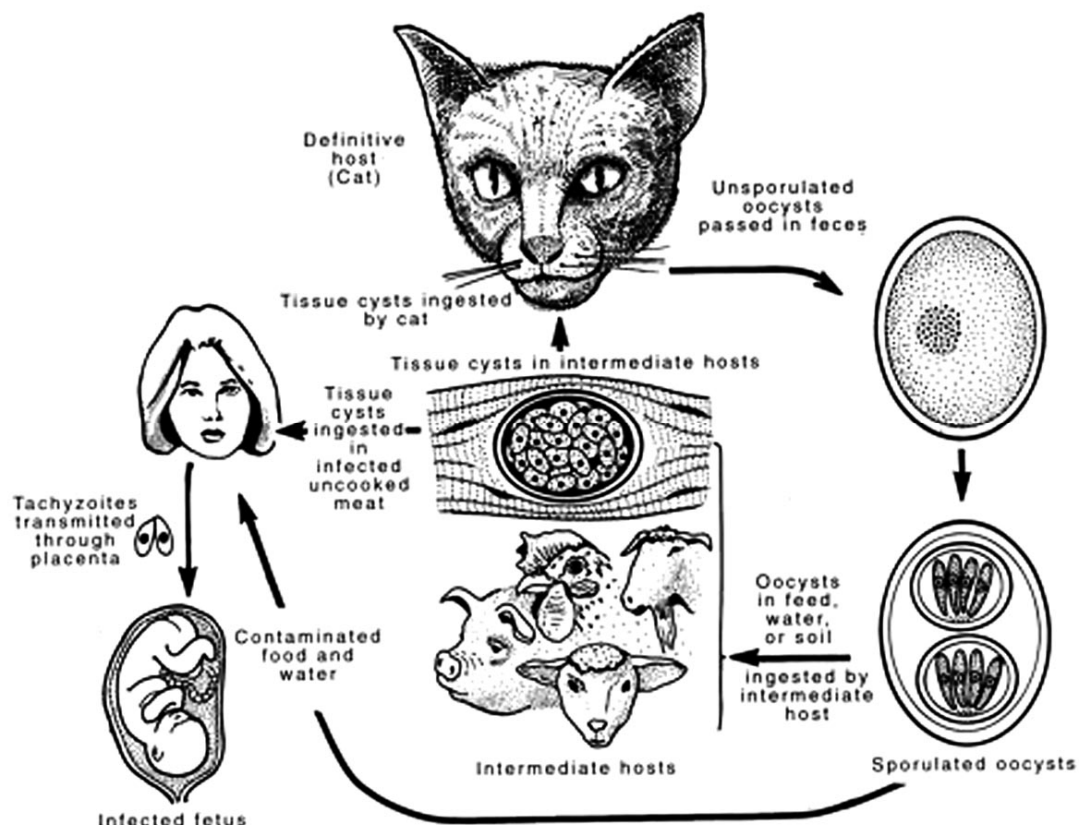
- Mature oocysts in food and drinks contaminated with cat's feces.
- Pseudocysts in raw/undercooked contaminated meat.
- Tachyzoites in unpasteurized goat's and cow's milk.

2. Inhalation of mature oocysts.

3. Blood transfusion and organs transplantation.

4. Contamination of mucous membrane, skin abrasions during handling and preparation of fresh infected meat, or accidentally for laboratory workers.

5. Transplacental route, where the tachyzoites can be transmitted from infected pregnant woman to the fetus via the blood stream (placenta).



Pathogenicity:

A) Sheep:

Tissue cyst causes abortion (in the 1st trimester), born dead offspring (in the 2nd trimester) or offspring born alive but with congenital abnormalities (in the 3rd trimester).

B) Cattle: No abortion, but hepatitis, myocarditis, pneumonia and encephalitis occur.

C) Humans:

1. Congenital toxoplasmosis:

A common form occurs when the mother gets infected for the first time during early pregnancy. Early infections end in stillbirth. Infants who survive are likely to be born with serious problems; seizures, hepatosplenomegaly, jaundice and severe eye infections. The next pregnancy gives normal infants.

2. Acquired toxoplasmosis:

It is asymptomatic in 80-90% of healthy people. However, people with acute infections develop signs similar to those of the flu; fever, fatigue (body aches), swollen lymph nodes and headache.

3. In immunosuppressed patients:

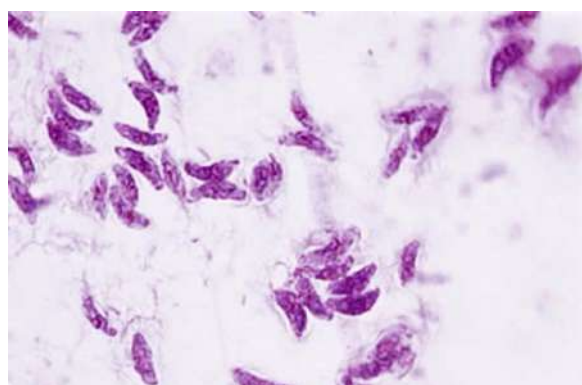
In AIDS, the chemotherapy may reactivate toxoplasmosis and more severe signs; headache, confusion, poor coordination, seizures and lung affections appear.

Diagnosis:

- Clinical signs.
- Detection of oocysts in feces of cats.
- Laboratory diagnosis:

- 1- **Sabin-Feldman dye test**: It depends on the presence of Ab in serum.
- 2- Antibody detection tests: ELISA, IFA, and IHA.
- 3- Detection of the parasite in stained smears from exudates and aborted fetuses.
4. Molecular diagnosis: By the detection of DNA in blood, CSF and different tissues.

Tachyzoite	Bradyzoite
4 µm long	
Central nucleus	Nucleus shifted to the posterior (rounded) end
Broader	Slender
Easily destructed by proteolytic enzymes	Highly resistant



Toxoplasma gondii tachyzoites

Control:

- Treatment with pyrimethamine and sulfadiazine with folinic acid for one year. Pregnant women given spiramycin (rovamycin) for 4 weeks to reduce the risk of transplacental transmission.
- Women (who become pregnant), children and immunocompromised individuals should avoid contacts with cats and/or their feces.
- Proper washing of hands, vegetables and fruits and proper washing of hands and utensils after handling raw meat.
- Proper freezing and cooking of meat.
- Never fed raw meat to cats. Only dry, cooked or canned meat should be fed.
- Cats' feces should be flushed down the toilet or burned.

2) Genus *Sarcocystis* (syn. *Sarcosporidia*)

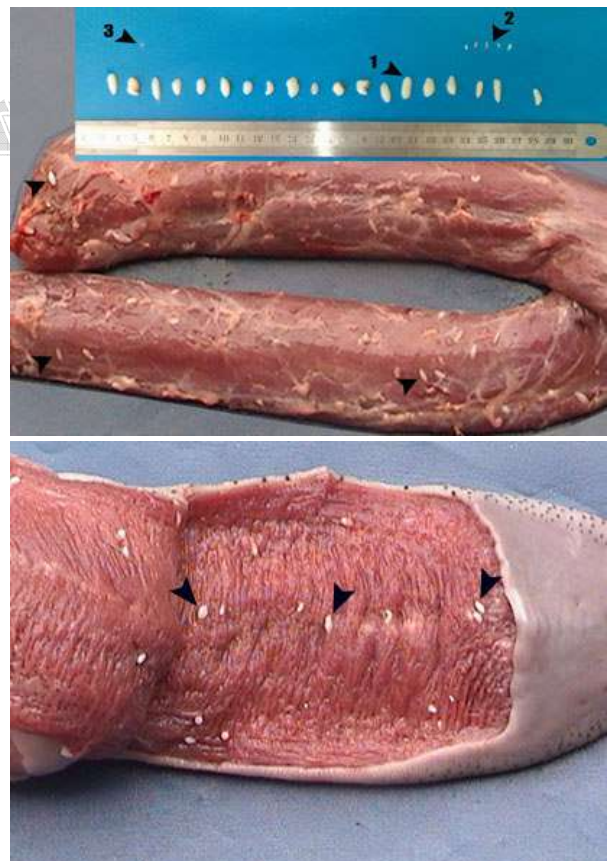
General characters:

It resembles *Toxoplasma* but:

- It is obligatory heteroxenous tissue cyst-forming coccidian parasites with a prey animals (herbivores) serving as the intermediate host and a predator (carnivores) serving as the definitive host. Humans act as incidental intermediate hosts.
- Sporulation occurs **within** the definitive host.
- The infective stage is **disporocystic tetrazoic oocyst**.

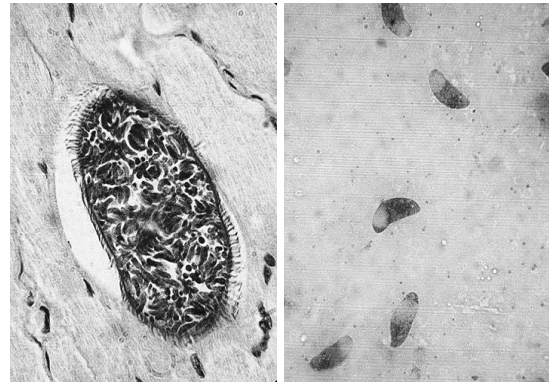
Life cycle:

- Infected cat sheds sporulated oocyst. The cyst wall is very thin (fragile) and easily to undergoes rupture, so, sporocysts, rather than oocysts, are often seen in feces. Moreover, sporocystic walls may rupture releasing sporozoites.
- Sporocysts/sporozoites are ingested by the proper intermediate host inside which sporozoites penetrate the intestinal wall with the formation of 2 or more schizont



generations, then merozoites circulate in blood reaching the endothelial lining of blood vessels of internal organs forming tachyzoites (**endopolygony**).

