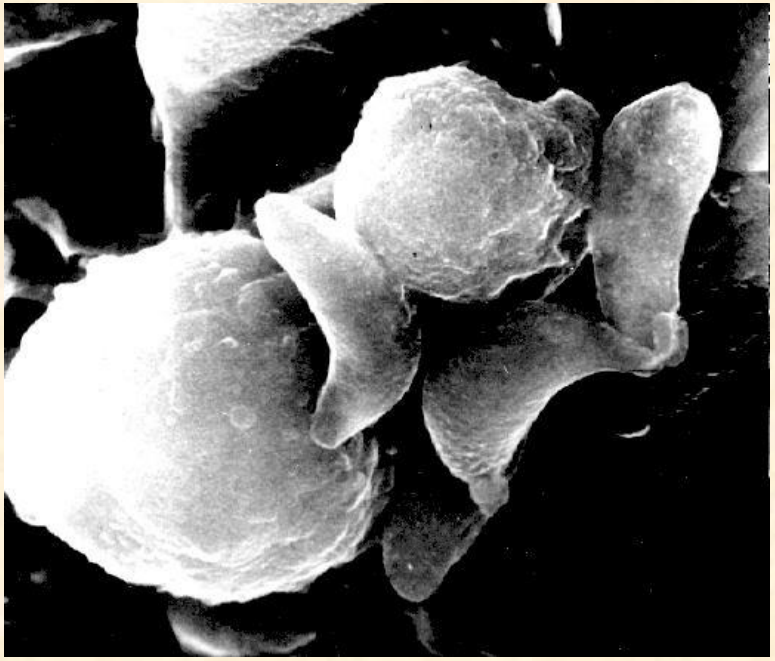
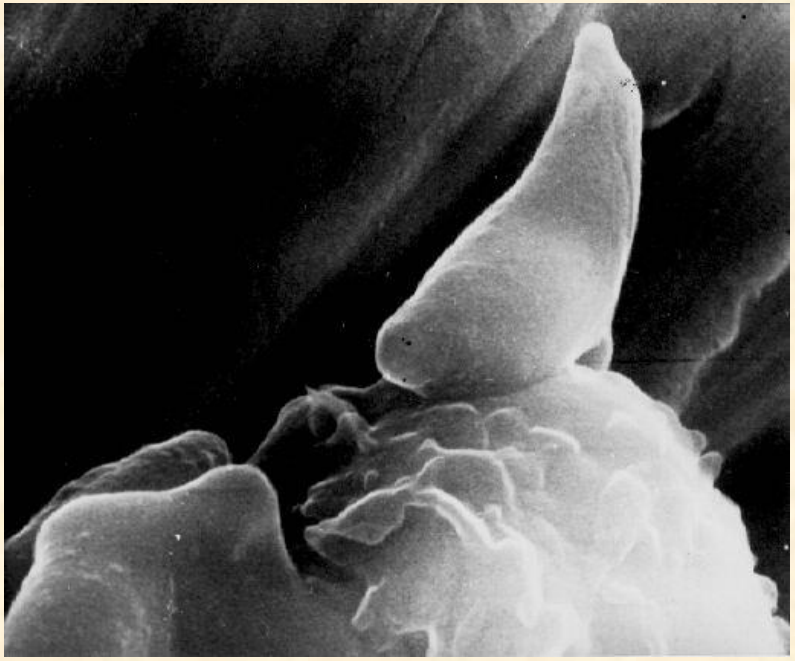
Toxoplasmosis

?

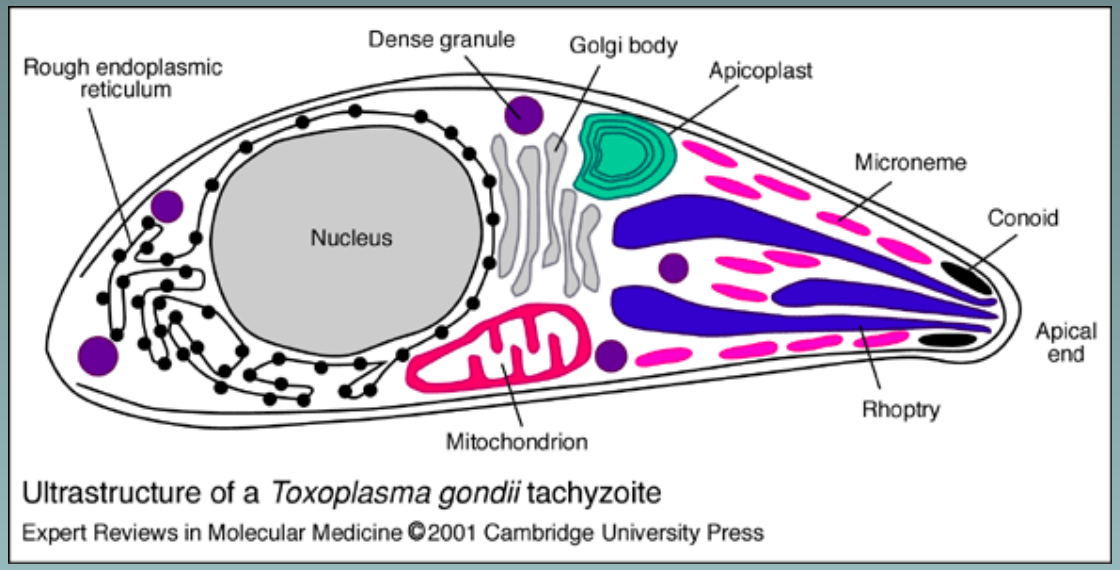




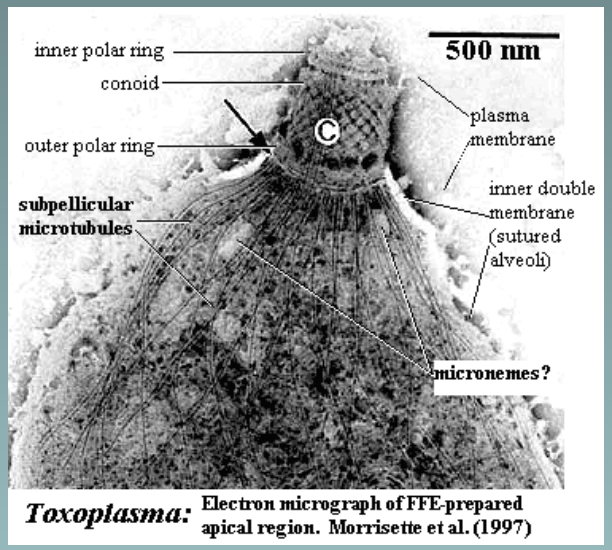
Toxoplasma gondii

- **intracellular protozoan**
- **classified in the phylum *Apicomplexa***
- ***T gondii* takes 3 forms during its life cycle:**
  1. **Tachyzoite**
  2. **Cyst (containing bradyzoites)**
  3. **Oocyst (containing sporozoites)**

Toxoplasma gondii tachyzoite



Ultrastructure of a *Toxoplasma gondii* tachyzoite



Toxoplasma: **Electron micrograph of FFE-prepared apical region. Morrisette et al. (1997)**

The invasive stages of apicomplexans:

- **apical complex**

  composed of specialized cytoskeletal & secretory organelles:

  - **micronemes (in attachement and penetration),**
  - **rhoptries (moving junction & establishment of parasitophorous vacuole (PV)),**
  - **dense granules (secrete proteins).**

Tachyzoite

invade any nucleated cell

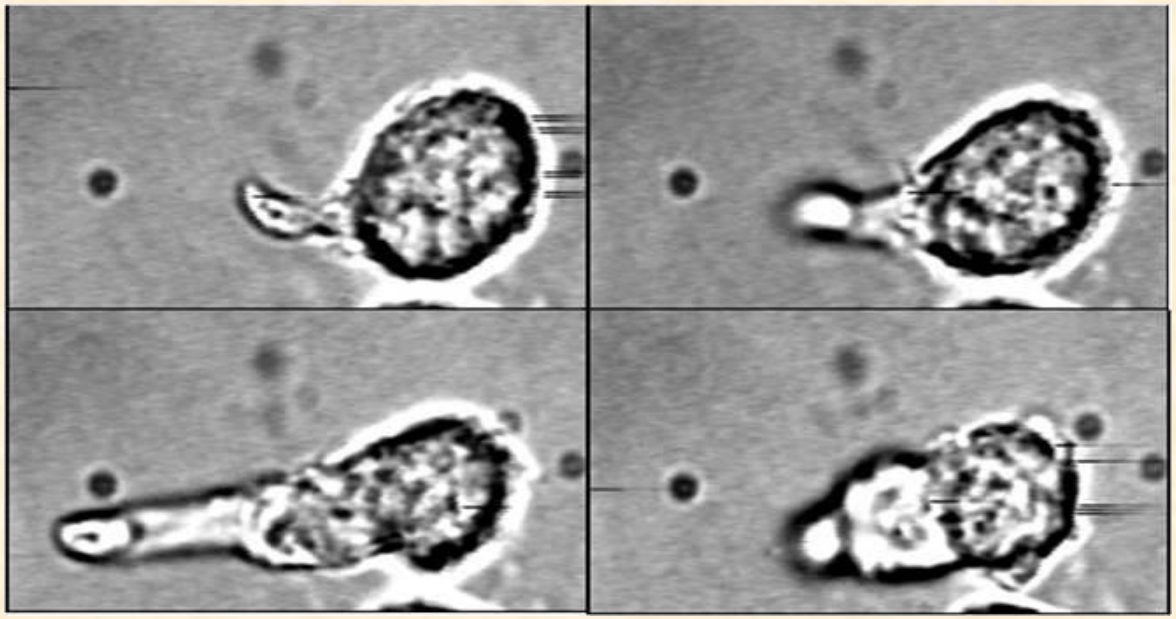
replicate

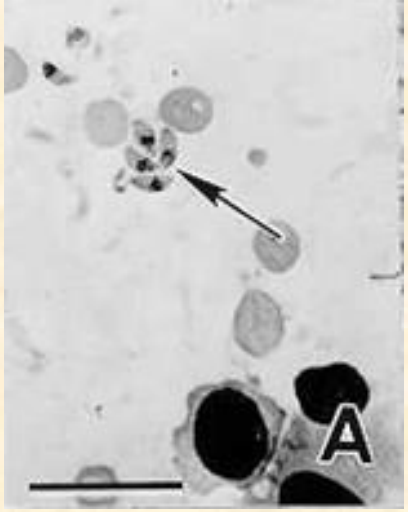
cytolytic infection

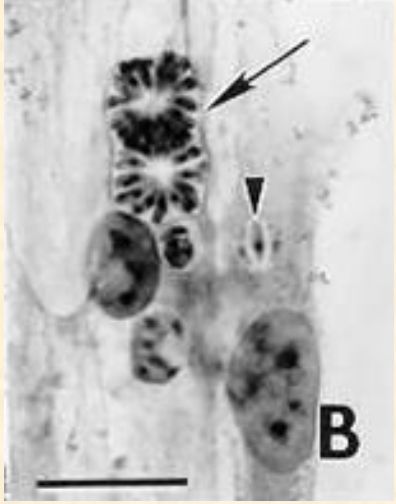
local necrosis accompanied by inflammation.

***T.* does not produce a toxin**

Hypersensitivity also plays a major role in inflammation reactions

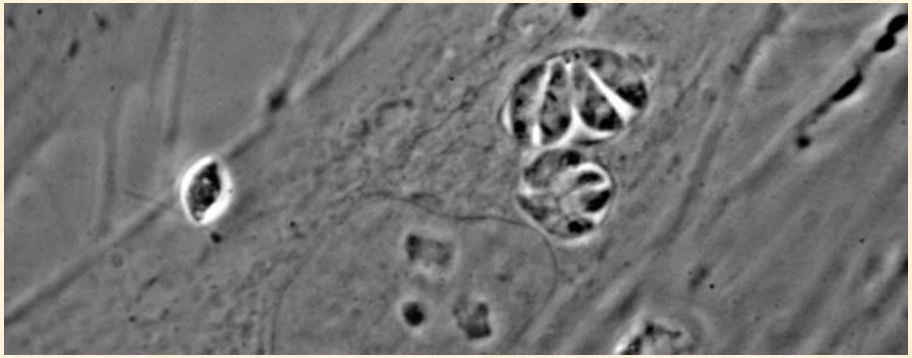






Extracellular T. released from host cells.
Compare their size with RBC / lymphocyte.

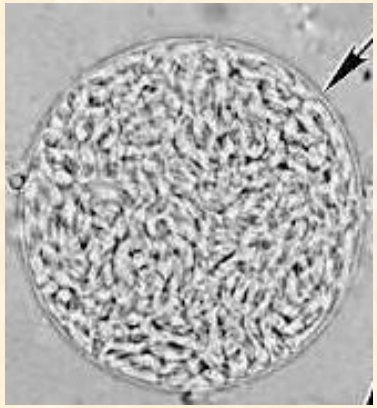
Intracellular T. in cell culture.
group arranged in a rosette;
vacuole around a tachyzoite.

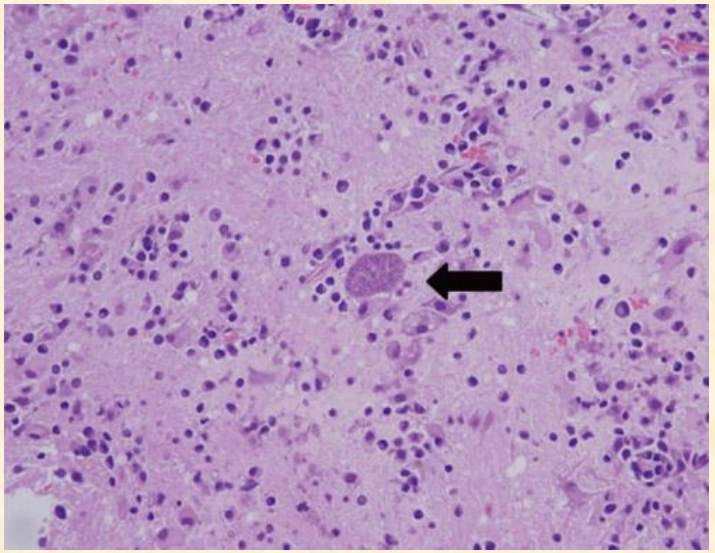


Parasitophorous vacuoles
is formed inside host cells from both parasite & host tissue.
T. is protected inside the parasitophorous vacuoles from the humoral immune response

Bradyzoite:

- **divide slowly** by endodyogeny within a tissue cyst,
- fail to provoke an inflammatory response.

