

IN SUMMARY
TOXOPLASMOSIS

TOXOPLASMOSIS

Figure removed due to copyright restrictions.

Types of Hosts

Intermediate Hosts

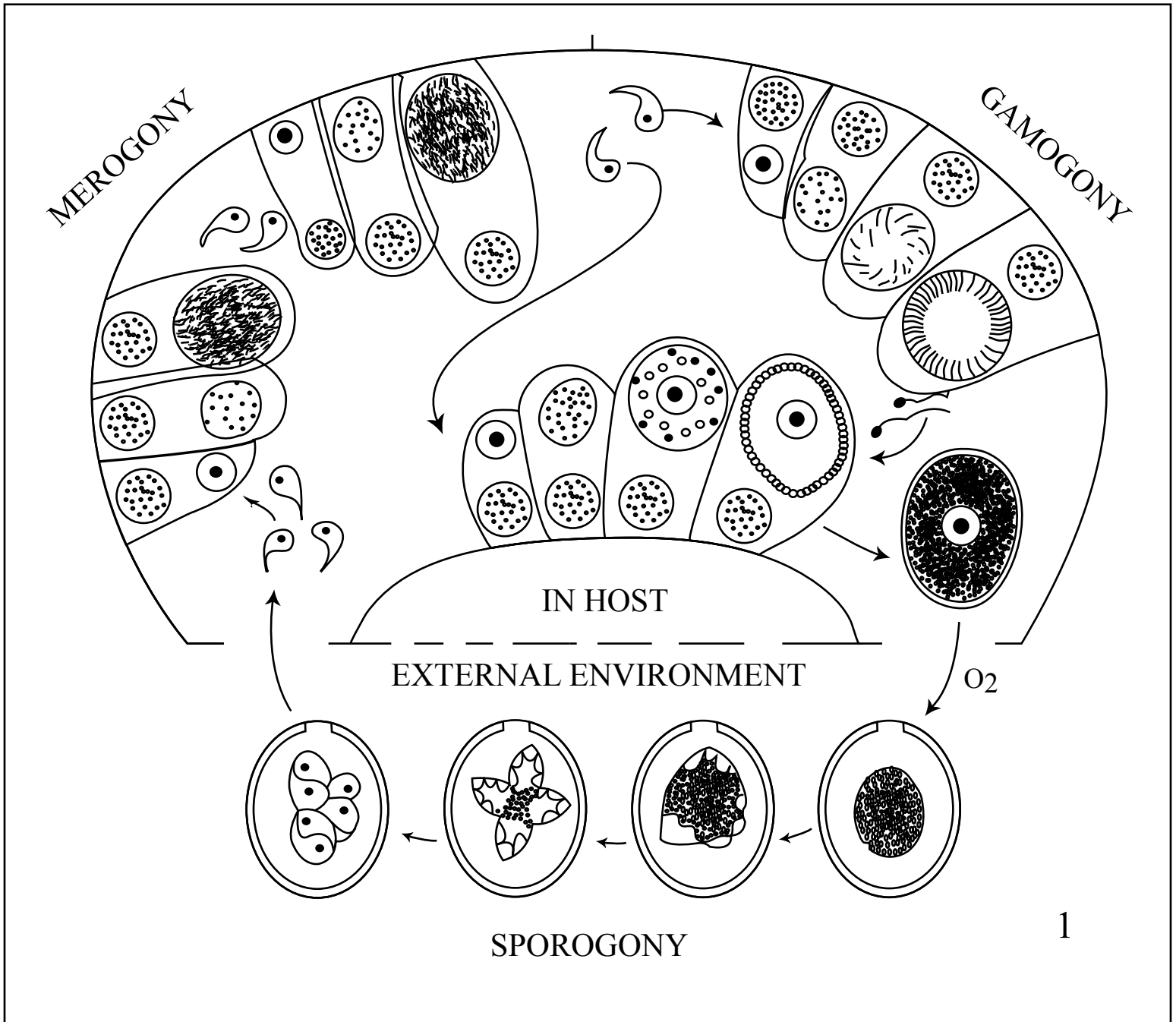
- Hosts in which asexual replications occurs
- If no replication occurs, the intermediate host is known as a transport or paratenic host
 - e.g. *Toxoplasmosis gondii* - various vertebrates
 - Plasmodium Spp.* - vertebrate blood and tissues

