Toxoplasma gondii
Classification

- **Phylum:** Apicomplexa
- **Class:** Sporozoea
- **Subclass:** Coccidia
- **Order:** Eucoccidia
- **Suborder:** Eimeriina
- **Genus:** *Toxoplasma*
- **Species:** *gondii*
**Toxoplasma gondii**

- Worldwide distribution
- Zoonotic parasite; Toxoplasma is an **opportunistic** pathogen.
- Infects animals, cattle, birds, rodents, pigs, and sheep.
- and humans.
- Causes the disease **Toxoplasmosis**.
- Toxoplasmosis is leading cause of abortion in sheep and goats.
- Intracellular parasite.
- Final host (Felidae family, cat)
- Intermediate host (mammals)

**Toxoplasmosis**

1. All parasite stages are infectious.
2. Risking group: Pregnant women, meat handlers (food preparation) or anyone who eats the raw meat
First Described: The organism that was ultimately named Toxoplasma gondii was first described in France in 1908 (Nicolle and Manceaux)
Affected Host Species: Cats are the definitive host and are the only species known to complete the sexual phase of T. gondii culminating in the passage of oocysts in feces. Cats and most other vertebrates can serve as intermediate hosts; invertebrates can serve as transport hosts by mechanical carriage of T. gondii oocysts
*Toxoplasma gondii* exists in three forms. All parasite stages are infectious.

1. Tachyzoites
2. Tissue cysts (bradyzoit)
3. Oocysts
Tachyzoite stage

- Rapidly growing stage observed in the early stage of infection. (Acute phase) habits in the body fluid.
- Crescent-shaped. One end is more pointed than the other subterminal placed nucleus.
- Asexual form.
- Multiplies by endodyogeny.
- It can infect phagocytic and non-phagocytic, cells.
Bradyzoites

- Are slow-growing stage inside the tissue cysts.
- Bradyzoites mark the chronic phase of infection.
- Bradyzoites are resistant to low pH and digestive enzymes during stomach passage.
- Protective cyst wall is finally dissolved and bradyzoites infect tissue and transform into tachyzoites.
- Bradyzoites are released in the intestine and are highly infective if ingested.
**Oocysts in the feces of cat**

- Cat ingests tissue cysts containing bradyzoites.
- Gametocytes develop in the small intestine.
- Sexual cycle produces the oocyst which is excreted in the feces.
- Oocysts appear in the cat’s feces 3-5 days after infection by cysts.
- Oocysts require oxygen and they sporulate in 1-5 days.
The Oocyst

- The oocyst is noninfectious before sporulation.
- Unsporulated oocysts are subspherical to spherical.
- Sporulated oocysts are subspherical to ellipsoidal.
- Each oocyst has two ellipsoidal sporocysts.
- Each Sporocyst contains four sporozoites.
- Shedding occurs 3-5 days after ingestion of tissue cysts.
- Sporulated oocyst remain infective for months.
Toxoplasma gondii

Cat's intestinal enterocytes

Bradyzoites infect cells and become tachyzoites.

Multiplication

Tissue cyst

Zygote

Gametocytes

Encapsulation of zygote within a rigid wall: oocyst

Oocyst

Gametes

Sporulated oocyst

Back to final hosts

INTERMEDIATE HOSTS

Oocyst ingested by intermediate hosts

Merozoites

Sporulated oocyst

Bradyzoite

Unsporulated oocyst

Final hosts

Cat's intestinal enterocytes
The life cycle of Toxoplasma can be divided into two stages;
I The asexual cycle with little host specificity i.e., the stage that occurs in sheep, humans, rodents and birds
II The sexual stage of the life cycle, confined to the intestinal epithelial cells of cats, which results in the production of oocysts.

