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Diagnosis, Treatment, and Prevention of Congenital Toxoplasmosis in the United States

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This Clinical Report has been reaffirmed with reference and data updates. New or updated references and datapoints are indicated in bold typeface. No other changes have been made to the text or content.
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EXECUTIVE SUMMARY

Congenital toxoplasmosis (CT) is a parasitic disease that can cause significant fetal and neonatal harm. Coordinated efforts by pregnant women, researchers, physicians, and health policy makers regarding potential primary and secondary preventive measures for CT and their implementation may lead to a lower incidence of CT as well as lower morbidity and mortality rates associated with CT. In the United States, the age-adjusted seroprevalence of *Toxoplasma gondii* among women of childbearing age (15–44 years) has declined over time (15%, 11%, and 9% in 1988–1994, 1999–2004, and 2009–2010, respectively; among US-born women only, the seroprevalence rates during these time periods were 13%, 8%, and 6%, respectively). Thus, approximately 91% of women of childbearing age in the United States are susceptible to *Toxoplasma* infection. Should these women become infected during pregnancy and remain undiagnosed and untreated, they could deliver an infant with CT. However, the incidence of acute primary infection is likely very low in the current era and is probably much lower than the 1.1 in 1000 pregnant women originally reported in 1960s.

There are 3 ways CT can occur. First, CT can develop through transmission of *T gondii* to the fetus from a previously seronegative, immunocompetent mother who acquired acute primary infection

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during pregnancy or within 3 months before conception. Second, CT can occur through reactivation of toxoplasmosis in a previously *T gondii*–immune pregnant woman who was severely immunocompromised during pregnancy. Third, CT can result after reinfection of a previously immune pregnant mother with a new, more virulent strain (eg, after international travel or after eating undercooked meat from areas where more virulent atypical strains predominate).

In cohorts of women who have been screened routinely during pregnancy and treated accordingly once primary infection was diagnosed, the mother-to-child transmission (MTCT) rate was <5% after an acute primary maternal infection very early in pregnancy, but MTCT rates were much higher with acute maternal infections acquired later in pregnancy (15%, 44%, and 71% after maternal seroconversions [acute primary infections] at 13, 26, and 37 weeks of gestation, respectively).[1] The risk of MTCT in untreated women may be higher. Factors (any or a combination) associated with an increased risk of MTCT are as follows: (1) acute *T gondii* infection during pregnancy, (2) immunocompromising conditions, (3) lack of antepartum treatment, (4) high *T gondii* strain virulence, and (5) high parasite load.

The incidence of CT, according to early cumulative published data from the New England Newborn Screening Program over a 12-year period (1988–1999), was 0.91 cases per 10 000 live births, which would have translated to the birth of approximately 365 infants with CT in the United States each year. The incidence of CT decreased after 1999 and, over the past 9 years (2006–2014), was approximately 0.23 cases per 10 000 live births. The true incidence of CT in the United States might be higher, because the sensitivity of the newborn screening test (blot-spot immunoglobulin [Ig] M test) is approximately 50% to 75%, and fetal losses attributable to severe CT were not counted.

Recent data from the National Reference Laboratory for Toxoplasmosis in the United States showed that 85% of 164 infants with CT identified over a period of 15 years were severely affected: 92% had chorioretinitis, 80% had intracranial calcifications, 68% had hydrocephalus, and 62% had all of these manifestations. These data were based on cases referred to reference toxoplasmosis centers in the United States, and they were not population based. The generalizability of these findings at a population level may be limited. The rate of symptomatic CT among infants diagnosed via the New England Newborn Screening program (1986–1992) was 40% (25% with eye disease at birth or follow-up and 29% with central nervous system [CNS] disease). However, in neonatal screening programs, spontaneous abortion, fetal demise, and pregnancy terminations attributable to severe CT are not captured. The rates of symptomatic CT in European cohorts are much lower than in the United States. Possible reasons for those disparities include differences in *T gondii* strains and the absence of antepartum screening and treatment of CT in the United States. In addition, differences between population-based, prospectively identified CT cases versus more selected cases referred to reference centers may bias reporting estimates.

Evidence in favor of antepartum treatment benefits to decrease the risk of MTCT of CT is variable and accumulated from several observational studies. One randomized therapeutic trial has been conducted in pregnancy.[2] European data from the Systematic Review on Congenital Toxoplasmosis (SYROCOT) international consortium, which performed a meta-analysis of individual patient data published in 2007,[1] suggested that the odds of MTCT were 52% lower (odds ratio [OR]: 0.48; 95% confidence interval [CI]: 0.28–0.80) when antepartum treatment was promptly initiated within 3 weeks after maternal seroconversion as compared with ≥8 weeks. The overall risk of transmission among women with primary infection in France decreased from 29% to 24% (P = .022) after 1992, when monthly antepartum screening became mandatory. These rates do not represent rates of MTCT without compared with prenatal treatment, because the majority of the women in France diagnosed with acute *Toxoplasma* infection, even in the earlier period when the frequency of prenatal screening was less intense, were prenatally treated. These rates may call into question the efficacy of treatment in preventing MTCT, because women in the postimplementation era were more likely to be identified by serologic testing alone rather than by ultrasonographic abnormalities in the setting of established infection. An alternative explanation for this paradoxical phenomenon is that early prenatal screening and treatment led to a decrease in fetal deaths, and thus the effect on the risk of MTCT was not very prominent. In other words, with prompt initiation of prenatal treatment, children who would have otherwise died of CT survive, making the decrease in MTCT less impressive. Evidence that supports this hypothesis includes data from the same prospective cohort study from Lyon, France, by Wallon et al,[3] published in 2013, which showed a significant reduction in symptomatic disease among infected pregnant women when comparing cases reported before 1995 with those after 1995, when amniotic fluid (AF) testing by polymerase chain reaction (PCR) was

initiated (from 11% before 1995 to 4% after 1995; $P < .001$). In Austria, where there is a nationwide antepartum screening program (the Austrian Toxoplasmosis Register), Prusa et al[4] reported a sixfold lower risk of MTCT in women who received antepartum treatment compared with untreated women (9% [87 of 1007] vs 51% [32 of 63]), but this finding may have also been related to the timing of identification during gestation. In a recently published retrospective cohort study by Hotop et al[5] from Germany, where spiramycin is given until the 16th week of pregnancy, followed by at least 4 weeks of combination therapy with pyrimethamine, sulfadiazine, and folinic acid (independently of the infection status of the fetus, with subsequent treatment determined according to the infection status of the fetus), very low rates of MTCT of CT (4.8% [33 CT cases infants/685 pregnant women]) were reported. An early Cochrane systematic review by Peyron et al[6] published in 2000 evaluated the effect of treatment in pregnancy and concluded that, despite more than 3200 articles, only 9 studies had evaluated the effectiveness of prenatal treatment on the risk of MTCT, and these results were conflicting, resulting in insufficient evidence to evaluate the efficacy of treatment.

Some observational studies have evaluated the association of antepartum treatment and symptomatic infant disease. Recent reanalysis of data from 14 European centers[7] suggested that antepartum treatment was associated with lower odds of severe neurologic sequelae in infants with CT (OR: 0.24; 95% CI: 0.07–0.71) (the authors advised caution in interpretation because the study included only 23 such very severely affected cases of CT and, of these, there were 9 terminations). In the Wallon et al[3] prospective cohort study mentioned previously, the risk of symptomatic CT among infected mothers in France decreased from 11% to 4% ($P < .001$) after 1995 when *T gondii* testing with AF PCR assay was initiated, but the increase in the mild maternal infections included in the latter group may have played a role as well. Recent data from Germany by Hotop et al[5] also showed a lower risk of symptomatic disease with early versus late initiation of maternal treatment (19% with early therapy versus 70% with late antepartum therapy; $P = .006$).

One cost-effectiveness model was recently developed by Stillwaggon et al[7] to evaluate the implementation of universal antepartum screening after the French protocol of monthly serologic screening during pregnancy (including confirmatory testing at a reference laboratory of any positive results) with antepartum treatment, fetal ultrasonography/AF PCR assay, and infant follow-up/treatment.[8] This decision analysis made a number of assumptions, including a cost of $12 per test, an estimated cost of fetal death of over $6 million, and an incidence of acute primary maternal infection during pregnancy of 1 in 1000 (including additional sensitivity analyses). It also assumed that treatment was highly efficacious and inexpensive. Although the study concluded that screening in the United States would be cost-effective, it remains unclear whether these conclusions would be reached if data were used assuming higher costs of screening, lower costs of loss, and less efficacy of treatment. A previous decision analysis did not arrive at the same conclusion.[9] An early Cochrane systematic review on treatments for toxoplasmosis during pregnancy published in 2000 suggested that in countries where screening or treatment is not routine, these technologies should not be introduced outside the context of a carefully controlled trial.[6]

Infants with suspected/proven CT may need to be managed in consultation with toxoplasmosis reference centers in the United States. The diagnostic criteria for CT include any of the following: (1) persistence of positive *Toxoplasma* IgG antibodies beyond 12 months of age (gold standard); (2) positive *Toxoplasma* IgG antibodies and positive *Toxoplasma* IgM antibodies and/or positive *Toxoplasma* IgA antibodies; (3) positive *Toxoplasma* PCR assay results from amniotic fluid, peripheral blood, cerebrospinal fluid (CSF), urine, or other body fluids; and (4) positive neonatal *Toxoplasma* IgG antibodies (but negative *Toxoplasma* IgM and IgA antibodies) and serologic evidence of acute maternal *T gondii* infection during pregnancy and evidence of clinical manifestations suggestive of CT.

At the National Reference Laboratory for Toxoplasmosis (Palo Alto, CA) and the Toxoplasmosis Center (Chicago, IL), clinical evaluation of infants with suspected CT includes detailed physical examination, neurologic evaluation, ophthalmologic examination (preferably by a retinal specialist), and brainstem auditory evoked responses. Imaging evaluation includes the following: (1) computed tomography of the head (or head ultrasonography) and (2) abdominal ultrasonography. If CT has not been confirmed but also has not been ruled out, an infant's workup includes complete clinical evaluation and serial IgG antibody titers every 4 to 6 weeks after birth until complete disappearance of *Toxoplasma* IgG antibodies.

At the National Reference Laboratory for Toxoplasmosis and the Toxoplasmosis Center, treatment of infants with suspected CT is continued for 12 months and

includes pyrimethamine plus sulfadiazine plus folinic acid.

1. Pyrimethamine: 2 mg/kg per day, orally, divided twice per day for the first 2 days; then from day 3 to 2 months (or to 6 months [considered for symptomatic CT]), 1 mg/kg per day, orally, every day; and after that, 1 mg/kg per day, orally, 3 times per week
2. Sulfadiazine: 100 mg/kg per day, orally, divided twice per day
3. Folinic acid (leucovorin): 10 mg, 3 times per week

In cases of severe chorioretinitis or elevated CSF protein concentration ≥1 g/dL, corticosteroids may be considered (after 72 hours of anti-*Toxoplasma* therapy).

INTRODUCTION

CT is a parasitic disease that can cause significant fetal and neonatal harm. Coordinated efforts by pregnant women, researchers, physicians, and health policy makers regarding potential primary and secondary preventive measures for CT and their implementation may lead to a lower incidence of CT as well as lower morbidity and mortality rates associated with CT. The purpose of this technical report is to summarize available information regarding the diagnosis, treatment, and prevention of CT.

Clinical Areas Targeted

The following clinical areas were targeted:

- evidence regarding the risk of maternal infection and MTCT and the risk of symptomatic disease in the United States and Europe;
- important differences between the North American and European literature that may need to be taken into consideration by practicing physicians and health care professionals in the United States;
- significant differences in the clinical spectrum and severity of CT seen in North America and Europe;
- diagnostic considerations in the mother, fetus, and infant;
- evidence from observational studies regarding the effectiveness of antepartum treatment to decrease MTCT and to prevent severe CT;
- prenatal and postnatal treatment protocols; and
- feasibility of routine antepartum screening and treatment of *T gondii* infections in the United States.

Search Strategy

First, a PubMed search for publications in English language with the use of the following search strategies was performed: (1) (congenital toxoplasmosis) AND (mother to child transmission OR mother to fetus transmission OR mother to infant transmission) (up to September 15, 2014), (2) (congenital toxoplasmosis [ti]) AND (outcome OR follow-up OR chorioretinitis OR eye disease OR ocular disease OR intracranial calcifications OR ventriculomegaly OR hydrocephalus) (up to August 31, 2014), and (3) (toxoplasmosis [TI] AND United States) (January 1, 2004, to September 15, 2014) (see Supplemental Table 14). Second, the reference lists of key publications were screened to identify additional pertinent articles. Third, the evidence from some early key publications from cohorts of infants with CT whose mothers had not received antepartum treatment was also reviewed, because such older cohorts are more similar to the cohorts of children with CT still seen in the United States, where the majority of infants with CT are born to mothers without antepartum treatment. Fourth, we perused the systematic reviews already performed by the European Toxoprevention Study (EUROTOXO) group, the European Initiative for the study of CT on the following topics: (1) burden of disease from CT,[10] (2) evaluation of diagnostic performance of diagnostic tests for CT,[11] (3) assessment of postnatal treatment effects,[12] (4) strategies for the prevention of CT,[13] and (5) evaluation of adverse effects from antepartum and postnatal treatment of CT.[14]

Search Strategy Results

A total of 457 articles were screened; 403 articles were identified with PubMed searches (288 with the first 2 searches and 130 with the third search strategy), 50 additional articles were identified by hand-screening the reference lists of key publications, and 225 articles were finally considered to be pertinent to this technical report and were included.

Grading of the Quality of Evidence

The identified evidence consisted of observational studies. Currently, there are no randomized controlled trials (RCTs) performed for the evaluation of different therapeutic approaches for CT, according to an early systematic review from the Cochrane Pregnancy and Childbirth Group (up to date as of February 2006) by Peyron et al.[6]

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system[15,16] was used to assess the quality of evidence. The GRADE system is a step toward recognition that evidence from observational studies might become the vehicle for generating evidence for policy makers, especially in situations in which RCTs are considered unethical and large treatment effects have been documented in observational studies. According to the GRADE system (Fig 1), the quality of evidence for the effectiveness of antepartum screening and treatment would be considered of high quality on the basis of the following 2 criteria: "exceptionally strong evidence from unbiased observational studies" and

Strength of Recommendation and Quality of Evidence	Clarity of Balance Between Desirable and Undesirable Effects	Methodological Quality of Supporting Evidence (Examples)	Implications
Strong recommendation, high-quality evidence	Desirable effects clearly outweigh undesirable effects, or vice versa	Consistent evidence from well-performed RCTs or exceptionally strong evidence from unbiased observational studies	Recommendation can apply to most patients in most circumstances. Further research is unlikely to change our confidence in the estimate of effect.
Strong recommendation, moderate-quality evidence	Desirable effects clearly outweigh undesirable effects, or vice versa	Evidence from RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from unbiased observational studies	Recommendation can apply to most patients in most circumstances. Further research (if performed) is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Strong recommendation, low-quality evidence	Desirable effects clearly outweigh undesirable effects, or vice versa	Evidence for at least 1 critical outcome from observational studies, RCTs with serious flaws or indirect evidence	Recommendation may change when higher-quality evidence becomes available. Further research (if performed) is likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Strong recommendation, very low-quality evidence (very rarely applicable)	Desirable effects clearly outweigh undesirable effects, or vice versa	Evidence for at least 1 critical outcome from unsystematic clinical observations or very indirect evidence	Recommendation may change when higher-quality evidence becomes available; any estimate of effect for at least 1 critical outcome is very uncertain.
Weak recommendation, high-quality evidence	Desirable effects closely balanced with undesirable effects	Consistent evidence from well-performed RCTs or exceptionally strong evidence from unbiased observational studies	The best action may differ depending on circumstances or patients or societal values. Further research is unlikely to change our confidence in the estimate of effect.
Weak recommendation, moderate-quality evidence	Desirable effects closely balanced with undesirable effects	Evidence from RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from unbiased observational studies	Alternative approaches likely to be better for some patients under some circumstances. Further research (if performed) is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Weak recommendation, low-quality evidence	Uncertainty in the estimates of Desirable effects, harms, and burden; desirable effects, harms, and burden may be closely balanced	Evidence for at least 1 critical outcome from observational studies, from RCTs with serious flaws or indirect evidence	Other alternatives may be equally reasonable Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Weak recommendation, very low-quality evidence	Major uncertainty in the estimates of desirable effects, harms, and burden; desirable effects may or may not be balanced with undesirable effects	Evidence for at least 1 critical outcome from unsystematic clinical observations or very indirect evidence	Other alternatives may be equally reasonable. Any estimate of effect, for at least 1 critical outcome, is very uncertain.

Abbreviation: RCT, randomized controlled trial.

[a] Based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system [1–6].

FIGURE 1

Grading system.[15] (Reprinted with permission from Chow AW, Benninger MS, Brook I, et al. IDSA clinical practice guideline for acute bacterial rhinosinusitis in children and adults. *Clin Infect Dis*. 2012;54:1043.)