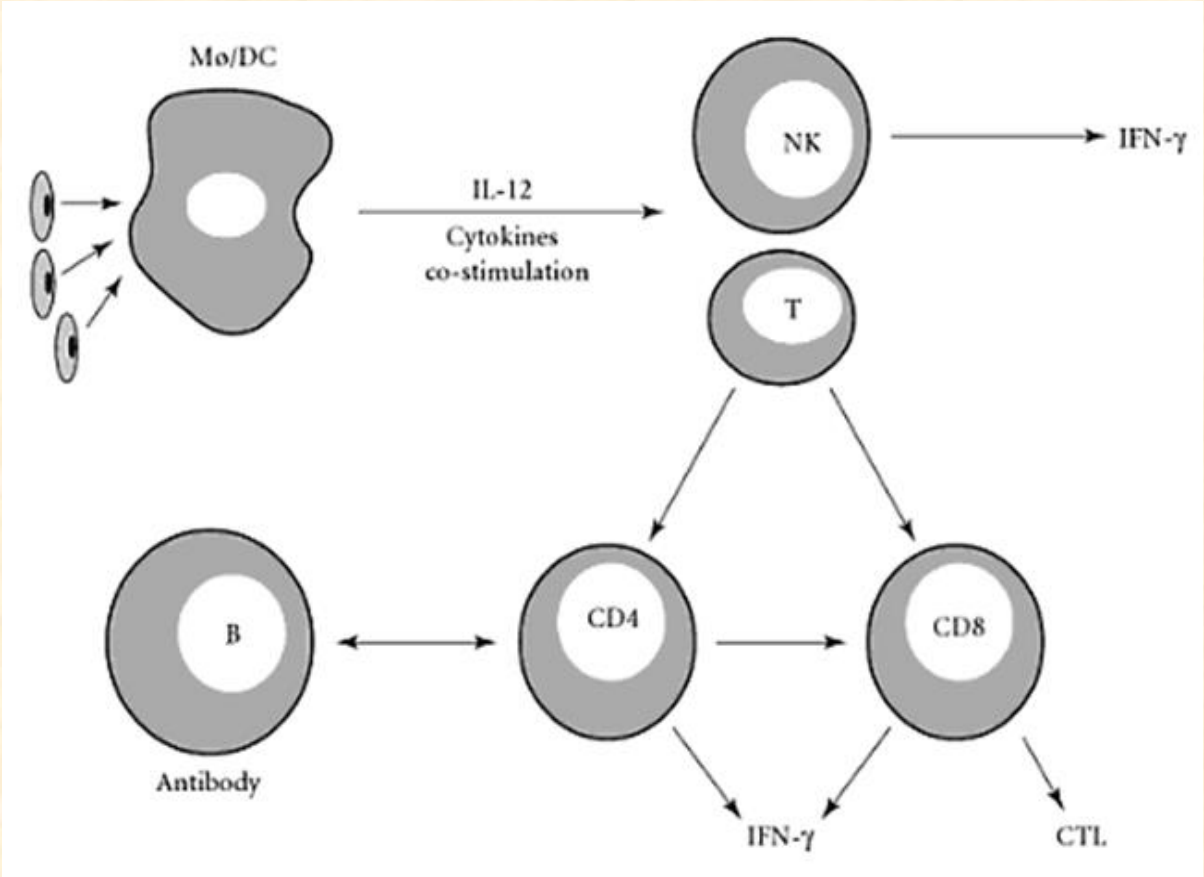




Tissue cyst
= a collection of bradyzoites surrounded by a well-defined host cell membrane.

CNS cysts have been reported in neurons, astrocytes, microglia, retinal cells

- The protective response is dominated by **cell-mediated immunity**
  - by the ability of accessory cells (macrophages, dendritic cells) to present Ag, provide co-stimulation, produce cytokines (IL-12).
  - by the production of the cytokine IFN-γ by T-cells (Th1-response),
- The production of IL-12 is critical for the development of protective immunity.
- **Encysted *T. gondii* bradyzoites are capable of inhibiting cellular apoptosis, so they can persist in host cells for long periods of time**



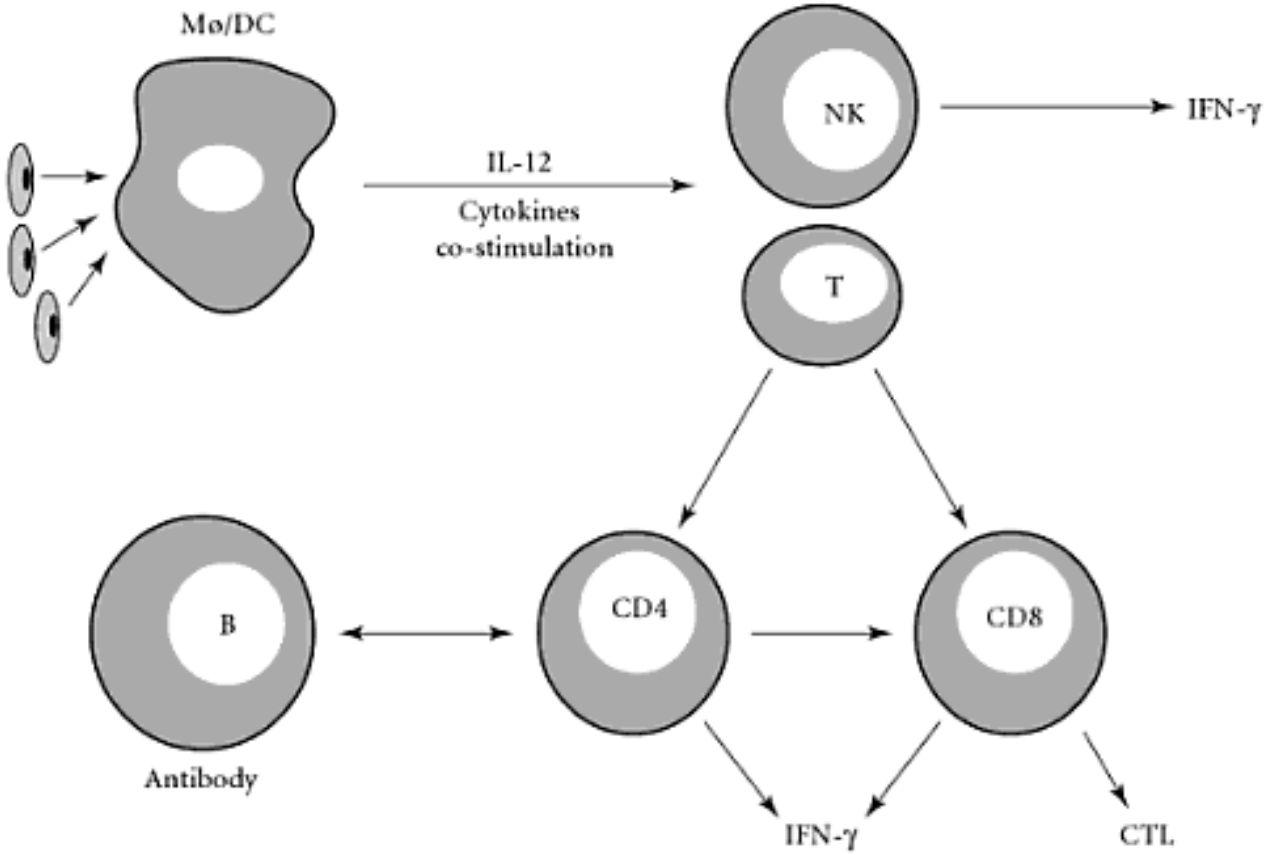
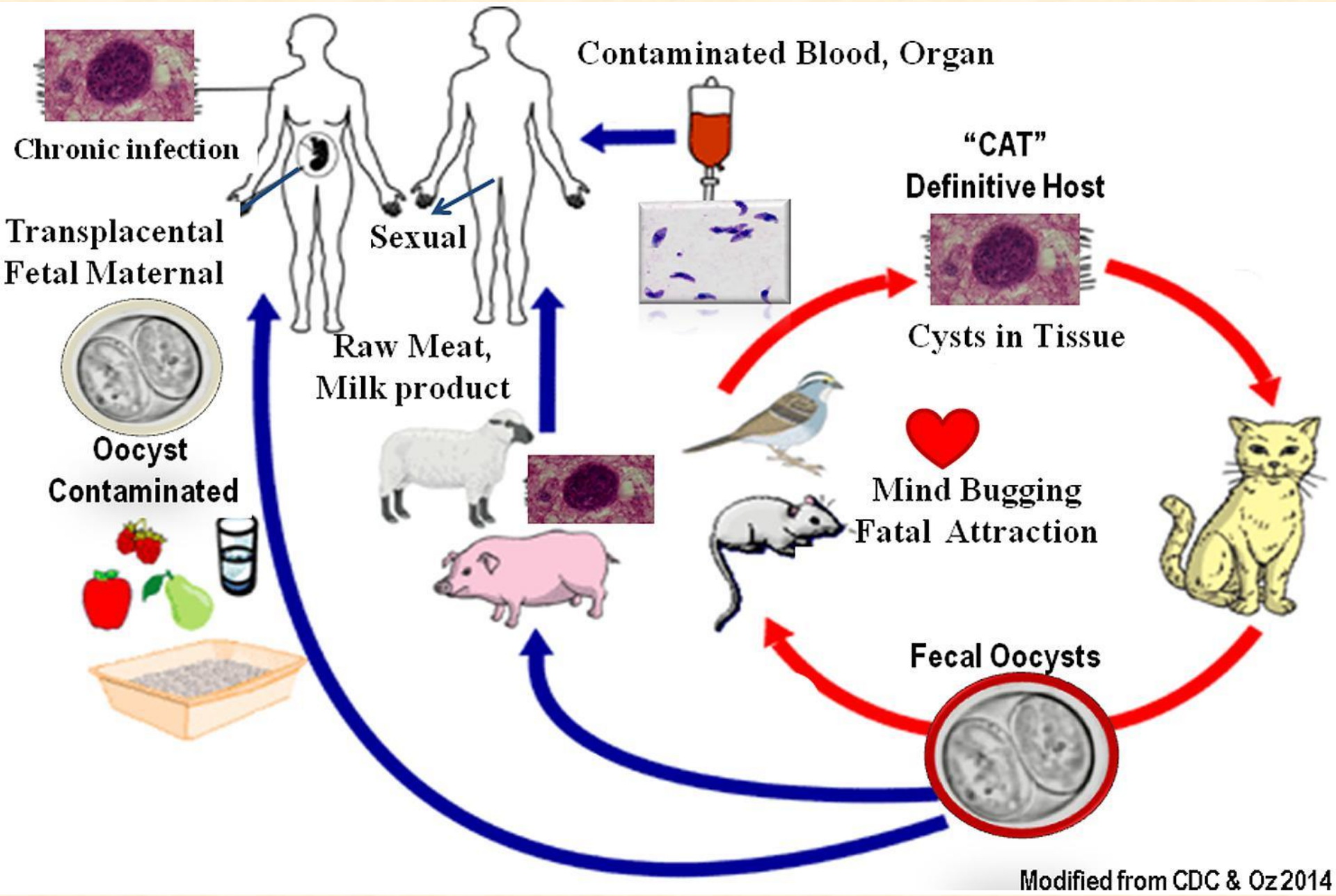
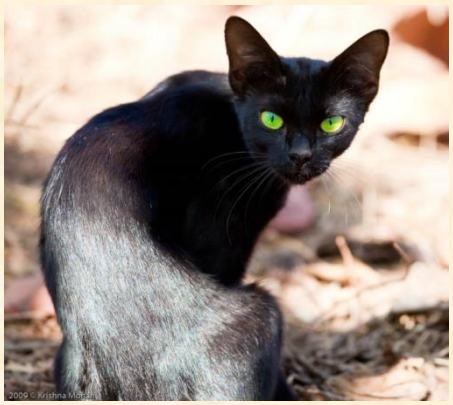


Figure 2.1 Development of the immune response to *Toxoplasma gondii*. Infection with *T. gondii* leads to the production of interleukin-12 (IL-12) by macrophages (Mϕ) and/or dendritic cells (DC). These cells also present parasite antigens and provide co-stimulatory signals and other proinflammatory cytokines required for optimal T-cell and natural killer (NK) cell responses. Together, these events lead to the production of interferon-γ (IFN-γ) by NK cells and directs the development of $CD4^+$ and $CD8^+$ T-cells into TH1 cells which produce IFN-γ. $CD4^+$ T-cells provide help for maximal $CD8^+$ T-cells responses as well as for the maturation of B-cell responses. CTL, Cytotoxic lymphocytes.


Chronic infection
Contaminated Blood, Organ
"CAT"
Definitive Host
Transplacental
Fetal Maternal
Sexual
Cysts in Tissue
Raw Meat,
Milk product
Oocyst
Contaminated
Mind Bugging
Fatal Attraction
Fecal Oocysts
Modified from CDC & Oz 2014



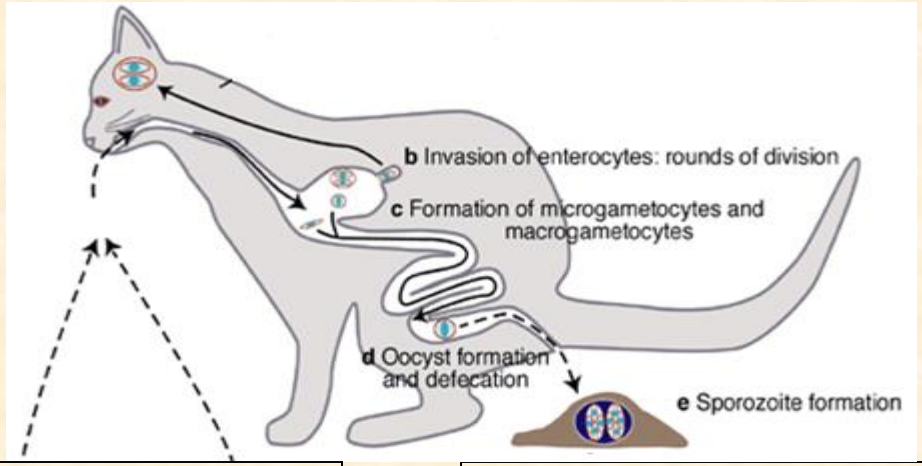
➢ **Definitive host - felidae cats it´s relatives**

➢ **Intermediate host - humans**

➢ **Intermediate host - warm-blooded animals**

Inf.→less prevalent in cattle than in sheep or pigs.
Cooking food to safe 71.1ºC= 160ºF

Reinfection does not seem to result in disease or in congenital transmission of the parasite



Cats acquire *T.* by ingesting:

1.tachyzoites

2.bradyzoites

3.oocysts

- **Unsporulated oocysts are shed in the cat's feces after 2 we**
- **Oocysts shed for 1-2 weeks**
- **Oocysts in 1-5 days sporulate in the environment →infective**
- **Oocyst survive 12-18 mo in moist sand // soil**

- **<50% cats shed oocysts after ingesting tachyz. or oocysts,**
- **All cats shed oocysts after ingesting tissue cysts.**

Cats are not the most common source of infection in humans due to:

- **their fastidious nature,**
- **the short term of oocyst shedding,**
- **the passing of non-infective oocysts**

Seroprevalence IgG against *T. gondii*, varies worldwide:

Common in warm climates, lower altitudes

Sero-prevalence for T. gondii in women of child-bearing age (1990–2000):

- **58% in Central European countries,**
- **51–72% in several Latin-American countries,**
- **54–77% in West African countries**
- **4–39% in southwest Asia, China, Korea**
- **11–28% in Scandinavian countries**

Infection with *T. gondii* has been shown to affect behavior in rats; specifically, in an open field arena infected rats showed:

- significantly reduced climbing,
- rearing (standing on hind paws), and grooming activity;
- significantly longer time spent in the center of the arena,
- reduced anxiety,
- to be attracted to the odour of cat urine.