Definitive Hosts

- Hosts in which the sexual cycle occurs
 - e.g. *Toxoplasmosis gondii* - wild and domestic cats
 - Plasmodium spp.* - mosquitoes (Diptera)

Major “Groups” of Apicomplexa

- Gregarines - septate & aseptate (considered “primitive”) - parasites of invertebrates - extracellular
- Coccidia - homoxenous and heteroxenous species
 - eimerid coccidia (*Eimeria*, *Lankesterella*, etc)
 - isosporoid coccidia (*Sarcocystis*, *Toxoplasmosis*, *Neospora*, etc.)
- *Cryptosporidium spp.*
- Haemosporinids - malarial parasites and their relatives
- Piroplasms - *Babesia*, *Theileria* and their relatives
- Haemogregarines - *Haemogregarina*, *Heptozoon*, etc.



1

Illustration courtesy of MIT OCW.

Apicomplexa Protista

- All members of the phylum Apicomplexa
- Single-celled organisms
- Possess a more or less developed “apical complex”
- Possess flattened subpellucular vesicles (share this feature with the dinoflagellates and ciliates)
- Usually complex life cycles

Typical Apicomplexan Zoite

- Eukaryotic, apical complex, trilaminar pellicle

Figure removed due to copyright restrictions.

Figure removed due to copyright restrictions. Please see:

Figure 3 in Schematic drawings of a tachyzoite (left) and a bradyzoite (right) of *T. gondii*.
The drawings are composites of electron micrographs.

Dubey, J. P., D. S. Lindsay, and C. A. Speer. "Structures of *Toxoplasma Gondii* Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts." *Clin Microbiol Rev.* 11, no. 2 (April 1998): 267-99.

- Tachyzoites can move by gliding, flexing, undulating, and rotating
- No visible means of locomotion such as cilia, flagella, or pseudopodia
- Functions of the conoid, rhoptries, micropores, and micronemes are not fully known
- Probably associated with host cell penetration
- The conoid can rotate, tilt, extend, and retract as the parasite probes the host cell plasmalemma immediately before penetration

- Rhoptries have a secretory function associated with host cell penetration, secreting their contents through the plasmalemma just above the conoid to the exterior
- Tachyzoites multiply asexually within the host cell by repeated endodyogeny
- Specialized form of reproduction - two progeny form within the parent parasite consuming it
- Golgi complex divides first - becoming two complexes at the anterior end of the nucleus The host cell ruptures when it can no longer support the growth of tachyzoites
- After the ingestion of tissue cysts by cats, the cyst wall is dissolved by proteolytic enzymes in the stomach and small intestine.
- Released bradyzoites penetrate the epithelial cells of the small intestine and initiate the development of numerous generations of *T. gondii*
- Five morphologically distinct types of *T. gondii* develop in intestinal epithelial cells before gametogony begins

Figure removed due to copyright restrictions. Please see:

Figure 1: Life cycle of *T. gondii* in Dubey, J. P., D. S. Lindsay, and C. A. Speer. "Structures of Toxoplasma Gondii Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts."

Clin Microbiol Rev. 11, no. 2 (April 1998): 267-99.