"further research is unlikely to change our confidence in our estimates." The quality of evidence for the effectiveness of postnatal treatment would be considered of moderate quality on the basis of the criterion "exceptionally strong evidence from unbiased observational studies"; however, "further research, if performed, is likely to affect our confidence in the estimate of efficacy and may change our estimate."

BACKGROUND

The organism and Epidemiology

Toxoplasma *gondii*

T gondii is an obligate intracellular parasite with worldwide distribution that infects approximately one-third of the human population and a wide range of animals and birds. Infection with *T gondii* in humans can have devastating consequences for fetuses, children, immunocompromised patients, and immunocompetent individuals infected with virulent strains. Although members of the *Felidae*

family are the definitive hosts, cat ownership per se is not correlated with the prevalence of human infection in most studies.[17–22] The parasite has 3 infectious stages: (1) tachyzoites, which are responsible for rapid spread of the parasite between cells and tissues and for the clinical manifestations of toxoplasmosis; (2) bradyzoites, which are within tissue cysts and stay dormant for the life of the host unless the individual becomes severely immunocompromised; and (3) sporozoites, which are within oocysts, are shed by members of the felid family, and widely disseminate the agent in the environment.[23] Approximately half of infected individuals do not exhibit the conventional risk factors for acute infection or report clinical symptoms suggestive of acute toxoplasmosis at the time of their primary infection.[24,25] A survey of 76 women who gave birth to infants with CT indicated that 61% of these women had no exposure to cat litter or raw meat, 52% had no acute toxoplasmosis-like febrile illness during pregnancy, and 52% had neither of the 2.[24,25] Serologic testing is the only method that can reliably establish whether an individual has ever been infected, is chronically infected, or is experiencing an acute infection.

Genotyping studies have allowed the identification of 3 main clonal lineages of *T gondii* (types 1, 2, and 3) in Europe, North America, and South America.[26,27] Atypical strains that do not fall within these 3 clonal lineages have been frequently reported in North America and South America. In Western Europe, the predominant *T gondii* strain implicated in human disease was type 2,[28] in North America all 3 main clonal lineages strains were implicated in human disease (types 1, 2, and 3), and an additional new fourth clonal lineage (type 12) was recently identified.[29] In South America, mainly types 1 and 3 and atypical strains were identified[28,30,31]; and in Africa, mainly type 3 and recombinant types 1/3, 1/2, and 1 were detected.[32] In North and South America, atypical and more virulent strains have been implicated in more aggressive clinical manifestations in immunocompetent individuals.[33–36]

It has been suggested that differences in *T gondii* strains may at least partially explain the observed differences in the clinical spectrum of CT in different parts of the world and particularly in Europe, North America, and South America. In the United States, more severe disease, and even preterm birth, has been associated with infections with non–type 2 strains[37]; and in South America, more severe ocular disease has been reported in children with CT as compared with that reported in Western European countries.[38]

Atypical *T gondii* strains have been reported from several areas of the world,[39–49] including, but not limited to, Central and South America, Australia, and Africa. This distribution of strains may need to be considered when clinical syndromes consistent with severe toxoplasmosis are encountered in individuals returning from these areas.

Routes of Transmission and Risk Factors for T gondii Infection

Humans are incidental hosts and become infected primarily by the oral route, that is, by ingestion of oocysts present in contaminated food, water, or soil or by ingestion of tissue cysts contained in infected meat[23] (Table 1). They also can become infected in utero by transplacental transmission of the parasite from an acutely infected mother to the fetus, from an infected organ donor in the setting of organ transplantation,[50–53] and rarely, by blood transfusion or laboratory accidents.[54] Recent studies suggested that oocysts were the predominant route of transmission of *T gondii* infections in the United States; 78% (59 of 76) of pregnant women with acute primary *T gondii* infections during pregnancy who gave birth to infants with CT had serologic evidence suggestive of being infected by oocysts.[24,55]

Different types of locally produced meat from retail meat stores in the United States (eg, pork, lamb, goat, wild game meat) have been found to be contaminated with *T gondii*.[40,47,57–60] Moreover, with the globalization of the food market, imported meats from other countries (that could be infected even with atypical, more virulent *T gondii* strains) also can be found in the United States and Europe.

TABLE 1 Routes of *Toxoplasma gondii* Infection

1. Contact with soil contaminated with *T gondii* from cat feces
Touching mouth with hands after gardening
Cleaning a cat's litter box
Touching anything that has come into contact with cat feces
Eating unwashed fruit or vegetables contaminated with soil
2. Contact with contaminated meat:
Eating undercooked meat from infected animals, especially pork, lamb, or venison
Touching mouth after handling raw contaminated meat
Using kitchen utensils or chopping boards that have been in contact with raw contaminated meat
3. Other
Drinking unpasteurized raw milk[56]
Drinking contaminated water
Receiving an infected organ transplant or blood transfusion (rare)

Adapted from ref 23.

Freezing (below –20°C [–4°F] for at least 48 hours)[61] and thorough cooking to at least 63°C (145°F) for whole cut meat (excluding poultry), 71°C (160°F) for ground meat (excluding poultry), and 74°C (165°F) for poultry (whole cut or ground)[62,63] have been shown to inactivate *T gondii* tissue cysts. Neither microwave cooking nor chilling at 5°C for 5 days is sufficient to kill tissue cysts (microwave cooking may not generate a homogenous temperature of 67°C [153°F]).[61,64]

The following risk factors for acute *T gondii* infection were reported in a US study[20]: eating raw ground beef, rare lamb, or locally produced cured, dried, or smoked meat; working with meat; eating raw oysters, clams, or mussels; drinking unpasteurized raw goat milk; or having ≥3 kittens in the household. Eating raw oysters, clams, or mussels is a novel risk factor that was recently identified; however, in selected populations in the United States or in other parts of the world,[65,66] additional/different risk factors (eg, contact with cat feces or drinking untreated water) also have been implicated. Untreated water has been found to be a source of major community outbreaks of acute toxoplasmosis in Canada and Brazil.[67,68]

In up to 50% of individuals in whom an acute *T gondii* infection was confirmed, it was not possible to identify a known risk factor for their infection.[24] Thus, toxoplasmosis should be considered in the differential diagnosis of ill patients with symptoms suggestive of toxoplasmosis, even in the absence of known risk factors for *T gondii* infection.

Outbreaks of Acute Toxoplasmosis

Community outbreaks of acute toxoplasmosis have been described in several parts of the world,[34,49,69–74] including North America.[67] Outbreaks of acute *Toxoplasma* infection within families (ie, with more than 1 family member being infected) have been documented in the United States,[74] and the prevalence of within-family clusters of acute *Toxoplasma* infections in the United States has also been studied.[75] Although a cost-benefit analysis of routine screening of additional family members of index cases with acute toxoplasmosis might be useful, until such a study is completed, it is important for physicians to be aware of this phenomenon and consider screening additional family members of index cases diagnosed with acute toxoplasmosis, especially if pregnant women, immunocompromised patients, or young children live in such households.[74] Young children living in such households who acquire acute *T gondii* infection might not be able to appropriately communicate problems with their vision if acute toxoplasmic chorioretinitis occurs.

Seasonal Variation in Acute Toxoplasmosis

A seasonal pattern for acute toxoplasmosis has been recently identified in Europe[76] and the United States.[77] In France, the first highest peak of acute *Toxoplasma* infections during pregnancy was observed between August and September, and the second highest peak was observed between October and December.[76] In the United States, a similar peak during December was observed for cases of acute toxoplasmic lymphadenopathy referred to the Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory (PAMF-TSL),[77] the National Reference Laboratory for the Centers for Disease Control and Prevention and the US Food and Drug Administration (FDA).

Seroprevalence of *T gondii* Infections

United States

In the United States, the rates of *T gondii* IgG-seropositive individuals has decreased over the past 2 decades. Data from the most recent NHANES for 2009–2010[78,79] showed an overall age-adjusted seroprevalence in people older than 6 years of 12.4% (95% CI: 11.1–13.7%) (The respective unadjusted seroprevalence rate was 13.2% [95% CI: 11.8–14.5%].) The age-adjusted seroprevalence among women of childbearing age (15–44 years) in the whole US population was 9.1% (95% CI: 7.2–11.1%) in 2009–2010 compared with 11% in 1999–2004 and 15% in 1988–1994. For US-born women of childbearing age, the respective seroprevalence rates were 6%, 8%, and 13% in 2009–2010, 1999–2004, and 1988–1994, respectively. Most US women of childbearing age are susceptible to *Toxoplasma* infection. If women become infected during pregnancy, they could give birth to an infant with CT. People born outside the United States were significantly more likely to be seropositive than people born in the United States (25.1% vs 9.6%, respectively).[78] Seroprevalence rates were also higher in persons with a Hispanic versus a non-Hispanic white racial background (15.8% vs 10.2%, respectively).[78] In addition, seroprevalence rates in children living on farms in Wisconsin have been reported to be fivefold higher than rates in children not living in farms (18% [29 of 159] vs 4% [8 of 184], respectively).[80]

In the United States, toxoplasmosis was found to be the second leading cause of death and the fourth leading cause of hospitalizations attributable to foodborne illnesses.[81,82] Recent cumulative US data over an 11-year study period (2000–2010) identified 789

toxoplasmosis-associated deaths, with a cumulative productivity loss attributable to toxoplasmosis of $815 million.[83] Black and Hispanic persons had the highest toxoplasmosis-associated mortality. HIV infection, lymphoma, leukemia, and connective tissue diseases were associated with increased risks of toxoplasmosis-associated deaths.[83] Population-based data estimated that *T gondii* infects approximately 1.1 million people each year in the United States[84]; toxoplasmic chorioretinitis is estimated to occur in approximately 2% of *T gondii*–infected individuals (approximately 21 000 people in the United States per year), and symptomatic chorioretinitis in approximately 0.2% to 0.7% of *T gondii*–infected individuals (approximately 4800 people in the United States per year).[84]

According to the 2010 report by the Council of State and Territorial Epidemiologists, reporting of toxoplasmosis is not mandatory in most of the United States.[85] As of 2010, toxoplasmosis was a reportable disease only in 19 states; in an additional 9 states, toxoplasmosis was previously a reportable disease, but not as of 2010.

Other Areas of the World

T gondii seroprevalence rates vary in different parts of the world[86] and can range from <10% in some northern European countries[87] to as high as 60% to 80% (eg, in Mexico[88] and Brazil[89,90]).

Incidence of Maternal Primary Infection During Pregnancy

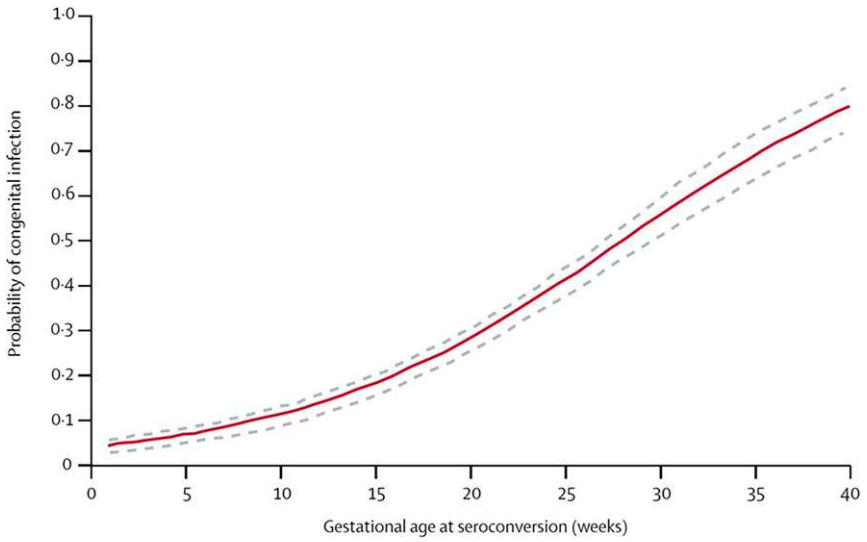
The incidence of acute primary *T gondii* infections during pregnancy in the United States, according to the National Institutes of Health–sponsored Collaborative Perinatal Project in 22 845 women (1959–1966) who were screened every 2 months during pregnancy, at birth, and at 6 weeks postpartum, was estimated to be 1.1 cases per 1000 pregnant women.[91] However, that is likely to be an overestimate of the true incidence of acute *T gondii* infections during pregnancy in the current era, with the overall decrease in *T gondii* seroprevalence. Extrapolating from recent data from the New England Newborn Screening Program of the incidence of CT of approximately 0.23 per 10 000 live births, and after taking into account that this value might underestimate the true incidence of CT by approximately 50%, the true incidence of CT could be as high as double that value (approximately 0.5 CT cases per 10 000 live births). The incidence of CT may also be higher in areas and in subpopulations in the United States with higher overall *T gondii* seroprevalence rates.[66,78] Then, assuming an overall MTCT rate of 25% (consistent with more recent data), the estimated incidence of acute primary infection during pregnancy in the United States would have been approximately 0.2 per 1000 pregnant women. If these numbers (0.2–1.1 acute cases per 1000 pregnant women) are extrapolated to the approximately 4 million pregnancies per year in the United States,[92] approximately 800 to 4400 women per year in the United States acquire acute *T gondii* infections during pregnancy.

Of note, when France first began antepartum screening for toxoplasmosis in the early 1980s, the incidence rate of acute primary *T gondii* infections during pregnancy at that time was 4 to 5 per 1000 (the population seropositivity rate at that time was very high, and the percentage of nonimmune, susceptible pregnant women was low).[93]

Recently reported rates of acute primary infections during pregnancy in other countries ranged from 0.5 in 1000 pregnancies in Sweden[94] to 2.1 in 1000 pregnancies in France (95% CI: 1.3–3.1).[95] However, chronologic and methodologic differences between such studies preclude accurate direct comparisons.

Risk of MTCT of *T gondii* Infection

Data regarding the risk of MTCT of *T gondii* infection come from studies in which almost all women were routinely screened during pregnancy and therefore received antepartum treatment once primary infection was diagnosed.[1,3,96] The SYROCOT meta-analysis of individual patient data from an international consortium, provided important information from 26 countries participating in the consortium. However, data for the effect of antepartum treatment on the MTCT risk were based only on European cohorts that had antepartum screening/treatment programs; neonatal cohorts from the United States, Brazil, and Colombia were excluded. Across the 26 cohorts, the overall risk of MTCT was <5% after seroconversion (maternal acute primary infection) early in pregnancy, 15% (13%–17%) after seroconversion at 13 weeks, 44% (40%–47%) after seroconversion at 26 weeks, and 71% (66%–76%) after seroconversion at 37 weeks (Fig 2).[1] Consistent rates for the MTCT risk were published from the Lyon, France, cohort for the past 2 decades (1987–2008).[3] For maternal seroconversion before 8 weeks of gestation, the MTCT risk was <8%. For maternal seroconversion between 8 and 10 weeks of gestation, the MTCT risk was <10%[3] (Fig 3). Other factors associated with an increased risk of MTCT included acute *T gondii* infection during pregnancy, immunocompromising conditions, lack of antepartum treatment, and *T gondii* strain virulence, higher parasite load, and parasite source (oocyst/sporozoite-related infections from contact with cat feces versus tissue cyst/bradyzoite-



[a] The vast majority of these mothers (94%) had received antepartum anti-*Toxoplasma* treatment.

FIGURE 2

Risk of MTCT of *T gondii*, according to gestational age at seroconversion. Data are from 26 international cohorts participating in the international SYROCOT study. The vast majority of these mothers (94%) had received antepartum anti-*Toxoplasma* treatment. (Reprinted with permission from Thiebaut R, Leproust S, Chene G, Gilbert R; SYROCOT Study Group. Effectiveness of prenatal treatment of congenital toxoplasmosis: a meta-analysis of individual patients' data. *Lancet*. 2007;369:118.)

related infections from ingestion of undercooked meat).