→ increase the incidence of cat predation → increasing the transmission

***T. gondii* is capable of altering the behaviour of intermediate hosts in order to favour transmission**.

In humans:

During digestion →release of
- – Bradyzoites from tissue cysts
- – of oocysts from sporozoites

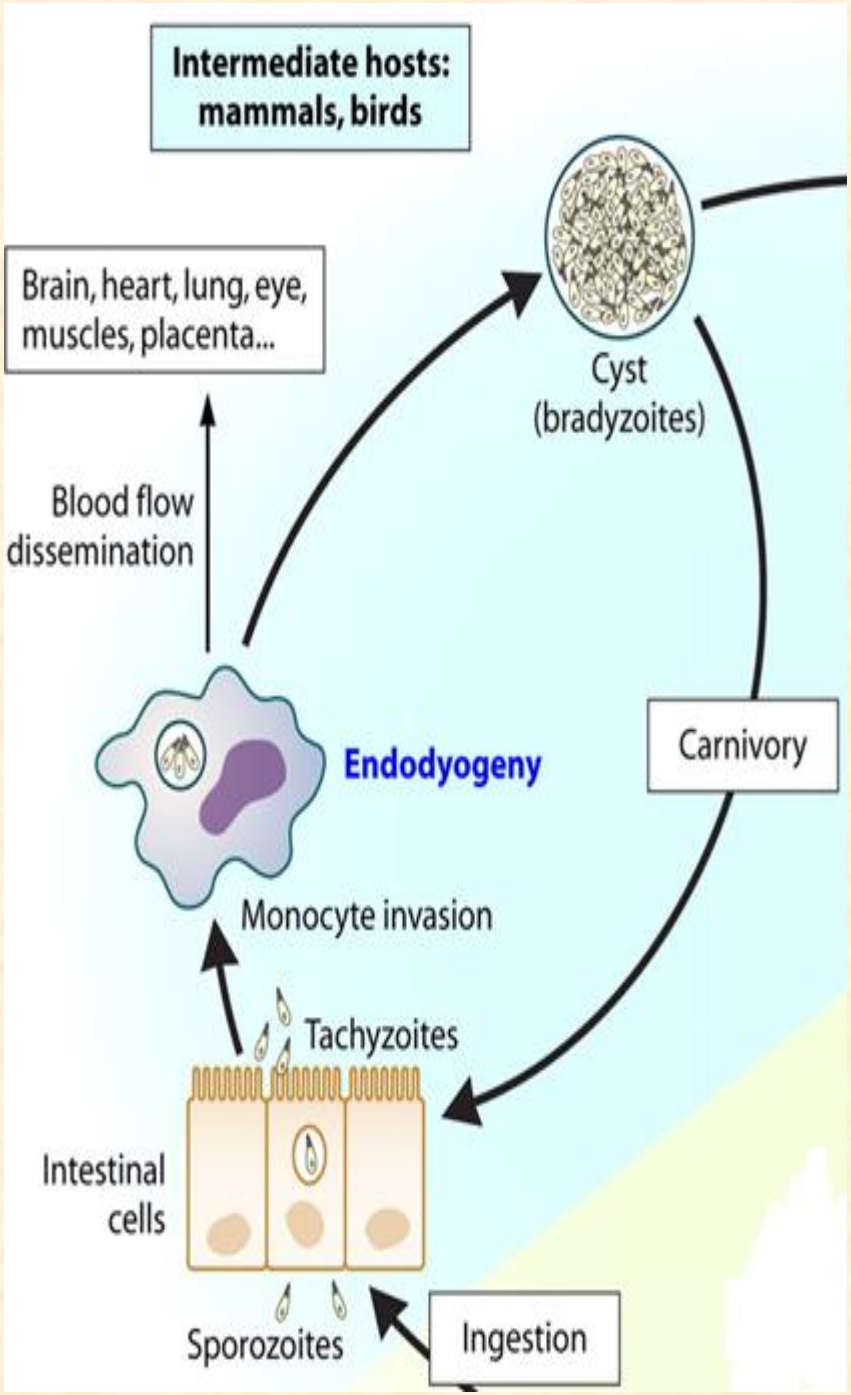
➢ Tachizoites **dissemination to**
- Lymphatic tissue
- Skeletal muscle
- Myocardium
- Retina
- Placenta
- CNS
- et al

By ~3 week after inf. tachyzoites disappear from visceral tissues →

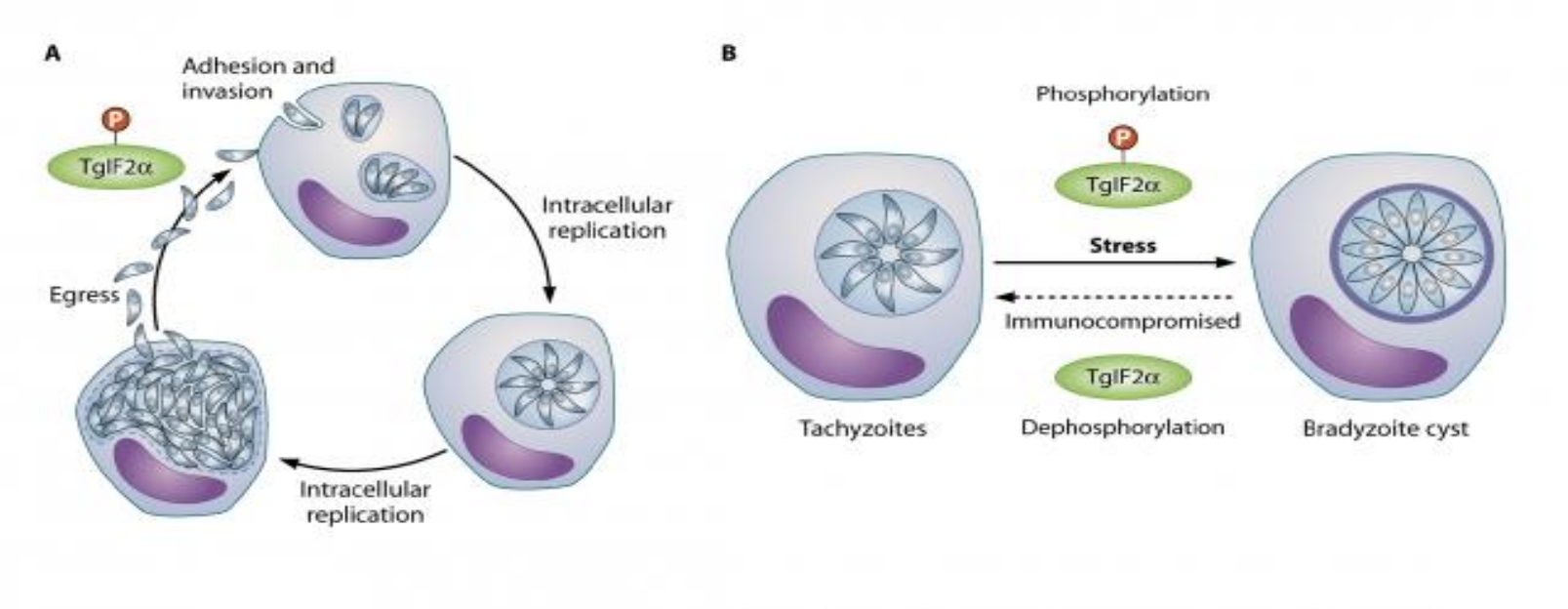
parasite enters the “resting” stage

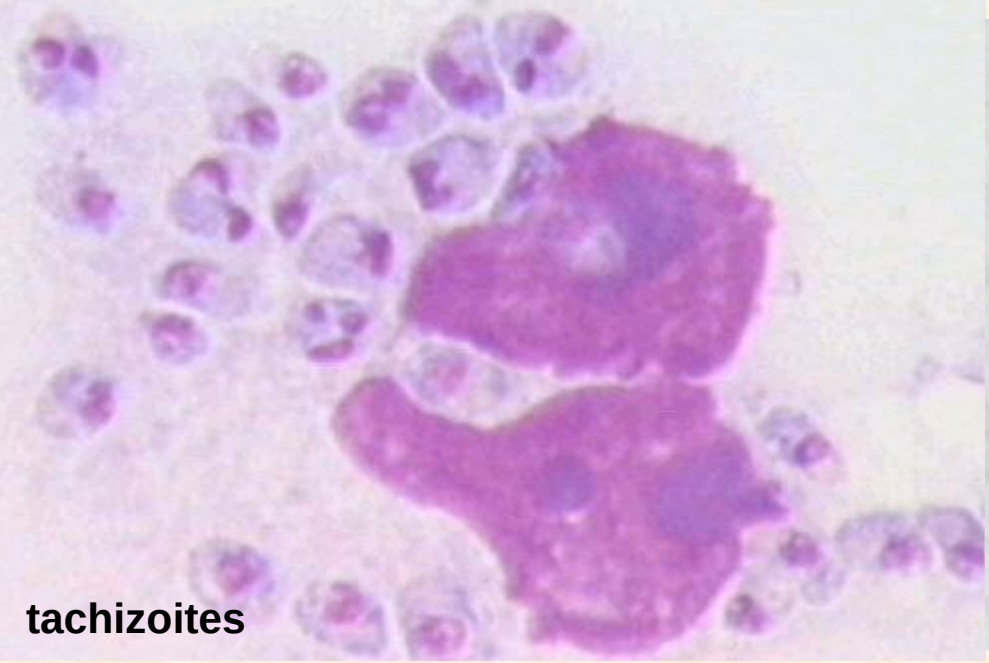
(tissue cysts > in **brain, liver, muscles**.).

Tahyzoites may persist longer in the spinal cord, brain ← immune responses are less effective in these organs.

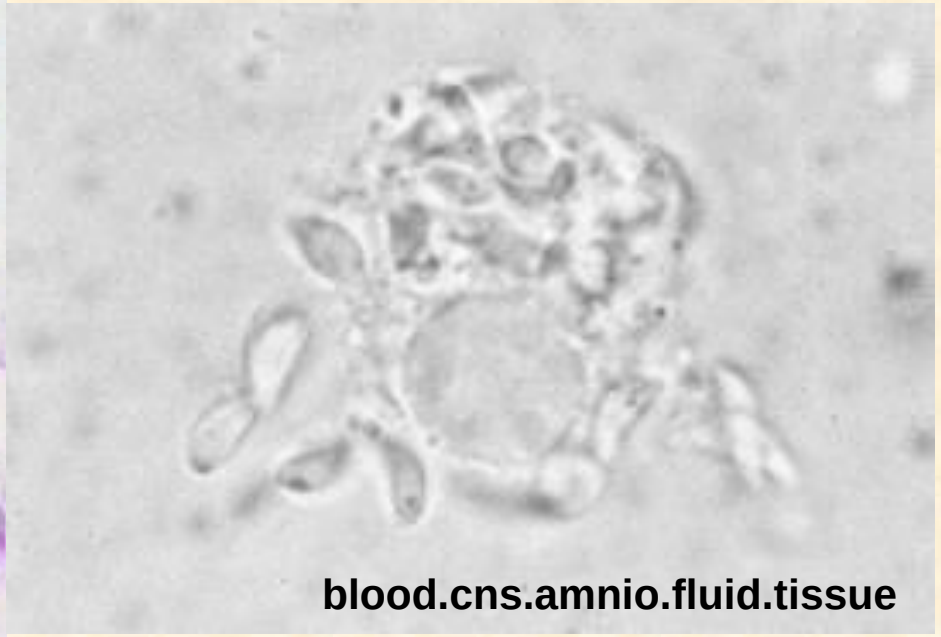


- **Tissue cysts usually cause no host reaction → infection persists lifelong even in immunocompetent hosts**
- **Lifelong infection usually remains subclinical**
- **Tissue cysts rupture periodically, the bradyzoites released are normally destroyed by the immune responses.**
- **In immunosuppressed patients, rupture of a tissue cyst may result in renewed multiplication of bradyzoites into tachyzoites**
- **Chronic infections may be reactivated locally (for example, in the eye).**



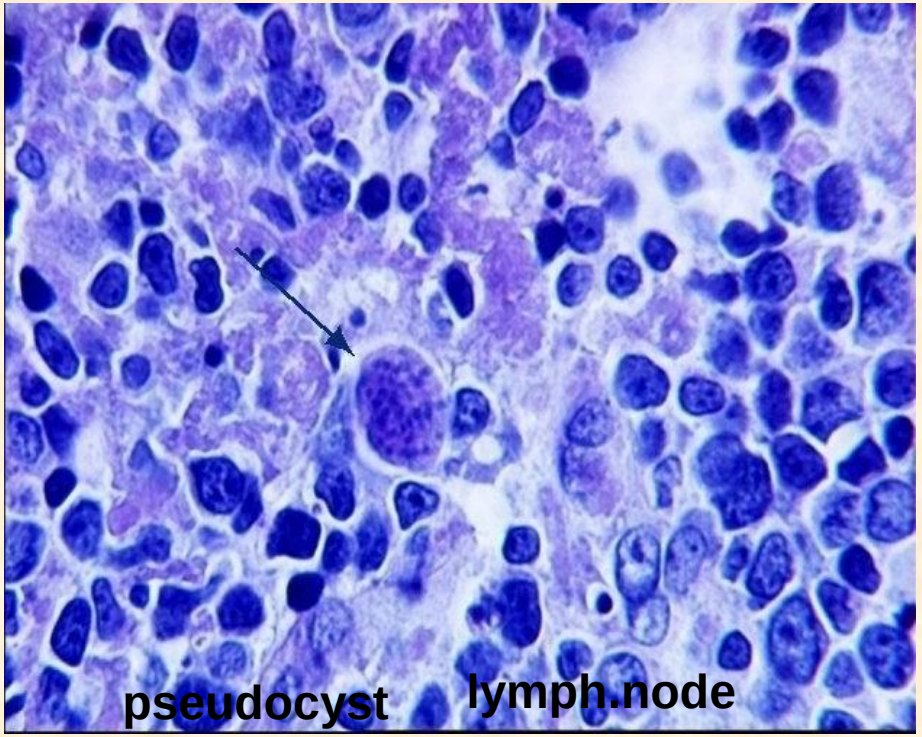


tachizoites



blood.cns.amnio.fluid.tissue

cyst.tissue.bradyzoit.



pseudocyst lymph.node

For clinical purposes, toxoplasmosis can be divided for convenience into five infection categories:

1. **acquired by immunocompetent patients**
2. **acquired during pregnancy**
3. **acquired congenitally**
4. **acquired by or reactivated in immunodeficient patients**
5. **ocular infections**

methods of diagnosis and
their interpretations may differ
for each clinical category

The incubation period of acquired inf. =4–21days (7 days on average)

In immunocompetent patients

- Acute disease - usually asymptomatic and self-limited.
  - unrecognized > 80%

- Most common symptoms:
  - Lymphadenopathy (most often involving a single site around the head and neck), generalized 20–30%
  - Headache
  - Malaise, fatigue
  - Fever, usually < 37- 38°C

- Less common:
  - Mononucleosis-like syndrome (fever, malaise, sore throat, headache, atypical lymphocytosis)
  - Abdominal pain
  - Maculopapular rash
  - Meningoencephalitis

- Symptoms resolve within several weeks
- Lymphadenopathy may persist for some months.