Factors Determining Expression of Disease

- The susceptibility of the host
- Age of the host
- The strain of the pathogen
- The degree of acquired immunity

Prevalence

- Overall world prevalence ~13%
- Diet Uncooked or poorly cooked meat is eaten
- Immuno-compromised host
 - Patient is undergoing suppressive therapy
 - Transplant patients
 - HIV

Spread by the Cat

- Only members of the cat family shed oocysts
- Cats become infected by ingesting
 - Oocysts from fecal contamination
 - A cat can excrete 10⁷ to 10⁹ oocytes per day
 - Tissue cysts present in the flesh of eaten animals

Digestive enzymes release the organisms

Invade the feline small intestine

The organisms reproduce

- Producing millions of noninfectious, unsporulated oocysts
- Excreted in feces of cats for up to 2 weeks

Outside the cat's body

- Sporogony occurs for up to 21 days
- Development of infectious oocysts
- Warm, moist soil - oocysts viable for more than 1 year

Intermediate Hosts

- Herbivores/omnivores -Type 1 Host
- Carnivores - Type 2 Host

Distinctive Differences in the life cycle stages

Herbivore/omnivore - intermediate host

- Hosts infected by eating sporulated oocysts
- Each oocyst contains 2 sporocysts with 4 sporozoites

Liberated sporozoites infect extra-intestinal sites

- Acute phase
 - Mesenteric lymph nodes
 - Liver
- Chronic
 - Brain
 - Heart
 - Skeletal muscles

Sporozoites - infect the intestinal epithelial cells
undergo schizogony

Only in the cat

- Sexual phase of the parasite (*gametogony*) observed
- Produces a single celled oocyte
- Cycles through the cat rapidly (3-5 days)
- Cat does not appear to suffer much discomfort
- Quickly replaces damaged epithelial cells
- Kittens - more susceptible and some die of the infection.

Sporozoites divide by *endodyogeny*

[*Endodyogeny - Formation of daughter cells each surrounded by its own membrane, while still in the mother cell*]

Form pseudocysts

- Contain merozoites (**tachyzoites**)
- Can infect other cells and form **tissue cysts**.

Pseudocyst and Tissue cysts

Require the host to be devoured by a carnivore (type 2 host) to continue life cycle

Both are infective to cats.

Human Spread

Spread of the merozoites usually prevented by the host immune response

- Predominately a cellular one
- Persisting as long as the host remains infected
- Usually for life

If tissue cysts eaten by a carnivore other than a cat

- Released merozoites
- Penetrate extra intestinal cells
- Form pseudocysts
- Produce more merozoites or **bradyzoites**
- Lead to the production of tissue cysts
- Transmission via the placenta in females to the fetus occurs

Insects

- Flies and cockroaches - can act as transport hosts
- Disseminating the oocysts

Review of Basic Facts About Toxoplasmosis

- *Toxoplasma gondii* - obligate intracellular parasite
- Properties similar to the pathogen that causes malaria
- One of the most common human parasites in the world
- First discovered in the gundi, a North African rodent
- Members of the cat family are the definitive hosts
- Domestic cats play a major role in transmission
- Congenital infection is the only form of human-to-human transmission.

Presenting History

- Acquired toxoplasmosis can present with a range of clinical manifestations, from **subclinical lymphadenopathy** (the most common presentation) to **fatal, fulminant disease**
- In healthy adults, infection with *T gondii* **usually is subclinical**.
- In the immunocompetent host, infection with *T gondii* may be indistinguishable from **infectious mononucleosis**.
- In immunocompromised hosts, toxoplasmosis may **mimic** other opportunistic infections, such as tuberculosis or infection with *P carinii*.
- In patients with **AIDS**, **CNS** involvement is the most common manifestation, followed by pulmonary involvement.

Physical Findings

- Toxoplasmosis cannot be diagnosed on clinical grounds alone
- May mimic a variety of other diseases.
- No clinical features are pathognomonic for toxoplasmosis
- Lymphadenopathy is the most common finding

Laboratory Studies

- The immunoglobulin (IgM) immunofluorescent antibody test (IgM-IFA)
- An IgM-IFA titer of 1:160 or greater
- IgM-enzyme-linked immunosorbent assay (IgM-ELISA) titer of 1:256 or greater
- Considered diagnostic of recently acquired *T gondii* infection

Imaging Studies

- MRI
 - Considered best diagnostic imaging modality
 - May detect lesion not visualized on C-T
 - May find single or multiple lesions
- Ring enhancing
- Basal ganglia
- Cortico-medullary regions