Effect of Antepartum Treatment on the Risk of MTCT

RCTs to assess the efficacy of toxoplasmosis antepartum screening and treatment programs for the reduction in MTCT risk have not been conducted, although data from prospective and retrospective observational studies provide information on the association of prenatal treatment and MTCT as well as symptomatic congenital disease. Observational studies have led to the uptake of routine screening in many countries for the purpose of providing treatment, making the conduct of needed RCTs difficult.[7]

The individual patient-data meta-analysis of the European data from the SYROCOT international consortium published in 2007[1] revealed that when antepartum treatment was promptly initiated within 3 weeks after maternal seroconversion, the odds of MTCT were 52% lower when compared with delayed treatment (≥8 weeks after seroconversion; adjusted OR: 0.48; 95% CI: 0.28–0.80). Decreases of similar magnitude in MTCT risk were observed when antepartum treatment was initiated between 3 and 5 weeks and between 6 and 8 weeks after seroconversion, when compared with delayed treatment (ORs [95% CIs]: 0.64 [0.40–1.02] and 0.60 [0.36–1.01], respectively). These analyses might have also underestimated the true effectiveness of antepartum treatment, because some of the women who received antepartum treatment who would have otherwise lost their fetuses because of *T gondii* infection might have eventually delivered infants with only mild CT. Of note, the overall risk of MTCT among women with primary infection in France decreased from 29% (125 CT cases per 424 infected pregnant women) before 1992 to 24% (388 CT cases per 1624 infected pregnant women) after 1992 (P = .022) when monthly antepartum screening became mandatory (as opposed to simply "recommended" without a specified frequency of screening before 1992). These rates may call into question the efficacy of prenatal treatment to prevent MTCT, because the women in the postimplementation era were more likely to be identified by serologic testing alone rather than by ultrasonographic abnormalities in the setting of established infection. An alternative explanation for this paradoxical phenomenon is that early prenatal screening and treatment led to a decrease in fetal deaths, and thus the effect on the risk of MTCT was not very prominent. In other words, with prompt initiation of prenatal treatment, children who would have otherwise died of CT survive, making the decrease in MTCT risk less impressive. Evidence that supports this hypothesis includes data from the same prospective cohort study from Lyon, France, by Wallon et al,[3] published in 2013, which showed a reduction in symptomatic disease among infected pregnant women when comparing cases reported before 1995 with those after 1995 when AF testing with PCR was initiated (from 11% [87 symptomatic CT cases per 794 infected mothers] before 1995 to 4% [46 symptomatic CT cases per 1150 infected mothers] after 1995; $P < .001$). With the routine use of AF PCR, fetal infections were properly diagnosed, and the initiation of treatment with pyrimethamine/sulfadiazine (P/S) was promptly instituted. Poor adherence to monthly screening by pregnant women might have also contributed to this phenomenon. A recent study by Cornu et al reported that only 40% of seronegative pregnant women in France had all ≥7 screening tests according to the French scheme.[97] However, this study was based on only a small sample of pregnant women in France.

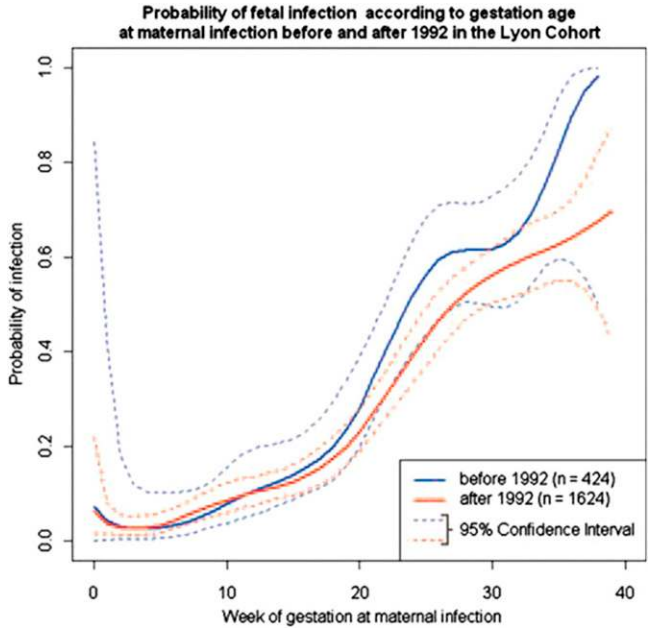


FIGURE 3

Probability of fetal infection according to gestational age at time of maternal infection before and after mid-1992, when the universal antepartum screening for toxoplasmosis in France in mid-1992 became mandatory. Shown are data from the Lyon cohort (1987–2008) from 451 mothers before mid-1992 and 1624 mothers after mid-1992. The vast majority of these women were treated during pregnancy. (Reprinted with permission from Wallon M, Peyron F, Cornu C, et al. Congenital toxoplasmosis infection: monthly prenatal screening decreases transmission rate and improves clinical outcome at age 3 years. *Clin Infect Dis*. 2013;56:1229.)

Prusa et al[4] reported in 2015 that, in Austria, where there is a nationwide antepartum screening program (the Austrian Toxoplasmosis Register), a sixfold lower risk of MTCT was observed in women who received antepartum treatment compared with untreated women, after also taking into account the gestational age at maternal infection (9% [87 CT cases per 1007 infected pregnant women] versus 51% [32 CT cases per 63 infected pregnant women]). However, their findings may have been related to the timing of identification during pregnancy.

Hotop et al[5] reported in 2012 that, in Germany, where spiramycin is given until the 16th week of pregnancy, followed by at least 4 weeks of combination therapy with pyrimethamine/sulfadiazine/folinic acid (independently of the infection status of the fetus, with subsequent treatment determined according to the infection status of the fetus), very low rates of MTCT of CT were seen (4.8% [33 CT cases per 685 infected pregnant women]).

An early Cochrane systematic review by Peyron et al[6] published in 2000 evaluated prenatal treatment in pregnancy and concluded that, despite more than 3200 articles, only 9 studies were identified by that time that had evaluated prenatal treatment and the risk of MTCT, and these results were conflicting, resulting in insufficient evidence to evaluate the efficacy of treatment.

Effect of Antepartum Treatment on the Risk of Symptomatic CT

Some observational prospective and retrospective cohort studies have shown the association of antepartum treatment (and particularly of prompt initiation of prenatal treatment as soon as possible after acute maternal infection) with the prevention of symptomatic infant's disease. Cortina-Borja et al,[7] in a recent analysis of data from 293 infants with CT from 14 European centers in 6 countries, suggested that antepartum treatment was associated with lower odds of severe neurologic sequelae or death (SNSD; OR adjusted for gestational age at maternal seroconversion: 0.24; 95% CI: 0.07–0.71; 10 cases of SNSD per 104 untreated CT cases versus 13 SNSD per 189 treated CT cases). (The authors advised caution in interpretation, because the study included only 23 such very severely affected cases of CT, and of these, there were 9 pregnancy terminations.) This composite outcome of SNSD included the following outcomes: pediatric report at any age of microcephaly; insertion of intraventricular shunt, an abnormal or suspicious neurodevelopmental examination that resulted in referral to a specialist, or seizures during infancy or at an older age that required anticonvulsant therapy; severe bilateral visual impairment in both eyes assessed after 3 years; cerebral palsy; or death from any cause before 2 years of age, including termination of pregnancy (Fig 4).

Wallon et al,[3] in their prospective cohort study from France (published in 2013), showed that the risk of symptomatic CT decreased in France after 1995, when testing for *T gondii* in AF by AF PCR was initiated, from 11% (95% CI: 9–13% [87 symptomatic CT cases per 794 infected mothers]) before 1995 to 4% (95% CI: 3–5% [46 symptomatic CT cases per 1150 infected mothers]) after 1995 ($P < .001$), but the increase in the mild maternal infections included in the latter group may have played a role as well. AF PCR testing allowed for the prompt initiation of effective

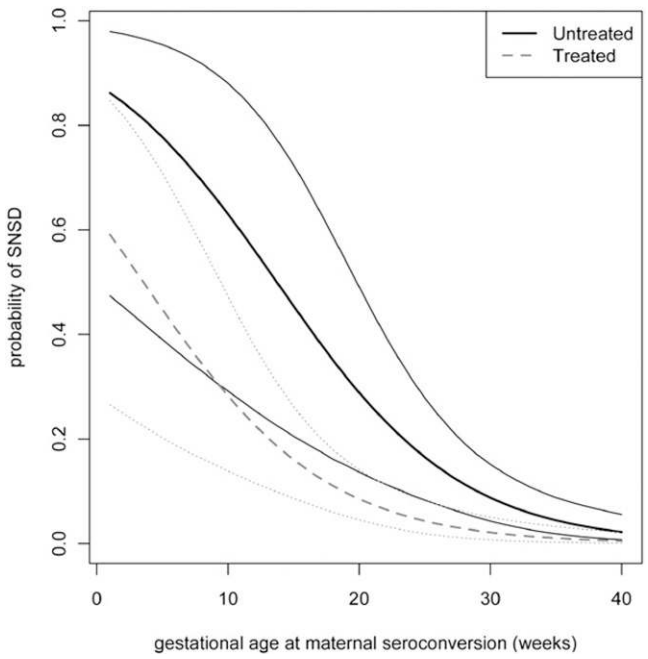


FIGURE 4

Risk of severe neurologic disease or death (SNDD) in children with CT according to antepartum treatment. Probability of SNDD according to imputed gestational age at seroconversion and 95% Bayesian credible limits. Dotted lines denote treated pregnancies; solid lines denote untreated pregnancies. (SNDD is a composite outcome comprising a pediatric report at any age of microcephaly; insertion of an intraventricular shunt; an abnormal or suspicious neurodevelopmental examination that resulted in referral to a specialist; seizures during infancy or at an older age that required anticonvulsant therapy; severe bilateral visual impairment [visual acuity of Snellen 6/60 or less in both eyes assessed after 3 years]; cerebral palsy; or death from any cause before 2 years of age including termination of pregnancy [the consistency of SSND findings was checked through multiple assessments].) Severe neurologic sequelae were assessed at a median of 4-year follow-up, death was assessed by 2 years of age, and severe bilateral visual impairment was included in the composite outcome of severe neurologic sequelae. SNDD, severe neurologic disease or death. (Reprinted under a Creative Commons License from Cortina-Borja M, Tan HK, Wallon M, et al. Prenatal treatment of serious neurologic sequelae of congenital toxoplasmosis: an observational prospective cohort study. *PLoS Med.* 2010;7:e1000351.)

antepartum treatment (P/S) for a greater number of women with infected fetuses. Earlier reports from France also had shown that the severity of clinical manifestations dramatically decreased after the introduction of antepartum screening and treatment (29% [68 of 234 CT cases] of CT cases were symptomatic between 1984 and 1992 versus 99% of 147 CT cases between 1949 and the 1960s).[98]

A recent retrospective cohort study in 685 infected pregnant women from Germany by Hotop et al,[5] published in 2012, also showed a lower risk of symptomatic disease with early versus late initiation of maternal treatment (fewer than 4 weeks versus more than 8 weeks after seroconversion; 19% with early antepartum therapy versus 70% with late antepartum therapy; $P = .006$). In this study,[5] after the diagnosis of acute primary maternal infection, spiramycin was initiated in all women up to week 16 of gestation, at which time treatment was changed in all women to P/S for at least 4 weeks. P/S was continued until further AF PCR and fetal ultrasonographic testing excluded or confirmed fetal infection; in the former situation, antepartum treatment was changed back to spiramycin until delivery, whereas in the latter situation, P/S was continued until delivery.

Kieffer et al,[99] in their prospective cohort study published in 2008 in 335 infants with CT from the Lyon, Paris, and Marseille cohorts, found that a delay of more than 8 weeks from the time of maternal seroconversion to initiation of maternal treatment was associated with an increased risk of chorioretinitis in the first 2 years of life (hazard ratio [HR]: 2.54; 95% CI: 1.14–5.65).

A previous report from the European Multicenter Study of Congenital Toxoplasmosis (EMSCOT) study by Gras et al[100] from 2005 also showed 72% lower odds of intracranial lesions in infants whose mothers initiated treatment less than 4 weeks after seroconversion (OR: 0.28; 95% CI: 0.08–0.75). However, the treatment benefit was lost when antepartum treatment was initiated more than 4 weeks after seroconversion.[100]

It should be noted here that the analysis of data from the European cohorts participating in the SYROCOT international consortium (550 infants with CT per 1745 women), published in 2007,[1] failed to show that prenatal treatment reduced the risk of clinical manifestations (adjusted OR for treated versus not treated: 1.11; 95% CI: 0.61–2.02). However, a characteristic of the SYROCOT analysis could explain this finding. Specifically, the 4 cohorts with the highest burden of disease from North and South America were excluded from their analysis (2 cohorts from Brazil, 1 from Colombia, and 1 from the United States [Massachusetts]). The justifications for this exclusion were as follows: (1) differences in the burden of the disease compared with the remaining cohort (eye disease was more frequent and

more severe in South American than in European cohorts), (2) differences in the *T gondii* strains, and (3) differences in the methods used for the diagnosis of intracranial calcifications (computed tomography versus head ultrasonography in Europe). It is not surprising that epidemiologic studies that excluded severe cases and attempted to estimate the risk of symptomatic disease in groups of treated infants with CT reported only a weak effect for antepartum treatment. Moreover, in the analyzed European cohorts, only 18% of the pregnant women (307 of 1745) across the 26 cohorts were untreated, and only one-third (164 of 550) of the infants with CT were born to mothers who had not received antepartum treatment. This finding might have limited the study power to detect statistically significant differences between prenatal treatment and no treatment on clinical manifestations of CT. Additional discussion on the relationship between prenatal treatment and the risk of symptomatic CT can be found in the Supplemental Information.

Incidence of CT

United States

Data regarding the disease burden of CT in the United States are limited, although this information is crucial for policy makers and public health decisions for routine antepartum screening.[101] At the time of this report, only Massachusetts and New Hampshire offer routine newborn screening for CT.[102] The New England Regional *Toxoplasma* Working Group, during an early 6.5-year period of neonatal screening in Massachusetts (1986–1992) and a 4-year period in New Hampshire (1988–1992), identified 52 cases of CT among 635 000 screened infants, and the estimated incidence of CT was 0.82 cases per 10 000 live births.[103–105] This estimated incidence translates (if extrapolated to the approximately 4 million infants born each year in the United States) to approximately 330 infants being born each year in the United States with CT. Subsequent cumulative published data from the New England Newborn Screening Program[106] over the 12-year period of 1988–1999 identified 93 cases of CT among 1 019 904 screened infants, and the estimated incidence of CT was 0.91 cases per 10 000 live births, which would translate to ~365 children being born each year in the United States with CT. The respective incidence among US-born pregnant women was 0.82 cases per 10 000 live births (H. W. Hsu, MD, Massachusetts Department of Public Health, personal communication, 2015). The incidence of CT has decreased since 1999 and, over the past 9 years (2006–2014), was approximately 0.23 cases per 10 000 live births (H. W. Hsu, MD, Massachusetts Department of Public Health, personal communication, 2015). This decrease of approximately 50% appears to be in line with the decrease in seroprevalence in adults in the United States since early 2000.[78] Older prospective studies, even before the 1970s, had estimated an incidence of CT of up to 10 cases per 10 000 live births.[103]

The true incidence of CT in the United States might be even higher, because the sensitivity of the newborn screening test (filter paper blot spot *Toxoplasma*-IgM) used in the New England Neonatal Screening Program is approximately 50% to 75%.[78,104,106] The incidence of CT may also be higher in areas of the United States where the seroprevalence of *T gondii* infections is higher. Moreover, neonatal screening programs might also underestimate the true incidence of CT, because fetal losses attributable to severe CT were not counted.

Neonatal screenings also exist in the United States for several inborn errors of metabolism, even though the numbers of affected infants are very small (annual incidence of phenylketonuria, which is the most common of these diseases, is 1 per 15 000 live births[108]), because treatment markedly alters the outcome in those infants. The role of antepartum screening and treatment of CT in the United States may need reevaluation in the light of recently accumulated evidence from several observational studies that showed that prompt initiation of treatment can alter the clinical course of the disease.[109–112]

France and Other European Countries

In France, the first National Surveillance System for CT was established in 2007, approximately 30 years after the initiation of the national antepartum screening programs. The prevalence of CT according to this system was estimated to be 3.3 per 10 000 live births (95% CI: 2.9–3.7; 272 CT cases per 818 700 births),[113] and the prevalence of symptomatic CT was 0.34 per 10 000 live births (95% CI: 0.2–0.5; 28 symptomatic CT cases per 818 700 births).[113] Moreover, among the 272 CT cases, there were 11 pregnancy terminations (4%) (6 abortions and 5 cases of fetal demise). The overall seroprevalence rates in the French population also decreased by nearly 50% (from 84% to 44%) between the 1960s and 2003, which could have also explained, in part, the observed low rates of CT in France. However, over the same time period, strong evidence has accumulated indicating that the universal antepartum screening/treatment program dramatically affected the clinical severity of the disease.[1,3,99,114]

Cumulative data from a recent 13-year period (1996–2008) from the Rhones-Alpes area of France revealed that approximately 80% of infants with CT are now asymptomatic at birth (161 asymptomatic infants per 207 CT cases[3]). For comparison, only 60% (95% CI: 45–74% [29 of 48]) of US infants with CT were asymptomatic at birth, according to an early report from the New England Newborn Screening Program.[104] However, because the screening methods used in France (antepartum screening and treatment) and in the New England Newborn Screening Program (only postnatal screening and treatment as indicated) were different, the utility of such a comparison is limited.

According to the 2006 EUROTOXO study report,[10] reported rates of CT vary across different European countries (ie, 0.7 in 10 000 in Sweden,[94] 0.8 in 10 000 in the United Kingdom,[115] 1 in 10 000 in Austria,[4] 1.6 in 10 000 in Denmark,[116] 5 in 10 000 in Switzerland, 11 in 10 000 in Poland,[117] and 13 in 10 000 in Germany[10]). However, the differences in those rates should be interpreted cautiously because of chronologic and methodologic differences.