Link to neuropsychiatric disorders

Increased incidence of toxo seropositivity among pts suffering from :

- **schizophrenia**
- **Parkinson`s disoders (PD)**
- **obsessive compulsive disoder**
- **depression**

Because:

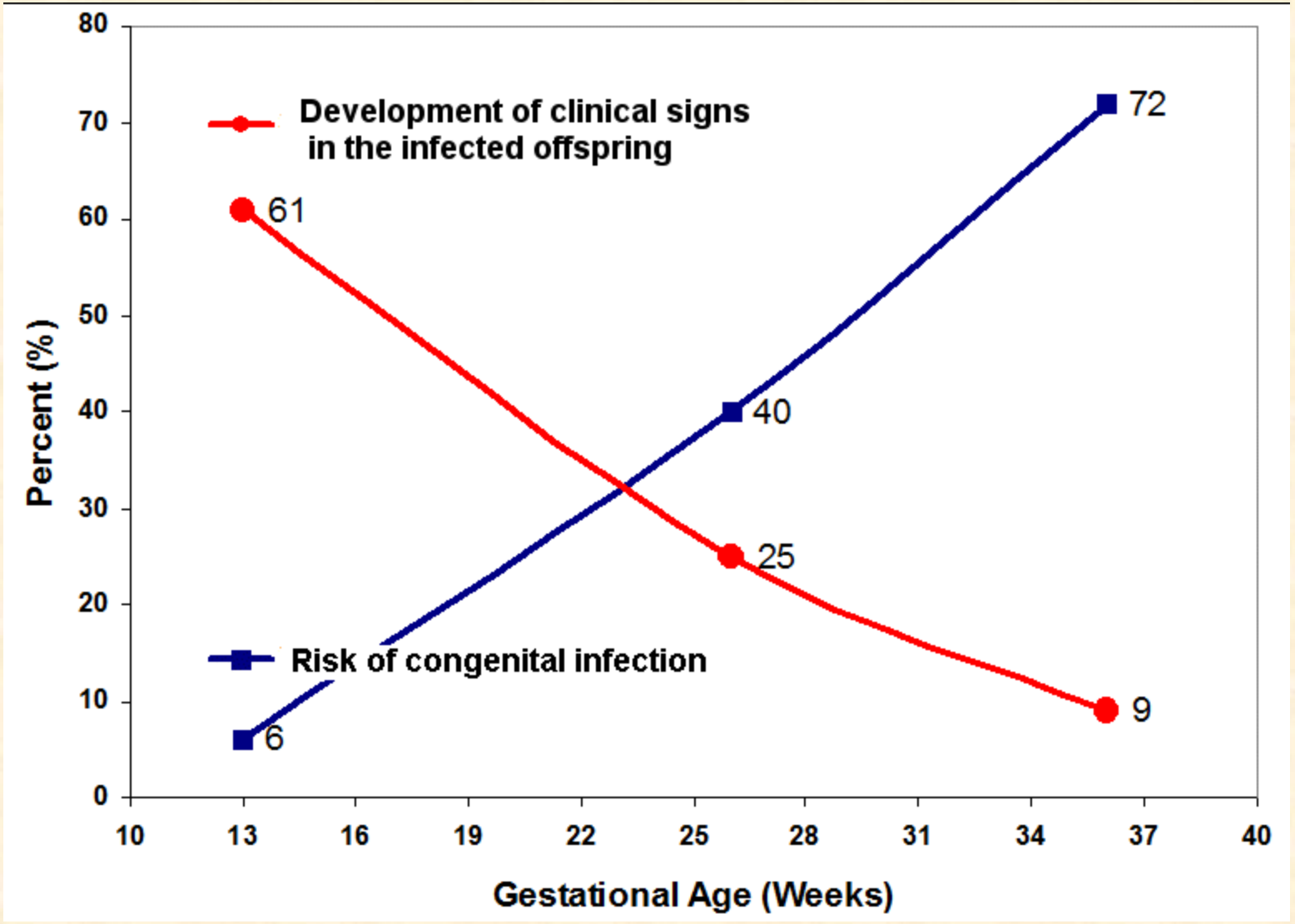
- **T. gondii affect dopamine in the brain, which is a common pathological process shared with schizophrenia and others,**
- **toxoplasmic encephalitis commonly affects the basal ganglia**
- **Treatment trails show that treatment of toxoplasmosis improves schizophrenia symptoms.**

Infection in pregnant women

Women seropositive ≥ 6 mo before pregnancy →
usually are protected against acute infection →
do not give birth to infected neonates.

- **1/3 women infected during pregnancy → transmit inf. to the fetus.**
- **Only 20% pregnant women inf. with *T.* develop clinical signs of inf.**

The severity of the fetal illness is inversely proportional to gestational age
Vertical transmission rate is directly proportional to the pregnancy stage



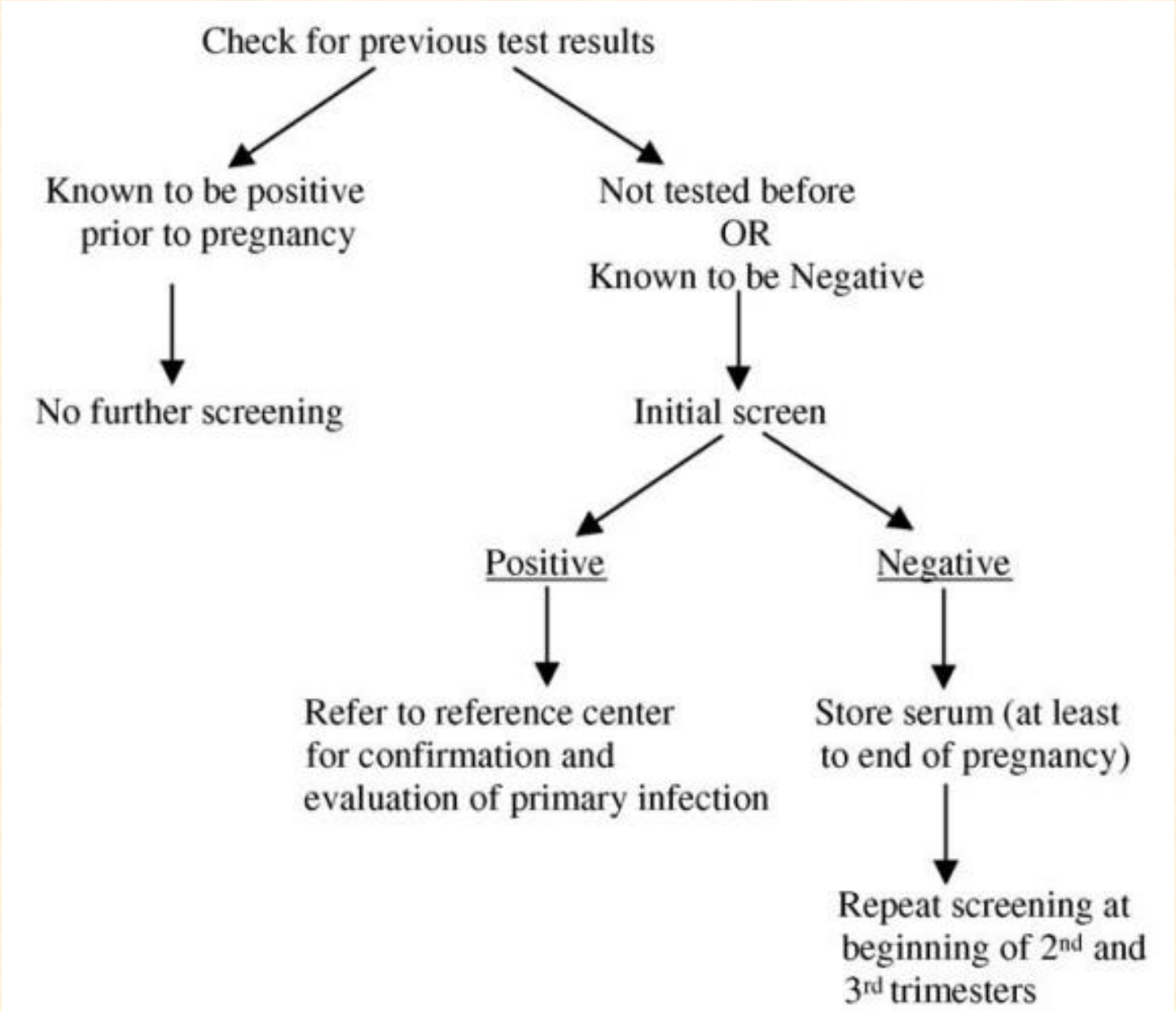
Maternal infection during the I trimester:

- **transplacental infection ~15%,**
- **severe neonatal disease ← immune system is poorly developed**

Maternal infection during the III trimester:

- **Increase blood supply to foetus during the later stages of pregnancy →**
  transmission most frequent during later stages = >65%
- **II or III trimester → foetal immune system is better developed →**
  can cope more efficiently with infection →
  inf. is more likely to be subclinical →
  infant usually asymptomatic at birth

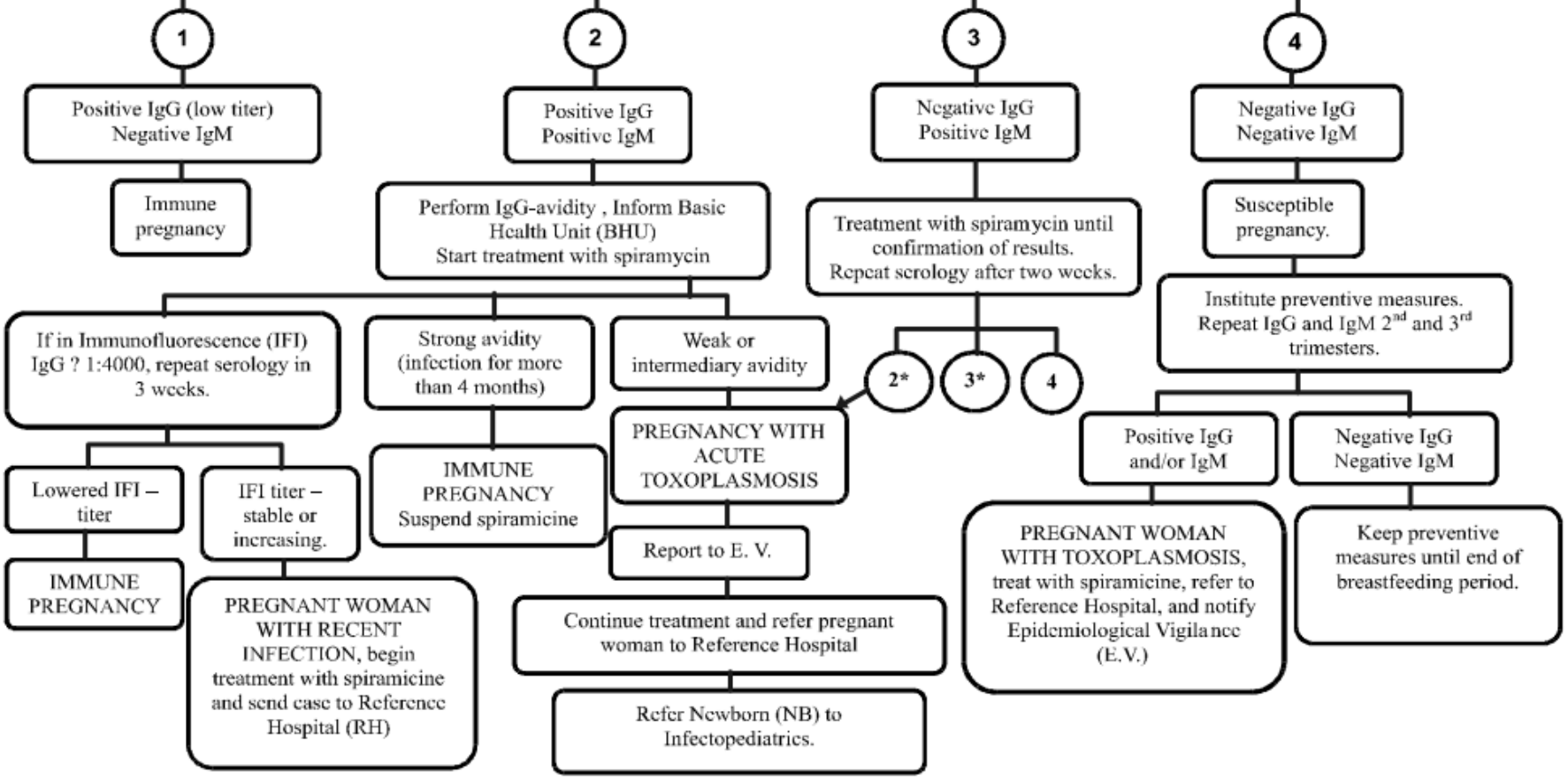
- Exposure of the developing immune system to T.gondii →
  result in tolerance of T-cells for parasite Ag →
  inability to deal efficiently with this infection →
  disease later in life →
  chorioretinitis in adults who have been affected by congenital toxoplasmosis



Laboratory diagnosis of congenital toxoplasmosis.

INTERPRETATION OF RESULTS AND CONDUCT FOR PREGNANT WOMEN FIRST THREE MONTHS OF PREGNANCY

Request serology for Toxoplasmosis IgG and IgM (capture ELISA, MEIA, ELFA, Chemilumi nescence Immunoassay)



1 If pregnant woman present suggestive symptoms or fetal ultrasound scan show alteration REPEAT SEROLOGY.
2* - There is no need to perform IgG-avidity, because reactive IgG and reactive IgM already confirm recent infection.
3* - If result is maintained (IgM was false-positive) SUSCEPTIBLE pregnancy.

