

NEW METHODS OF INVESTIGATION IN TOXOPLASMOSIS

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Abstract

Toxoplasmosis is a zoonosis of epidemiological major importance, an obligate intracellular protozoan. It is acquired perorally, transplacentally, or rarely, parenterally in laboratory accidents, by transfusion or from a transplanted organ.

Diagnosis of acute Toxoplasma infection can be established by culture of Toxoplasma gondii from blood or body fluids, identification of tachyzoites in section or in tissues, identification of cysts in the placenta or tissue of a fetus. The serologic tests are important like Sabin-Feldman dye test, IgG-IFA, IgM-IFA, IgM-ELISA, IgM-ISAGA, IHA, ELIFA test and PCR that is used to identify the DNA of Toxoplasma gondii in amniotic fluid CSF, urine or infant peripheral blood.

Key words: toxoplasmosis, Sabin-Feldman dye test, tachyzoites, amniotic fluid

INTRODUCTION

Toxoplasmosis is a zoonosis of epidemiological major importance, caused by an obligate intracellular protozoan, *Toxoplasma gondii*. It is acquired perorally, transplacentally, or rarely, parenterally in laboratory accidents, by transfusion or from a transplanted organ (Jones J.L., Deanna Kruszon-Moran, †, Marianna Wilson, 2003; Iqbal J, Khalid N., 2007)

The parasite is very common in cat feces, raw vegetables, and the soil. It is also common in raw meat, especially pork, lamb, or deer meat.

Etiology

The life cycle of *Toxoplasma gondii* is complex and involves two types of host—definitive and intermediate (Cook AJ, Gilbert RE, Buffolano W., 2000; Popescu M., 2008)

Cats, both wild and domestic, are the only definitive hosts for *Toxoplasma gondii*. This means that the parasite can only produce oocysts (eggs) when infecting a cat. When a cat ingests an infected prey (or other infected raw meat) the parasite is released into the cat's digestive tract. The organisms then multiply in the wall of the small intestine and produce oocysts during what is known as the intrainestinal infection cycle.

These oocysts are then excreted in great numbers in the cat's feces. Cats previously unexposed to *T. Gondii* will usually begin shedding oocysts between three and 10 days after ingestion of infected tissue, and continue shedding for around 10 to 14 days, during which time many millions of oocysts may be produced. Oocysts are very resistant and may survive in the environment for well over a year.

During the intrainestinal infection cycle in the cat, some *T. gondii* organisms released from the ingested cysts penetrate more deeply into the wall of the intestine and multiply as tachyzoite forms. These forms then spread out from the intestine to other parts of the cat's body, starting the extraintestinal infection

cycle. Eventually, the cat's immune system restrains this stage of the organism, which then enters a dormant or "resting" stage by forming cysts in muscles and brain. These cysts contain bradyzoites, or slowly multiplying organisms.

Other animals, including humans, are intermediate hosts of *Toxoplasma gondii*. These hosts can become infected but do not produce oocysts. Oocysts passed in a cat's feces are not immediately infectious to other animals. They must first go through a process called sporulation, which takes one to five days depending on environmental conditions. Once sporulated, oocysts are infectious to cats, people, and other intermediate hosts.

Intermediate hosts become infected through ingestion of sporulated oocysts, and this infection results in formation of tissue cysts in various tissues of the body. Tissue cysts remain in the intermediate host for life and are infectious to cats, people and other intermediate hosts if the cyst-containing tissue is eaten.

Contact with oocyst-contaminated soil is probably the major means by which many different species—rodents, ground-feeding birds, sheep, goats, pigs, and cattle, as well as humans living in developing countries—are exposed to *Toxoplasma gondii*.

In the industrialized nations, most transmission to humans is probably due to eating undercooked infected meat, particularly lamb and pork. People also become infected by eating unwashed fruits and vegetables.

The organism can sometimes be present in some unpasteurized dairy products, such as goat's milk.