Global Burden of CT

A systematic review of the global burden of CT,[118–122] which was part of a larger study of the global burden of foodborne toxoplasmosis coordinated by the World Health Organization, has been published.[122] Nine major databases from published and unpublished sources were systematically reviewed, and their authors were also directly contacted. The global yearly number of CT cases was estimated to be 190 100 (95% CI: 179 300–206 300). Cumulative incidence rates of CT were reported according to geographic region: Africa, 20 to 24 in 10 000 births; North America, 6 in 10 000 births; South America, 18 to 34 in 10 000 births; European countries, 5 in 10 000 births; Eastern European countries, 15 to 16 in 10 000 births; Southeast Asia, 8 to 13 in 10 000 births; and West Pacific regions (Australia and New Zealand), 12 to 14 in 10 000 births.[122] The highest incidence rates were reported in South America and were considered to be driven by the more virulent genotypes circulating in that region.[29,122] In addition, the global burden of disease from CT was estimated to be equivalent to 1.2 million disability-adjusted life-years.[122]

Clinical Manifestations of CT

Significant differences in the prevalence and severity of major manifestations (eg, chorioretinitis, brain calcifications, and hydrocephalus) have been observed in the United States, France, other Western European countries, and South America. A compendium of ocular, neurologic, and other manifestations reported in the literature to be associated with CT is shown in Table 2.

The US Experience: Clinical Manifestations

In the United States, CT is notable for its severity in presentation,[124] but CT is not on the list of nationally notifiable diseases.[125] Over a period of 15 years (1991–2005) at the PAMF-TSL,[124] 84% of infants diagnosed with CT were severely affected (116 of 138 CT cases with clinical information, with their clinical findings confirmed at the NCCCT study center)[124]; 92% had chorioretinitis (119 of 129 CT cases with this information), 80% had intracranial calcifications (94 of 118 CT cases with this information), 68% had hydrocephalus (67 of 99 CT cases with this information), and 62% had all 3 manifestations (53 of 86 CT cases with this information).[124] Accurate estimates of additional clinical manifestations, such as microcephaly, cerebral atrophy, hepatomegaly, splenomegaly, skin rashes, including purpura, jaundice, thrombocytopenia, elevated CSF protein, and CSF pleocytosis, were not possible, because information regarding whether all infants had been evaluated/tested for these outcomes was not available.

It is possible that children who were eventually referred to the PAMF-TSL for laboratory confirmation of their diagnosis were a select group of infants and not representative of the true spectrum of CT in the United States. On the other hand, these rates could also possibly underestimate the severity of CT in the United States, because they did not capture the CT cases that resulted in spontaneous abortion, fetal deaths, or pregnancy termination because of severe disease.

Similar high rates of severe CT were reported by the National Chicago-Based Collaborative Study on Congenital Toxoplasmosis (NCCCT) in its cumulative report from 1981 to 2004 (85% with chorioretinitis, 85% with intracranial calcifications, and 50% with hydrocephalus).[112] These reported rates pertained only to the 96 children with CT in the "severe disease stratum" and not all of the 120 children with CT in the cohort (including 24 CT cases who were asymptomatic or with only mild clinical manifestations). For the vast majority of infants in the NCCCT study, their mothers have not received antepartum treatment. These high rates are also similar to those reported in the 1950s by Eichenwald[126] from an early Danish cohort of untreated infants (94% of CT cases had chorioretinitis, 50% had intracranial calcifications, and 28% had hydrocephalus). Additional clinical manifestations reported in the NCCCT study[112] included

TABLE 2 Compendium of Clinical Manifestations/Signs Reported in the Literature to Be Associated With CT

Ocular signs[98,109,110,112]

1. Amblyopia*
2. Cataract*
3. Chorioretinitis* (bilateral or unilateral, posterior pole macular lesions or peripheral lesions; yellowish-white cotton-like patches in the fundus; solitary or small clusters; lesions of various ages; sharp borders in older lesions)
4. Chorioretinal scars* (macular, peripheral, juxtapapillary)
5. Chorioretinal edema
6. Choroidal neovascular membranes (vision-threatening)
7. Detachment of posterior hyaloid membrane
8. Iritis (if associated with posterior pole lesions)
9. Leukocoria
10. Macular edema
11. Microphthalmia-microcornea
12. Nystagmus*
13. Necrotizing retinitis
14. Optic nerve atrophy*
15. Papilledema
16. Retinal detachment
17. Strabismus* (convergent or divergent)
18. Visual impairment*
19. Vitritis (active; exudation of cells in overlying vitreous)

CNS signs[98]

1. Brain masses
2. CSF pleocytosis, elevated protein, eosinophilia, hypoglycorrhachia*
3. Delay in developmental milestones* (psychomotor retardation)
4. Hypotonia* (or abnormal muscle tone)
5. Intracranial calcifications* (1–3 mm; scattered in white matter, in periventricular areas of occipitoparietal and temporal regions, curvilinear streaks in basal ganglia, nodular calcifications, linear calcifications)
6. Macrocephaly or microcephaly*
7. Obstructive hydrocephalus*
8. Palsies*
9. SNHL*
10. Seizures*
11. Spasticity*
12. Wide spectrum of manifestations from massive acute encephalopathy to subtle neurologic signs

Additional signs[98]

1. Anemia*
2. Disseminated intravascular coagulation
3. Hepatitis*
4. Hepatic calcifications*
5. Hepatomegaly or hepatosplenomegaly*
6. Jaundice
7. Lymphadenopathy
8. Myocarditis*
9. Pneumonitis
10. Preterm birth* (associated with certain non–type II *T gondii* strains in the United States[37])
11. Rash* (petechial, blueberry muffin rash, purpura, maculopapular)
12. Sepsis-like illness* (disseminated disease with multiple organ failure, acute respiratory distress syndrome, disseminated intravascular coagulation) (rare)
13. Splenomegaly*
14. Temperature instability (hypothermia or hyperthermia/fever)
15. Thrombocytopenia*

Fetal ultrasonographic findings[123]

1. Ascites*
2. Echogenic bowel*
3. Fetal demise*
4. Hydrocephalus*
5. Hydrops fetalis
6. Hepatosplenomegaly*
7. Intracranial densities/calcifications*
8. Intrahepatic densities/calcifications*
9. Intrauterine growth retardation*
10. Pericardial and/or pleural effusions
11. Placenta hyperdensities, placenta increased thickness*

Clinical manifestations/signs are listed alphabetically. Signs considered to be encountered more frequently according to the PAMF-TSL are indicated by an asterisk (*). Accurate estimates of the frequency of all of these clinical signs are not available.

microcephaly (15%), seizures (20%), microphthalmia (20%), hepatosplenomegaly (30%–35%), thrombocytopenia (40%), anemia (20%) skin rashes (25%), and jaundice (60%).

For newborn infants with CT diagnosed by the New England Newborn Screening Program (1986–1992),[104] 40% (19 of 48 CT cases) had either chorioretinitis or CNS abnormalities. More specifically, 19% (9 of 48) had eye disease diagnosed at birth (4% [2 of 48] had active chorioretinitis at birth and 15% [7 of 48 CT cases] had retinal scars at birth without active inflammation). Another 3 infants, among the 30 infants with CT without eye disease at birth, had eye disease diagnosed during the follow-up at ≥1 year. Overall, 25% (12 of 48) of infants with CT had eye disease at birth or during follow-up and 29% (14 of 48 CT cases) had CNS abnormalities (elevated CSF protein: 25% [8 of 32 CT cases]; intracranial calcifications: 20% [9 of 46 CT cases]; enlarged ventricles identified on computed tomography or ultrasonography: 2% [1 of 47 CT cases]). According to the New England Newborn Screening Program, only 60% of infants were asymptomatic at birth, compared with 80% of infants with CT who were reported as asymptomatic in Europe.[3] As noted previously, spontaneous abortion, fetal demise, or pregnancy terminations because of severe CT were not captured in the New England Newborn Screening Program.

There are possible explanations for the observed differences in the reported rates of severe CT between the European Union and United States. The lower rates of severe CT in the European Union cohorts might reflect the following: (1) differences in the spectrum of CT observed in population-based, prospectively identified consecutive CT cases versus more selected cases referred to reference centers such as the PAMF-TSL and the University of Chicago Toxoplasmosis Center; (2) differences in the implicated *T gondii* strains; or (3) differences in the use of antepartum treatment of CT (mothers of infants in the US cohorts did not receive antepartum treatment). Approximately 61% of *T gondii* strains implicated in CT in the United States are non–type 2 strains,[37] whereas in France, 95% of cases are caused by type 2 strains.[29,127] It is possible that the non–type 2 strains found in the United States are more pathogenic than the type 2 strains commonly found in Europe.[128,129]

Effect of Postnatal Treatment on Eye Disease

In 1 report, only 31% (34 of 108 CT cases; 95% CI: 23%–41%) of infants with CT in the United States who were treated during their first year of life developed new chorioretinal lesions during long-term follow-up.[110] However, in another report, 72% (18 of 25 CT cases; 95% CI: 51%–89%) of US children with CT who were diagnosed after infancy (and did not receive any postnatal treatment during infancy) developed new chorioretinal lesions during follow-up.[109] When postnatal treatment was started at ≤2.5 months of age and continued for 12 months, recurrent eye disease developed in 9% of children without substantial neurologic disease at birth and in 36% of children with moderate to severe neurologic disease at birth.[112]

Effect of Postnatal Treatment on CNS Disease

Initial studies in untreated children with CT revealed unfavorable long-term neurologic outcomes in approximately ∼50% of children.[98] Subsequently, cumulative reports from the NCCCT study (1981–2004) with data on 120 children with CT (24 of whom were asymptomatic or had only mild findings and 96 of whom had severe clinical findings) reported relatively favorable neurologic outcomes after 1 year of postnatal treatment[112] (Table 3). However, long-term follow-up was not reported for all of the children in the 2 groups. More analytically, treatment of the 24 children without substantial neurologic disease at birth resulted in the following: (1) abnormal cognitive and neurologic outcomes in all but 1 child (4%), (2) interval decrease in IQ (≥15 points) in 4% of children (1 of 24), (3) abnormal auditory outcomes in no children, (4) vision impairment in 15% of children (2 of 13), and (5) recurrences of eye diseases in 9% of children (1 of 11). For comparison, treatment of the 96 children with severe CT at birth resulted in (1) abnormal neurologic and/or cognitive outcomes in 27% of children (18 of 66), (b) interval decrease in IQ (≥15 points) in 16% of children (9 of 55), (3) abnormal auditory outcomes in no children, (4) vision impairment in 85% of children (47 of 55 [with the retinal lesion causing the visual impairment already present at birth]), and (5) recurrence of eye disease in 36% of children (17 of 47). In the above estimates of clinical outcomes, 11 additional children who died between 3 months and 7 years of age were not considered. The majority of these children already had severe neurologic manifestations at birth. Although in this cohort there were children with severe and likely irreversible brain and eye disease, overall, these treated children had substantially better outcomes than did children from historical cohorts in the 1960s–1980s who were untreated or treated for only 1 month.

Earlier reports from the NCCCT study in 36 postnatally treated CT cases (1981–1991) also provided

TABLE 3 Outcomes of Postnatal Treatment of CT According to the NCCCT Study

	Treatment Outcome for Infants With CT With No/Mild Findings at Birth (n = 24), % (n/n)	Treatment Outcome for Infants With CT With Severe Findings at Birth (n = 96),[a] % (n/n)
Normal motor-neurologic outcomes	100 (13/13)	80 (44/55)
Normal cognitive outcome (IQ >70)	100 (13/13)	73 (48/66)
No decreased IQ of ≥15 points between any 2 consecutive evaluations	92 (12/13)	84 (46/55)
Normal auditory outcome	100 (13/13)	100 (55/55)
No vision impairment	85 (11/13)	15 (8/55) (in the remaining 85% with vision impairment, the responsible lesion was already present at birth)
No recurrence of eye disease	91 (10/11)	64 (30/47)

Adapted from ref 110. Follow-up outcome ascertainment was not available for all children in each of the 2 groups. (Different outcomes were ascertained at different time points: for example, the endpoint of IQ <70 was evaluated at ≥3.5 y of age; the new eye lesions endpoint was evaluated at ≥7.5 y of age; all other endpoints were evaluated at ≥5 y of age.)
[a] Infants were classified in the severe stratum if there were ≥3 punctate focal calcifications or abnormal density of white matter in initial CT scan findings, seizures, motor abnormalities, hydrocephalus, microcephalus, elevated CSF protein, hypoglycorrhachia, macular scarring, optic nerve atrophy, visual impairment, microphthalmia, a CSF *T gondii*–specific antibody production ratio >1, or hearing loss.

evidence for reversible disease or later neurologic adaptation.[130] Several of those infants had CNS abnormalities attributable to *T gondii* infection (including CSF pleocytosis, hypoglycorrhachia, and CSF protein elevation) that resolved during postnatal therapy. Some additional favorable neurologic outcomes reported in this earlier report included the following: (1) resolution of seizures in 50% (4 of 8 children) within the first months of life (however, there were 2 other children who developed new seizures between 3 and 5 years of age), (2) resolution of tone and motor abnormalities by 1 year of age in 60% (12 of 20 children), (3) IQ improvement in 79% (23 of 29 children [normal Mental Developmental Index] at 1 year of age; however, 21% [6 of 29 children] had a Mental Developmental Index <50 but without significant deterioration over time), and (4) normal/near-normal neurodevelopmental outcome in 94% (17 of 18 children) without hydrocephalus and in 75% (6 of 8 children) with obstructive hydrocephalus with shunt. However, because of the small numbers, there was large uncertainty around those estimates for the long-term prognosis of these infants. Moreover, in children with hydrocephalus ex vacuo and elevated CSF protein, the outcome was poor.[130]

A small case series from the United States described resolution of intracranial calcifications in postnatally treated infants with CT.[111] In 75% (30 of 40) of cases, brain lesions resolved or diminished in size by 1 year of age, but in 25% (10 of 40) of cases, lesions remained the same. The diminution or resolution of intracranial calcifications was also associated with improved neurologic functioning.[111] However, an increase in the size and/or number of calcifications has also been reported during months to years after the initial infection, but this finding might reflect healing.[98]

The European Experience

In Europe, the vast majority of pregnant women undergo routine *Toxoplasma* antepartum screening and treatment if needed. Thus, the rates of clinical manifestations and the severity of CT reported over the past 30 years from Europe pertain primarily to children whose mothers received antepartum treatment and who also received postnatal treatment. Data on the effect of postnatal treatment are more limited in European cohorts, because this effect is more difficult to separate from the effect of antepartum treatment.

Clinical Manifestations

The overall risk for "any" clinical manifestations in the European cohorts of the SYROCOT study group report was 19% (105 of 550 CT cases) in infants followed up to 1 year of age. (The SYROCOT study also included non-European cohorts from the United States, Brazil, and Colombia, but these were excluded from the analysis of the effect of prenatal treatment on clinical manifestations.) For comparison, the overall risk for "any" clinical manifestations across the 26 cohorts was 24%, and in the 2 Brazilian and the 1 Colombian cohorts the overall risk was 38% to 77% (3 of 8 CT cases, 17 of 22 CT cases, and 3 of 8 CT cases, respectively).[1] Reported rates in the SYROCOT study might have underestimated the true rates of clinical manifestations because of the relatively short period of follow-up (up to 1 year of age).

In Lyon, France, only approximately 20% of infants with CT were symptomatic at birth (46 of 207 CT cases) during the study period 1996–2008.[3] These results were consistent with earlier reports from European cohorts. Berrebi et al,[131]

in their 20-year prospective study from Toulouse, France (1985–2005), reported that only 27% (29 of 107) of infants with CT were found to be symptomatic during a median follow-up of 8 years (range: 1–20 years).

The reported rates of eye disease and intracranial calcifications during the first year of life in 22 European cohorts of SYROCOT were low (14% and 9%, respectively).[1,124] (The eye disease and neurologic outcomes of CT are discussed separately.) Other severe manifestations, such as hydrocephalus, splenomegaly, and pneumonia, have rarely been reported.[132]

The odds of developing "any" clinical manifestation (chorioretinitis and/or intracranial lesions) in the SYROCOT study decreased by 4% per additional week of gestation at seroconversion (OR: 0.96; 95% CI: 0.93–0.99 per week). The decrease in the incidence of eye disease and intracranial calcifications according to gestational age at seroconversion is shown in Figs 5, 6, and 7.