Prenatal congenital toxoplasmosis

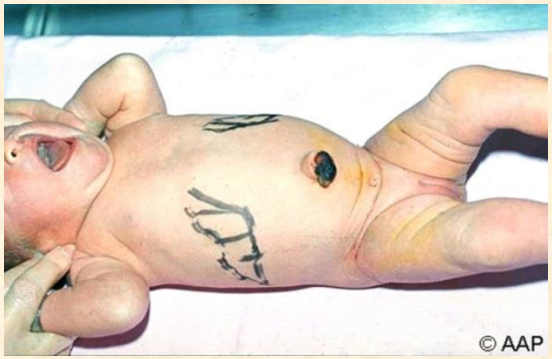
Prenatal ultrasound → usually no abnormality in fetuses

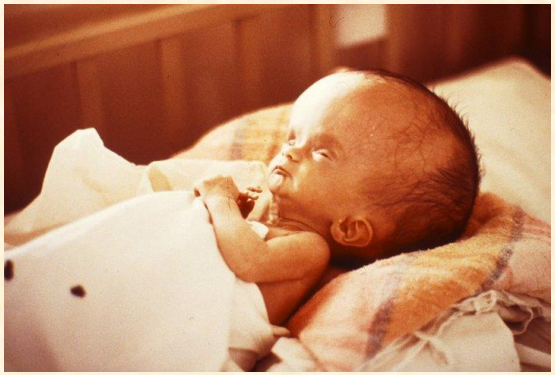
When ultrasonographic findings are present:

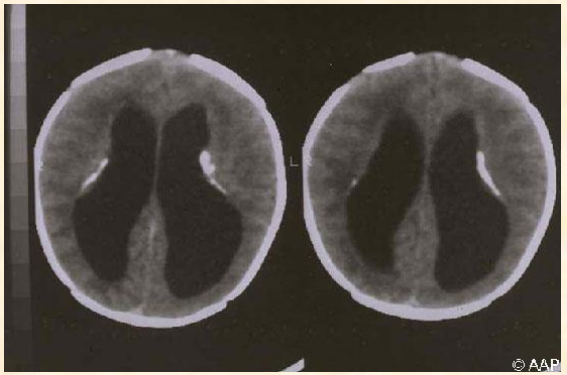
- **intracranial calcifications,**
- **ventricular dilatation,**
- **hepatic enlargement,**
- **ascites,**
- **increased placental thickness**

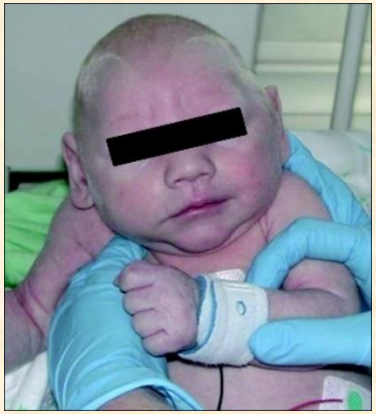
Neonatal congenital toxoplasmosis

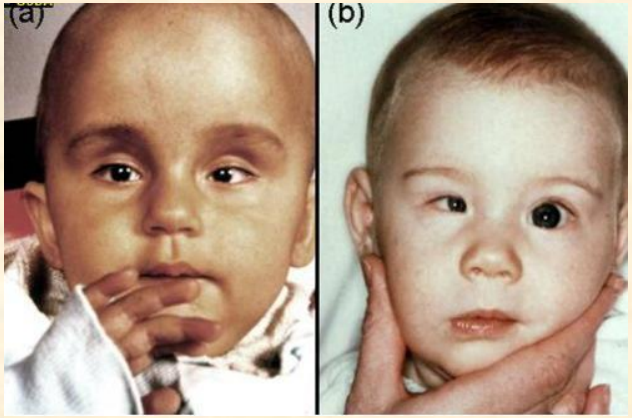
- hydrocephalus, microcephaly, ventricular dilatation
- intracranial calcifications,
- cerebral palsy, nerve deafness
- epilepsy, psychomotor or mental retardation
- maculopapular rash,
- generalized lymphadenopathy,
- hepatomegaly, splenomegaly, hyperbilirubinemia
- petechia associated with thrombocytopenia, and anaemia







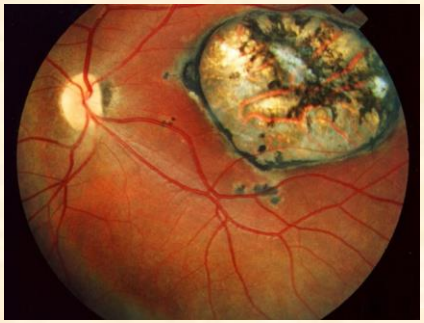




A) bulging forehead,
B) b) Microopthalmia of the left eye.

Neonatal congenital toxoplasmosis

- **>80% children with sub-clinical toxo present ocular sequels at some point in their lives.**
- **chorioretinitis 92%,**



←Macular scar secondary to congenital toxo

- **strabismus, microphthalmia**
- **~ iridocyclitis, cataracts, glaucoma, diminished visual acuity**

Congenital toxo signs can be mimicked by TORCH pathogens (Toxo, Other, Rubella, CMV, HSV)

Ocular toxoplasmosis

1. congenital 75-80%

2. postnatally acquired infection 1-3%???. (in a Canadian epidemic of toxo, 21% who were affected developed ocular lesions)

3. patients with the HIV 1-2% (recurrent toxo)

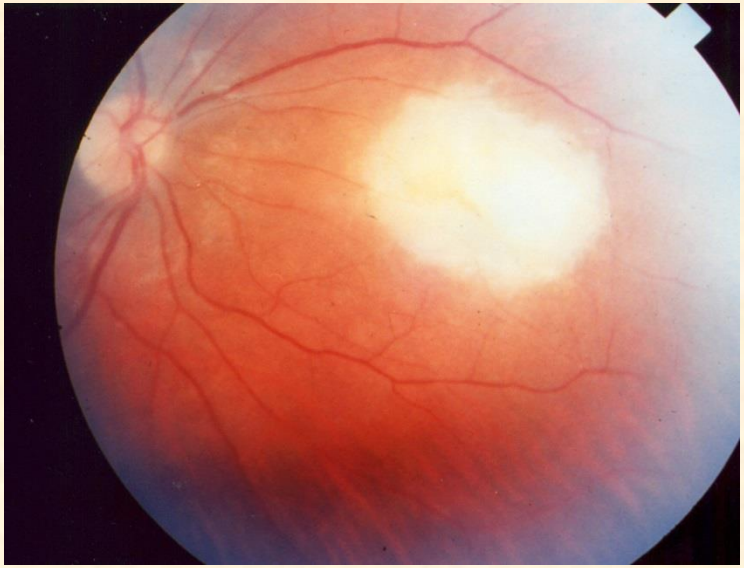
The retina is the primary site for the multiplying parasites, while the choroid and the sclera may be the sites of contiguous inflammation

Ocular toxoplasmosis

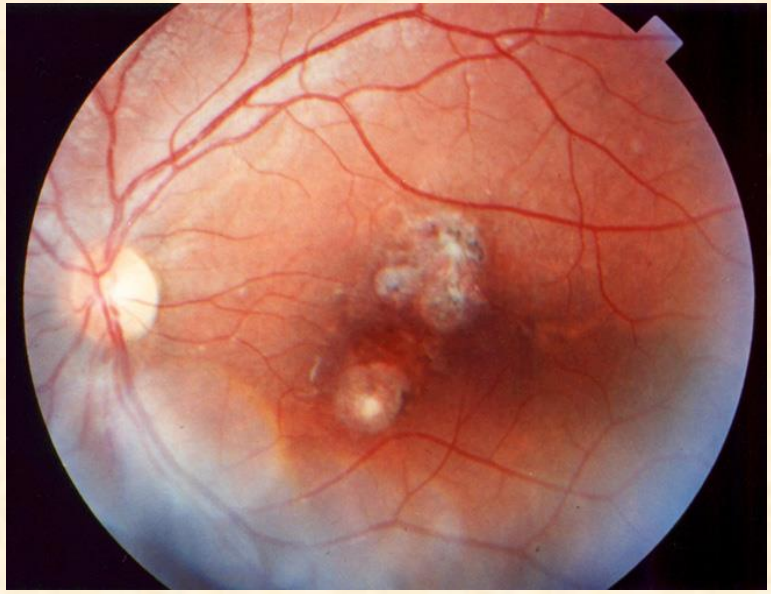
- Most common in congenital toxo →

  necrotizing retinochoroiditis, with predilection for the posterior pole described as a whitish, fluffy lesion surrounded by retinal edema

- ~ **optic neuritis or papillitis** associated with edema (Jensen diseas).
- ~ **posterior vitreous detachment** → precipitates of inflammatory cells on the posterior vitreous face, referred to as vitreous precipitates.
- ~ **retinal vasculitis & granulomatous or nongranulomatous anterior uveitis** ←*toxo Ag* are responsible for a hypersensitivity reaction



Acute macular retinitis associated with primary acquired toxo



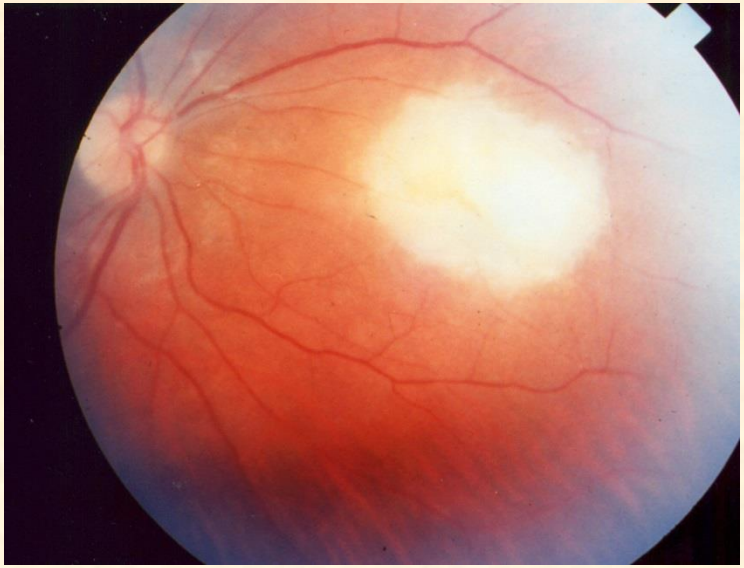
Peripapillary scars secondary to toxo

In primary ocular toxo → unilateral focus in >50% cases.

Lesions may occur during the acute or latent (chronic) stage

- **Floaters, blurred vision, decreased visual acuity** ← as a result of macular involvement or severe vitreous inflammation
- **Photophobia**
- **Scotoma, loss of central vision**
- **Nystagmus**
- **Disorders of convergence/strabismus**

- **Ocular toxoplasmosis** :
  - **Acute toxo:** yellow-white, cotton-like patches with indistinct margins of hyperemia
  - **As the lesions heals:** punched-out scar, revealing white, underlying sclera (results from extensive retinal and choroidal necrosis surrounded by variable pigment proliferation)
  - **Recurrent ocular toxo**: the areas of newly active necrotizing retinitis are usually adjacent to old scars (so-called satellite lesions).
  - **In AIDS**: retinal lesions are often large, with diffuse retinal necrosis.



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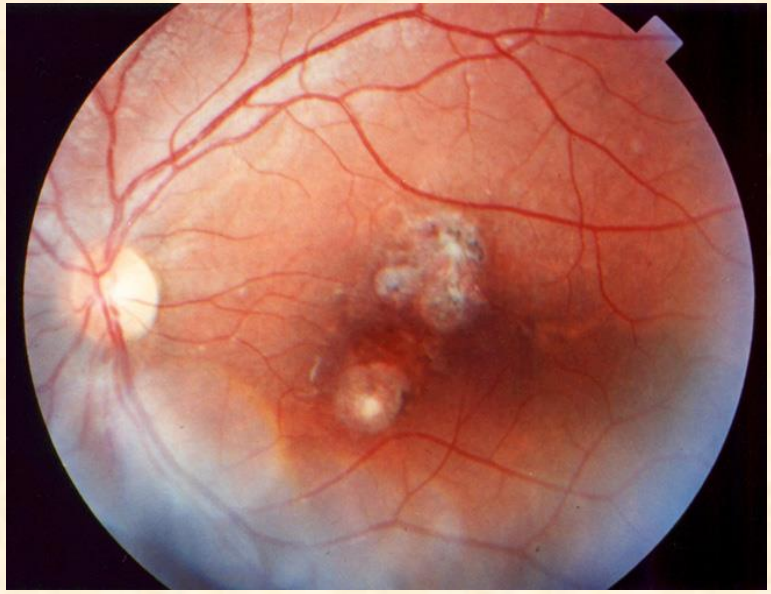
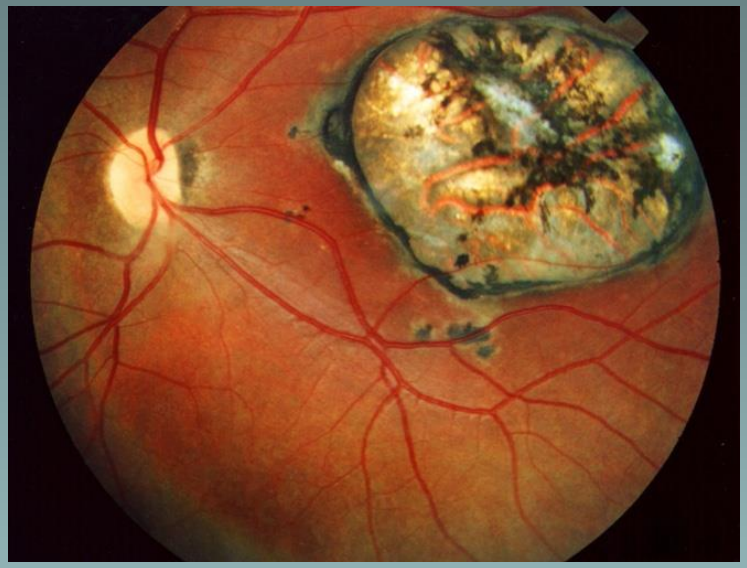
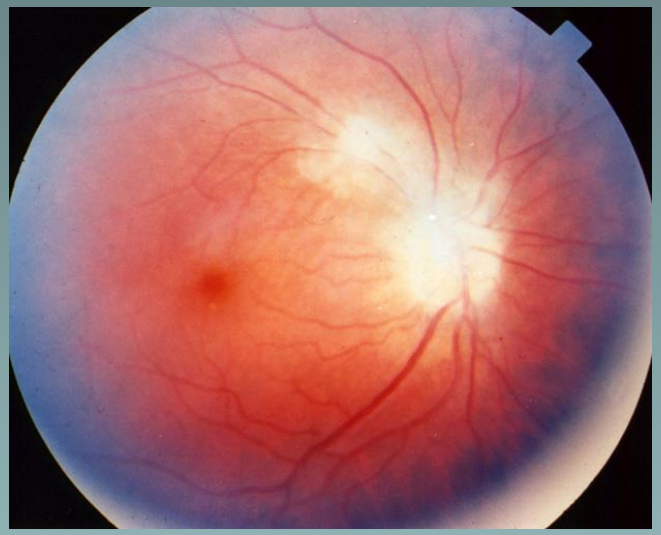