Toxoplasma gondii can also be transmitted directly from pregnant woman to unborn child when the mother becomes infected during pregnancy. There are two populations at high risk for infection with *Toxoplasma gondii*; pregnant women and immunodeficient individuals. Congenital infection is of greatest concern in humans. About one-third to one-half of human infants born to mothers who have acquired *Toxoplasma* during that pregnancy are infected. The vast majority of women infected during pregnancy have no symptoms of the infection themselves.

The majority of infected infants will show nosymptoms of toxoplasmosis at birth, but many are likely to develop signs of infection later in life.

Loss of vision, mental retardation, loss of hearing, and death in severe cases, are the symptoms of toxoplasmosis in congenitally infected children.

In immunodeficient people—those undergoing immunosuppressive therapy (e.g., for cancer or organ transplantation) or those with an immunosuppressive disease such as AIDS—enlargement of the lymph nodes, ocular and central nervous-system disturbances, respiratory disease, and heart disease are among the more characteristic symptoms. In these patients— especially those with AIDS—relapses of the disease are common, and the mortality rate is high.

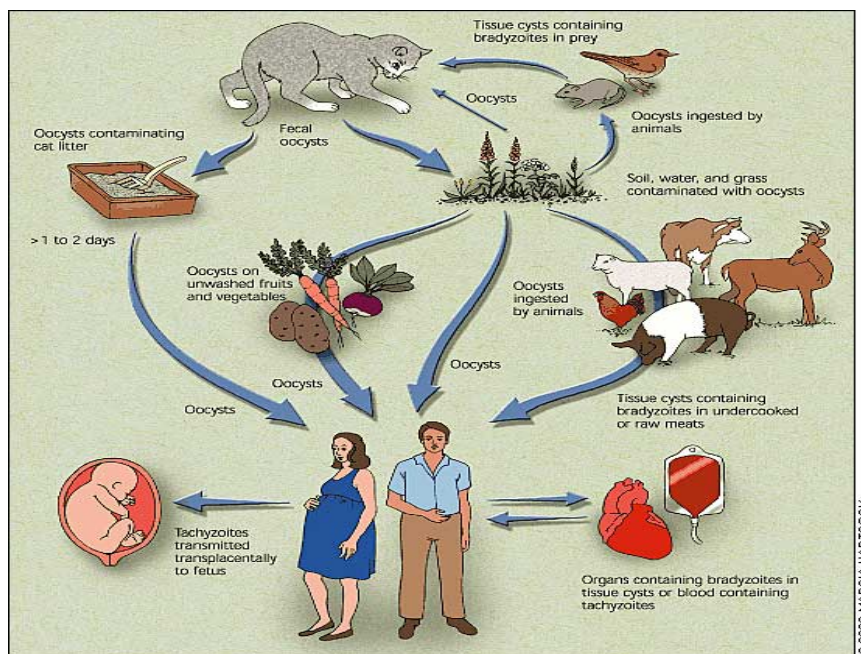


Fig. 1. Life cycle of *Toxoplasma gondii*
 (after Marcia Harstock cited in Popescu M., 2008)

Epidemiology

Disease with universal spread, the toxoplasmosis is noted from the polar to the equator region (Ciursas O, 2004; Szénási Z, Ozsvár Z, Nagy E, Jeszenszky M, Szabó J, Gellén J, Végh M, Verhofstede C, 1997).

The bigger incidence was noted to the zoo technicians and veterinaries as well as to the mothers whose children present neuropsychical and congenital malformations. The specific antibodies was noticed at 55% of women from fertile group of age South and Central America, West Europe, West and Central Africa.

In the USA was noticed yearly 3500 cases of congenital toxoplasmosis with a prevalence of 0.6 cases at 1000 pregnant women. In the region with high sero prevalence for toxoplasmosis to 50% of cases with HIV infection develops toxoplasmotic encephalitis (Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, 1999).

Clinical table

There are four categories of toxoplasmosis (Remington JS, McLeod R, Thulliez P, Desmonts G., 2001; Jones JL., Lopez A., Wilson M, Schulkin J, Gibbs R, 2001; Jones J.L., Deanna Kruszon-Moran,†, Marianna Wilson, 2003; Holland G.N., 2003 et 2004; Montoya JG, Liesenfeld O, 2004):

1. toxoplasmosis on immunocompetent
2. the ocular toxoplasmosis on immunocompetent
3. the immunodepressed people's toxoplasmosis
4. the congenital toxoplasmosis

1. The incubation is 10-21 days when the transmission was realized by infested meat consumer, or at 5-20 days when the transmission was produced by ingestion of oocysts.