In the Dunn et al[96] study from Lyon, France, the risk of symptomatic CT decreased with increasing gestational age at maternal seroconversion (at 13 weeks: 61%; 95% CI: 34%–85%; at 26 weeks: 25%; 95% CI: 18%–33%; at 36 weeks: 9%; 95% CI: 4%–17%). If the gestational age at the time of acute primary maternal infection was known, then the risk of delivering a symptomatic infant could be calculated by multiplying the risk of MTCT for that gestational age by the risk of symptomatic disease for that gestational age. For example, for acute primary maternal infections (seroconversion) at 26 weeks of gestation, there would be a 40% risk of MTCT and a 25% risk of symptomatic disease in the infant, which would translate to a 10% risk (0.40 × 0.25) of symptomatic CT in the infant.[96] In this study,[96] the maximum risk of delivering a symptomatic infant was considered to be 10% (8%–14%), which occurred at approximately 24 to 30 weeks of gestation. It is important to note that all of these estimates of MTCT risks and risk of symptomatic CT in the infant were based on a maternal cohort in which approximately 95% of women received antepartum anti-*Toxoplasma* treatment during

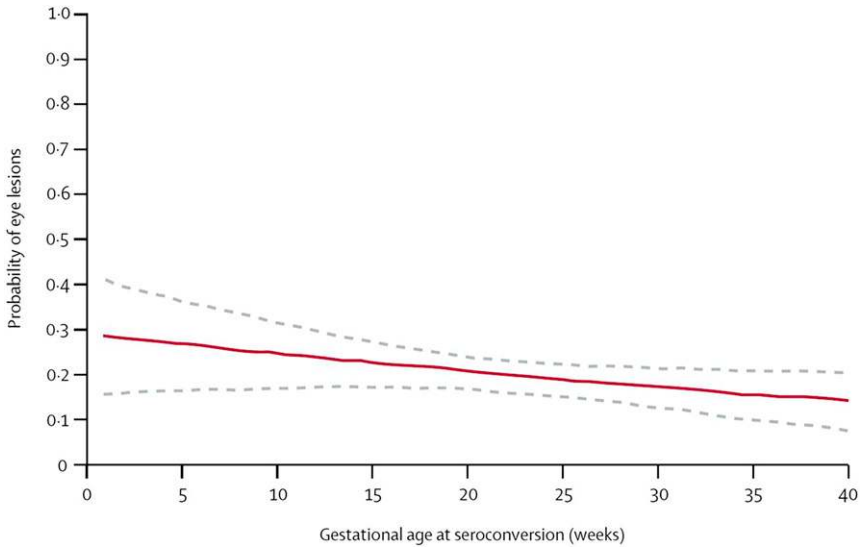


FIGURE 5
Risk of eye disease in infants with CT according to gestational age at maternal seroconversion. Data shown are from 526 infants with CT. The vast majority of these infants' mothers had received antepartum anti-*Toxoplasma* treatment. (Reprinted with permission from Thiebaut R, Leproust S, Chene G, Gilbert R; SYROCOT Group. Effectiveness of prenatal treatment of congenital toxoplasmosis: a meta-analysis of individual patients' data. *Lancet*. 2007;369:120.)

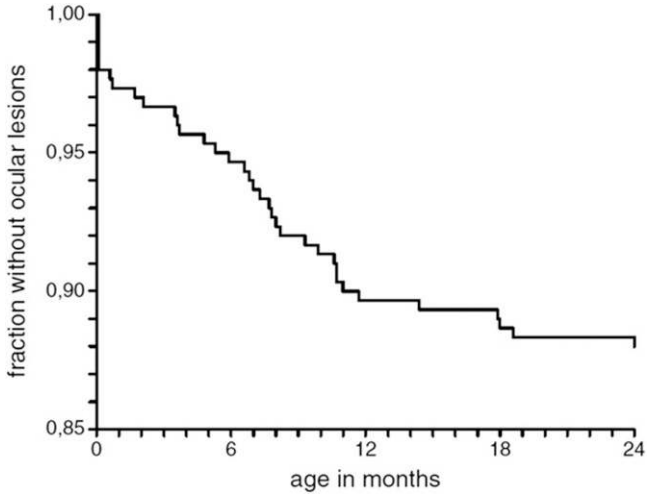


FIGURE 6
Age at first diagnosis of chorioretinitis during the first 2 years of life among 300 children with CT (Paris, Lyon, Marseille). The cumulative incidence of eye disease at 2 years of age was 12% [36 of 300 children]). The vast majority of these women were treated during pregnancy, and the vast majority of the infected children were also treated. (Reprinted with permission from Kieffer F, Wallon M, Garcia P, et al. Risk factors for retinochoroiditis during the first 2 years of life in infants with treated congenital toxoplasmosis. *Pediatr Infect Dis J*. 2008;27:28.)

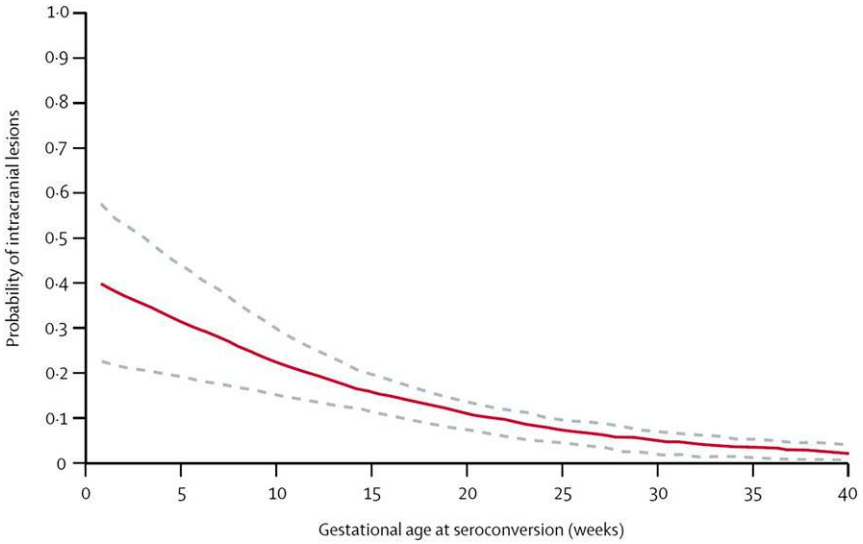


FIGURE 7

Risk of intracranial lesions in infants with CT according to gestational age at maternal seroconversion. Data shown are from 473 infants with CT. For the vast majority of these infants, their mothers had received antepartum anti-*Toxoplasma* treatment. (Reprinted with permission from Thiebaut R, Leproust S, Chene G, Gilbert R; SYROCOT Study Group. Effectiveness of prenatal treatment of congenital toxoplasmosis: a meta-analysis of individual patients' data. *Lancet*. 2007;369:120.)

pregnancy, so these numbers would presumably underestimate the true risks in the United States.

A recent analysis of earlier data from France focusing on infants whose mothers were infected in the first trimester revealed favorable outcomes for infants whose mothers had routine antepartum screening and treatment.[133] Among 36 such infants with normal fetal ultrasonography and with antepartum and postnatal treatment, 97% were asymptomatic or mildly symptomatic (without major vision loss) at up to 4 years of age (range of follow-up: 1–12 years).[133]

Eye Disease

Risk of eye disease and of visual impairment

For European cohorts participating in the SYROCOT international consortium, the risk of eye disease diagnosed in the first year of life was 14% (79 of 550 CT cases).[1] For comparison, across the 26 cohorts, the risk was 18% (125 of 524), and for the South American cohorts, the risk was 47% (18 of 38 CT cases).[1]

Data from the EMSCOT[134] revealed that 12% of children with CT (33 of 281) had their eye disease diagnosed during infancy, and by 3 years of age 17% (49 of 281) of children with CT had at least 1 chorioretinal lesion. Two-thirds of children with eye disease had a lesion at the posterior pole, and almost 50% of children with posterior pole lesions had visual impairment in the affected eye; this condition occurred in only 17% of those with peripheral lesions.

Visual impairment in the most affected eye has been reported to occur in 29% (20 of 69) of children with CT who have chorioretinitis,[38] but severe bilateral impairment was rare and occurred in only 9% of children with CT.[134,135] However, according to the EMSCOT, more than 90% of children with chorioretinitis had normal vision in their best eye.[134]

Data from the long-term follow-up cohort study from Toulouse[131] indicated that chorioretinitis occurred in 26% of CT cases (28 of 107) when follow-up was extended to 20 years (median follow-up: 8 years; range: 1–20 years). The location of the lesions was unilateral peripheral in 71%, bilateral peripheral in 7%, and unilateral macular in 21%. Results were similar in an earlier report by the same team.[133]

Another recent cumulative report from the Marseille cohort (1995–2010), with a median follow-up of 4 years (maximum follow-up: 12 years), revealed a cumulative prevalence of eye disease of 19% (24 of 127 CT cases); visual impairment occurred in only 6% (8 of 127) of CT cases.[136]

Age at diagnosis of the first eye lesion

A French CT cohort from Lyon and Marseille[98] with 335 CT cases (1996–2002) revealed that chorioretinitis was diagnosed at birth in approximately 2% of CT cases, at 6 months in approximately 5% of CT cases, at 12 months in approximately 10% of CT cases, and at 24 months in approximately 12% of CT cases (Fig 6). A delay of more than 8 weeks between maternal seroconversion and initiation of antepartum treatment and the presence of intracranial calcifications was significantly associated with an increased risk of a chorioretinitis before 2 years of age.[99] Freeman et al,[137] in the EMSCOT, found that nearly 50% of children with only eye disease (22 of 50) had their first eye lesion detected before 4 months of age, and nearly 80% of children with additional clinical manifestations of CT had their eye disease diagnosed before 4 months of age.

Long-term risk of eye disease

The risk of eye disease increases from 10% early in infancy, to 16% by 4 years of age,[135,137,138] to 20%

by school age,[137] and to 30% by 12 years of age.[98,135] In a large French cohort study by Berrebi et al[131] with up to 20 years of follow-up, 39% of children with chorioretinitis (11 of 28 chorioretinitis cases among 107 CT cases) had their eye disease diagnosed at birth, 85% (24 of 28) had eye disease diagnosed before 5 years of age, and 96% (27 of 28) had eye disease diagnosed before 10 years of age.[131]

Risk of recurrence in the Lyon cohort

Wallon et al[139] described the long-term follow-up of 477 infants with CT from the Lyon cohort (1987–2008), with 81.5% of infants having mothers who received antepartum treatment. Almost all infants (99.8%) received postnatal treatment for 12 months.

Over a period of 12 years, approximately 30% of treated children experienced at least 1 recurrence of eye disease or developed a new chorioretinal lesion.[139] This proportion increased to almost 50% when the follow-up was extended to 18 years of age. The eye lesions were first seen at a median age of 3 years (range: 0.0–20.7 years). Lesions were mainly unilateral in 69% of cases and did not cause visual loss in 81% of cases. This promising long-term prognosis for ocular disease in children with CT might not apply to children with CT in the United States, where different *T gondii* strains are implicated and there is no routine antepartum screening/treatment.

The probability of recurrent chorioretinitis by 4 years of age increased significantly when the first episode of chorioretinitis was diagnosed before 4 months of age (probability: 30%; 95% CI: 0%–66%) and when additional clinical manifestations also were present before 4 months of age (probability: 70%; 95% CI: 31%–98%).[137]

Although chorioretinitis could occur at any time in the child's life, children who did not have chorioretinitis before 4 months of age were at low risk of developing eye lesions by 4 years of age.[137] In children with CT without chorioretinitis before 4 months of age, the probability of developing chorioretinitis by age 4 years was low (8%) whether or not additional clinical manifestations were present during early infancy. Most children with CT (92% [230 of 249]) in the EMSCOT cohort did not have eye disease diagnosed before 4 months of age.[137]

Predictors of chorioretinitis

The risk of chorioretinitis decreased by 3% for any additional week of gestation when maternal primary infection occurred (OR: 0.97; 95% CI: 0.93–1.00 per week; $P = .04$).[1] One study estimated that the risk of chorioretinitis was 2.1 times higher if the maternal primary infection occurred before compared with after 20 weeks of gestation.[137] Delay in maternal treatment of more than 8 weeks after maternal seroconversion and the presence of intracranial calcifications at birth were independent predictors of chorioretinitis (HRs [95% CIs]: 2.54 [1.14–5.65] and 4.3 [1.9–10], respectively).[99] The risk of chorioretinitis was 3.6 times higher if the infant with CT had additional clinical manifestations of CT at birth (ie, head ultrasonographic abnormalities, serious neurologic findings, lymphadenopathy, or hepatosplenomegaly).[137]

Long-term consequences of eye disease

Long-term follow-up by survey questionnaire of 126 adults with CT from the Lyon cohort (follow-up range: 18–31 years) revealed that treated CT had little effect on quality of life and visual function.[140]

Effect of postnatal therapy on eye disease (the European experience)

There are limited data from European cohorts on the incremental benefit of postnatal treatment on the risk of eye disease. The EMSCOT study[137] did not find an increased risk of eye disease with delayed initiation of infant treatment. However, the study was underpowered to detect such a difference, and moreover, all infants had already received the benefit of maternal treatment during pregnancy. The early 2005 EUROTOXO systematic review of studies evaluating postnatal treatment effect did not identify good-quality published studies to address this question.[12]

Neurologic Disease

The overall risk of intracranial lesions diagnosed during the first year of life for the European cohorts in the SYROCOT study was only 9% (49 of 550).[1] For comparison, across the 26 cohorts in the SYROCOT study, the risk of intracranial lesions diagnosed during the first year of life was 13% (88 of 691), and for the Brazil/Colombian cohorts the risk was 53% (20 of 38). For the European SYROCOT cohort, the risk of intracranial lesions decreased by 9% for any additional week of gestation at maternal primary infection (OR: 0.91; 95% CI: 0.87–0.95[1]; Fig 7).

Possible Reasons for Differences in the Clinical Spectrum of CT Between the United States and South American and European Countries

First, the most important difference is likely the fact that there were no routine antepartum screening/treatment programs in the United States and South America, so infants did not receive the benefit of

antepartum therapy. Initiation of postnatal therapy could also be delayed for weeks to months. The use of routine antepartum screening/treatment programs best explains the decrease in clinical severity in European cohorts over time. Second, in Western Europe, the predominant strain was type 2,[28] which seems to be the least virulent,[128,141,142] whereas in North America, all of the 3 main types (1, 2, and 3) were encountered, in addition to the newly identified more virulent type 12 strains.[29,143] In South America, type 1 and type 3 strains were the predominant strains, type 2 strains were rarely found, and atypical, more virulent strains were also detected in South America[28,30,31] and appeared to be responsible for the higher incidence of severe ocular disease in children with CT.[38] Atypical strains have also been implicated in severe CT cases in South America and in Europe.[46,144] Genotyping data from Central America were more limited, with type 1 and type 1–related strains being detected in cases of CT[145]; type 1 and type 3 strains found in chickens,[146] and atypical strains found in wild animals.[145] Third, although there has been evidence of genetic predisposition for symptomatic CT,[147,148] it is not known whether allelic differences between European and certain US populations could also account for host-pathogen interaction differences that could lead to detected differences in disease severity. Fourth, possible differences in gestational age at the time of acute primary maternal infections between the United States and Europe could contribute to differences in disease severity. Although the time of acute primary maternal infections in the United States is not known for the majority of CT cases,[124] recent data from France showed that there were differences in the trimester during which acute primary infections were diagnosed (42% [684 of 1624] diagnosed during the first trimester, 31% [497 of 1624] diagnosed during the second trimester, and 27% [443 of 1624] diagnosed during the third trimester).[3]

Differences in Eye Disease Between Europe and South America

A recent study[38,149] of 281 CT cases from the EMSCOT and 30 cases from Brazil showed that *T gondii* causes more severe ocular disease in Brazil. Children in Brazil (1) developed chorioretinitis more frequently than children in Europe by their first year of life (50% [15 of 30 CT cases] versus 10% [29 of 281 CT cases]), (2) had a fivefold higher risk of chorioretinitis than in Europe by 4 years of age, (3) more frequently had multiple eye lesions, and (4) more frequently had larger lesions and lesions that were more likely to be located in the posterior pole and cause visual impairment and threaten vision than in Europe (visual impairment occurred in 87% of cases in Brazil versus 29% in Europe).[38]

The observed differences in the frequency of chorioretinitis and in the number and size of the ocular lesions could be attributed to the lack of antepartum treatment and the higher frequency of more virulent strains infecting children in South America when compared with Europe.[38] It is important for US physicians to appreciate that there are geographic differences in the spectrum and severity of clinical manifestations between geographic areas, especially when evaluating children from such international settings.

Sensorineural Hearing Loss

In a systematic review by Brown et al,[150] the prevalence of sensorineural hearing loss (SNHL) associated with CT ranged from 0% to 26%. In subgroup analysis of children who received no postnatal treatment or only limited treatment (less than 1 year), the prevalence of SNHL was 28% (8 of 29 CT cases), whereas in children who were prescribed 12 months of postnatal therapy but in whom treatment was not confirmed to have started before 2.5 months of age, the prevalence of SNHL was 12% (2 of 17 CT cases). In children treated for 12 months postnatally and with therapy initiated before 2.5 months of age, the prevalence of SNHL was 0% (0 of 69 CT cases).[150]

The largest study in US children who received treatment of 12 months suggested that SNHL was a rare occurrence in treated children.[112] Nevertheless, it has been suggested that children with CT who received anti-*Toxoplasma* therapy initiated before 2.5 months of age for a total duration of 12 months may need to have follow-up audiometric evaluation at 24 to 30 months of age. In contrast, children who had received no treatment, received partial treatment, or had delayed onset of treatment (eg, after 2.5 months of age) may need to undergo annual audiologic monitoring until they are able to reliably self-report hearing loss.[150] A recent otopathologic evaluation of temporal bones from 3 infants who died of CT identified *T gondii* in the dormant cystic form as well in the tachyzoite form with a surrounding inflammatory response, suggesting that the mechanism of hearing loss seen in CT can be sensory, neural, or sensorineural[151] and is directly mediated by the parasite and host immune response.