- Tachyzoites circulate to the skeletal muscles, particularly oesophagus, heart and diaphragm and multiply by **endodyogony** and change to bradyzoites. A mass of the later is called a true cyst



(**sarcocyst** or **Miescher's tubule**). It is either macroscopic (few centimeters) or microscopic (few micrometers), spindle-shaped, parallel to the longitudinal axis of muscle fibers. The covering is either thin or thick wall consisting of an outer homogenous layer and inner nucleated layer. From the later, a number of septa (trabeculae) derived to divide the internal cavity into several compartments of different sizes. Metrocytes (mother cells of bradyzoites) derived from the nucleated layer and give rise to bradyzoites (merozoites). The number of the later decreases towards the center. Each bradyzoite (**Rainey's corpuscle**) is banana-shaped, the anterior end is more pointed containing granules surrounding a vacuole, the posterior end is broader and the nucleus is either centrally located or shifted posteriorly. A mature cyst is formed within 4 months.

- The tissue of intermediate host containing infective sarcocyst is ingested by the appropriate definitive host, within which the sarcocyst ruptures with liberation of bradyzoites that penetrate the **lamina propria** of the small intestine and form gametes (**no schizonts**). Fertilization between macrogametes and flagellated microgametes occurs with the production of zygote forming unsporulated oocysts which soon sporulate (infective stage) endogenously followed by shedding of sporocysts in feces.

Commons species:

Cattle

- *S. bovicanis* (*S. cruzi*).
- *S. bovifelis* (*S. hirsuta*).
- *S. bovi hominis*.

Sheep

- *S. ovicanis*.
- *S. ovifelis* (*S. tenella*).

Buffaloes

- *S. fusiformis* (cats-buffaloes). Macroscopic.
- *S. levinei* (dogs-buffaloes). Microscopic.

Equines

- *S. equicanis*.

Rats

- *S. muris*.

Humans

- *S. lindemanni*.

Pathogenesis:

Sarcocystis spp. are not generally considered highly pathogenic. However, sarcocysts destroy the part of the muscle fibers which it occupies, and as it grows, it may cause pressure atrophy of adjacent cells.

Diagnosis: Detection of sarcocysts via meat inspection in abattoirs.

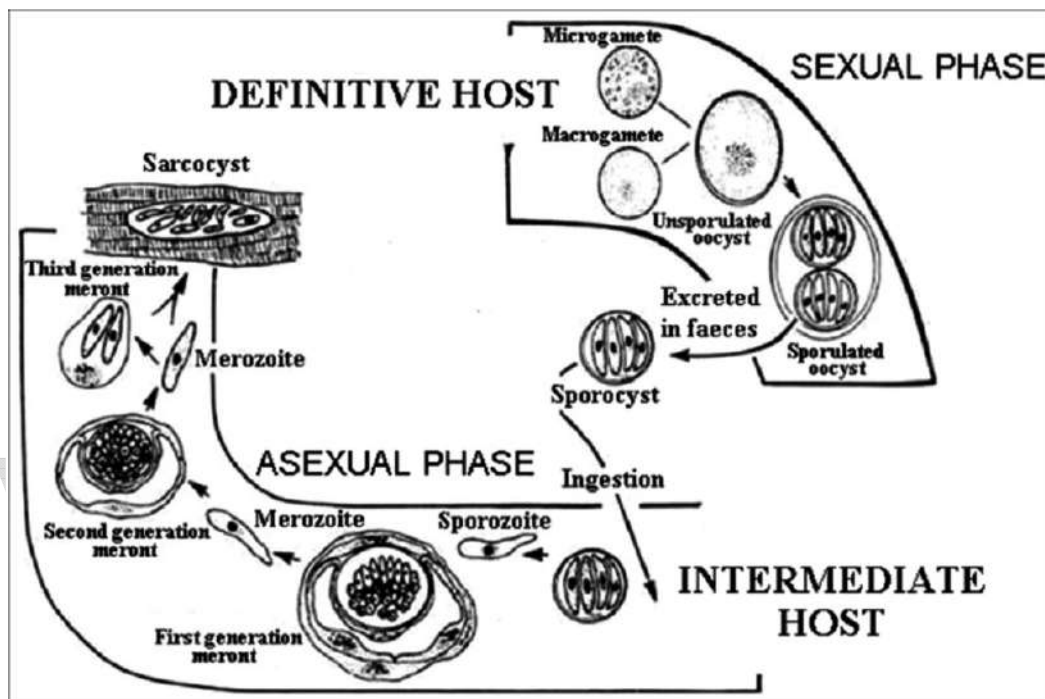


Image at: Prakas P, Butkauskas D (2012). Ekologija 58(1): 45-58.
DOI: 10.6001/ekologija.v58i1.2349

3) Genus *Besnoitia*

Species *B. besnoiti*

It resembles *Sarcocystis* spp. but:

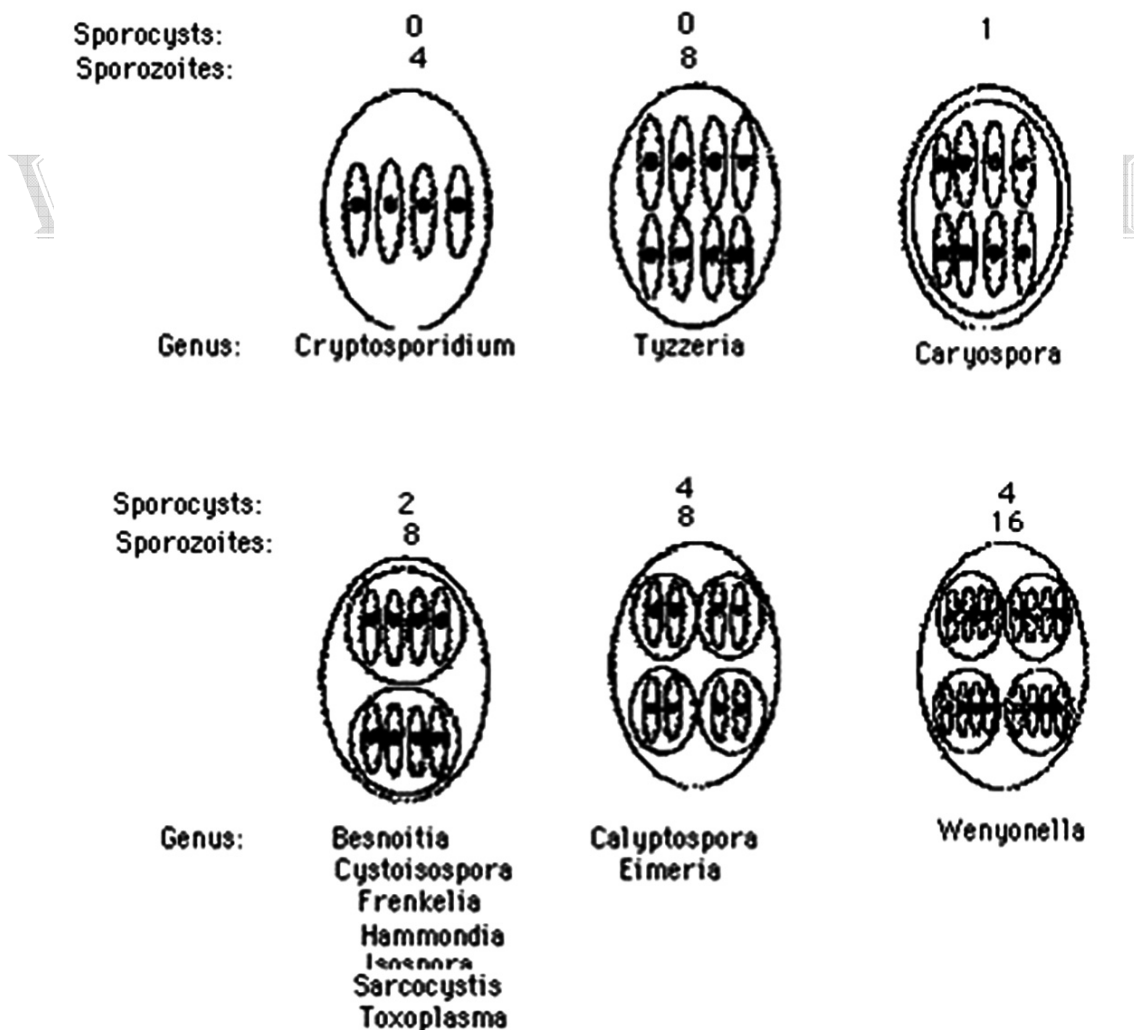
- The definitive host is cats, and the intermediate host is cattle, horses and goats.
- Sporulation occurs **outside** the host (within 24 days), and the infective stage is **disporocystic tetrazoic oocyst**.
- The 1st generation of schizont is formed in the endothelial cells of blood.
- The tissue cyst is formed in the skin, subcutis, mucosa of nose, trachea and other tissues. The cyst is spherical, with an outer thick wall and inner layer with several flattened giant cells. It has no septa.
- In cats, several generations of schizonts are formed.

- Macrogametocytes are formed in goblet cells, while microgametocytes are difficult to be seen.
- In cattle, the parasite causes a serious disease that appears in 2 forms:
 - 1- Febrile form: fever, lymphadenitis, skin wrinkling and photophobia.
 - 2- Seborrheic form: affected skin is covered with a thick scaly layer.

4) Genus *Neospora*

Species *N. caninum*

- The definitive host is dogs, and intermediate hosts are cattle, sheep and goats.
- Sporulation occurs **outside** the host, and the infective stage is **disporocystic tetrazoic oocyst**.
- Tissue cysts are similar to those of *Toxoplasma*.
- It causes abortion in cattle (in mid-gestation).