The symptoms of acute infection are adenopathy, asthenia, myalgia, fever, hepatosplenomegalia exanthemas fugacea.

The chronic infection exists at all infected persons after the resolution of the acute phase.

2. It is the consequence of the congenital infection's reactivation but may also occur in the acute infection got or as a result of the chronic forms of toxoplasmosis. Ocular findings of congenital toxoplasmosis also include strabismus, nystagmus, microcornea, microphthalmia, cataracta, glaucoma, optic neuritis and optic atrophy.

3. The highest risk of severe toxoplasmosis are the people with immunosuppressors agents in leukemia, collagenoses, drug consumers and with organ transplants.

4. The congenital toxoplasmosis is usually incompatible with life.

It is considered that the transplacental transmission is realized only on 55% cases, the congenital toxoplasmosis severity and incidence varying with the pregnancy semester.

In the first semester the disease occurs very seldom, but it is severe leading to the spontaneous abortion, birth of dead fetus or severe form of disease.

In the second semester the toxoplasmosis occurs in 30-45% of cases with hepatosplenomegalia and limfadenopatia.

In the third semester, the disease is more frequent (60-65%) of cases but often benign.

METHODS IN PARACLINICAL INVESTIGATIONS

Diagnosis of acute *Toxoplasma* infection can be established by culture of *Toxoplasma gondii* from blood or body fluids, identification of tachyzoites in section or in tissues, identification of cysts in the placenta or tissue of a fetus (Piergili Fioretti D, 2004; Reiter-Owona Ingrid, 2005).

Organism are isolated by inoculation of body fluids, leukocytes or tissue specimens into mice or tissue cultures. Body fluids should be processed and inoculated immediately, but *Toxoplasma gondii* has been isolated from tissue and blood that have been stored overnight or even for 4-5 days at 4 C.

There are many serologic tests to confirm the diagnosis of congenital or acutely acquired *Toxoplasma* infection (Foulon W, Pinon JM, Stray-Pedersen B, Pollak A, Lappalainen M, Decoster A, Villena I, Jenum PA, Naessens A., 1999; Liesenfeld O., Montoya J., Kinney S., Press C., Remington J.: 2001; Czuba B, Pendzich J, Gola J, Mazurek U, Sławska H, Kamiński K, Wilczok T, 2001; Reiter-Owona Ingrid, 2005; Logar J, Petrovec M, Novak-Antolic Z, Premru-Srsen T, Cizman M, Arnez M, Kraut A, 2003):

- The Sabin-Feldman dye test is sensitive and specific. It measures primarily IgG antibodies.
- The Remington test is an agglutination test which uses formalin-preserved whole parasites to detect IgG antibodies. The Remington test is accurate, simple to perform and inexpensive.
- The IgM – IFA (indirect fluorescent antibody) test is useful for the diagnosis of acute infection with *Toxoplasma gondii* in the older child because IgM antibodies appear earlier, often by 5 days after infection, and diminish more quickly than IgG antibodies. The IgM – IFA test may yield false-positive results as a rheumatoid factor.
- The IgG - IFA (indirect fluorescent antibody) test measures the same antibodies as the dye test, and the titers tend to be parallel. These antibodies appear 1-2 week after infection, reach high titers after 6-8 weeks, and then decline over months to year.
- The IgM – ELISA test (enzyme-linked immunosorbent assay) is more sensitive and specific than the IgM – IFA test for detection of *Toxoplasma* IgM antibodies. IgM – ELISA avoids both the false-positive results from rheumatoid factor and false-negative results from high levels of passively transferred maternal IgG antibody in fetal serum, as may occur in the IgM – IFA test. The IgA ELISA is also a sensitive test for detection of maternal and congenital infection and results may be positive when those of the IgM-ELISA are not.