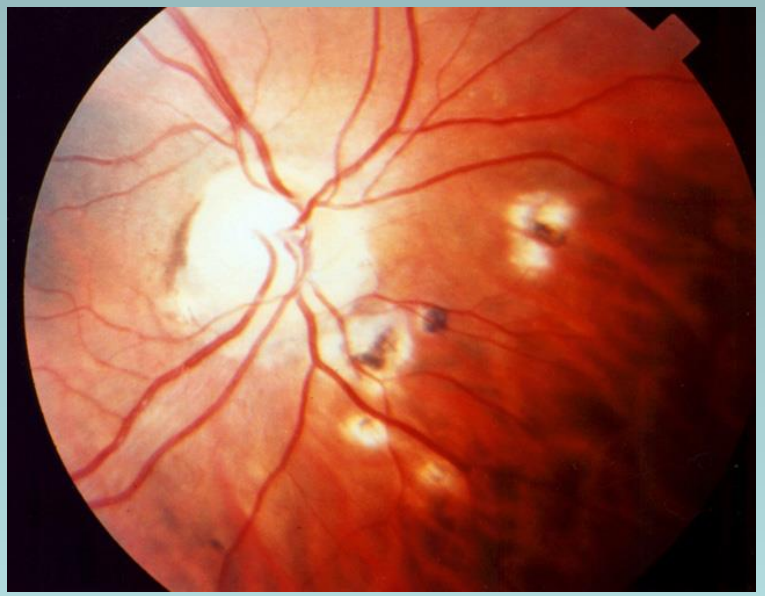
Peripapillary scars secondary to toxo



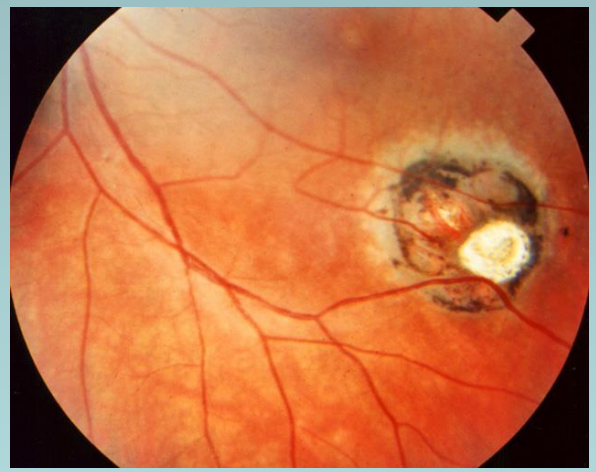
Macular scar secondary to congenital toxo



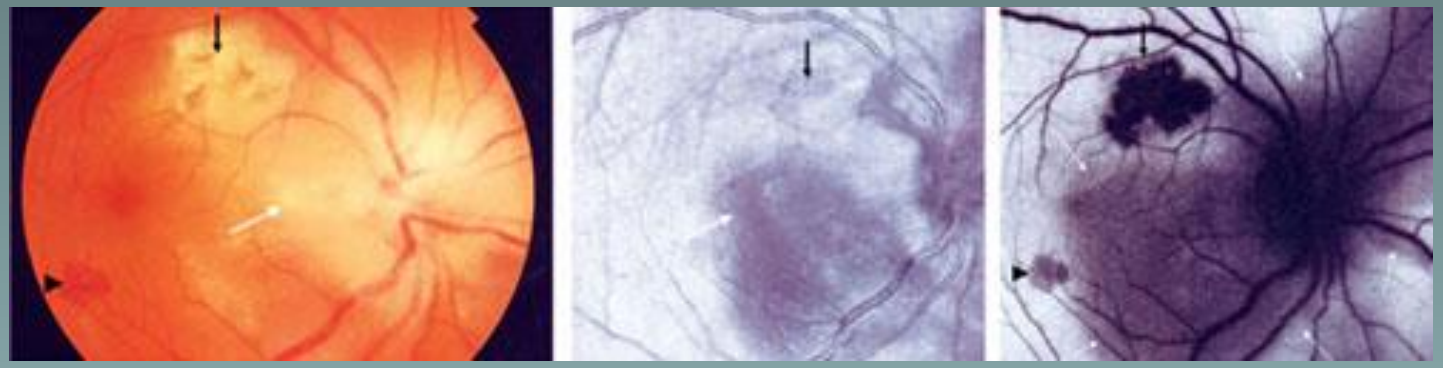
Papillitis secondary to toxo



Peripapillary scars secondary to toxo



Inactive chorioretinal scar secondary to toxo



Color fundus (left), infrared (center) and autofluorescence (right) photo. of a 27-year-old man:

- an acute diffuse neuroretinitis (white arrows).
- Color fundus photographs show a pre-retinal hemorrhage (arrow head) and
- an old toxoplasma retinochoroiditis scar superior to the macula (black arrow).
- The corresponding autofluorescence image shows the extent of hyper-autofluorescence (white arrows), which clearly demonstrates the lesion border, which may not be detectable clinically.

D.D.: Necrotizing retinitis due to CMV, HSV, HZV, candidiasis, blastomycosis, septic retinitis, toxocariasis, sarcoidosis, syphilis, tuberculosis

In immunocompromised patients

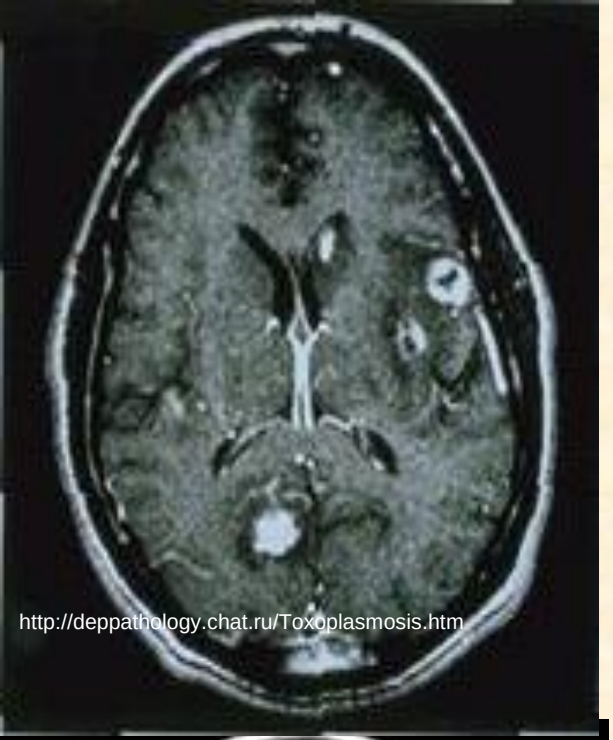
- Infection of macrophages with HIV → inhibits the ability to kill T.gondii
- peripheral blood mononuclear cells (PBMC) produce reduced level of IL-12 and IFN γ in response to T.gondii infection

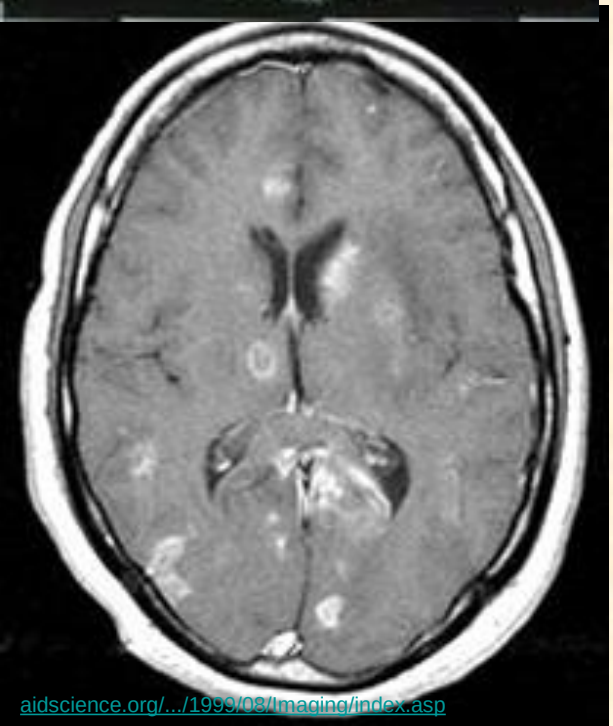
Toxoplasmic **encephalitis** occurs in 10-50% of HIV-inf. pts who are seropositive for antibodies to *T gondii* & $CD4^+$ <100/µL

1. **Reactivation of latent infection**
2. **Acquisition of new infection**

In immunocompromised patients

- Symptoms and signs principally involve the **CNS**:
  - insidious (several weeks) or acute
  - altered mental status 75%
  - fever 10–72%
  - seizures 33%
  - headaches 56%
  - focal neurologic findings 60%:
    - motor deficits, movement disorders
    - cranial nerve palsies, visual field loss
    - aphasia
- **Rarely** - ocular
- **Rarely** – respiratory
  - fever, dyspnea, nonproductive cough
  - bilateral
  - may rapidly progress to ARDS
- **Rarely** - infection of the heart
  - can be associated with cardiac tamponade or biventricular failure

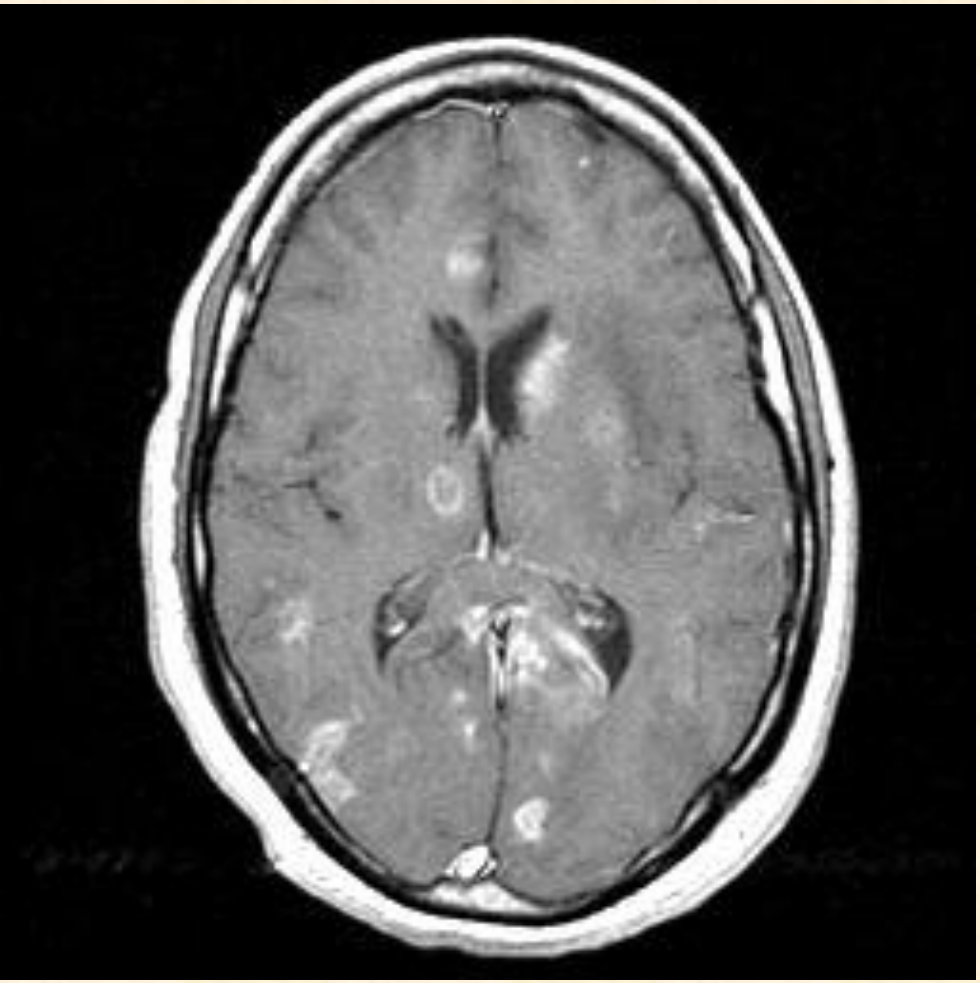


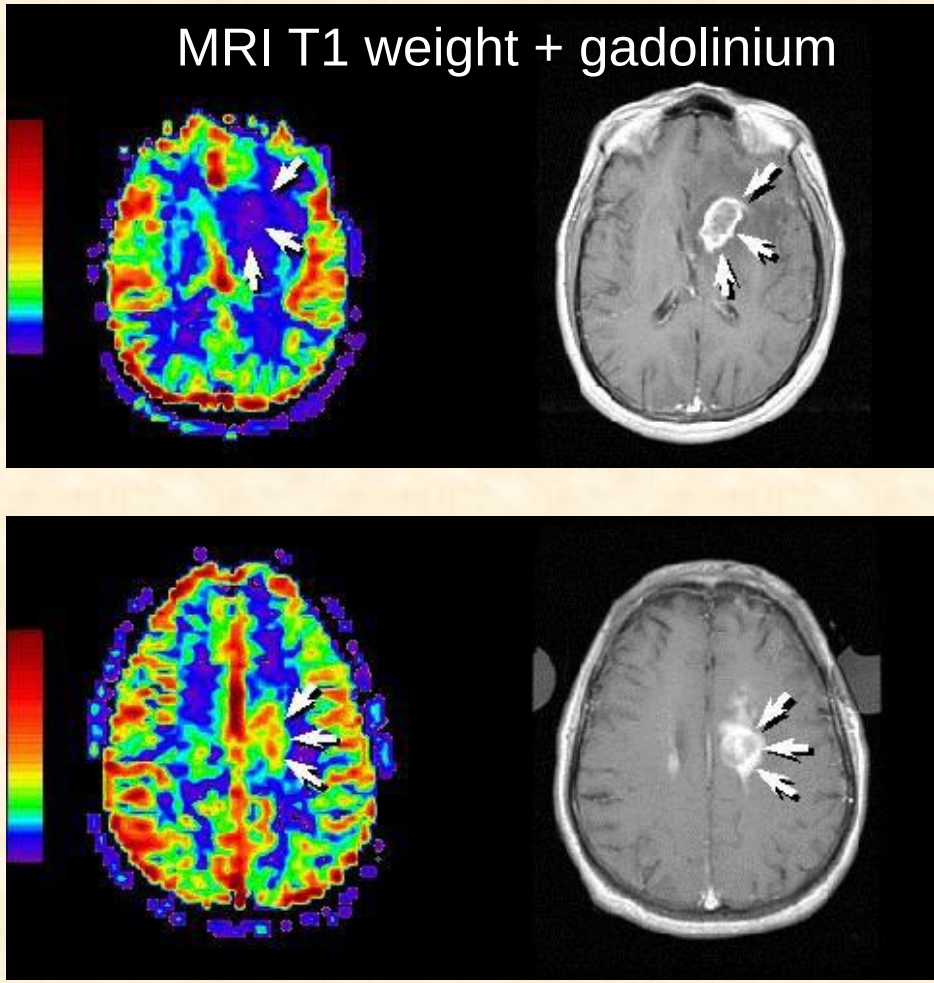


brain abscesses → central avascular area is surrounded by a region of necrosis and inflammatory cells that may also contain free and intracellular tachyzoites

- **multiple lesions 70-80% cases**
- **focal signs**
- **adjacent edema, different intensity**
- **CT: lesions iso- /hypodense, mm-4 cm**
- **RMN:**
  - **în T1: izo-/hypointense**
  - **în T2: different intensity**
- **CT & RMN + contrast:**
  - **ring enhancing**
  - **rarely more homogeny, nodular, or without accumulation**
  - **ring – thin, good contrasted**
  - **in big affection – can be thick ring**

Cerebral toxoplasmosis





MRI is more sensitive than CT m

multiple ring-enhancing lesion surrounded by variable degrees of vasogenic edema

predilection for cortex and deep gray-matter structures such as the basal ganglia

cerebellum and brain stem are less commonly involved

Cutaneous toxoplasmosis

Rare

- roseola
- erythema multiforme-like eruptions,
- prurigo-like nodules,
- urticaria,
- maculopapular lesions.