Item	<i>Toxoplasma</i>	<i>Sarcocystis</i>	<i>Besnoitia</i>	<i>Neospora</i>
FH	Cats	Carnivores & humans	Cats	Dogs
IH	Mammals, birds	Herbivores	Cattle, horses, goats	Cattle
Multiplication	Macrophages	Endothelial lining of internal organs	Macrophages and endothelial cells	Various body cells
Tissue cyst	CNS, muscles, uterus	Muscles	Cutis and subcutis	CNS, uterus
Shed oocysts	Unsporulated	Sporulated	Unsporulated	Unsporulated
Infection via	Tissue cysts, sporozoites, bradyzoites, tachyzoites	Tissue cyst	Tissue cyst	Tissue cysts
Pathogenesis	Abortion in women & sheep	May be cattle	Skin lesions	Abortion in cattle

Oder: Haemosporida

General characters:

- Members are heteroxenous with schizogony in vertebrate hosts while gametogony and sporogony in invertebrates (blood sucking arthropods).
- Motile zygote (ookinete). Absent conoid.
- Naked sporozoites and pigments granules (hemozoin) are formed by the host cell Hb.
- Flagellated microgametes.

Family: Plasmodidae

1) Genus *Plasmodium*

General characters:

The same features plus:

- Protozoa causing malaria in both mammals and aves.
- Vector is mosquitoes (*Anopheles* in mammalian spp. and *Culex* and *Aedes* in avian sp.). Schizonts occur in vertebrate RBCs.
- Common species including both mammalian (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*) and avian (*P. gallinaceum* in chicken and *P. durnae* in turkeys) species.

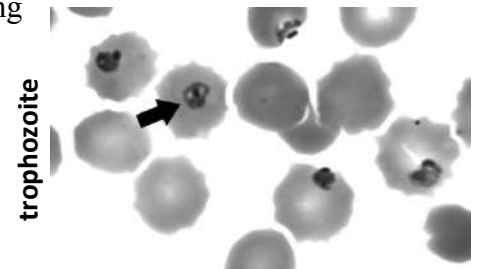
Life cycle and morphology of stages:

- Mosquitoes (invertebrate host) are the final host (gametogony and sporogony)
- Humans and birds (vertebrate host) are the intermediate host (schizogony).
- The infective female mosquito has the sporozoites in its salivary glands. The sporozoites are long thread-like or spindle shaped with a chromatin dot (nucleus) in the middle.

In vertebrate host:

I. Pre-erythrocytic (exo-erythrocytic) stage:

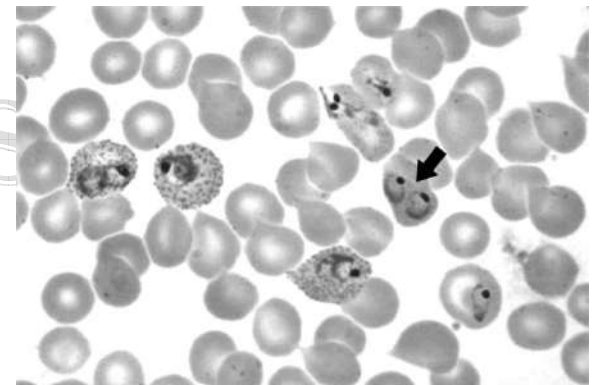
- During the bite of an infected female mosquito, sporozoites (long thread-like/spindle with one central chromatin dot, the nucleus) are injected, with saliva, into the skin and find their way to the circulation.



- Within 15-30 min, they reach the liver parenchyma, where they rapidly multiply forming hidden merozoites (merozoites-like)

known as **cryptozoites** forming **primary exo-erythrocytic schizonts**. These merozoites undergo multiple fissions to form **metacryptozoites**.

- The later invade new hepatic cells to form the **secondary exo-erythrocytic schizonts**, from which another multiple fissions occur to form new metacryptozoites.

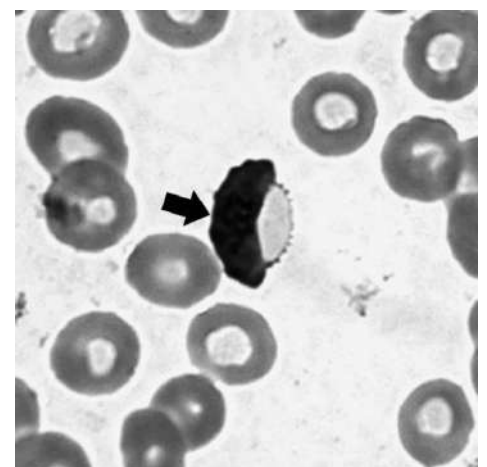


Signet-ring form

- All hepatocytes morphologically altered with their nuclei are often shifted to the periphery as a result to the presence of large number of metacryptozoites. The number of schizont generations differs according to the species.

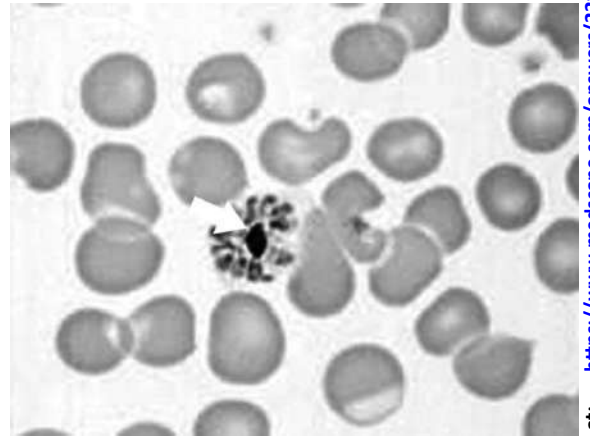
II. Erythrocytic (ring) stage:

- Following the schizogony, cryptozoites break out hepatocytes to reach the blood stream in which each metacryptozoite, inside RBCs, is rounded up forming a **trophozoite** leaving a large central vacuole and consists of a red nucleus shifted at one pole and a thin ring of blue cytoplasm (with Romanowsky stain), so called a **signet-ring form**. The later changes to the amoeboid form with nuclear



fragmentation, each is surrounded by a portion of cytoplasm forming **merozoites** which feed on the infected host cells Hb producing pigment granules (**hemozoin**) and other toxic secretions.

- Hemolysis occurs with the release of merozoites and schizonts that invade another intact RBCs with a frequent liberation of hemozoin and toxins. These products are responsible for symptoms of chilling and fever of malaria. The duration differs according to species (2 days for *P. falciparum* and *P. vivax* and 3 days for *P. malariae*).



- Repeated divisions occur to produce several generations of merozoites [merozites may re-invade the hepatocytes in mammalian species or RES in avian species inducing a recurrent infection, the **post-erythrocytic stage**] and the production of gametocytes starts (gametogony). Macrogametocyte (**macrogamont**) is ovoid (long and slender) with a compact nucleus, deep blue cytoplasm and a large number of pigment granules. Microgametocyte (**microgamont**) is ovoid (short and broad) with a diffuse nucleus, pale blue cytoplasm and few pigment granules at poles.

In an invertebrate host:

- Once the adult female mosquito attacks an infected human, gametocytes are ingested. Inside the insect midgut, rupture of RBCs with the release of gametocytes. By the process of exflagellation, each microgametocyte gives rise to 6-8 long and thin flagella-like microgametes (while each macrogametocyte gives rise to **one** macrogamete) which still attach to the parent cell till fertilize the macrogamete. Note that, inside the insect midgut, all *Plasmodium* stages are digested except gametocytes. Fusion occurs, in the midgut, to form a worm-like motile zygote, **ookinete**, that penetrate the gastric wall to form a spherical-shaped structure, **oocyst**, in the basement membrane.
- Nucleus and cytoplasm divide frequently to produce thousands of spindle-shaped **sporozoites**. Accordingly, the oocyst is renamed **sporocyst**. Rupture of the sporocystic wall occurs with the release of sporozoites in the hemocoel via the hemolymph to each all tissues, particularly **salivary glands**.
- Sporozoites are inoculated into a new host during getting blood meals. Infected mosquitoes still carries the infection during its life span.

2) Genus *Haemoproteus*

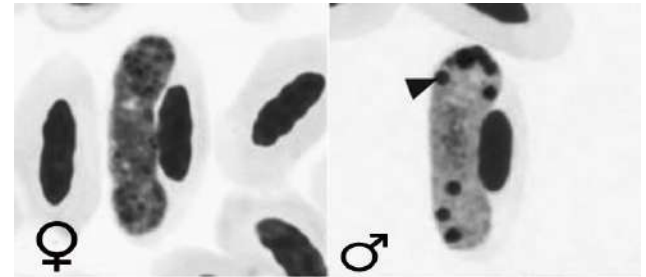
General characters:

It resembles *Plasmodium* spp. but:

- They are parasites of domestic/wild pigeons, doves, ducks, geese, turkeys and other wild birds.
- Gametogony occurs in peripheral blood, while schizogony occurs in the endothelial cells of blood vessels, particularly those of lungs.

Macrogametocyte:

It is kidney or sausage-shaped, more **slender** and **completely** encircling the host nucleus. On staining, it has a dark-blue cytoplasm with small, dark red and compact nucleus. Pigment granules are **dispersed** throughout the cytoplasm.



Microgametocyte:

It is kidney or sausage-shaped, more **broad** and **partially** encircling the host nucleus. On staining, it has a pale-blue to pinkish cytoplasm with large, pale pink and diffuse nucleus. Pigment granules are **condensed** in polar spherical masses at both **poles**.

- Only **one generation of schizont**.
- Macrogametes and microgametes (one microgametocyte produce **4** snake-like microgametes) present in erythrocytes range from tiny ring forms to elongate crescent shapes.
- Insect vectors are *Pseudolynchia canariensis* (*H. columbae*) and *Culicoides* (*H. nettioni*).