- The ISAGA test (immunosorbent agglutination assay) combines trapping of a patient's IgM, IgA or IgE to a solid surface and use of formalin-fixed organism or antigen-coated latex particles. The IgM ISAGA is more sensitive than the IgM – ELISA and may detect specific IgM antibodies before and for longer periods than the IgM – ELISA.
- The avidity test can be helpful to time infection. A high avidity test result indicates that infection began > 16 weeks earlier. A low avidity test result may be present for many month and is not diagnostic of recent acquisition of infection.
- The IHA test (indirect hemagglutination test) measures different *Toxoplasma gondii* antibodies from those measured in IFA and dye tests.
- The (HS/AC) differential agglutination test compares antibody titers obtained with formalin-fixed tachyzoites (HS antigen), with titers obtained using acetone or methanol-fixed tachyzoites (AC antigen) to differentiate recent and remote infection in adults and older children.
- PCR is used to amplify the DNA of *Toxoplasma gondii* , which than can be detected by using a DNA probe. Detection of a repetitive *Toxoplasma gondii* gene , the B1 gene , in amniotic fluid is the PCR target of choice for establishing the diagnosis of congenital *Toxoplasma* infection in the fetus. Sensitivity and specificity of this test in amniotic fluid obtained to diagnose infection aquired between 17 and 21 weeks of gestation are approximately 95%.
- The ELIFA (enzyme-linked immunofiltration assay) using micropore membranes permits simultaneous study of antibody specificity by immunoprecipitation and characterization of antibody isotypes by immunofiltration with enzyme-labeled antibodies. This method may be capable of detecting 85% of cases of congenital infection in the 1 st few days of life.
- The comparative Western immunoblot test of sera from a mother and infant may detect congenital infection. Infection is suspected when the mother 's serum and her infant 's serum contain antibodies that react with different *Toxoplasma gondii* antigens.
- Lymphocyte blastogenesis to *Toxoplasma gondii* antigens, has been used to diagnose congenital toxoplasmosis when the diagnosis is uncertain and other test results are negative. However a negative result does not exclude the diagnosis because many infected newborns do not respond to *Toxoplasma gondii* antigens.

RESULTS IN TREATMENT

Pyrimethamine plus sulfadiazine act synergistically against *Toxoplasma*, and combination therapy is indicated for many of the forms of toxoplasmosis. However use of pyrimethamine contraindicated during the 1st trimester of pregnancy (Montoya JG, Liesenfeld O., 2004; Jones J., Lopez A., Wilson M., 2003; Logar J, Petrovec M, Novak-Antolic Z, Premru-Srsen T, Cizman M, Arnez M, Kraut A., 2003; x x x RAPORT TEHNIC Contract nr. 361/8.12.2006).

Spyramicine should be used to attempt to prevent vertical transmission of infection to the fetus of acutely infected pregnant women, and to treat congenital toxoplasmosis.

All patients treated with inhibits platelet and leukocyte count twice weekly. Folinic acid as calcium leukovorin, should always be administrated concomitantly and for 1 week after treatment with pyrimethamine is discontinued to prevent bone marrow suppression.

Different preparations used in different associations: Pyrimethamina+sulfadiazine, Pyrimetamina+Sulfadoxina, Pyrimethamina+Sulfametaxodiazina or Cotrimoxazol.

CONCLUSIONS

Toxoplasmosis is a widespread disease in a very great number of countries, in all continents (Jones J.L., Deanna Kruszon-Moran,†, Marianna Wilson, 2004). The causal agent, *Toxoplasma gondii* is present in Romania and in our county (Claponea A., 2007; Ciursas O. 2004, Popescu M., 2008).

The presence and the consequences of this disease, are very important especially in pregnant women and children.

More than 60 million people in the world probably carry the *Toxoplasma* parasite, but very few have symptoms because the immune system usually keeps the parasite from causing illness. However, expectant mothers should be cautious because an infection can cause problems in pregnancy. Toxoplasmosis also affects persons with weakened immune systems.

These are the reasons who motivate new investigations in our country and in county Bihor, these investigations will obtain better efficacy if they will be made in international cross-border cooperations.

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