The asexual life cycle of Toxoplasma
Tachyzoite stage:
1. Cats shed millions of unsporulated oocysts in their faeces, these take 1-5 days to sporulate depending on the climatic conditions
2. The sheep ingests a sporulated oocyst
3. In the gut the sporozoites are released and they penetrate the intestinal wall and migrate via the lymphatic and portal systems
Cats (young strays in particular) are the primary source of infective oocysts as they shed millions in their faeces.
4. Tachyzoites penetrate host cells and become surrounded by a vacuole – Toxoplasma gondii can infect cells in the reproductive system, central nervous system, lung, liver and muscle tissue
5. The tachyzoites multiply asexually by a process called ‘budding’ 6. Once 8 – 16 tachyzoites have accumulated, the cell ruptures and new cells are infected
Some cases result in the death of the host, but more usually the host develops immunity to the infection and chronic infection is established, which is called the bradyzoite stage.

Bradyzoite stage:
1. Antibodies are produced by the host’s immune system and any extracellular parasites are eliminated.
2. The antibodies limit the invasiveness of intracellular tachyzoites to new cells, resulting in the formation of cysts which are found most frequently in the brain and skeletal muscle.
3. These cysts contain between a few and many thousands of organisms called bradyzoites, which grow very slowly – this is the latent form. If immunity wanes, cysts may rupture releasing bradyzoites.

The sexual stage of the life cycle of Toxoplasma
The sexual stage of the life cycle starts when a (usually) young cat ingests food containing cysts, such as a rodent. The walls of the cysts dissolve in the stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine and form gametocytes over the 3-15 days following infection. The formed microgametes are released and swim to and penetrate macrogametes. The resulting oocysts, each containing a fertilised gamete, are passed out of the cat and sporulate within 1 – 5 days.
Transmission
Toxoplasma gondii in the Feline host

Sporulated Oocyst (infective)

Sporogony

Asexual Cycle

Merogony

Tachyzoites

Sporulated Oocyst

Sexual Stage

Fertilization

Cyst Wall Formation

Unsporulated Oocyst

Passed in the faeces.
**T. gondii in the Intermediate host**

1. **Ingestion of Sporulated Oocyst**

2. **Excystation**
   - Sporozoites

3. **Asexual Cycle**
   - Tachyzoites
   - Rapid multiplication and dissemination of tachyzoites.
   - Tachyzoites invade brain, muscle & liver cells. Then become bradyzoites that slowly divide & form cysts.

4. **Some sporozoites invade deep tissues**

5. **Transplacental transmission to fetus.**

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[http://chirograteapp.com/bioscience.html]
Toxoplasma gondii in the Feline host

Start here

Paratelic Host

Ingestion of Paratelic host

Asexual Cycle

Bradyzoites

Rapid multiplication and dissemination of tachyzoites.

Some bradyzoites invade deep tissues.

Tachyzoites

Tachyzoites invade brain, muscle & liver cells. Then become bradyzoites, that slowly divide & form cysts.

Sexual Stage

Fertilization

Cyst Wall Formation

Unsporulated Oocyst

Passed in the feces.

(Sporogony)
Direct Life Cycle: *T. gondii*  
Cat to Cat

**Direct Life Cycle** (homoxenous)
- Definitive host = Felids only

**Transmission** -- fecal-oral, ingestion of oocyst

**Invasion** -- Sporozoites excyst from oocyst and invade enterocyte

**Asexual reproduction (in intestinal cells)**
- Endodyogeny AND Merogony
  - ~ 5 cycles
- Causes either no or mild pathology in the cat
- Some sporozoites invade deep tissues (CNS, muscle, viscera) multiply & disseminate as tachyzoites, and eventually form bradyzoite cysts
Pathogenesis: Toxoplasma gondii

- Intestinal phase in felids → minimal

- Systemic disease (extra-Intestinal phase) in felids, paratenic hosts or humans
  - Explosive replication of tachyzoites causes:
    - massive direct destruction of host cells
    - acute immune response
  - Most often affects brain, liver, lungs and striated muscles.
  - Tissue cysts cause physical cell/tissue damage and are a source for latent disease
Clinical Disease: *Toxoplasma gondii*

**cats only**

**Intestinal/Acute Disease** – usually no complaint
- Oocysts noticed on routine fecal
- 10-20% of cats develop self-limiting small bowel diarrhea