Common species:

H. columbae (pigeons), *H. nettioni* (ducks, geese) and *H. meleagridis* (turkeys).

Pathogenesis:

Only *H. columbae* is slightly pathogenic. In heavy infections, birds (particularly squabs) may appear restless, off food, anemic (due to hemolysis). The liver and spleen may be enlarged and congested.

Diagnosis: Detection of gametocytes in the peripheral blood.

3) Genus *Leucocytozoon*

Species *L. simondi*

General characters:

It resembles *Plasmodium* spp. but:

- Parasites of ducks and geese as a vertebrate host and the insect vector is *Simulium* (Black fly).
- Schizogony occurs in the endothelial and parenchymatous cells of liver, heart, kidneys and other organs of avian hosts (not in RBCs).
- Gametocytes occur in circulating blood, mainly **lymphocytes**. Mature gametocytes are elongated, oval or spindle bodies of 14-15 μm long. The infected host cell becomes greatly distended and elongated. The nucleus of the infected host cell is elongated and forms a long thin dark crescent along one side of the parasitized cell. Macro- and microgametes could not be differentiated, however, the former appears with dark blue vacuolated cytoplasm and a compact nucleus, while the later is slightly smaller with pale blue cytoplasm and a diffused nucleus. Rounded forms of the parasite may occur in undistorted cells.

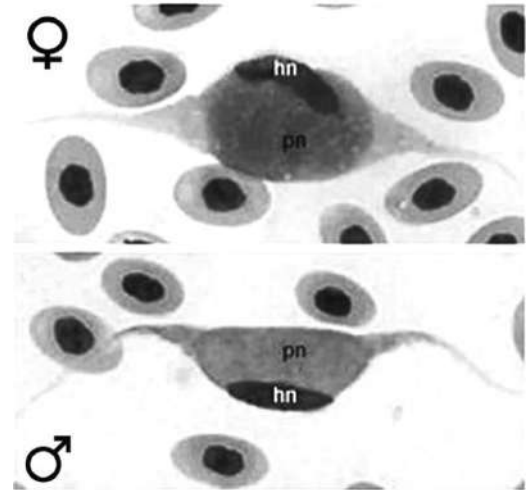


Image at: Zhao et al. (2015). PLoS ONE 10(7):e0133478.
DOI: 10.1371/journal.pone.0133478

Pathogenesis:

It is more pathogenic for ducklings, particularly in the evening, with cessation of feeding and rapid respiration due to occlusion of pulmonary capillaries with schizonts. Splenomegaly and a hepatic hypertrophy are common.

Family: Haemogregarinidae

- Heteroxenous hemoparasites with active ookinetes.
- Merogony and gametogony occur in vertebrates, while sporogony in the invertebrates.
- One microgametocyte produces 1-4 microgametes.

Genus *Hepatozoon*

Species *H. canis*

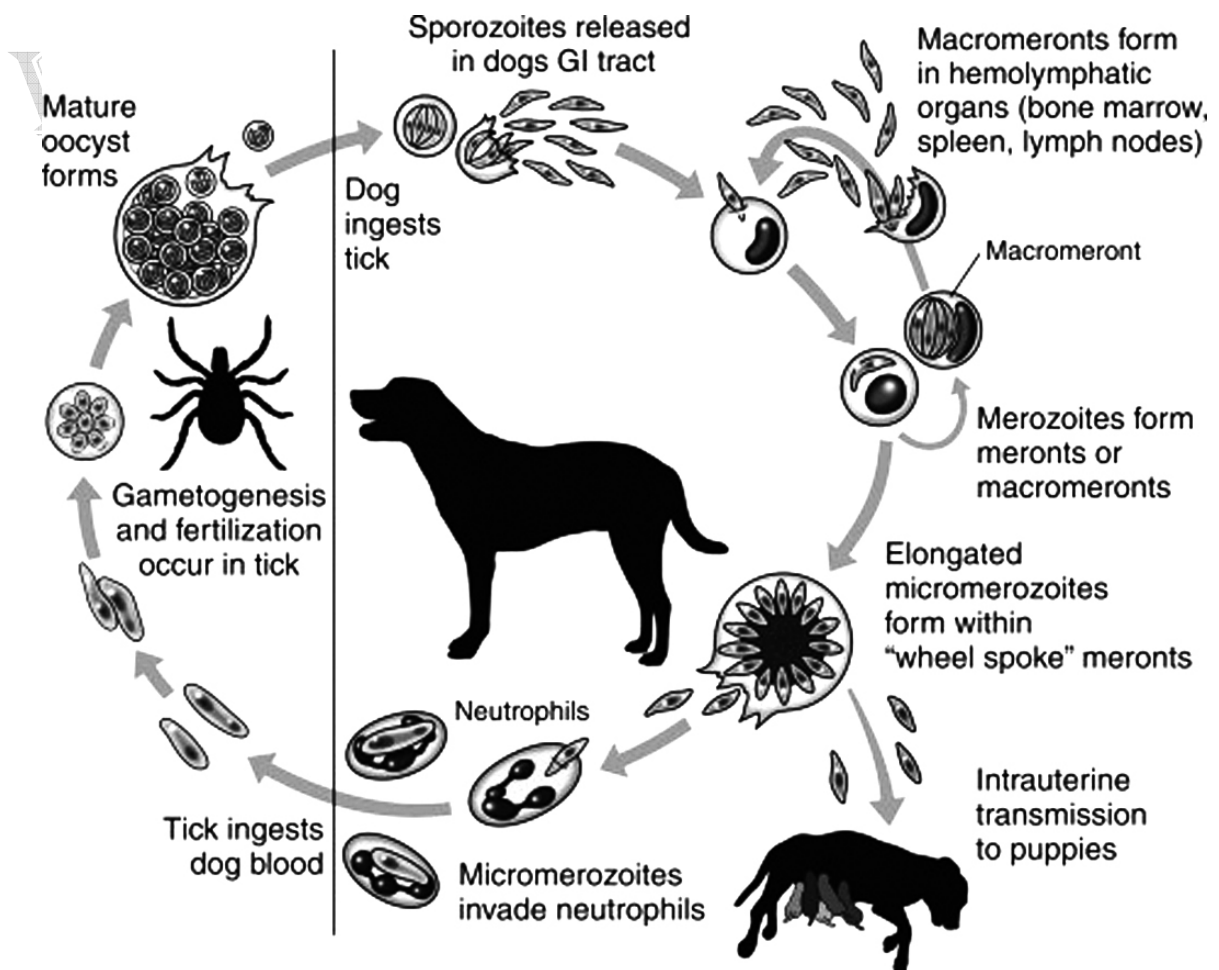
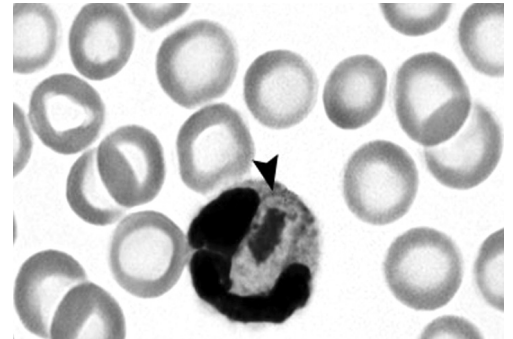
General characters:

- The insect vector is the hard tick, *Rhipicephalus sanguineus* (**brown dog tick**). Dogs are infected by **ingestion** of infected ticks.

- Schizogony occurs in viscera, while gametogony takes place often in **neutrophils** (or may be in RBCs).

Life cycle:

- Following the **ingestion** of infected ticks, sporozoites are released in the intestine penetrating its wall, via blood, to viscera like liver, lungs, spleen and bone marrow.
- In those tissue, the parasite undergoes multiple fission forming schizonts with numerous merozoites. Several generations are produced. By the end of this process, gametocytes are produced.
- During attacking dogs to get blood meals, the brown dot ticks are infected. Fertilization occurs giving the ookinete that penetrate the vector intestinal wall to the hemocoel forming oocysts containing sporocysts and sporozoites.
- Dogs are infected when ingesting infected ticks with a rupture of both oocysts and sporocysts releasing sporozoites.



Biology and characteristics of Haemosporidia:

Item	<i>Plasmodium</i> spp.	<i>Haemoproteus columbae</i>	<i>Leucocytozoon simondi</i>
Vertebrate host	Humans and birds	Pigeons	Ducks and geese
Vector	<i>Anopheles</i> , <i>Culex</i> , <i>Aedes</i>	<i>Pseudolynchia canariensis</i>	<i>Simulium</i>
Schizogony	Pre-erythrocytic (liver, spleen, B.M) and erythrocytic stages	Endothelial cells of internal organs (liver, lung and spleen)	Parenchymatous organs mainly liver and lung
Diagnostic stage	Signet-ring and gametocytes in RBCs	Gametocytes in RBCs	Gametocytes in neutrophils
Gametogony and sporogony	Inside the vector		

Oder: Piroplasmida

General characters:

- Intra-erythrocytic pyriform, rounded, rod-shaped or amoeboid heteroxenous parasites of vertebrates. Few species are found in WBCs or other cells.
- Conoid, hemozoin, spores, oocysts, flagella and cilia are absent. The most predominant complex structures are polar ring and rhoptries.
- Locomotion occurs by gliding.
- Vectors are ixodid ticks.
- Schizogony in vertebrates and sporogony in ticks.