DIAGNOSIS

Laboratory tools for the diagnosis of *T gondii* infection include serologic tests, such as the *Toxoplasma* IgG, IgM, IgA, IgE, IgG-avidity, and differential agglutination (AC/HS)

tests; PCR assays; histologic and cytologic examination of tissue and body fluids; and attempts to isolate the parasite with mice subinoculation (this test is only performed in reference laboratories). Commercial laboratories in the United States usually offer *Toxoplasma* IgG and IgM testing; some of them also offer *Toxoplasma* IgA and IgG-avidity tests (FDA approved since 2011) and PCR assays. In reference laboratories for toxoplasmosis (eg, http://www.pamf.org/serology/), panels of serologic tests are offered that include different combinations of all of the aforementioned serologic tests (according to the clinical indication), and in addition, they include the IgG dye test (the gold standard for the detection of IgG, a sensitive and specific neutralization test in which live tachyzoites are lysed in the presence of the patient's IgG *T gondii*–specific antibodies and complement), the AC/HS differential agglutination test (which uses 2 antigen preparations that express antigenic determinants found early after acute infection [AC] or in the later stages of infection [HS]; AC/HS ratios are interpreted as acute, equivocal, nonacute patterns of reactivity or nonreactivity), and well-standardized *T gondii* PCR assays. In consultation with a medical expert, the toxoplasmosis reference laboratory in the United States offers appropriate interpretations of serologic and PCR test results for each case.

Case Definitions of *T gondii* Infection Status

Classification systems for *T gondii* infection in pregnant women used in Europe[152] differ from those used in the United States. In Europe, classification is based on routine antepartum screening (every month [eg, France and Italy] or every 2–3 months [eg, Austria, Lithuania, Slovenia, and Germany[10]]) throughout pregnancy. Frequent screening allows for the identification of previously seronegative pregnant women who seroconvert during pregnancy.[153] In contrast, in the United States, the interpretation of maternal infection status has typically been based on testing at a single time point (performed usually because of fetal ultrasonographic abnormalities or maternal signs or symptoms suggestive of toxoplasmosis). The classification system for *T gondii* infections that has been used by the PAMF-TSL for maternal, fetal, and infant infectious status is depicted in Table 4.

Diagnosis of *T gondii* Infection During Pregnancy

When there is clinical suspicion of acute toxoplasmosis during pregnancy (eg, in the setting of certain ultrasonographic abnormalities or a high clinical suspicion of acute infection on the basis of symptoms), samples should be sent to a reference laboratory for toxoplasmosis to avoid any unnecessary delays in the establishment of diagnosis and initiation of prenatal treatment. Prompt initiation of prenatal treatment as soon as possible after acute maternal infection has been recently shown in observational studies to decrease MTCT and ameliorate the severity of clinical manifestations.

There is variation in the diagnostic performance of serologic screening tests for toxoplasmosis in commercial versus reference laboratories. Calderaro et al[162] evaluated the diagnostic performance of 4 commercially available tests for toxoplasmosis. For all 4 commercial IgG tests, the sensitivity (point estimates) was 100% and the specificity (point estimates) of these different IgG tests ranged between 97% and 100%. The sensitivity (point estimates) of the different commercial IgM tests ranged between 82.3% and 100%, and the specificity (point estimates) of these different IgM tests ranged between 99.7% and 100%. There were commercially available tests that had 100% sensitivity and 100% specificity for both of their *Toxoplasma* IgG and IgM tests (eg, the Vidas Biomerieux). In this validation study, the "status" of samples as positive or negative was defined on the basis of concordant results obtained by at least 3 of the 4 assays compared in this study. Moreover, according to Wilson et al,[163] who performed a study sponsored by the FDA for the validation of 6 commercial IgM tests, the sensitivity (point estimates) of these 6 different IgM tests ranged between 93.3% and 100% and the specificity (point estimates) ranged between 77.5% and 98.6%. In this validation study, the reference gold standard for the *Toxoplasma* IgM was the double-sandwich enzyme-linked immunosorbent assay (ELISA) of the National Reference Laboratory for Toxoplasmosis (the PAMF-TSL). In an area of low seroprevalence, tests with a high specificity (but <100%) could lead to a large number of false-positive results. In reference laboratories, the specificity of the confirmatory testing (for the diagnosis of a recently acquired acute infection) is 100%.[163]

Interpretation of Maternal Serologic Results at the PAMF-TSL

Women With Negative Toxoplasma *IgG and IgM Test Results Obtained at Commercial, Hospital-Based, Clinic-Based, or Nonreference Laboratories*

These results generally exclude maternal *T gondii* infection. It is useful to order *Toxoplasma* IgM tests concomitant with IgG tests. It is possible to have a negative *Toxoplasma* IgG test result and a positive

TABLE 4 Case Definitions of Maternal, Fetal, and Infant *T gondii* Infection, According to the PAMF-TSL

Patient Group and Infection Status	Case Definition
Maternal infection status	
Not infected	Seronegative mother (negative *Toxoplasma* IgG and negative *Toxoplasma* IgM[a]) (provided that the mother is capable of producing immunoglobulins)
Chronically infected	Serologic test results suggestive of a maternal *T gondii* infection acquired in the distant past, before pregnancy (>12 mo before testing).[b]
Recently infected (definite/probable acute primary *T gondii* infection acquired during pregnancy or close to conception[c])	Serologic evidence of a recently acquired *T gondii* infection, likely to have occurred during pregnancy or very close to conception[c,d]
Unclear infection status (cannot rule out an acute primary *T gondii* infection acquired during pregnancy or close to conception[c])	Mother was *T gondii* infected, but the estimation of the exact time of infection (whether it occurred during pregnancy or before pregnancy) was not possible and complete exclusion of a primary infection acquired during gestation or close to conception also was not possible. For mothers tested very late in pregnancy or at delivery, the characterization of the infection status can occasionally be unclear (unless the maternal serologic test results were either clearly suggestive of an "acute recent infection" or clearly suggestive of a "chronic infection," acquired >12 months before the time of testing). In such cases, and without having any additional serologic results from early pregnancy, acute maternal primary infections early in pregnancy or close to conception[c] cannot be excluded. (Maternal serologic profiles at the time of testing might have been significantly changed from the early serologic responses, which could have been indicative of an acute primary infection.) Moreover, in cases in which the serologic test results do not exclude a maternal infection acquired within 6 to 12 mo before the date of testing (eg, if the woman was tested for the first time during the late second or third trimester of pregnancy), an accurate estimate of whether the patient acquired the infection before or during pregnancy might not be possible. In those cases, testing of a saved serum sample that might have been obtained during the first 16 weeks of pregnancy would be helpful to determine the timing of infection.
Additional rare scenarios	
Reactivation of *T gondii* infection in a chronically infected pregnant woman because of an immunocompromising condition[154,c,e]	Laboratory confirmation of local or systemic reactivation is difficult with testing at a single time point.
Reinfection with a new, more virulent strain in a previously chronically infected woman[151,155]	The currently available serologic tests cannot differentiate between infections from typical versus atypical or more virulent *T gondii* strains.
Fetal infection status	
Not infected	Seronegative mother (negative maternal *Toxoplasma* IgG and *Toxoplasma* IgM [provided that the mother was able to produce immunoglobulins])
Unlikely CT	Mother with evidence of chronic *T gondii* infection acquired in the distant past and before pregnancy (in the absence of any immunocompromising condition or immunosuppressive medications[b]) or AF PCR negative (with acute maternal infections estimated to have occurred in the first or second trimester) and normal fetal ultrasonograph. However, if the acute maternal infection is estimated to have occurred in the third trimester, even a negative AF PCR and a normal fetal ultrasonograph cannot exclude CT. For infections acquired in the third trimester, the NPV of AF PCR has been shown to be lower than the NPV for infections acquired earlier in gestation.
Infected; definite/probable CT	AF PCR positive and/or fetal ultrasonographic findings highly suggestive of CT (ventriculomegaly/ hydrocephalus, intracranial calcifications, intrauterine growth retardation, etc) and serologic evidence of acute maternal *T gondii* infection acquired during gestation or near the time of conception.[c] Infant's infection status>
Not infected	Negative *Toxoplasma* IgG dye test and negative *Toxoplasma* IgM ISAGA and negative *Toxoplasma* IgA ELISA (provided that the infant is able to produce immunoglobulins) and mother also negative for *Toxoplasma* IgG dye test antibodies and *Toxoplasma* IgM and IgA ELISA (provided that the mother is capable of producing immunoglobulins).

TABLE 4 Continued

Patient Group and Infection Status	Case Definition
CT could not be completely ruled out (in infant without postnatal treatment)	Positive *Toxoplasma* IgG dye test (with titers equal or lower to the maternal titers) and negative *Toxoplasma* IgM ISAGA and negative *Toxoplasma* IgA ELISA and no evidence of CT after complete clinical, radiologic, and laboratory evaluation. Even with the above scenario, if maternal serology was suggestive of a recently acquired primary infection during gestation and suspicion for CT was high, the initially negative *Toxoplasma* IgM ISAGA and IgA ELISA results at birth may not completely exclude CT. Repeat testing 2 to 4 weeks after birth and every 4 weeks thereafter until 3 months of age could be considered in such cases to rule out the delayed appearance of IgM or IgA antibodies. In these infants, monthly IgG screening should be continued (until complete disappearance of IgG antibodies) according to the PAMF-TSL. Of note: disappearance of *Toxoplasma* IgG antibodies in an infant with suspected CT, in the presence of anti-*Toxoplasma* treatment, does not rule out CT. In postnatally treated infants, repeat serologic testing 1–3 months after neonatal seroconversion from positive *Toxoplasma* IgG to negative *Toxoplasma* IgG and after treatment was discontinued, might be needed to rule out CT.[f]
CT ruled out (in infant without postnatal treatment)	Infants with initially positive *Toxoplasma* IgG dye test (with titers equal or lower to the maternal titers) and negative *Toxoplasma* IgM ISAGA and *Toxoplasma* IgA ELISA and no evidence of CT after complete clinical, radiologic, and laboratory evaluation and in whom monthly serial serologic follow-up (approximately every 4-6 wk after birth; with serial *Toxoplasma* IgG dye tests tested in parallel with most recent previous test) documented a decrease in the *Toxoplasma* IgG dye test antibodies by ≥50% every 30 days and eventual disappearance of *Toxoplasma* IgG antibodies (confirming that previous positive *Toxoplasma* IgG dye test antibodies were transplacentally transferred maternal antibodies).
Definite CT; asymptomatic at birth	Positive *Toxoplasma* IgG dye test and positive *Toxoplasma* IgM ISAGA (after 5 d of life) or positive *Toxoplasma* IgA ELISA (after 10 d of life) but without any clinical findings of CT after complete clinical, radiologic, and laboratory evaluation (as described in Table 9). (The role of *T gondii* DNA PCR in CSF, blood, and urine in the diagnosis is discussed separately in Table 9.)
Definite CT; symptomatic at birth	Positive *Toxoplasma* IgG dye test and positive *Toxoplasma* IgM ISAGA or *Toxoplasma* IgA ELISA and clinical findings consistent with CT, after a complete clinical, radiologic, and laboratory evaluation (as described in Table 9). (The role of *T gondii* DNA PCR in CSF, blood, and urine in the diagnosis is discussed separately in Table 9.)

a According to the PAMF-TSL, all pregnant women should be screened for both *Toxoplasma* IgG and IgM. This approach will help capture cases of early seroconversion in which the *Toxoplasma* IgG might be negative (*Toxoplasma* IgG did not have the time to increase at the time of testing) and only the *Toxoplasma* IgM might be positive. In those cases, serial maternal serologic testing within 2–4 weeks from the first testing could help differentiate whether the positive *Toxoplasma* IgM in the absence of a positive *Toxoplasma* IgG was attributable to a very early infection (IgG would be expected to increase in the follow-up sample) versus a false-positive *Toxoplasma* IgM.

b Unless the woman is immunocompromised or taking immunosuppressive medications, the risk of reactivation of a chronic infection during pregnancy is close to zero.

c Within 2–3 months from conception.[156]

d Serologic testing at the PAMF-TSL with the appropriate panel of tests and interpretation of serologic test results by the medical consultants at PAMF-TSL help in the estimation of the most likely time that the acute primary maternal infection had occurred. Serologic evidence of acute infection: Criteria for the diagnosis of recently acquired *T gondii* infections, within 6 months from sample collection dates, have been previously published by investigators at PAMF-TSL.[74,157–160] Those criteria (see following) have been used mainly for research purposes. In routine clinical practice, slightly less strict criteria may be used. (A) *Toxoplasma* IgG dye test titer ≥1:1024 and *Toxoplasma* IgM ELISA ≥5.0 and acute pattern in the AC/HS test; (B) *Toxoplasma* IgG dye test titer ≥1:1024 and *Toxoplasma* IgM ELISA ≥3.0 and acute pattern in the AC/HS test and either *Toxoplasma* IgA ELISA ≥5.0 or a low *Toxoplasma* IgG avidity (<10); (C) *Toxoplasma* IgG dye test titer ≤1:512 and *Toxoplasma* IgM ELISA ≥5.0 and acute pattern in the AC/HS test and either *Toxoplasma* IgA ELISA ≥5.0 or low *Toxoplasma* IgG avidity (<10).

e Recent data from a survey in The Netherlands showed that, contrary to previous beliefs, the incidence rates of recurrent eye disease in pregnant women were not higher than in nonpregnant women (incidence rate ratio: 0.54; 95% CI: 0.22–1.29).[161]

f Postnatal treatment can affect the kinetics of *Toxoplasma* IgG antibodies. There are reports of infants with CT who seroconverted to *Toxoplasma* IgG negative during treatment but eventually had a rebound after the discontinuation of the anti-*Toxoplasma* therapy.[98]

Toxoplasma IgM test result when testing is performed shortly after acute primary infection (eg, fewer than 2 weeks from time of infection).

The pregnancy-specific serologic panels of tests for a gestational age less than 16 weeks versus more than 16 weeks used by PAMF-TSL[164] help estimate the most likely time of maternal infection and thus differentiate chronic infections (acquired in the distant past, before conception) from acute infections. The majority of the tests in those panels are not available in nonreference laboratories, including the *Toxoplasma* IgG dye test, the *Toxoplasma* IgM immunosorbent agglutination assay (ISAGA), the *Toxoplasma* IgE ELISA, and the AC/HS differential agglutination test. Recently, the *Toxoplasma* IgG avidity test was approved by the FDA and currently also is performed by some nonreference laboratories.[165] However, the *Toxoplasma* IgG avidity test alone cannot differentiate between an acute and chronic infection, because there are cases in which low *Toxoplasma* IgG avidity can persist for years after primary infection. In such cases, a comprehensive panel of tests generally is necessary.

For pregnant women at ≤16 weeks of gestation, the PAMF-TSL panel contains the *Toxoplasma* IgG dye test, the *Toxoplasma* IgM ELISA, and the *Toxoplasma* IgG-avidity test. (Referring physicians can obtain the specific laboratory request forms online from the PAMF-TSL Web site at: http://www.pamf.org/serology/SpecimenRequirements.pdf). They can also contact the laboratory directly (telephone: 650-853-4828; fax: 650-614-3292; e-mail: toxolab@pamf.org) regarding the specimens to be tested (volume, shipping temperature, turnaround times, etc) (Tables 5, 6, 7).

For women at a gestational age of >16 weeks, the PAMF-TSL panel includes the *Toxoplasma* IgG dye test, the *Toxoplasma* IgM ELISA, and the AC/HS differential agglutination test (an acute AC/HS indicates an infection acquired <12 months from the time of testing, and a nonacute AC/HS is suggestive of a chronic infection acquired >12 months from the time of testing).

Toxoplasma IgG avidity is included in the routine panel only for women at ≤16 weeks of gestation, because a high avidity can exclude an acute infection acquired during pregnancy or close to conception. However, a high avidity in a woman at >16 weeks of gestation may not exclude an infection acquired early in pregnancy or very close to conception (IgG avidity becomes high 16 weeks after a primary infection).

In certain situations, additional reflex testing sometimes is needed. Reflex testing includes the *Toxoplasma* IgA ELISA, the *Toxoplasma* IgE ELISA, and the *Toxoplasma* IgG-avidity test, even for women at >16 weeks of gestation. A guide for the interpretation of the individual serologic test results performed at PAMF-TSL can be found on the PAMF-TSL Web site (http://www.pamf.org/serology/clinicianguide.html).[164,166]

Women With Negative Toxoplasma *IgG and Positive IgM Results From Tests Performed at Commercial, Hospital-Based, Clinic-Based, or Nonreference Laboratories*

In general, such results are confirmed in a reference laboratory for toxoplasmosis.[166] A positive *Toxoplasma* IgM could indicate either a very recently acquired infection or a false-positive IgM result.[167] Additional tests performed in a reference laboratory[166] and follow-up testing within 2 to 3 weeks will help differentiate between these 2 possibilities. If the *Toxoplasma* IgG test result becomes positive during follow-up, then this finding would be consistent with seroconversion and of a very recently acquired infection during pregnancy. If the *Toxoplasma* IgG test result remains negative, then the *Toxoplasma* IgM result probably was a false-positive result.