Diagnosis of cutaneous toxoplasmosis is based on the tachyzoite being found in the epidermis.

It can be identified by electron microscopy or by Giemsa staining tissue where the cytoplasm shows blue, the nucleus red

The diagnosis of T. gondii infection or toxoplasmosis may be established by:

1.serologic tests
2.amplification of specific nucleic acid sequences (PCR)
3.histologic demonstration of the parasite and/or its Ag (i.e. immunoperoxidase stain)
4.isolation of the organism

A combination of serologic tests is usually required to establish whether an individual has been most likely infected in the distant past or has been recently infected

Serologic patterns in Toxoplasma gondii infection

ELISA	Key points
IgG	Appears **in 1 to 2 weeks.** Peak 1-2 mo
	FDA **warns against using as the sole method** of determining infection during pregnancy
	Declines over 1-2 years but **may persist for life**
	Passively transferred maternal Autonomous IgG Ab in a congenitally inf. untreated newborn begins in about 3 mo
IgM	Appears **early in infection** & decline more rapidly than IgG Ab
	Neg test in immunocompetent pts generally excludes acute infection
	May persist for 1 or more years following infection
	Do not use assay for sole determination of acute inf. in pregnant women or adults
IgA	More sensitive than IgM ELISA for detection **of inf. in fetus & newborn** Test should be performed during first few days of life; if positive, retest after 10 d
	May persist for months or years following acute infection = of little additional assistance for diagnosis of acute infection in the adult.
	Avidity assay useful in inf. during first trimester of pregnancy
IgE	In adults, presence usually indicates **acute inf.** Pos for briefer period than IgM or IgA (for less than 4 mo after infection)
	Pos in patients with toxoplasmic **encephalitis**
	Present in congenital infection
	Absence does not exclude infection

IgM antibodies

pos. IgM test in a single serum sample can be interpreted as:

1.a true-positive result in the setting of a recently acquired infection,

2.a true-positive result in the setting of an inf. acquired in the distant past,

3.a false-positive result

→confirmatory testing should be done for all cases

True-neg. IgM test rules out recent inf.

The FDA has issued a warning not to use *T gondii* IgM commercial test kits as the sole method of diagnosis during pregnancy.

IgG antibodies

✓DT (Sabin-Feldman dye test),
✓ELISA,
✓IFA,
✓modified direct agglutination test.

- **appear within 1–2 weeks of acquisition of the inf.**
- **peak within 1–2 months,**
- **decline gradually over the next 1 to 2 years, persist for life.**

Rarely IgG Ab → not detected within 2–3 weeks after initial exposure
Rarely toxo chorioretin. & encephalitis in immunocomprom. pts → neg. IgG Ab

Passively transferred maternal IgG →half life of approximately 1 month, generally disappearing completely within one year

Appearance of autonomous IgG Ab in a congenitally infected newborn begins, in an untreated pts about 3 months after birth

Anti-parasitic therapy may delay Ab production for about 6 months and, occasionally, may completely prevent antibodies production

IgG antibodies

Discriminate between recently acquired and distant infection:

Avidity test of Toxo IgG-Ab → affinity increases during subsequent weeks & months by Ag-driven B cell selection. Inf. acquired in the recent 3–4 months is ruled out in the presence of high avidity antibodies.

Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex. The avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated samples.

HS/AC test. Agglutination titers to formalin-fixed tachyzoites (HS antigen) are compared with titers against acetone- or methanol-fixed tachyzoites (AC antigen). Acute pattern → high AC and HS titers, nonacute pattern → high AC titers and low HS titers.

IgM antibodies

✓IgM-ELISA kits,
✓IFA test (false-pos due to RF & ANA are not detected by IgM-ELISA)
✓immunosorbent agglutination assay (IgM-ISAGA)

➢Appear earlier & decline more rapidly than IgG Ab
➢Become negative within a few months
➢~ can still be observed during the chronic phase
➢~ can be detected as long as 12 years after the acute infection
➢May yield frequent **false-pos or false-neg results (pregnants**, immunocomprom., highly endemic.)

pos. IgM test in a single serum sample can be interpreted as:
1.a true-positive result in the setting of a recently acquired infection,
2.a true-positive result in the setting of an inf. acquired in the distant past,
3.a false-positive result
→confirmatory testing should be done for all cases

True-neg. IgM test rules out recent inf.

The FDA has issued a warning not to use *T gondii* IgM commercial test kits as the sole method of diagnosis during pregnancy.

IgA antibodies.

✓ELISA
✓immunosorbent agglutination assay (IgM-ISAGA)

IgA antibodies may be detected in sera of:

- acutely infected adults
- congenitally infected infants
- may persist for many months or more than a year → are of little additional assistance for diagnosis of acute infection in the adult.
- **increased sensitivity of IgA assays over IgM assays for diagnosis of congenital toxoplasmosis**

~newborns with congenital toxo. neg IgM Ab→diagnos by pos IgA+IgG Ab

During the first trimester of pregnancy determine recent infection

ELISA rarely detects IgA Ab in AIDS pts with toxoplasmic encephalitis.

IgE antibodies.

✓ELISA
✓immunosorbent agglutination assay (IgM-ISAGA)

- increases rapidly in acutely infected adults,
- **remains detectable for less than 4 months after infection**
- congenitally infected infants,
- children with congenital toxoplasmic chorioretinitis.

Their demonstration does not appear to be particularly useful for diagnosis of T. gondii infection in the fetus or newborn when compared with IgA tests.

The duration of **IgE seropositivity is briefer than that with IgM or IgA Ab and hence appears useful for identifying recently acquired infections.**

IgE-Ab have been detected in pts with toxoplasmic encephalitis

In pregnant women.

IgM IgG IgE

The important issue is whether the inf. was acquired before or after conception.

Diagnosis of acute *T gondii* infection requires:

- **neg → pos** or a significant rise in Ab levels,
- collected at least 3 weeks apart and tested in parallel.

IgM ELISA could give a **false-positive** result →
confirmatory testing is highly recommended

- neg. IgM in first 6 mo of gestation & low IgG test = ?
- neg IgM in the III trimester, = ?

In pregnant women.

➢neg. IgM in first 6 mo of gestation & low IgG test = acquisition prior to gestation.

➢neg IgM in the III trimester, = chronic maternal inf. or acute inf. acquired early in pregnancy but with rapid decline in IgM titers → use other serologic tests

When an acute infection is suspected during pregnancy →
prenatal diagnosis is advised →
ultrasonography & amniocentesis **18 we gestation** →
Amniotic fluid PCR analysis
specificity = 99.7%
sensitivity - 64%

At least 4 weeks should elapse after acute disease → possibility of a false-neg result.

PCR is insufficient by itself → must be done in conjunction with other testing methods

When ordering and interpreting maternal serological screening tests:

- question lab regarding its methods of **quality assurance;**
- **should not rely on a single sample test !!!**
- **seek confirmatory testing** through a nationally recognized reference laboratory if results are pos.

- Testing of serial specimens obtained 3–4 weeks apart (in parallel) provides the best discriminatory power if the results in the initial specimen are equivocal.

- Serologic tests **should not be considered useful** for measuring response to therapy.

Congenital toxoplasmosis

- **IgA and IgM Ab is the foundation of diagnosis in the newborn.**
- IgA Ab → more sensitive than IgM Ab in newborn
- IgA may be present when there is no IgM, and the vice versa
- Fetal IgA or IgM Ab may not be produced before 22 weeks of gestation

Umbilical cord serum samples → contaminated with maternal blood !

Western blots → compare newborn versus maternal antibodies

Ocular Toxoplasmosis

- Low titers of IgG Ab are usual in pts with active chorioretinitis due to reactivation of congenital T. gondii infection; IgM antibodies usually are not detected.
- In most cases, toxoplasmic chorioretinitis is diagnosed by ophthalmologic examination, and empiric therapy
- In unclear clinical diagnosis and/or inadequate clinical response → detection of increased Ab response in ocular fluids
- parasite isolation, histopathology, PCR in both aqueous and vitreous fluids

- **The PCR of toxoplasmosis DNA from amniotic fluid has been deemed the most reliable and safe method of prenatal diagnosis:**
  - sensitivity (ability to find true positives) - 64%
  - specificity (ability to find true negatives) -100%

PCR has enabled detection of T. gondii DNA in:

- **brain tissue, CSF,**
- **vitreous and aqueous fluids,**
- **bronchoalveolar lavage (BAL) fluid,**
- **Blood**

PCR is insufficient by itself → must be done in conjunction with other testing methods

Histologic Diagnosis

It is often difficult to demonstrate tachyzoites in conventionally stained tissue sections.

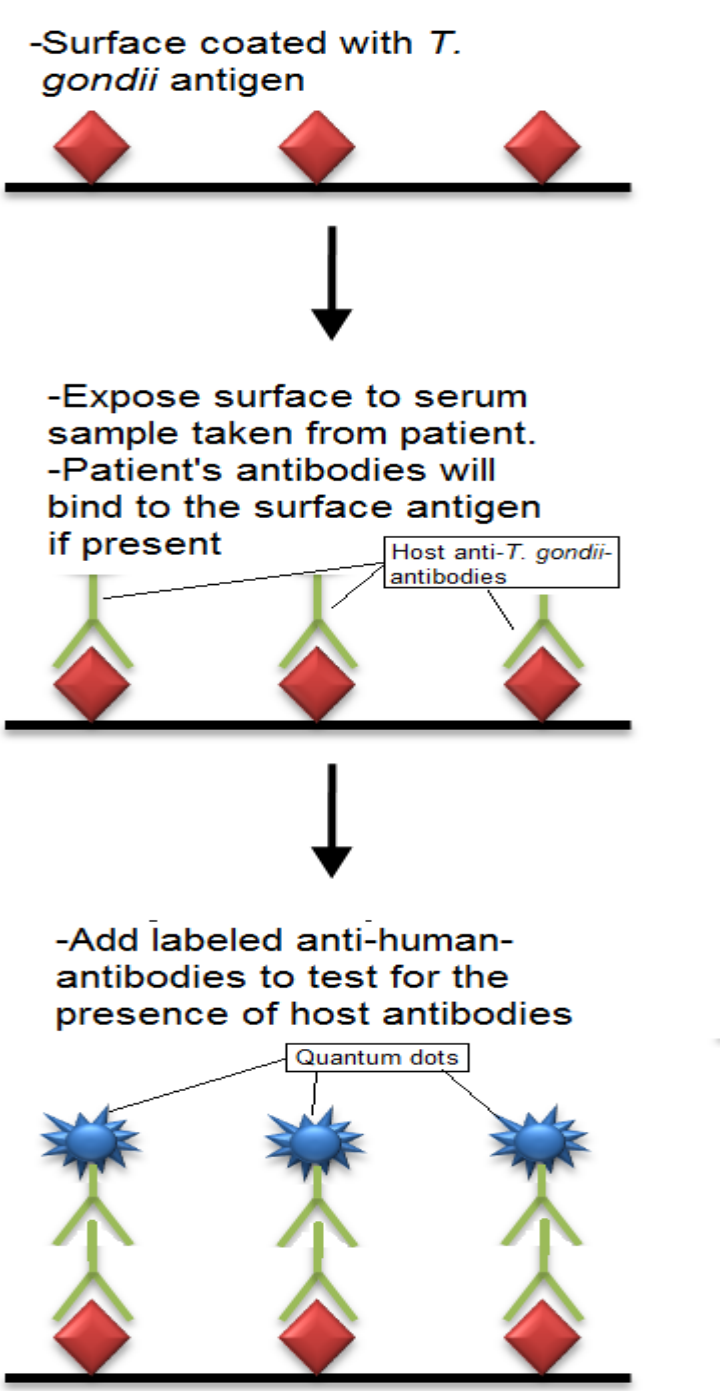
The immunoperoxidase technique, which uses antisera to T.gondii, has proven both sensitive and specific.

The immunoperoxidase method is applicable to unfixed or formalin-fixed paraffin-embedded tissue sections.

A rapid, technically simple, and under-used method is the detection of T. gondii in air-dried, Wright-Giemsa–stained slides of centrifuged (e.g., cytocentrifuge) sediment of CSF or of brain aspirate or in impression smears of biopsy tissue.

Isolation of T. gondii

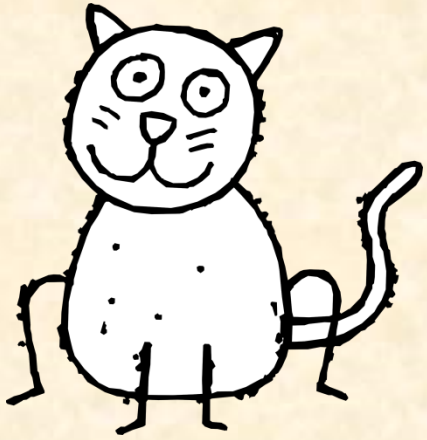
Attempts at isolation of the parasite can be performed by mouse inoculation or inoculation in tissue cell cultures of virtually any human tissue or body fluid.