**Systemic Disease** -
- **Non-specific disease:** Fever, anorexia, vomiting, diarrhea, myositis, uveitis, enlarged lymph nodes, pneumonia (especially for FIV+ cats), encephalitis, nephritis, death
- Can transmit congenitally to kittens
Development of clinical toxoplasmosis is dependent on both host and parasite. Some strains of T. gondii may be more pathogenic than others, and some strains may have specific tissue affinities, such as a tendency to cause ocular disease in cats. If a poor immune response is mounted after primary infection, overwhelming tachyzoite replication that results in tissue necrosis is the major cause of disease. Eosinophilic fibrosing gastritis was recently described in a T. gondii–infected cat. Fatal extraintestinal toxoplasmosis in cats can develop from overwhelming intracellular replication of tachyzoites following primary infection; hepatic, pulmonary, CNS, and pancreatic tissues are commonly involved. Kittens infected by the transplacental or transmammary routes develop the most severe signs of extraintestinal toxoplasmosis and generally die of pulmonary or hepatic disease. Common clinical signs in cats with disseminated toxoplasmosis include lethargy, anorexia, and respiratory distress.
**Toxoplasma gondii**

**Non-felid hosts**

- **Cattle**
  - Congenital toxoplasmosis (abortion, but very rare), ingest oocyst from cat feces.

- **Horse**
  - Systemic toxoplasmosis, low pathology, ingest oocyst from cat feces

- **Rodents**
  - Systemic toxoplasmosis, ingest oocyst from cat feces or tissue cysts from prey
  - Decreased fear of cats
  - **Major source of infection for cats and pigs**
Toxoplasma gondii
Non-felid hosts

- Dogs
  - Systemic toxoplasmosis, ingest oocyst from cat feces or tissue cyst from prey
    - Less commonly develop clinical disease
    - May develop nonspecific signs: fever, neurological, ocular or respiratory signs
    - Rule-out Neospora caninum infection

- Sheep & Goats
  - Systemic & Congenital toxoplasmosis, ingest oocyst from cat feces
    - Systemic – CNS signs (circling, etc.)
    - Congenital – abortion
    - Toxovac S48 live vaccine available
Toxoplasmosis in Sheep

The signs of toxoplasmosis in sheep manifest following the exposure of a naive pregnant ewe to oocysts. The sporozoites ingested excyst in the digestive tract and penetrate the intestinal epithelium, before reaching the mesenteric lymph nodes around day 4 post-infection. Here, they cause lymphomegaly and focal necrosis before contributing to a parasitaemia from day 5. Pyrexia is associated with the development of parasitaemia. Following dissemination of *T. gondii* in the blood, many tissues become infected. Parasitaemia ends when the maternal immune response becomes effective, and protozoa start to encyst as bradyzoites. In pregnant animals, the uterus is an immunoprivileged site, and the outcome of foetal infection is influenced by the stage of gestation. In early pregnancy, the foetus is unable to mount any immune response, and so cannot inhibit parasite multiplication. The foetus rapidly dies and is resorbed. In a flock, this is visible clinically as large numbers of barren ewes. In mid-gestation (70-120 days), infection can again be fatal. This causes a mummified foetus which is often twinned with a lamb that is stillborn or weak. Abortion due to infection at 70-120 days gestation tends to occur in very late pregnancy. Because the foetal immune system is well developed in late pregnancy, infection at this stage will be resisted, and the lamb will be born transiently infected but alive.
Sources of infection:
- Contaminated water or food by oocysts
- Ingestion of tachyzoites and bradyzoites (cysts) in flesh of infected host.
- Undercooked meat.
- Mother to fetus.
- Organ transplant (rare).
- Blood transfusion (rare).
**Toxoplasma gondii**

*Non-felid hosts (important sources of human infections)*

**Swine**
- Systemic toxoplasmosis, ingest oocyst from cat feces or tissue cyst from prey.
  - Fever, respiratory signs
  - Highly prevalent in free-range pigs
  - **Important source of infection for humans**

**Poultry**
- Systemic toxoplasmosis, ingest oocyst from cat feces
  - Prevalent in free-range and back-yard chickens
  - **Important source of infection for humans**
Many Humans at risk with Toxoplasmosis
Implications on Human Health

- In Humans produces
  1. Congenital Toxoplasmosis
  2. Post natal Toxoplasmosis
Events on Development in man