TABLE 5 Specimen Requirements (for Specimens Sent to the PAMF-TSL)

Specimen Type	Specimen Age	Container	Shipping Conditions	Recommended Volume, mL
Serum	Up to 1 week when stored refrigerated; indefinite when stored frozen	Serum-separator tube or red-top tube (centrifuge specimen and, if possible, send serum only)	Cold pack or frozen preferred 20°–25°C acceptable; overnight delivery	3
CSF	Up to 1 week when stored refrigerated; indefinite when stored frozen	Sterile tube	Cold pack or frozen preferred 20°–25°C acceptable; overnight delivery	1

(1) Specimen requirement for serologic testing is 3 mL of serum from a serum-separator tube or a red-top tube (minimum of 0.5 mL, but this quantity may be insufficient for repeat testing). The specimen should be centrifuged and, if possible, only serum should be sent. Grossly hemolyzed, icteric, lipemic, and bacterially contaminated specimens cannot be tested. Serum specimens can be sent at ambient temperature. (2) The patient's name and collection date must appear on specimen label. Unlabeled specimens will not be tested. (3) The PAMF-TSL should be contacted at (650) 853-4828 for specimens that do not meet these requirements. For specimens not meeting our requirements, testing can still be performed when authorized by the client. (4) The PAMF-TSL should be contacted at (650) 853-4828 for specimens that are less than the recommended volume. Specimens received with less than recommended volumes may be insufficient for repeat testing. (Also refer to http://www.pamf.org/serology/SpecimenRequirements.pdf for the most recent updates on specimen requirements and testing information.)

TABLE 6 Conditions for Processing, Storing, and Shipping Specimens for PCR Testing (for Specimens Sent to the PAMF-TSL)

Specimen Type	Specimen Age	Shipping Conditions	Volume	
			Minimum	Preferred
AF (collected at >18 weeks' gestation)	Up to 1 month when stored refrigerated; indefinite when stored frozen	2°–8°C on ice or cold packs preferred; frozen acceptable; overnight delivery	3 mL	10 mL
Other body fluids: ascitic, peritoneal, pleural, bronchial lavage	Up to 1 month when stored refrigerated; indefinite when stored frozen	2°–8°C on ice or cold packs; frozen acceptable; overnight delivery	3 mL	10 mL
CSF	Up to 1 month when stored refrigerated; indefinite when stored frozen	Frozen preferred; 2°–8°C on ice or cold packs acceptable; overnight delivery	0.4 mL	1 mL
Ocular fluids (vitreous and aqueous)	Up to 1 month when stored refrigerated; indefinite when stored frozen	Frozen preferred; 2°–8°C on ice or cold packs acceptable; overnight delivery	0.1 mL	0.5 mL
Whole blood, bone marrow	Up to 2 days	EDTA or citrate tube; 20°–25°C; overnight delivery	1 mL	5 mL
Urine	Up to 1 week when stored refrigerated; indefinite when stored frozen	2°–8°C on ice or cold packs preferred; frozen acceptable; overnight delivery	6 mL	10 mL
Solid tissues (a referral to PAMF-TSL consultants is recommended)	Up to 24 hours when stored refrigerated; indefinite when stored frozen; no preservatives	Frozen preferred; 2°–8°C on ice or cold packs acceptable; overnight delivery	25 mg	50 mg

The PAMF-TSL will test specimens that deviate from these conditions. However, sensitivity might be compromised. Contact the laboratory at (650) 853-4828 for any questions about testing specimens that do not conform to these conditions. (Also refer to http://www.pamf.org/serology/SpecimenRequirements.pdf for the most recent updates on specimen requirements and testing information.)

Women With Positive Toxoplasma *IgG and Negative* Toxoplasma *IgM Test Results From Commercial, Hospital-Based, Clinic-Based, or Nonreference Laboratories*

These results obtained during the first half of the pregnancy (<20 weeks' gestation) could exclude, in most patients, an infection acquired during pregnancy, particularly if the *Toxoplasma* IgG titers are "low" (eg, IgG dye test titers ≤512 [value refers to a dilution of 1:512]). However, for results at ≥20 weeks' gestation or when there is high clinical suspicion of CT (ie, abnormal fetal ultrasonographic findings), these results should be confirmed in a reference laboratory for toxoplasmosis[166] with an additional panel of tests according to gestational age.

In addition, more recently, there have been some reports of atypical seroconversion with negative or transient IgM responses, but the clinical significance and how common this phenomenon is remain unclear.[168] (Among 4500 documented maternal seroconversions across 12 centers in France, negative IgM or only transient IgM responses were reported to occur in 0.58% of cases [n = 26 of 4500] and this was considered to represent an atypical seroconversion serologic profile.[168]) Even though these are rare cases, they could be associated with significant clinical implications if not appropriately managed.

Women With Positive Toxoplasma *IgG Test Results and Either Positive or Equivocal IgM Test Results From Commercial, Hospital-Based, Clinic-Based, or Nonreference Laboratories*

In general, such results are confirmed in a reference laboratory before being interpreted as evidence of acute primary infection. Approximately 60% of cases with positive *Toxoplasma* IgM results from nonreference laboratories were not associated with recently acquired infections when tested at the PAMF-TSL with the use of the comprehensive pregnancy panel of tests.[103,154] These positive *Toxoplasma* IgM assays represented either false-positive IgM assays (20%) or positive IgM assays in the context of chronic infections (40%).[167,169] The persistence of IgM antibodies in low titers beyond 1 year is not uncommon.[154] In those cases, reflex testing with additional tests at a toxoplasmosis reference laboratory will help confirm or exclude that possibility.

Prenatal Diagnosis of Congenital *T gondii* Infection in the Fetus

Ways in Which CT Can Occur

CT can occur in 3 ways: (1) transmission of *T gondii* infection to the fetus from a previously seronegative, immunocompetent mother who acquired acute primary infection during pregnancy or shortly before conception (even within 3 months before conception)[156]; (2) reactivation of a *Toxoplasma* infection in previously *T gondii*–immune women who have been immunocompromised during gestation (eg, women with altered immune function attributable to HIV infection or immunosuppressive medications); and (3) reinfection of a previously immune mother with a new, more virulent strain[144] (eg, after international travel or after eating undercooked meat from areas where more virulent atypical strains predominate[29,33,141,142,144]).

Amplification of Toxoplasma *DNA by PCR Assay*

AF PCR assay has many advantages as a method of diagnosis of fetal

TABLE 7 Instructions for Specimens Sent for PCR Testing (for Specimens Sent to the PAMF-TSL)

Shipment	Send specimens for PCR testing to this address: *Toxoplasma* Serology Laboratory Attention: PCR PAMF Research Institute Ames Building, 795 El Camino Real Palo Alto, CA 94301 Ship samples for PCR testing separately from other samples. All samples received for PCR testing will be prepared for testing and will not be suitable for return. A serum specimen for serologic testing must accompany PCR test requests for any patient not recently tested at PAMF-TSL. The minimum request for serologic testing must include *Toxoplasma* IgG and IgM. There is an additional charge for testing this serum.
Amniotic fluid	It is recommended that amniocentesis for toxoplasmosis PCR be performed at a minimum of 18 weeks' gestation. A serum sample from the mother must accompany the AF unless she has been previously tested at PAMF-TSL.
Specimen	DNA for the PCR procedure is obtained from pelleted AF.
Volume	10 mL
Shipment	Ship AF on wet ice or cold packs sufficient to maintain a temperature of 2°–8°C during shipment; frozen acceptable. Include enough dry ice to keep specimen frozen during shipment.
Cerebrospinal, vitreous, or aqueous fluid	
Specimen	A serum sample from the patient must accompany the fluid unless serum has been recently tested in at PAMF-TSL.
Volume	1 mL
Shipment	Freeze sample immediately after collection. Ship sample on dry ice by overnight courier. Include enough dry ice to keep specimen frozen during shipment.

Refer to http://www.pamf.org/serology/SpecimenRequirements.pdf for the most recent updates on specimen requirements and testing information.

infection. The evidence for the diagnostic performance of AF PCR assay is summarized in Table 8, according to the results of the most recent studies published over the past 10 years.[114,170,171] The clinical value of AF PCR assay results in estimating a woman's risk of delivering an infant with CT varied according to gestational age at maternal seroconversion. Figure 8 shows how the probability of fetal infection (posttest probability) changed (as compared with the pretest probability) after a positive or a negative AF PCR assay result according to the gestational age at which the maternal primary infection most likely had occurred.

Special Considerations for the AF PCR (According to the PAMF-TSL)

A negative AF PCR assay result for maternal primary infections thought to be acquired in the first or second trimester may be reassuring with regard to excluding fetal infection. The negative predictive value (NPV) of AF PCR assay for such early maternal primary infections was very high (Table 8). On the contrary, for infections thought to be acquired in the third trimester, the NPV was lower, and a negative AF PCR assay result in such cases should be interpreted cautiously and would not exclude fetal infection (Fig 8).[171,172] (The exact reason for this situation is not known. Some possible explanations could be a dilution effect attributable to a larger amount of AF in the third trimester and/or maternal treatment before AF PCR testing [eg, in cases in which P/S was initiated immediately after the diagnosis and before the AF was tested].)[114,170,171] Moreover, it probably takes several weeks for the infection to cross the placenta from the mother to the fetus, in large enough quantities to spill into the AF. *T gondii* likely first infects the placenta, replicates, then crosses to the fetus.

The positive predictive value (PPV) of an AF PCR assay result for maternal infections acquired at any time during pregnancy was very high (Table 8), and a positive AF PCR assay result would indicate CT and could be used to recommend treatment for fetal infection (assuming there is no laboratory contamination). (Estimates of the diagnostic performance of AF PCR assay always refer to the gestational age at which maternal infection was acquired and not to the time the amniocentesis was performed.)

In general, AF PCR assay should be performed at least 4 weeks after acute primary maternal infection and at ≥18 weeks of gestation. The majority of the available data on the diagnostic performance of an AF PCR assay came from women who had amniocentesis performed at ≥18 weeks of gestation, and there were no data regarding the interpretation of negative results of AF PCR assay performed at <18 weeks of gestation (false-negative results cannot be excluded, for example, because of the small amounts of AF that can be obtained at this gestational age).

Data from France revealed that only 28% of women infected at >24 weeks of gestation underwent amniocentesis,[114] either because it was not offered to them (because of concern for preterm birth) or because of patient refusal. In such cases, it is possible that physicians elected to treat those women empirically with P/S because of the high risk of MTCT with *T gondii* infections during the third trimester of pregnancy.

TABLE 8 Diagnostic Performance of AF PCR According to the Time of Acute Maternal Primary Infection

First Author, Year	CT Cases/ AF Tested (Prevalence)	Sensitivity (Positive Test Results/CT Cases) (95% CI)	Specificity (Negative Test Results/Non-CT Cases) (95% CI)	PPV[a] (CT Cases/Positive Test Results) (95% CI)	NPV[b] (No CT Cases/Negative Test Results) (95% CI)
First-trimester seroconversion					
Sterkers, 2012[172]	11/154 (7%)	91% (10/11) (59%–100%)	100% (143/143) (97%–100%)	100% (10/10) (69%–100%)	99% (143/144) (96%–100%)
Wallon, 2010[114]	4/127 (3%)	75% (3/4) (19%–99%)	100% (NR) (97%–100%)	100% (NR) (29%–100%)	99% (NR) (96%–100%)
Rabilloud, 2010[170,c]	7/234 (3%)	57% (4/7) (14%–86%)	100% (226/227) (99%–100%)	80% (4/5) (NR)	99% (226/229) (NR)
Thalib, 2005[171]	9/357 (3%)	33% (3/9) (7%–70%)	99% (347/348) (98%–100%)	75% (3/4) (22%–98%)	98% (347/353) (96%–99%)
Second-trimester seroconversion					
Sterkers, 2012	27/114 (24%)	78% (21/27) (62%–94%)	100% (87/87) (96%–100%)	100% (21/21) (84%–100%)	94% (87/93) (89%–99%)
Wallon, 2010	30/108 (28%)	97% (29/30) (83%–100%)	100% (NR) (95%–100%)	100% (NR) (88%–100%)	99% (NR) (93%–100%)
Rabilloud, 2010[c]	39/182 (21%)	67% (26/39) (51%–82%)	99% (141/143) (97%–100%)	93% (26/28) (NR)	92% (141/154) (NR)
Thalib, 2005	46/200 (23%)	80% (37/46) (47%–85%)	97% (149/154) (92%–99%)	88% (37/42) (74%–96%)	94% (149/158) (89%–97%)
Third-trimester seroconversion					
Sterkers, 2012	12/23 (52%)	100% (12/12) (74%–100%)	100% (11/11) (72%–100%)	100% (12/12) (74%–100%)	100% (11/11) (72%–100%)
Wallon, 2010	17/26 (65%)	88% (15/17) (67%–99%)	100% (NR) (66.4%–100%)	100% (NR) (78.2%–100%)	82% (NR) (48%–98%)
Rabilloud, 2010[c]	34/65 (52%)	74% (25/34) (59%–88%)	100% (31/31) (89%–100%)	100% (25/25) (NR)	78% (31/40) (NR)
Thalib, 2005	25/36 (69%)	68% (17/25) (66%–81%)	91% (10/11) (57%–99%)	94% (17/18) (71%–99%)	56% (10/18) (31%–78%)
Overall diagnostic performance					
Sterkers, 2012[d]	51/295 (17%)	86% (43/50) (77%–96%)	100% (241/241) (99%–100%)	100% (43/43) (92%–100%)	97% (241/248) (95%–99%)
Wallon, 2010[e]	51/261 (20%)	92% (47/51) (81%–98%)	100% (NR) (98%–100%)	100% (NR) (93%–100%)	98% (NR) (95%–99.5%)
Rabilloud, 2010[c]	80/481 (17%)	69% (55/80) (59%–79%)	99% (398/401) (98%–100%)	95% (55/58) (NR)	94% (398/423) (NR)
Thalib, 2005	80/593 (13%)	71% (57/80) (61%–81%)	98% (506/513) (97%–99%)	89% (57/64) (78%–95%)	96% (506/529) (93%–97%)

Note that, in these studies, the majority of the women had continued treatment throughout pregnancy once the diagnosis of acute *T gondii* infection was made. Differences in the percentages of women on treatment and the duration of treatment before amniocentesis could explain differences in the reported sensitivity of the AF PCR across these studies. NR, not reported.

a NPV is the probability that the fetus would not be infected if the AF PCR result is negative.

b PPV is the probability that the fetus would be infected if the AF PCR result is positive.

c For the Rabilloud et al study, the PPV and NPV values listed represent the PPV and NPV at 6, 18, and 30 wk of gestation, respectively.

d In the Sterkers et al study, 7 of 50 infants with CT had a negative AF PCR, giving an overall sensitivity for the AF PCR of 86% (all the 7 cases that were not detected in utero were subclinical cases).

e In the Wallon et al study, 4 of 51 infants with CT had a negative AF PCR (all 4 infants were asymptomatic up to 1 y of age, likely suggesting a milder infection with a lower parasite load). Factors contributing to the increased sensitivity of the AF PCR method in this study included (1) larger volumes of AF (10 mL), (2) use of a real-time PCR method, (3) 529 gene targets, and (4) fluorescent probes.

All infants born to mothers with suspected/confirmed *T gondii* infection during pregnancy, even if they had negative AF PCR assay results, will need a complete clinical, radiologic, and laboratory diagnostic evaluation to rule out CT as well as serial serologic monitoring every 4 to 6 weeks during their first year of life to document disappearance of *Toxoplasma* IgG antibodies.