Example ELISA for detecting *T. gondii*

Acute toxoplasmosis can be differentiated from chronic toxoplasmosis using flow-cytometry or VIDAS avidity assays to quantify the presence of different types of antibodies

Acute patients are positive for IgM antibodies but have a low IgG avidity index; whereas, chronic patients can be positive or negative for IgM antibodies but will have a high IgG avidity index.



- Serologic investigation of a cat to establish whether it is a potential source of the infection should be discouraged → Seropositivity in the cat does not predict shedding of oocysts.

- **Universal pregnant screening is mandatory in France and Austria:**
- **Screening prior to conception & follow-up during pregnancy (monthly – France, 3 times/pregnancy - Austia)**
- Germany, Switzerland and Belgium?

- **Bader et al. used decision analysis to compare three screening methods:**
- **no screening,**
- **targeted screening based on prenatal history of maternal risk factors,**
- **universal maternal screening.**

Universal screening reduced the total disease incidence the most, but had an unacceptable fetal loss ratio compared with both no screening and targeting screening.

IgG	IgM	Report/Interpretation for All Except Infants
Neg	Neg	No serological evidence of infection
Neg	Equiv	Possible early acute infection or false-positive IgM reaction. Obtain a new specimen for IgG and IgM. If new specimen result remains the same = pts is probably not infected
Neg	Pos	Possible acute infection or false-positive IgM. Obtain a new specimen for IgG and IgM. If results remain the same = IgM reaction is probably a false-positive.
Equiv	Neg	Indeterminate: obtain a new specimen for testing or retest this specimen for IgG in a different assay.
Equivocal	Equivocal	Indeterminate: obtain a new specimen for both IgG and IgM testing.

IgG	IgM	Report/Interpretation for All Except Infants
Equiv	Pos	Possible acute infection. Obtain a new specimen for IgG and IgM. If results with the new specimen remain the same or the IgG becomes positive, both specimens should be send to a reference laboratory.
Pos	Neg	Infected for more than 1 year.
Pos	Equiv	Infected with *Toxoplasma gondii* for probably more than 1 year or false-positive IgM reaction. If second specimen remain the same, send to a reference laboratory
Pos	Pos	Possible recent infection within the last 12 months. Send the specimen to a reference laboratory

TREATMENT

Effective treatment: **combination of 2 agents**

- **Pyrimethamine (Daraprim)** =Antiprotozoal Agents = folic acid antagonist that selectively inhibits plasmodial dihydrofolate reductase
- **Atovaquone (Mepron)** = Antiprotozoal Agents inhibits the mitochondrial electron transport chain

- **Sulfadiazine** = competitive antagonism of PABA, sulfadiazine interferes with microbial growth
- **Trimethoprim and sulfamethoxazole (Bactrim DS, Septra DS)** = competitive antagonism with PABA

- **Dapsone** = bactericidal and bacteriostatic against mycobacteria = a competitive antagonist of PABA, preventing the formation of folic acid and inhibiting bacterial growth.
- **Clindamycin (Cleocin)** = lincosamide antimicrobials. Block the dissociation of peptidyl transfer ribonucleic acid (t-RNA) from ribosomes, causing RNA-dependent protein synthesis to arrest.
- **Azithromycin (Zithromax, Zmax)** = macrolide antibiotic = binding to the 50S ribosomal subunit of susceptible microorganisms, thereby interfering with microbial protein synthesis.
- **Spiramycin** = macrolide antibiotic

Treatment nonpregnant patients

Immunocompetent, nonpregnant typically do not require treatment.

Treatment → **6-week regimen**:

- **pyrimethamine** 100mg loading dose orally → 25-50 mg/day +
- + **folinic acid** (leucovorin) 10-25 mg/day +
- + **sulfadiazine** 1 g x qid

OR

- pyrimethamine + folinic acid + clindamycin 300 mg x qid

Alternative treatment:

- TMP-SMX 10/50 mg/kg/day for 4 weeks

Sulfadiazine or clindamycin can be substituted for:

- + azithromycin 500 mg daily
- + atovaquone 750 mg x bid

A dosing regimen for pregnant patients → 50% decrease in fetal infection

➢**Spiramycin** 1 g every 8 hours

Pyrimethamine →not used before 12 weeks' gestation = teratogenic

If the amniotic fluid test result for *T gondii* is positive:

3 weeks of:

- pyrimethamine 50 mg/day +
- + Leucovorin 10-25 mg/day orally to prevent bone marrow suppression
- + sulfadiazine 3 g/day orally in 3 divided doses

alternating with a **3-weeks** course of:

- spiramycin 1 g 3 times daily for maternal treatment

OR

- Pyrimethamine 25 mg/day orally +
- + Leucovorin 10-25 mg/day orally to prevent bone marrow suppression
- sulfadiazine 4 g/day orally divided 2 or 4 times daily until delivery

- Spiramycin prophylaxis followed by a 4-week course of pyrimethamine plus sulfadiazine at 17 weeks of gestation in Austria and Germany

Treatment	Dosage	Comments
Spiramycin	1 g (3 million U) every 8 h (for a total of 3 g or 9 million U per day)	Not teratogenic; does not treat infection in the fetus; indicated for pregnant women suspected of having acquired the infection at <18 weeks of gestation. Spiramycin treatment should be continued until delivery in women with low suspicion of fetal infection or those with documented negative results of amniotic fluid PCR and negative findings on ultrasounds at follow-up. Available in the United States only through the Investigational New Drug process at the FDA. Prior consultation with medical consultants[a] is required.
Pyrimethamine, sulfadiazine, and folinic acid	Pyrimethamine: 50 mg every 12 h for 2 days followed by 50 mg daily; sulfadiazine: initial dose of 75 mg/kg, followed by 50 mg/kg every 12 h (maximum, 4 g/day); folinic acid[b] (leucovorin): 10–20 mg daily (during and 1 week after completion of pyrimethamine therapy)	Pyrimethamine is teratogenic; therefore, this combination should not be used before week 18 of gestation (in some centers in Europe, it is used as early as week 14–16). Indicated for women suspected of having acquired infection at ≥18 weeks of gestation and those with documented fetal infection (positive result of amniotic fluid PCR) or abnormal ultrasound findings suggestive of congenital toxoplasmosis, given when patient is at ≥18 weeks of gestation

NOTE. FDA, US Food and Drug Administration.

[a] Palo Alto Medical Foundation Toxoplasma Serology Laboratory, telephone number (650) 853-4828, or US (Chicago, IL) National Collaborative Treatment Trial Study, telephone number (773) 834-4152.

[b] Folic acid should not be used as a substitute for folinic acid.

Treatment of congenital toxoplasmosis

- **Drug therapy is usually continued for one year**
- **Active and recurrent toxoplasmic eye disease also frequently responds to antiparasitic drugs, which may be given with steroids**

- **Proper evaluation of treatment in the asymptomatic infected infant is not possible because of insufficient data.**

  Nevertheless, treatment for such infants should be undertaken in the hope of preventing the remarkably high incidence of late untoward sequelae seen in children who receive inadequate or no treatment.

Treatment *AIDS*:

- **pyrimetamine** 200 mg orally initially, followed by 50-75 mg/day orally **+**
- **leucovorin 10 mg/d +**
- **sulfadiasine 1-1,5g qid**

x 3-6 weeks after resolution of signs/symptoms

followed by

- **pyrimetamine 25mg/d + leucovorin 15mg/d + sulfadiasine 500mgx qid indefinitely (or until immune reconstitution).**

- **Alternatives: pyrimetamine 50-100mg/d + leucovorin 10-20mg/d + clindamycin 600mg qid (PO or IV).**
- **Atovaquone 750mg PO q6h, azithromycin 1200-1500 mg PO daily, or dapsone 100 mg PO daily instead of sulfa when pt. is sulfa-intolerant.**

Primary or Reactivation

- Acute disease in immunocompetent, non-pregnant patients: usually no treatment, unless visceral disease or symptoms severe or persistent.
- Treatment (AIDS, preferred): pyrimethamine 50-100mg/d + leucovorin 10-20mg/d + sulfadiazine 1.1.5g qid all PO x 3-6 weeks after resolution of signs/symptoms; followed by pyrimethamine 25mg/d + leucovorin 15mg/d + sulfadiazine 500mg PO qid indefinitely (or until immune reconstitution).
- Folinic acid (leucovorin) 15-20mg PO daily prevents bone marrow suppressive effect of pyrimethamine.
- Alternatives: pyrimethamine 50-100mg/d + leucovorin 10-20mg/d + clindamycin 600mg qid (PO or IV).
- Atovaquone 750mg PO q6h, azithromycin 1200-1500 mg PO daily, or dapsone 100 mg PO daily instead of sulfa when pt. is sulfa-intolerant.
- Treatment needed in immunocompromised patients and pregnant woman.
- Spiramycin 3-4 g/d in divided doses for pregnant woman in first trimester to prevent transmission if mother seroconverts.
- As prophylaxis against recurrence of toxoplasmic retinochoroiditis: trimethoprim 60 mg + sulfamethoxazole160 mg given every 3 days
-

Ocular toxoplasmosis

Classic therapy: 4-6 weeks

- pyrimethamine 75-100 mg daily for 2 days followed by 25-50-mg dose daily
- sulfadiazine 1 g x qid
- folinic acid 5 mg daily
- prednisolone 1 mg/kg daily from the 3 day of therapy & tapered 2-6 weeks (as an adjunct to minimize collateral damage from the inflam. response)

Alternative treatment regimens:

- classic regimen + clindamycin (as single or combined use of clindamycin, TMP-SMX, spiramycine, minocycline, azithromycin, atovaquone, clarithromycin).
- Treatment intravitreal injection of 1.5 mg → 1mg clindamycin (a half-life of 5.6 days) & dexamethasone (400 μg) = promising effects.
- Topical corticosteroids are used depending on the anterior chamber reaction.
- Depot steroid therapy is absolutely contraindicated. *(The high-dose medication in close proximity to ocular tissues apparently overwhelms the host's immune response, leading to rampant necrosis and the potential for a blind, phthisical globe.)*

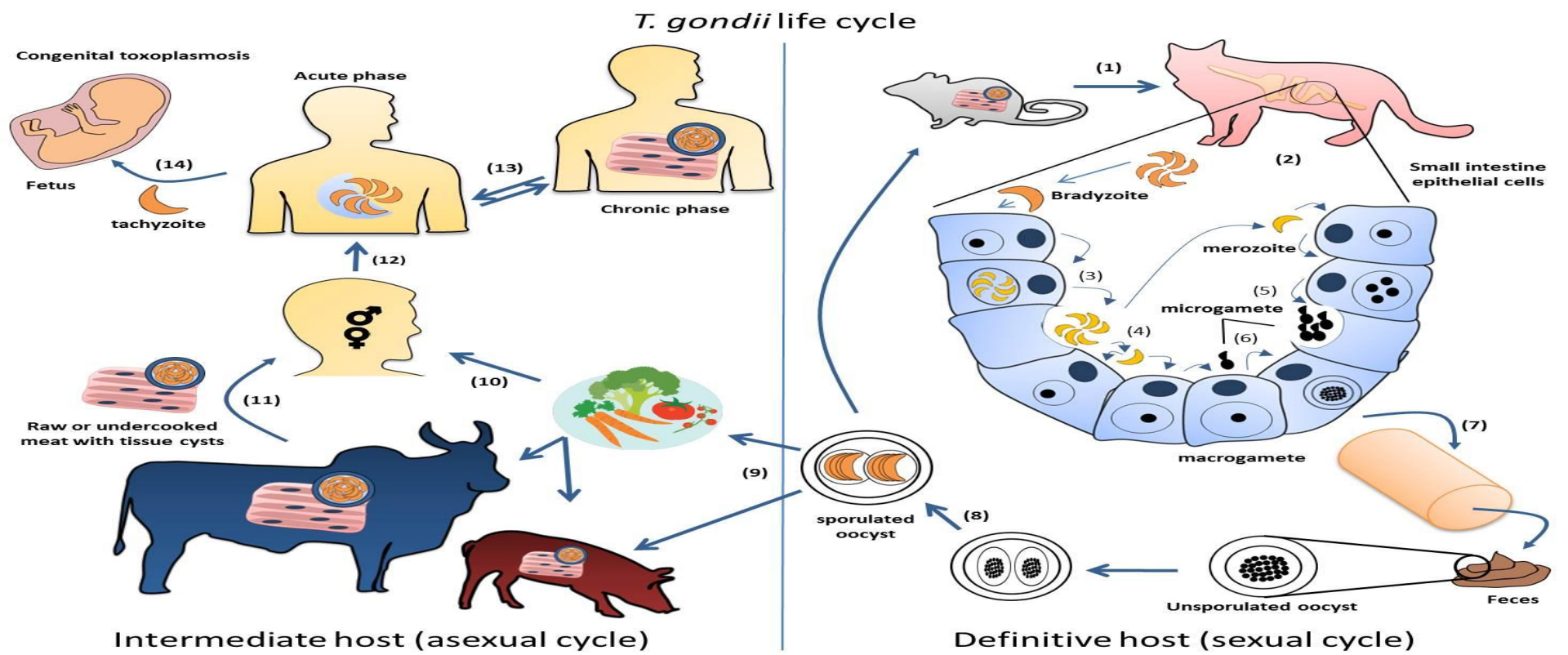
How to Prevent Toxoplasmosis

- Change litter box daily
- Feed cat commercial dry or canned food
- Cover outdoor sandboxes
- Keep indoor cats indoors
- Don't get new cat while pregnant

How to Prevent Toxoplasmosis (cont'd)

Don't forget to . . .

- Clean
  Fruits and vegetables thoroughly
- Separate
  Raw foods from ready-to-eat foods
- Cook
  Meat thoroughly



(1) Definitive host;
(2) Cyst disruption and intestinal epithelial cell;
(3) Formation of merozoites;
(4,5) Start sexual phase with macrogametes and flagellate microgametes formation;
(6) Fusion of microgamete & macrogamete;
(7) Oocyst release in the faeces;
(8) Unsporulated oocysts become infective and contaminate the environment; (9) Sporulated oocysts cause infection of animals & humans(10,11) Human inf. occurs by the ingestion of raw or undercooked meat of infected animals (12) *T. gondii* tachyzoite multiplication in the intermediate host; (13) Tachyzoite-bradyzoite differentiation and formation of tissue cysts; (14) Transplacentary transmission

- **Toxoplasmosis: A Comprehensive Clinical Guide,** David H. M. Joynson,Tim G. Wreghitt
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- http://www.revophth.com/content/d/retinal_insider/c/26716/
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- http://emedicine.medscape.com/article/1000028-clinical