- When man ingests Oocysts with eight Sporozoites excreted in Cats feces, can establish an infection and reproduces Asexually
- In humans Oocysts open in duodenum and releases eight Sporozoites which pass through the gut wall
- Circulate in body and invade various cells
Toxoplasmosis in Pregnancy

In 1st Trimester
May lead to still birth
- Major central nervous system anomalies
- In 2nd Trimester
  Less severe complications
  Birth Anomalies still common
**Congenital Infection**

**Lead to**
- Still Birth
- Chorioretinitis
- Intracellular calcification
- Psychomotor disturbances
- Hydrocephaly
- Microcephaly
- Prenatal toxoplasmosis may manifest with blindness apart from congenital defects
Babies infected with congenital Toxoplasmosis manifest with

- brain damage
- enlarged spleen and liver
- eye damage
- jaundice
- poor motor coordination
- unusually small head
- rash
Late Anomalies in Toxoplasmosis

- Clinical manifestation in infected fetus may be delayed until long after birth.
- Even may present during early childhood.
- Neurological problems of learning difficulties may be caused by long delayed effects of late prenatal infection.
Invasion of Lymphnodes lead to chronic infections

- Sporozoites invade various cells especially macrophages where they form Trophozoites further multiply break out and spread the infection to lymphnodes and other organs.
- The rapidly multiplying Crescentric cell (Tachyzoites) initiate the acute stage of disease.
Invade Organs

Brain involvement carries higher Morbidity and Mortality

- In further development they penetrate new cells especially Eye and Brain.
- Further development slows down in these organs called ad Bradyzoites to form a quiescent tissue cysts
- The event lead to chronic stage of disease
Systemic Toxoplasmosis

Immunocompetent Person

- 1st exposure
  - “flu-like” illness that may last for weeks
  - Fever, myalgia, sore throat, lymphadenopathy
  - Often asymptomatic

- Future exposures
  - immune-protected, no pathology

- Can remain latently infected with cysts
Toxoplasmosis - Immunosuppressed individuals

- Varying degrees of disease may occur in Immunosuppressed individuals results in
  - Retinitis
  - Chorioretinitis
  - Pneumonias
  - Other non specific manifestations
Other Human Infections

- Toxoplasmosis produces severe Human infections in patient with AIDS.
- The chronic infection is altered to Acute manifestations.

AIDS patients would suffer less if pain-relieving drugs were more widely available.
Laboratory diagnosis of *Toxoplasma gondii*

**Microscopy**
- Tachyzoites and tissue cysts detected in blood, sputum and bone marrow aspirates
- Stains used:
  - Giemsa
  - PAS
  - GMS

**Serodiagnosis**
- Antibody detection:
  - Test for detecting IgG antibody:
    - ELISA
    - IFAT
    - Latex fagglutination test
    - Sabin-Feldman dye test
  - Test for detecting IgM antibody:
    - Double sandwich IgM ELISA
    - IgM-ISAGA
  - Test for detecting IgA antibody:
    - Double sandwich IgA ELISA
  - Antigen detection:
    - by ELISA

**Molecular diagnosis**
- PCR

**Imaging**
- MRI and CT scan for central nervous system involvement
- USG for congenital toxoplasmosis

**Others**
- Animal inoculation
- Skin test of frenkel
Diagnosis:

- Direct microscopy → Detection of tachyzoites in blood and tissue cyst in tissue biopsy

Staining methods:
1. Giemsa
2. PAS
3. Silver stains
4. Immunoperoxidase stain
Serology:

- Detection of Toxoplasma antigen by ELISA

- Detection of Toxoplasma antibody by
  1. Sabin feldman dye test
  2. IgM ELISA
  3. IgG ELISA
  4. IgG avidity test
  5. TORCH test in Newborn
- Molecular diagnosis
- Animal inoculation
- Tissue culture
- Imaging methods

Frankel's Intracutaneous test for epidemiological purpose
Microscopic Examination of Tissues

- Smears and sections stained with Giemsa’s stain
- Periodic acid Schiff method preferred
- The densely packed cysts seen in the brain or other parts of nervous system suggest chronic infection
**Diagnosis: System Disease**