Family: Babesiidae

1) Genus *Babesia*

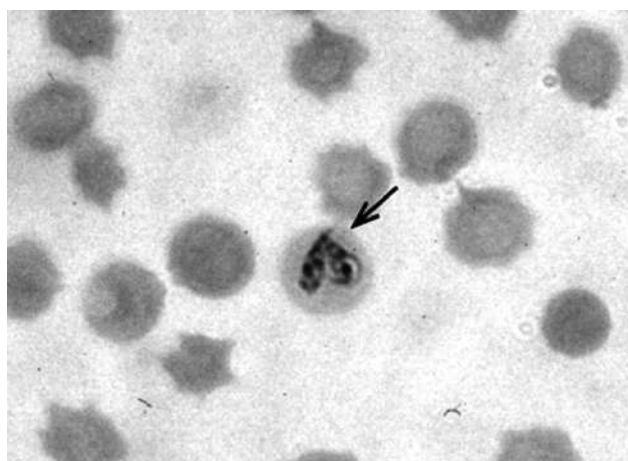
- Babesiae are cosmopolitan intraerythrocytic parasites affecting a variety of mammals including humans (*Babesia microti* and *B. divergens* are incriminated in human infection) with ticks are the main vector (usually, *Rhipicephalus*, formerly *Boophilus*, ticks are responsible for transmitting the infection).
- Bovine babesiosis (BB) is the most common highly fatal and economically important tick-borne protozoal disease, **babesiosis (piroplasmosis, Red-water disease, Texas fever or tick fever)**.

- Victor Babes (1888) was the first who described outbreaks of hemoglobinuria in Romanian cattle. He believed the causative piroplasm in blood is a bacterium and named it *Haematococcus bovis* which later described as *Babesia bovis*. Shortly afterwards, in Texas, Smith and Kilborne (1893) described a similar piroplasm causing Texas fever and named it *Pyrosoma bigeminum* which later redescribed as *Babesia bigemina*. The infection was closely associated with the occurrence of the ticks, *Boophilus annulatus*.
- Babesiosis is widespread whenever ticks are common. Although *B. bigemina* is the most widespread species in Africa, outbreaks of babesiosis referred to *B. bovis* are more common.
- Organisms are pear-shaped with an angle inside the RBCs. When stained, they show a blue cytoplasm and a red chromatin mass usually at one pole.
- They multiply in the erythrocytes by asexual division, producing two non-pigmented amoeboid parasites.

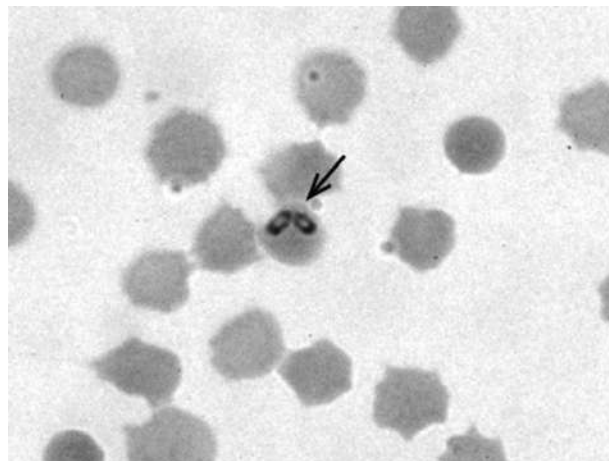
Common species of *Babesia*:

Species	Host	Morphology	Vector
<i>B. bigemina</i>	Cattle, buffaloes	Trophozoite is large (4-5 μm), pyriform, rounded, oval or irregular. Pyriform members occur in pairs with an acute angle. They are highly pathogenic.	<i>Boophilus microplus</i> <i>B. annulatus</i> <i>B. decoloratus</i>
<i>B. bovis</i>	Cattle	Similar to <i>B. bigemina</i> but smaller, in the center of RBCs. Vacuolated (signet-ring shape) forms are common.	<i>B. annulatus</i> <i>B. decoloratus</i>
<i>B. divergens</i>		Similar to <i>B. bovis</i> but smaller, tend to locate at the periphery with a relatively large angle. European sp.	<i>Ixodes ricinus</i> <i>Haemaphysalis longicornis</i>
<i>B. major</i>		Similar to <i>B. bovis</i> but larger.	<i>H. punctata</i>
<i>B. motasi</i>	Sheep, goats	Similar to <i>B. bigemina</i> . Paired pyriform trophozoites are	<i>H. punctata</i> <i>Rhipicephalus</i> spp.

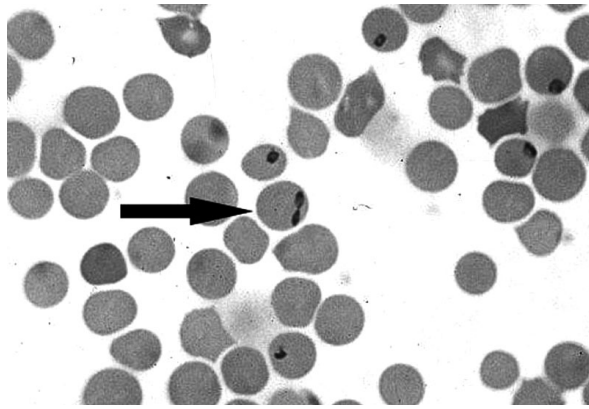
		common. Highly pathogenic in sheep.	
<i>B. ovis</i>	Sheep	Small babesiae. The majority are rounded and tend to be at the periphery. Less pathogenic.	<i>Rh. bursa</i>
<i>B. caprae</i>	Goats	Similar to <i>B. ovis</i> .	<i>Rhipicephalus</i> spp.
<i>B. caballi</i>	Equines	Large-sized, resembling <i>B. bigemina</i> with an acute angle	<i>Dermacentor</i> spp. <i>Rh. bursa</i>
<i>B. equi</i> (<i>N. equi</i>)		Small-sized, mostly are rounded. Pyriform shapes found in tetrads forming maltese-cross shape.	
<i>B. canis</i> - <i>B. canis canis</i> - <i>B. canis rossi</i> - <i>B. canis vogeli</i>	Dogs	Large -sized, usually pyriform/amoeboid containing vacuole. Multiple infections of up to 16 merozoites are found.	<i>Rh. sanguineus</i>
<i>B. gibsoni</i>		Small -sized and pleomorphic (mostly rounded).	<i>Rh. sanguineus</i> <i>Haemaphysalis</i> spp.
<i>B. felis</i>	Cats	Small-sized, mostly rounded/irregular (rarely pyriform).	<i>H. leachi</i>
<i>B. microti</i>	Rodents	Similar to <i>B. equi</i> .	<i>Ixodes</i> spp.



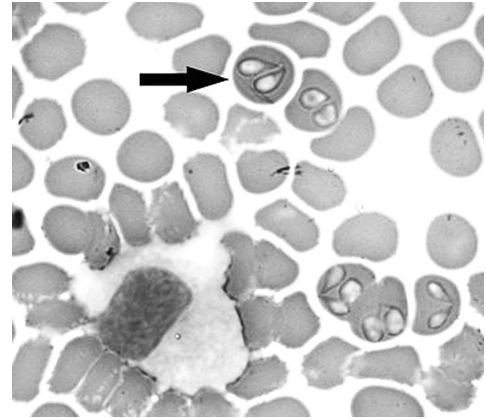
B. bigemina



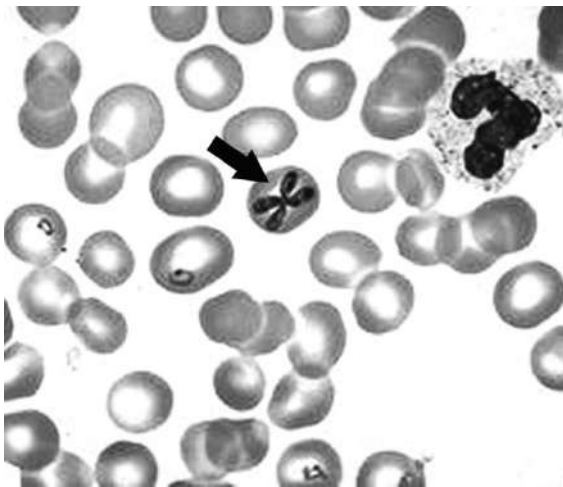
B. bovis



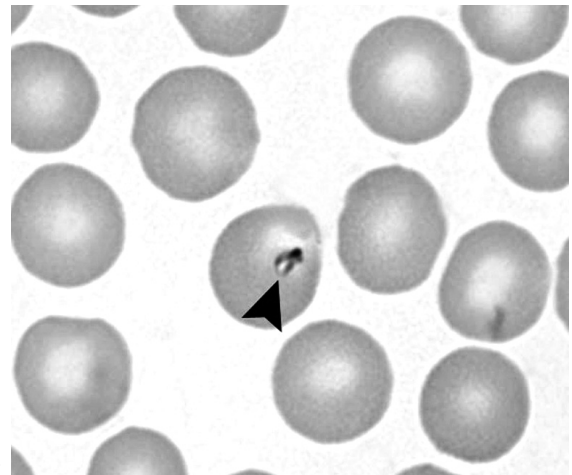
B. divergens



B. canis



B. microti (B. equi)



B. gibsoni

Image at: <https://www.wikidata.org/wiki/Q1002578>

Image at: <https://microbewiki.kenyon.edu/index.php/Babesia>

Image at: <https://commons.wikimedia.org/wiki/File:Ehrlichia%2BBabesia-dog.jpg>

Image at: El-Dakhly et al. (2015). J. Parasitol. 101(2): 160-166.