Other Tests

- Cerebrospinal fluid
- Mononuclear pleocytosis
- Elevated protein
- Normal glucose
- Presence of tissue cysts is diagnostic for toxoplasmosis
- Does not distinguish between acute and chronic infection
- Presence of either tachyzoites or toxoplasmal antigens in tissue or smears confirms acute infection
- Brain biopsy
- May reveal presence of tachyzoites

Histologic Diagnosis

- Tachyzoites in tissue sections or smears of body fluid
 - Difficult to demonstrate tachyzoites in conventionally stained tissue sections immunoperoxidase technique
 - Uses antisera to *T. gondii*,
 - Sensitive and specific
 - Presence of the parasite in the central nervous system (AIDS)
 - Applicable to unfixed or formalin-fixed paraffin-embedded tissue sections
 - A rapid and technically simple method
- Air-dried, Wright-Giemsa-stained slides
- Sediment of CSF
- Brain aspirate
- Impression smears of biopsy tissue
- The presence of multiple tissue cysts

Ocular Toxoplasmosis

- Acquired toxoplasmosis may be seen
- Majority of cases are felt to be a reactivation of a congenital toxoplasmosis
- Characterized by an acute retino-choroiditis
- Marked vitreous reaction overlying the active infection
- "headlight in the fog"
- Healed lesion leads to a large scar

Sabin-Feldman Dye Test

- IgG antibodies are primarily measured by the Sabin-Feldman Dye Test Sensitive and specific neutralization test
 - Live organisms are lysed
 - Presence of complement
 - The patient's IgG *T. gondii*-specific antibody
 - Becomes positive 1 to 3 weeks after infection
 - Titer increases for many months
 - Gradually declines over a period of 5 years or more
 - May remain positive for life
 - Titers of 1:4 are significant
 - Titers of 1:8 or 1:16 usually should be considered as positive.
- Rising titers - 2 weeks apart indicate recently acquired infection

IgG antibodies

- Usually appear within 1 to 2 weeks of the infection
- Peak within 1 to 2 months
- Fall at variable rates
- Persist for life
- Titer does not correlate with the severity of illness

A positive DT = patient has been exposed to the parasite

A negative DT essentially rules out prior exposure to *T. gondii*

Small number of patients

- IgG antibodies might not be detected within 2 to 3 weeks
- Toxoplasmic chorioretinitis
- Toxoplasmic encephalitis) in immuno-compromised patients

Differential Agglutination Test

- Also known as the "AC/HS test")
- Two antigen preparations
 - Early following acute infection (AC antigen)
 - Later stages of infection (HS)
- Ratios of titers using AC versus HS antigens
 - Acute (may persist for one or more years)
 - Equivocal
 - Non-acute
- Useful in helping differentiate acute from chronic infections
- Best used in combination with a panel of other tests

Avidity Testing

- Functional affinity of specific IgG antibodies
- Initially low after primary antigenic challenge
- Increases during subsequent weeks and months
- Protein-denaturing reagents (urea) used to dissociate the antibody-antigen complex.
- Avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated serum.
Used as additional confirmatory diagnostic tool
 - patients with a positive and/or equivocal IgM
 - acute and/or equivocal pattern in the AC/HS test
- Confirmatory test
- Not the ultimate test for decision-making
- Low or equivocal IgG avidity antibody results should not be interpreted as diagnostic of recently acquired infection
- Low or equivocal avidity antibodies can persist for months to one year or longer.