**Aspirates or Necropsy**

Tachyzoites from effusion or aspirate

Bradyzoite cyst from Necropsy / Histology

[https://www.cdc.gov/dpdx/toxoplasmosis/index.html](https://www.cdc.gov/dpdx/toxoplasmosis/index.html)
Gold standard antibody detection test
Done only in reference laboratories

Complement mediated neutralization test that requires live tachyzoites

Live tachyzoites are incubated with complement and test serum
Alkaline methylene blue dye is added and reincubated
Toxoplasma antibodies in the serum bind to the antigens in the live tachyzoites → killed due to complement mediated lysis

Killed tachyzoites → thin, distorted and colourless

The dilution of the test serum at which 50% of the tachyzoites are killed → antibody titer of the test serum
**Diagnosis:** *Toxoplasma gondii*

**cats only**

**Intestinal/Acute Disease**
- Oocysts in feces
  - Fecal float centrifugation (Zinc Sulfate)

**Systemic Disease**
- Serologic tests – measure IgG and IgM antibodies
  - good to rule-out if seronegative; not as useful at proving Toxo is cause of disease.
- Thoracic radiographs if lung involvement (pneumonia)
- Definitive diagnosis – detection of tachyzoites in effusions, tissue aspirates or biopsy samples
**Diagnosis:** Fecal Float Centrifugation
cats only

https://www.cdc.gov/dpdx/toxoplasmosis/index.html

Diagnosis of congenital toxoplasmosis:

- Toxoplasma antigens in amniotic fluid
- PCR
- IgM antibodies in fetal blood by ELISA
- Role of IgG antibodies in diagnosing congenital toxoplasmosis?
- Ultrasound of fetus at 20 to 24 weeks of gestation
Immunity

- Acquired immunity in women is particularly protective to the fetus.
- In immunosuppressed and AIDS patients, changes the host resistance and causes chronic infection becoming fulminating acute Toxoplasmosis.
**Treatment:** Toxoplasma gondii

cats only

**Intestinal / Acute Disease (shedding oocysts)**
- Pyrimethamine plus triple sulfa drugs
- Clindamycin
- Ponazuril
- Hospitalize cat during oocyst shedding to reduce zoonosis

**Systemic Disease (clinically ill)**
- **Clindamycin** for at least 4 weeks
- Supportive treatment depending on clinical signs
- No good treatment to clear tissue cysts
Treatment

- Combination of Pyramethamine and Sulphadiazine or Trisulfapyramidines
- Other alternative Drugs
  - Spiramycin
  - Clindamycin
  - Trimethoprim – Sulphmethoxazole
  - In pregnancy – Spiramycin is recommended drug
Control

It is difficult to control toxoplasmosis because of wide range of animal reservoirs. Currently, there is no effective vaccine available for humans. Primary control measure should be to protect feedstuffs from access by cats. A ‘clean’ flock should be prevented from ingesting food and bedding contaminated by cats, particularly young cats. A genetically engineered vaccine is under development for use in cats. Proper disposal of cat's litter and also the carcass
Control of Toxoplasmosis

- Avoidance of human contact with Cat feces is highly important measure.
- Changing of Cat litter and safe disposal can prevent transmission.
- **Pregnant women should avoid contact with kittens**
Screening of pregnant women

- Periodic screening of pregnant women with high risk for IgG and IgM antibodies to Toxoplasmosis is recommended
**Care of the Meat**

- Avoid eating raw or undercooked meat.
- Freezing < -20°C
- Heating at 50°C for 4-6 minutes destroys the cysts and sterilizes the meat.
**Prophylaxis**

1. Individuals at risk, particularly pregnant women, children, and immunocompromised persons should avoid contact with cat and its feces.
2. Proper cooking of meat.
3. Proper washing of hands and washing of vegetables and fruits before eating. Blood or blood products from seropositive persons should not be given and screening for *T. gondii* antibody should be done in all blood banks.
4. For primary prophylaxis, Trimethoprim-sulfamethoxazole is the drug of choice.
5. Toxovac S48 live vaccine available for sheep.