Mode of transmission:

In tick vectors, the development and transmission of babesiae is either:

A) Transovarian (transovarial) transmission: (vertical transmission)

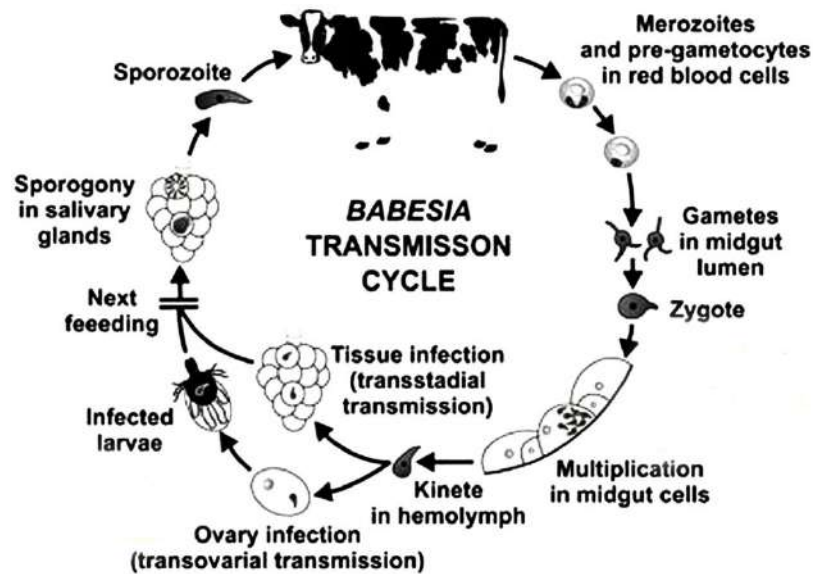
- It occurs for all *Babesia* spp. **except** *B. microti* and *B. equi*.
- It occurs in one-host ticks.
- It means that uninfected female ticks acquire the infection from the infected animals with the subsequent invasion and multiplication in **ovaries**. Thus, all the subsequent **progeny** will be infected.

Acquire infection

Uninfected female tick → multiplication in ovaries, oocytes and oviduct → several multiplications → all eggs become infected with dormant parasites → after a full engorgement, female drops on the ground to lay eggs → eggs hatching and larval stages infest an appropriate host → babesiae are activated due to stimulation of the heat released from the warm-blooded host.

- As ova develop to larvae, babesiae (organisms) repeatedly multiply forming small multinucleated amoeboid vermicules that later turn into sporozoites migrating to salivary glands to be injected into the host during obtaining blood meals. Accordingly, larvae, nymphs and adults can transmit the infection.

- Ticks can remain infected for several generations **without** the need to further blood meals.



B) Transtadial (stage to stage) transmission: (horizontal transmission)

- It occurs only in *B. microti* and *B. equi*.
- A similar development occurs with **no ovarian** involvement. By this way, one infected larva/nymph develops to one nymph/adult.
- Ticks become **cleansed** after transmission and a **further need** to blood meals from an infected host is **mandatory** for reinfection.

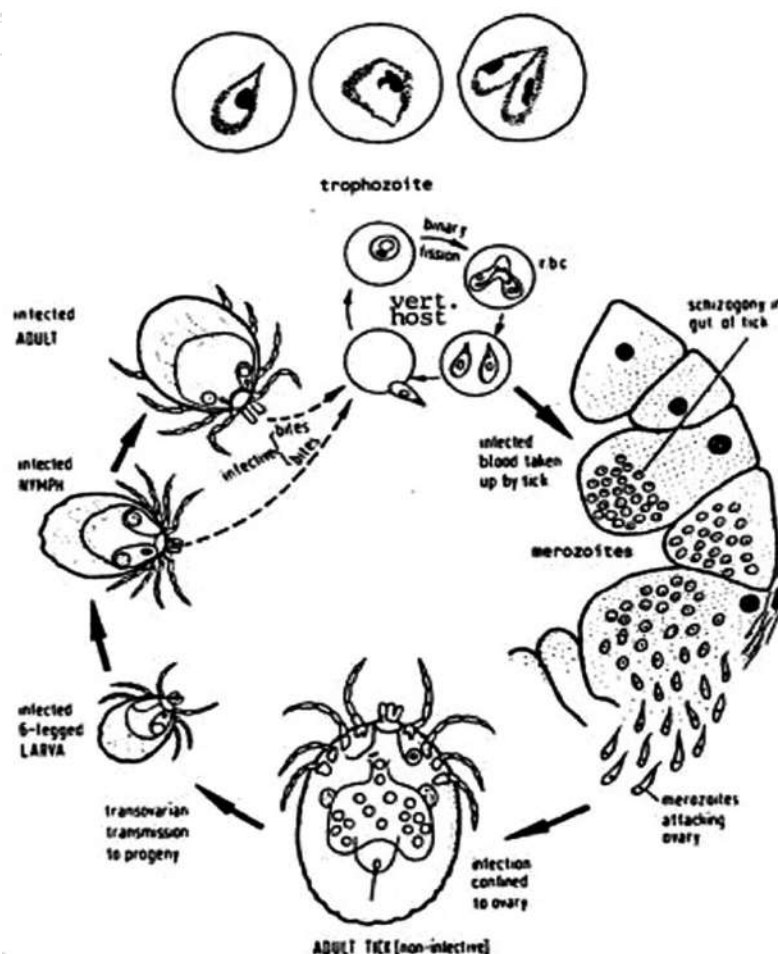
Life cycle: (*B. bigemina*)

- Following inoculation by ticks vector (*Boophilus* spp.), released **sporozoites** (often in transovarian species) penetrate the cell membrane of erythrocytes.
- Once inside, they become **trophozoites** which undergo binary fission to give 2-4 **merozoites**.
- The later break out RBCs wall and immediately invade another intact cells with repeated schizogony and consequently multiple merozoites. This asexual cycle occurs indefinitely until the host dies or eliminates the parasites.
- Some merozoites remain unchanged except taking an unusual shape and act as **gamonts** (gamont precursors). They are called ray bodies (**Strahlenkorper** in dogs). They produce gametes often in the gut lumen of tick vector.
- During the passage from the host to the midgut, 2 populations of ray bodies develop from the gamonts. Ray bodies undergo further multiplication to form large aggregations of multinucleated cells. Once the division is completed, uninucleated ray bodies with thorn-like structure are differentiated into micro- and macrogametes.

- After tick engorgement (within 2-4 days), gametes fuse together in pairs (initial syngamy) forming a spherical zygote that will be motile, **ookinete** or **primary kinete**.
- The later invades the gut epithelium with further multiplication (asexually) to form more club-shaped ookinetes (**vermicules**, **sporokinetes** or **large merozoites**) which escape into the hemolymph and invade a variety of tissues (**including uterus, ovaries and oocytes**) where successive series of secondary schizogony occur to form more vermicules. These cycles continue till the infected tick **dies**.
- Kinetes invade salivary glands and acini and undergo asexual division (**sporogony**) to form multinucleated stages (**sporozoites** or **small merozoites**) which are released in the salivary gland lumen mixed with saliva. Thousands of sporozoites (broad anteriorly and narrow posteriorly) are formed and become active within 5 days after taking the blood meal.

Note:

In *B. bigemina*, the development of infective sporozoites lasts for **9** days, while it takes 2-3 days in *B. bovis*. Moreover, the infective forms of *B. bigemina* are inoculated into cattle by both nymphal and adult stages, while those of *B. bovis* are injected via larvae.



Pathogenesis:

- Babesiae produce a highly pathogenic disease with the mortality rate is much higher in adults (young animals often get a subclinical infection. Recovered cases remain symptomless carriers with a low parasitemia for years.
- Clinical signs depend on the parasite species and vaccination status (host immunity):

1- Acute form: 3-7 days

- A high fever ($>40^{\circ}\text{C}$) for several days, depression, weakness, increased respiratory rates, hemoglobinuria (due to severe destruction of RBCs as a result of parasite multiple fission), anemia (due to decreased numbers of RBCs) with a release of Hb pigments in the circulation, dehydration, jaundice, swollen liver and splenomegaly.
- Death may occur due to anemia, oedema, jaundice and blocking of capillaries by infected RBCs.
- During pregnancy, fever may lead to abortion. The temperature decreases to subnormal just before death.

2- Chronic form:

Animals with fewer parasites and no clinical signs except weakness and emaciation. In humans, signs resemble those of malaria i.e. fever and chilling.

Diagnosis:

- Clinical signs and history of ticks on animals.
- Microscopic examination of Giemsa-stained thin blood films.
- Serological techniques.

Control:

- Prophylaxis via vaccination and eradication of ticks.
- Treatment using appropriate drugs. i.e. Imizole, Acaprine, Berenil.

2) Genus *Nuttallia*

Species *N. equi* (*B. equi*)

It closely resembles genus *Babesia* except:

- It occurs in equines.
- The parasite is found as **tetrads** i.e. in the form of 4-jointed merozoites forming a **maltese-cross** shape. In acute infections, more than 4 merozoites are formed.

3) Genus *Aegyptianella*

Species *A. pullorum*

- Haemoparasites of chickens, ducks and geese.
- The trophozoite is small rounded, oval or pyriform, often found in 8-16 merozoites intraerythrocytes with several generations of schizogony.
- The vector is the fowl soft tick, *Argas persicus*.