IgM Antibodies

- Measured by the "double-sandwich" or "immuno-capture" IgM-ELISA
- Avoids false positive results
 - Rheumatoid factor
 - Antinuclear antibodies
- In recently acquired infection
- IgM *T. gondii* antibodies are detected initially
- Become negative within a few months
- Occasionally positive IgM *T.gondii*-specific titers can be observed during the chronic stage of the infection
- IgM antibodies have been reported to persist as long as 12 years
- Persistence has no clinical relevance – chronically infected
- The FDA recommends: sera with positive IgM test results obtained at non-reference laboratories should be sent to a Toxoplasma reference laboratory (Such as Remington or CDC Labs)

Polymerase Chain Reaction (PCR)

Used to detect *T. gondii* DNA in body fluids and tissues

- Congenital
- Ocular
- Disseminated

Performed on amniotic fluid

- Revolutionized the diagnosis of fetal *T. gondii* infection
- Enabling an early diagnosis to be made
- Avoiding the use of more invasive procedures (PUBS)

Allowed detection of *T. gondii* DNA in brain tissue

Cerebrospinal fluid (CSF)

Vitreous and aqueous fluid

Bronchoalveolar lavage (BAL) fluid

Urine

Amniotic fluid

Peripheral blood.

Treatment

- Pyrimethamine (Daraprim)
- Folic acid antagonist
- Selectively inhibits plasmodial dihydrofolate reductase
- Highly selective against plasmodia and *T. gondii*.
- Does not destroy gametocytes
- Arrests sporogony in mosquito
- Possesses blood schizonticidal and some tissue schizonticidal activity against malaria parasites of humans.
- Extend regimens to include suppressive cure through any characteristic periods of early recrudescence and late relapse for at least 6-10 wks in each case.

Treatment

- Spiramycin - macrolide antibiotic
 - Used effectively in the treatment of pregnant women
 - Now is recommended for congenital infection
- Clindamycin plus pyrimethamine
- Clarithromycin
- Azithromycin
 - More potent than spiramycin and clindamycin

Drugs with Human Activity in Human Toxoplasmosis

- Pyrimethamine (inhibits DHFR)
- Sulfadiazine (inhibits dihydropterolate synthetase)
- Trimethoprim-sulfmethoxazole (Bactrim)
- Clindamycin (binds to ribosome - inhibits protein synthesis)
- Spiramycin (binds to ribosome - inhibits protein synthesis)
- Atovaquanon (inhibits mitochondrial cytochromes)
- Azithromycin, Clarithromycin, Trovafloxacin

Treatment is a Function of Immune Status

- Infants
 - Normal immune system
- Only with lymphadenopathy
- No treatment is required
- Organ damage
 - Pyrimethamine initiated in loading dose
 - Maintenance
 - Potent folic acid antagonist
- Bone marrow suppression
- Leukopenia
- Anemia
- Thrombocytopenia
- Leucovorin (Folinic acid)

Serologic

- Indirect fluorescent antibody (IFA)
 - Measures the same antibody as Sabin-Feldman Dye Test
 - IFA titers of 1:8 or 1:16 are believed to represent infection
- IgM fluorescent antibody (IgM-IFA)
 - Based on the known early rise of IgM
 - Precedes IgG
 - Used for early diagnosis of congenital infection
 - Acute infection in the acquired disease
 - Reactivation of latent toxoplasmosis
 - Titers of 1:10 to 1:1000 or higher are seen
 - May persist for a variable period
 - Usually becomes negative by the third or fourth month
 - Persists as low titers for a year

Table removed due to copyright restrictions.

[Multivariate analysis of risk factors for *Toxoplasma gondii* infection adjusted for age, location, period between diagnosis of infection and interview, and all other exposures shown]

FUNDAMENTAL QUESTIONS

1. Why is toxoplasmosis important to a pregnant woman?
2. List all the known intermediate hosts for toxoplasmosis?
3. What should a pregnant woman be told about toxoplasmosis avoidance?
4. What effect does Toxo have on a fetus?
5. Describe the life cycle of Toxo?
6. What drugs may be used in a pregnant woman who has acquired Toxo? How do they work?
7. What laboratory tests had been used to diagnose toxo until recently?
8. What is the Sabin-Feldman dye test? How is it done?
9. What is the current test used to diagnose toxo?
10. How long can toxo live in the muscle of an infected human? What conditions threaten the life of an adult patient infected with toxo?