Family: Theileriidae

Genus *Theileria*

General characters:

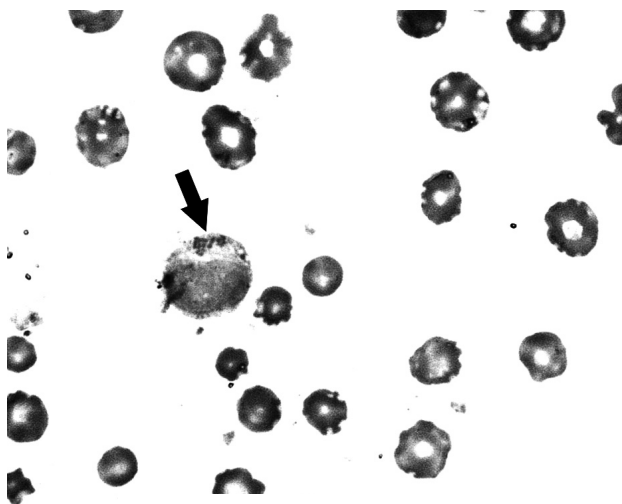
- Small, rounded, ovoid, irregular or bacilliform (rod) parasites.
- Schizogony occurs in **lymphocytes** followed by invasion of RBCs (similar to that occurs in *B. equi* and *B. microti*).
- Intraerythrocytic stages may (2-4 merozoites) or may not multiply.
- Apical complex structures are absent except rhoptries and polar ring.
- Pigments are **not** produced.
- Vectors are ixodids affecting mainly cattle, sheep and goats.

Item	(1) <i>Th. parva</i>	(2) <i>Th. annulata</i>
Disease	East Coast fever, African Coast fever, bovine theileriosis	Egyptian fever, Mediterranean Coast fever, tropical theileriosis
Hosts	Cattle, water buffaloes	Cattle, water buffaloes, sheep, goats
Distribution	Eastern, central and southern Africa	North Africa, South Africa
Location	Lymphocytes and RBCs	
Structure	• Rod/comma-shaped are usually inside RBCs. Rounded forms may be found. • Multiplying forms in lymphocytes and cells of RES (spleen, L.Ns) are called Koch's blue bodies (Koch's bodies). Each is circular or irregular with blue cytoplasm and numerous nuclei appear as red chromatin granules on staining with Romanowsky stain. Two types of schizonts: macroschizonts	• Rounded/oval forms are often found inside RBCs. Rod/comma-shaped organisms may be found. • Multiplying forms in lymphocytes of L.Ns and spleen produce Koch's blue bodies either intracellular or in their lumen. • Inside RBCs, parasite multiplication produces 2-4 daughter merozoites.

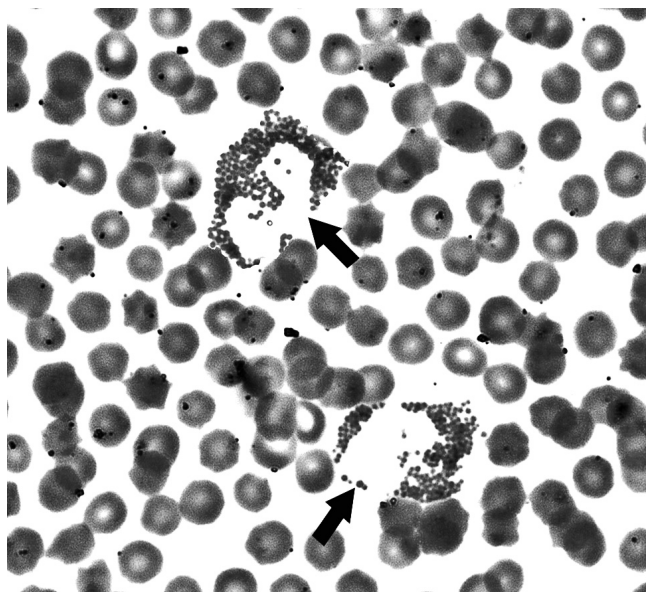
	(macromerozoites) and microschizonts (micromerozoites). Inside RBCs, parasites multiply by binary fission forming 2 merozoites.	
Vectors	<i>Rh. appendiculatus</i> , <i>Hyalomma excavatum</i>	<i>Hyalomma excavatum</i>
Pathogenesis	It is highly pathogenic for cattle (particularly imported species). Adults are more affected showing enlargement of L.Ns and spleen, hemorrhages on mucous and serous membranes (due to release of toxic metabolic products) with ulcers on the abomasal wall and red streaks on the intestinal walls.	• Mortality rate of 10-90%. • Enlargement of of L.Ns, spleen and liver. • Marked anemia, bloody diarrhea and icteric mucous membranes. • The disease may appear in: - Peracute form: death within 2-4 days. - Acute form: weakness, decreased milk production and enlargement of L.Ns. - Chronic form: irregular fever, anemia, icterus and severe emaciation.

(3) *Th. mutans* (*Gonderia mutans*)

- It produces benign bovine gonderiosis (theileriosis) in cattle and water buffaloes.
- It resembles *Th. annulata*.
- Mainly rounded/oval-shaped, and comma or *Anaplasma*-like forms are found.
- Few Koch's blue bodies in lymphocytes.
- Slightly pathogenic or non-pathogenic.
- Vectors are ixodids (*Amblyomma* spp.)



Theileria spp. macroschizont



Theileria spp. microschizont

Life cycle: Trans-stadial transmission (no ovarian involvement)

- Infective comma-shaped sporozoites are inoculated with saliva of infected tick during blood meals (activation of sporozoites occurs within 2-3 days), invade lymphocytes and enlarge by ingesting the host cell cytoplasm.

- The nucleus of sporozoite divides by binary fission to form large schizonts (macroschizont, macromeront or Koch's blue bodies) which contain 13-50 nuclei. Each produced organism is called macromerozoite. The duration of this process is 8-10 days in *Th. annulata* and 12-14 days in *Th. parva*.

- The schizont becomes rosette-like and macromerozoites change to micromerozoites, so, the schizont is termed microschizont (micromeront). The later rupture and merozoites become free and actively penetrate RBCs and multiply by binary fission forming 2 merozoites leading to hemolysis (so, anemia develops).

- Merozoites change to ovoid forms, gamonts, which are taken by

ticks. Inside the later, some change, within 2-4 days in the gut, to spindle-shaped ray bodies with 4 nuclei and several flagella-like appendages, ready to produce 4 microgametes in the gut epithelium. Other forms are considered as macrogametes.

- Syngamy occurs to produce a spherical zygote which change to a club-shaped motile stage, **ookinete**. The later penetrates the intestinal wall and reach salivary glands via the hemolymph. No multiplication in other tissues. Ookinete proliferate to produce sporonts that give sporoblasts which in turn give the comma-shaped sporozoites again.

Diagnosis: As in babesiosis.

Control: As in babesiosis, but oxytetracyclines are the drugs of choice for treatment. Vaccination against certain species does **not** give a protection against other species.

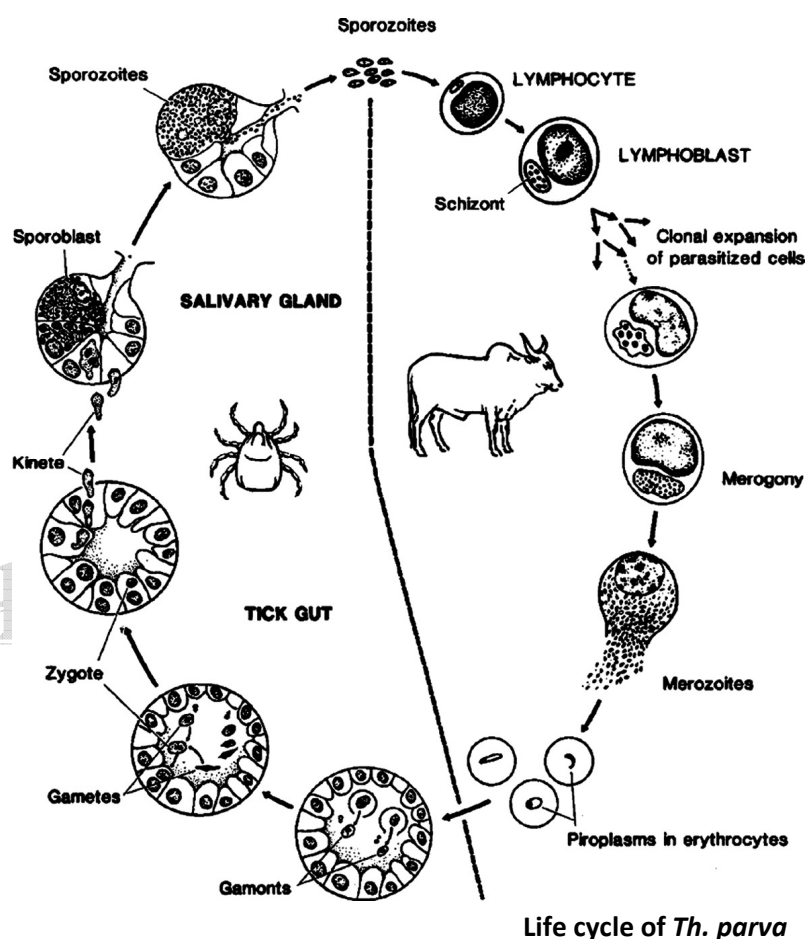


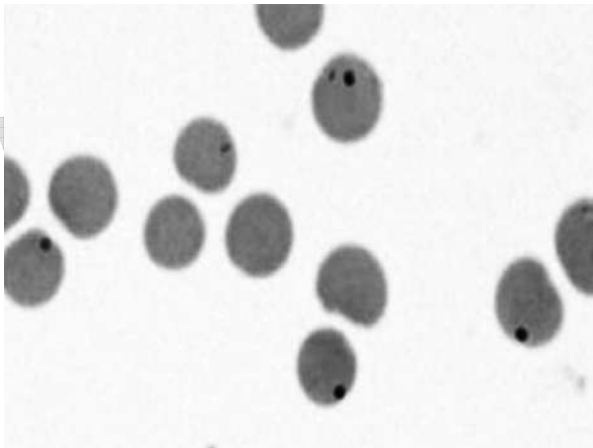
Image at: Marcelino et al. (2012). J. Proteomics., 75: 4232-4250.

Organisms of uncertain classification

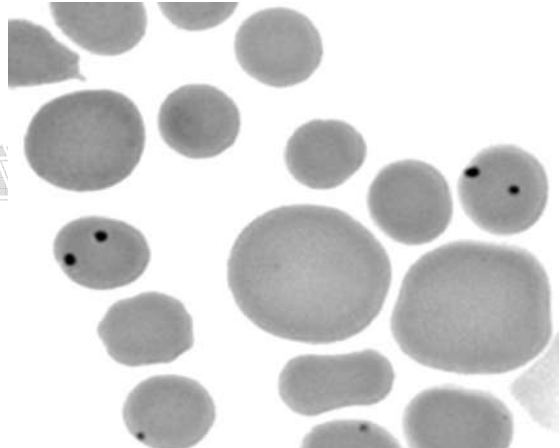
Organisms described in this section were initially grouped as protozoa, but nowadays, they are categorized as rickettsia.

Genus *Anaplasma*

- Small spherical intraerythrocytic bodies of cattle, buffaloes, sheep and goats. They appear as dark red granules without cytoplasm and surrounded with a faint halo when stained with Giemsa.
- They are transmitted by blood sucking vectors; ticks, flies, mosquitoes and *Tabanus*.
- Two forms of *Anaplasma* are known:
 - 1) *A. marginale*: The parasite occurs near to the **margin** of the red blood cell. It causes bovine anaplasmosis, a mild disease in cattle with fever and severe anemia. In sheep and goats, it is called *A. ovis*.
 - 2) *A. centrale*: The organism occurs near the **center** of the red blood cell. It is less pathogenic.



A. marginale



A. centrale

Phylum: Ciliophora

Class: Ciliata

- Members are characterized by the presence of cilia for locomotion as well as a macronucleus and a micronucleus. The macronucleus is large, compact and responsible for the cytoplasmic activities (vegetative functions), while the micronucleus is vesicular and is concerned with the reproductive process.
- The great majority of ciliates are free-living/commensal with a few is parasitic.

Family: Balantitidae

The same characters plus:

- The cytoplasm contains food and contractile vacuoles.
- Reproduction is either by transverse binary fission (asexual) or by conjugation (sexual).

Genus *Balantidium*

A parasite of the large intestine. One parasitic species is of veterinary medical importance in pigs and humans is *Balantidium coli*.

Balantidium coli

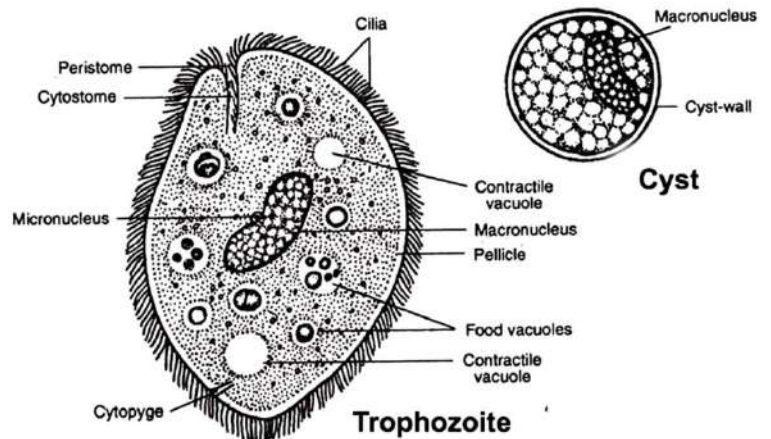
It is the only member of the ciliates known to be pathogenic to humans.

Hosts and habitats:

It is present in nests within tissues of caecum and colon of humans (zoonotic) and pigs.

Morphology:

- It has two developmental stages, trophozoite and cyst stages.
- The trophozoite is oval, ovoid, pear or ellipsoidal in shape (the anterior end is narrower than the posterior one) with varying sizes (30-300 μm long). It has two nuclei, the macronucleus is large, kidney- or sausage-shaped, and the micronucleus is spherical nested next to and often hidden by the macronucleus.

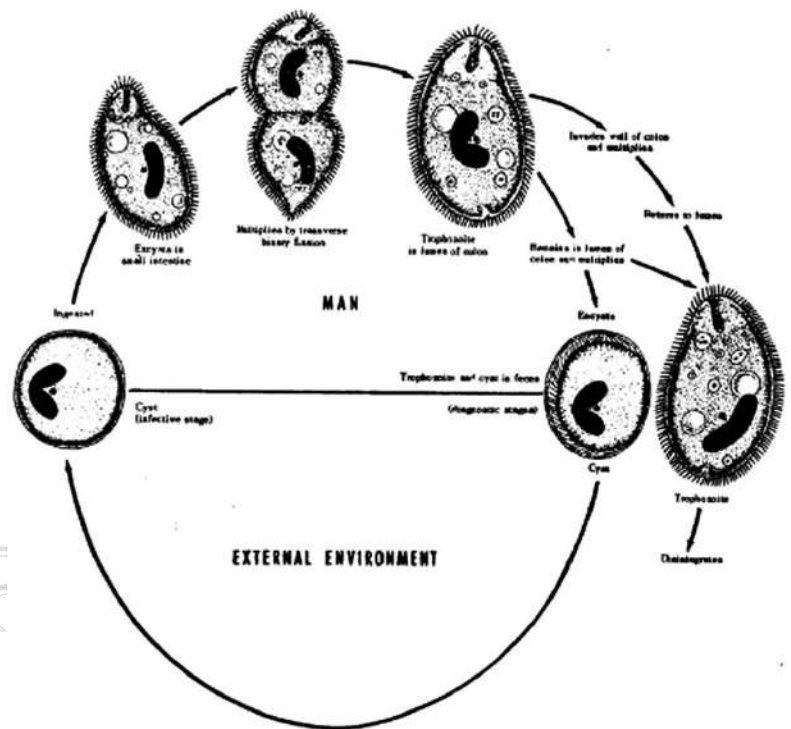


- At the pointed anterior end, the opening, the **peristome**, leads to the **cytostome** (the mouth) which lead to a narrow canal, **gullet** (cytopharynx), that open directly into the first third of the cytoplasm. The later is enclosed in a thin wall (**pellicle**).

- There are two contractile vacuoles, one terminal and the other near the center of the body. The cytoplasm has numerous food vacuoles which contain starch granules, cell fragments, bacteria and erythrocytes.
- Cysts are smaller (50 μm in diameter), rounded with a tough, thick non-ciliated cyst wall made of one/two layers. Micronucleus is absent.

Life cycle:

- Infection occurs when a host ingests a cyst via consumption of contaminated water/ food. Once ingested, it passes through the host's digestive system. In the small intestine, trophozoites are produced and colonize in the large intestine, where they live in the lumen and feed on the intestinal flora. Some of them invade the wall of the colon using proteolytic enzymes and multiply, and others return to the lumen inside which they may disintegrate or undergo encystment.



- Encystment is triggered by dehydration of the intestinal contents and usually occurs in the distal large intestine, but may also occur outside of the host in feces. Mature cysts are released into the environment where they can go on to infect a new host.

Pathogenesis:

- In pigs, it is a commensal in the lumen of the large intestine. Occasionally, it may invade the mucosa and cause superficial and even deep ulcerations associated with a mild to severe enteritis.
- In humans, it produces superficial to deep ulcers associated with dysentery. The early lesions resemble those produced by *Entamoeba histolytica*. Human infection is a zoonosis and is usually acquired from swine through the contamination of food stuffs with pig feces.

Clinical signs: Symptoms, if present, include diarrhea, dysentery, and abdominal pain.

Diagnosis: Microscopic examination of fecal samples reveals cysts/trophozoites.

Phylum Microspora

- Parasitic members of invertebrates except genus **Encephalitozoon** (vertebrates).
- Spores are unicellular with a single nucleus and cytoplasm. (**sporoplasm**) and a unique **polar tube** (polar filament) and a **polar capsule**.
- Vesicular nucleus.

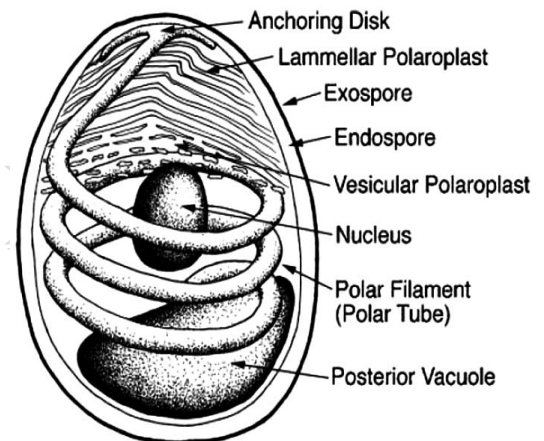
Family: Nosematidae

Genus *Encephalitozoon*

Species *E. cuniculi*

General characters:

- It is found in rabbits, domestic rats, dogs and probably humans.
- Spores are ellipsoidal or ovoid consisting of sporoplasm, coiled polar tube and a polar capsule (sporoplasm divides into sporoblasts and into spores).
- It resembles *Toxoplasma* but:
 - Absent apical complex.
 - Smaller trophozoite and rod-like.
 - Unclear when stained with HE and red-colored with carbol-fuchsin (blue in *Toxoplasma*).
 - Trophozoites are intracellularly in brain, kidneys, liver and macrophages.
 - The trophozoite is straight to slightly curved with both ends bluntly rounded and one end is little larger than the other. Slight constriction is found at the middle.
 - Oval/rounded, central and compact nucleus.
- The pseudocyst consists of approximately 100 trophozoites.



Life cycle: 2 days

- Trophozoites multiply by binary fission.
- The mode of natural infection is unknown (may be congenital).

Pathogenesis:

Nephritis, ascites, paralysis and death of rabbits may occur.