Quantitative AF PCR Assay

Quantitative AF PCR assay still is considered experimental and is not used routinely in commercial or even reference laboratories for the diagnosis of CT. In a retrospective study conducted in 88 consecutive AF samples that tested positive for *T gondii* by real-time quantitative PCR assay,[173] after adjusting for gestational age at maternal seroconversion, higher AF parasite concentrations were associated with more severely symptomatic CT cases (OR: 15.38 per parasite log [parasites per mL AF]; 95% CI: 2.45–97.7).[173] Pregnant women infected before 20 weeks of gestation with a parasite load ≥100/mL determined by AF PCR assay had the highest risk of giving birth to infants with severe CT.[173]

Fetal Ultrasonography

In addition to maternal serologic testing and AF PCR assay, fetal ultrasonography also may help determine whether the fetus has been affected, although a normal fetal ultrasonogram cannot exclude a fetal infection. Fetal ultrasonographic findings reported to be associated with CT include ventriculomegaly, intracranial calcifications, increased placental thickness, intrahepatic calcifications, ascites, fetal growth restriction, and echogenic bowel. Of note, microcephaly has not been reported in fetal ultrasonographic findings of CT, possibly because of the associated ventriculomegaly.[174] Crino[174] reported a pooled sensitivity of 49% and a pooled specificity of 99% of any fetal ultrasonographic findings for CT on

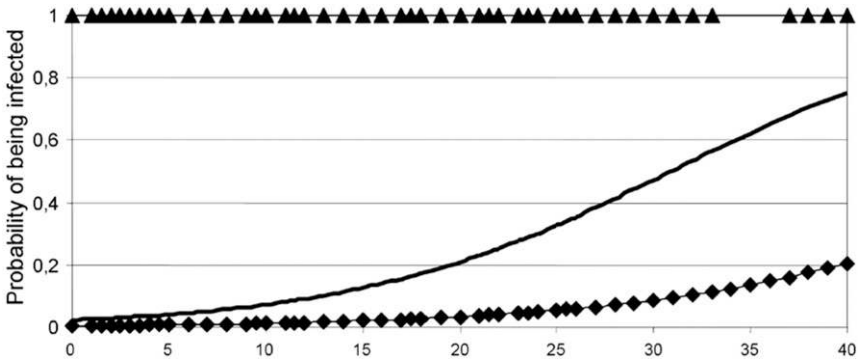


FIGURE 8

The probability of giving birth to an infant with CT according to gestational age at maternal infection and according to AF PCR assay results. The bold curved line shows the prevalence of CT (pretest risk) according to gestational age at the time of maternal infection. The solid triangles show the posttest probability of CT given a positive AF PCR result (PPV). The solid diamonds show the posttest probability of CT given a negative AF PCR result (NPV). For example, for a maternal infection at 25 weeks' gestational age, a negative AF PCR result allowed estimation of a posttest probability of CT of 5% instead of a pretest risk estimate of 33%. (The vast majority of these women were treated during pregnancy.) (Modified with permission from Sterkers Y, Pratlong F, Albaba S, et al. Novel interpretation of molecular diagnosis of congenital toxoplasmosis according to gestational age at the time of maternal infection. *J Clin Microbiol.* 2012;50:3948.)

the basis of results from 4 studies.[123,175–177]

Diagnosis of *T gondii* Infection in the Infant

Persistence of Positive Toxoplasma *IgG in the Child Beyond 12 Months of Age Is Considered to Be the Gold Standard for the Diagnosis of CT*

In general, any maternal *Toxoplasma* IgG antibodies transferred transplacentally are expected to decrease by 50% per month after birth and usually disappear by 6 to 12 months of age.[98] Maternal treatment during pregnancy and/or postnatal treatment could affect the production and kinetics of *Toxoplasma* IgG antibodies in the infant. In infants with CT, postnatal therapy might decrease the *Toxoplasma* IgG titers to below detectable levels; however, rebound will occur after discontinuation of therapy.[98] Disappearance of *Toxoplasma* IgG antibodies in the presence of postnatal treatment cannot be used as an indication that the child does not have CT. (Berrebi et al[131] used as a criterion the persistence of *Toxoplasma* IgG antibodies after 18 months of age.)

Diagnostic Criteria That Exclude CT

The disappearance of *Toxoplasma* IgG before 12 months of age in an infant capable of producing IgG antibodies and who has not received anti-*Toxoplasma* treatment essentially excludes the diagnosis of CT.

Positive Toxoplasma *IgG and Positive* Toxoplasma *IgM ISAGA Test and/or Positive* Toxoplasma *IgA Test in the Neonate*

A positive *Toxoplasma* IgM ISAGA result (at or after 5 days of age) and/or a positive *Toxoplasma* IgA test result (at or after 10 days of age) is considered diagnostic of CT in infants with a positive *Toxoplasma* IgG. Because IgM and IgA antibodies do not cross the placenta, they reflect the infant's response to *T gondii* infection. Of note, positive *Toxoplasma* IgM antibodies before 5 days of age or positive *Toxoplasma* IgA antibodies before 10 days of age also could represent false-positive results resulting from contamination of the infant's blood with maternal blood because of a materno-fetal blood leak, and the tests should be repeated after 5 and 10 days, respectively. Moreover, false-positive *Toxoplasma* IgG and IgM test results can occur after recent transfusion of blood products or receipt of immune globulin intravenously, and they should be repeated at least 1 to 2 weeks after the last transfusion. However, consideration should be given to testing infants with high suspicion for CT as soon as possible after birth.

These neonatal *Toxoplasma* IgM and/or IgA tests could fail to identify approximately 20% to 50% of CT cases, with the highest false-negative rates being reported in Western European countries,[178] where infants have been exposed to antepartum treatment, and the lowest false-negative rates being reported in the United States, where the majority of infants with CT had mothers who did not receive antepartum treatment.[124] False-negative *Toxoplasma* IgM and *Toxoplasma* IgA test results in infants with CT could also occur in cases of fetal infection early in gestation. In those cases, the early transient positive *Toxoplasma* IgM and/or IgA antibody responses might have disappeared by the time of delivery.[178] Moreover, if maternal infection was acquired very late in pregnancy, initially negative *Toxoplasma* IgM ISAGA and IgA ELISA results at birth could be attributable to delayed production of those antibodies and would not exclude the diagnosis of CT. Repeat testing 2 to 4 weeks after birth and every 4 weeks thereafter until 3 months of age could be considered in such cases.

In the study by Olariu et al,[124] the reported rates of false-negative results in the United States for neonatal *Toxoplasma* IgM ISAGA, IgA

ELISA, and IgM or IgA tests were much lower (false-negative IgM ISAGA results occurred in 13% of CT cases [22 of 164]; 95% CI: 9%–20%; false-negative IgA ELISA results occurred in 23% of CT cases [37 of 164]; 95% CI: 16%–30%; false-negative IgM ISAGA and IgA ELISA results occurred in 7% of CT cases [11 of 164]; 95% CI: 3%–12%) when infants were tested within the first 6 months of age.

According to the EUROTOXO 2006 systematic review of diagnostic tests, in 8 of 19 diagnostic studies that had the best study design and used appropriate reference standards for the ascertainment of the infant's infection status,[11] the reported false-negative rates for neonatal *Toxoplasma* IgM or IgA tests in infants with CT (the majority of whose mothers received antepartum treatment) were 19% to 45% (when both tests were performed and if tests were performed within the first 2 weeks after birth) and 21% to 37% if performed within 2 weeks to 3 months after birth. The reported false-negative rates for the neonatal *Toxoplasma* IgA test were 29% to 48% if the test was performed within 2 weeks after birth and 40% to 43% if performed within 2 weeks to 3 months of age. The reported false-negative rates for neonatal *Toxoplasma* IgM ISAGA were 33% to 56% if performed within 2 weeks after birth and 29% to 39% if performed 2 weeks to 3 months after birth.[11,179]

The observed differences in the sensitivity of these tests between the European and US cohorts could be attributed to the presence of antepartum treatment in most CT cases in the European Union. Such treatment could have affected the production of *Toxoplasma* IgM and IgA in those infants. In the US cohort,[124] all infants had mothers who did not receive antepartum treatment. There was no statistically significant difference in the rates of false-negative results whether these were performed at or before 1 month of age versus at 1 to 6 months of age.[124] However, the number of cases was small.

The role of *Toxoplasma* IgE antibodies for routine screening of infants has been considered to be limited,[124,180,181] and currently, IgE antibodies are not included in the recommended infant diagnostic panel for CT at the PAMF-TSL.[164]

Positive PCR Assay Results From Peripheral Blood, CSF, or Urine or Other Body Fluid, According to Clinical Presentations

In the largest cohort of CT cases without antepartum treatment in the United States[124] over the past 15 years (N = 164), results of PCR assays of peripheral blood, CSF, and urine were reported in 7, 58, and 10 infants, respectively. The sensitivity was 29% (95% CI: 4%–71%) for blood PCR assay, 46% (95% CI: 33%–60%) for CSF PCR assay, and 50% (95% CI: 19%–81%) for urine PCR assay.[124] The uncertainty around these estimates was large for blood and urine and is attributable to the small sample sizes.[124] The CSF PCR assay result was positive in 71% (22 of 31) of infants with CT and hydrocephalus, in 53% (23 of 43) of infants with intracranial calcifications, and in 51% (26 of 51) of infants with eye disease.[182] CSF PCR assay had the potential to increase the frequency of cases in which the diagnosis of CT was confirmed (95% of CT cases [55 of 58] had a positive IgM ISAGA, a positive IgA ELISA, or a positive CSF PCR assay result), and CSF PCR assay was successful in detecting CT in 3 infants who had negative *Toxoplasma* IgM and IgA test results at birth (3 of 6 infants with CT and negative IgM ISAGA and IgA ELISA results had positive CSF PCR assay results).[182]

Role of Placental Testing

The 2 most recent studies on the diagnostic performance of placental PCR from European cohorts of women who had received antepartum treatment showed a sensitivity ranging between 71%[183] and 79%[184] and a specificity ranging between 92%[184] and 97%[183] (Robert-Gangneux et al[183] tested 102 placentas [28 CT cases]; Sterkers et al[184] tested 238 placentas [39 CT cases]). The reported PPV of placental PCR assay was 67% (95% CI: 54%–81%) and the NPV was 96% (95% CI: 93%–99%).[184] A positive placental PCR assay result may provide some evidence of CT but is not diagnostic of CT per se. Whole placentas of at least 200 g were used, and several samples were obtained for testing from different placental sites.[183] Robert-Gangneux et al also reported the diagnostic performance of placental testing by mice subinnoculation and reported a sensitivity of 67% and a specificity of 100%.[183]

The diagnostic performance of placental PCR assay in the United States, where the majority of pregnant women with *T gondii* infection are not treated, is unknown. In general, for PCR assay, the specimen could be sent frozen, whereas for mice subinnoculation (ie, for isolation and serotyping of the *T gondii* strain), the specimen should not be frozen and should be sent at 4°C. Cord blood PCR assay does not appear to be clinically useful, because its sensitivity was shown to be very low (16% [4 positive cord blood PCR assay

results/25 CT cases])[172]; however, its specificity was 100%.[172]

Positive Neonatal Toxoplasma *IgG Test Results With Negative* Toxoplasma *IgM and IgA Test Results but Serologic Evidence of Acute Maternal T gondii Infection During Pregnancy and Evidence of Clinical Manifestations Suggestive of CT (ie, Eye Disease, Intracranial Calcifications, Hydrocephalus, Etc) (According to the PAMF-TSL)*

This situation is rare, but these newborn infants would be considered to be infected and be treated until proven otherwise, assuming that other etiologies for the clinical manifestations have been reasonably excluded. In the Olariu et al[124] study, 7% (11 of 164) of infants with CT had negative *Toxoplasma* IgM and IgA test results. As mentioned previously, initially negative neonatal *Toxoplasma* IgM ISAGA and IgA ELISA results could be attributable to delayed production of those antibodies and would not exclude the diagnosis of CT. Repeat testing at 2 to 4 weeks after birth and every 4 weeks thereafter until 3 months of age could be considered in such cases.

Diagnostic Workup of Infants With Suspected/Confirmed CT (According to the PAMF-TSL)

A detailed depiction of the diagnostic workup of infants with suspected CT, according to the PAMF-TSL, is shown in Table 9. In addition, technical details for the preparation and shipping of clinical specimens to toxoplasmosis reference laboratories are provided in Tables 5–7.

TREATMENT (ACCORDING TO THE PAMF-TSL)

Antepartum Management of Pregnant Women (According to Serologic Test Results)

The antepartum treatment algorithm (according to the PAMF-TSL) based on gestational age at which primary infection most likely occurred is shown in Fig 9 and Table 10 .

Maternal Serologic Test Results Suggestive of a Recently Acquired Infection, During Pregnancy or Within 3 Months of Conception (According to the PAMF-TSL)

Consideration should be given to initiating treatment of the pregnant woman as soon as possible, either with spiramycin, if maternal primary infection was acquired close to conception and up to 18 weeks of gestation (the request for spiramycin should be made to the FDA; details regarding how to obtain spiramycin are provided in the footnote of Table 10), or with P/S and folinic acid, if maternal infection was acquired at >18 weeks of gestation. (Spiramycin does not readily cross the placenta and thus is not reliable for treatment of infection in the fetus; P/S crosses the placenta and, if fetal infection has occurred, can provide treatment of the fetus.[154])

In addition, an AF PCR assay should be performed, if feasible and safe, as soon as possible after 18 weeks of gestation and sent to a reference laboratory. The risk of complications for amniocentesis performed at >24 weeks of gestation should be taken into consideration. In all cases, treatment should be continued until delivery, but the AF PCR assay result will determine whether optimal treatment for the duration of pregnancy is spiramycin or P/S plus folinic acid. Monthly fetal ultrasonographic monitoring throughout pregnancy is indicated.

Spiramycin should be continued throughout pregnancy to prevent MTCT, even if the AF PCR assay result is negative, because the placenta still might be infected and fetal infection through an infected placenta can occur at any time during pregnancy.

Fetal Infection Confirmed by a Positive AF PCR Assay Result

Treatment with P/S and folonic acid should be initiated in the pregnant woman. If the pregnant woman already was receiving spiramycin, spyramycin should be stopped and treatment should be switched to P/S. A French randomized clinical trial (TOXOGEST) was launched to compare the efficacy of P/S versus spiramycin in preventing MTCT. Enrolled pregnant women are those at ≥14 weeks of gestation with confirmed acute primary infection during pregnancy. The anticipated date of completion was 2015, and the target sample size is 330 pregnant women. Secondary study endpoints include the safety of therapy and the severity of CT.[203]

Maternal Serologic Test Results Consistent With an Infection Acquired Before Pregnancy and More Than 3 Months Before Conception

The incidence of CT in the children of such women has been shown to be extremely rare unless the woman is severely immunocompromised (eg, advanced HIV disease, receiving corticosteroids or immunosuppressive drugs). Anti-*Toxoplasma* treatment is not indicated in this case unless the patient is immunocompromised. However, there are a few exceptions to this dictum, illustrated by case reports of presumed *T gondii* reinfections during pregnancy with a different *T gondii* strain in previously immune women with serologic evidence of chronic *T gondii* infection.[144,204]

T gondii Infection in an HIV-Infected Pregnant Woman

There are a few published cases reports for HIV-infected, previously immune pregnant women with serologic evidence of chronic *T gondii* infection who transmitted the infection to their fetuses.[205–207] This

TABLE 9 Laboratory, Radiographic, and Clinical Evaluation of Fetuses and Infants With Suspected CT, According to the PAMF-TSL

Fetus

1. Maternal *Toxoplasma* serology and amniocentesis at or after 18 weeks of gestation for AF *Toxoplasma* PCR
2. Fetal ultrasound every month until delivery

Newborn infant

Laboratory workup

- Peripheral blood for *Toxoplasma* IgG, IgM, and IgA (if IgA also available at commercial laboratory) can be sent to commercial laboratories as soon as possible after birth. However, testing of infants with suspected congenital toxoplasmosis should be performed at a toxoplasmosis reference laboratory, where the *Toxoplasma* IgG dye test, the *Toxoplasma* IgM ISAGA, and the *Toxoplasma* IgA ELISA (and also additional testing with *Toxoplasma* PCR in blood/urine/CSF) are performed.
- Neonatal testing should be performed at a reference laboratory for toxoplasmosis. The more sensitive *Toxoplasma* IgM ISAGA test (performed only in reference laboratories) should be ordered. Negative *Toxoplasma* IgM ELISA at commercial hospital-based, clinic-based, or any other nonreference laboratories cannot exclude the diagnosis of CT, even in the absence of positive *Toxoplasma* IgA results.
- *Toxoplasma* IgG
- Neonatal *Toxoplasma* IgG antibodies should be tested in parallel with maternal *Toxoplasma* IgG antibodies. Without maternal serologic test results, appropriate interpretation of neonatal serologic test results may be tenuous unless the infant is found to be completely seronegative for *Toxoplasma* IgG, IgM ISAGA, and IgA (provided that the infant and the mother were capable of producing immunoglobulins).
- *Toxoplasma* IgM (ISAGA)
- Whenever a *Toxoplasma* IgM ISAGA test result is positive, reasons for false-positive test results should be considered (eg, caused by blood product transfusion or IVIg infusion), and the test should be repeated at least 7 days after last transfusion.
- Moreover, if there is concern for false-positive *Toxoplasma* IgM results because of possible contamination of infant's blood with maternal blood during labor, the test should be repeated at least 5 days after birth (half-life of *Toxoplasma* IgM antibodies is ∼5 days).[185]
- *Toxoplasma* IgA
- Whenever a *Toxoplasma* IgA test result is positive, reasons for possible false-positive test results should be considered (eg, caused by blood product transfusion or IVIg infusion), and the test should be repeated at least 7 days after last transfusion.
- Moreover, if there is concern for false-positive *Toxoplasma* IgA results because of possible contamination of infant's blood with maternal blood during labor, the test should be repeated at least 10 days after birth (half-life of *Toxoplasma* IgA antibodies is ∼10 days).[185]
- If maternal serologic test result was suggestive of an acute primary infection acquired very late in gestation, initially negative *Toxoplasma* IgM and IgA results at birth could also be attributable to delayed production of those antibodies, and repeat testing 2 to 4 weeks after birth and every 4 weeks thereafter until 3 months of age might be considered in such cases.
- *Toxoplasma* PCR
- Peripheral blood *Toxoplasma* PCR, urine *Toxoplasma* PCR, and CSF *Toxoplasma* PCR should be performed when there is strong suspicion of CT, as soon as possible after birth.
- Specifically, CSF *Toxoplasma* PCR should be considered in the following cases[a]:

a. infants with positive *Toxoplasma* IgM ISAGA and/or IgA ELISA;

b. infants born to mothers with confirmed acute *T gondii* infection during pregnancy who had a positive *Toxoplasma* AF PCR and/or abnormal fetal ultrasonographic findings suggestive of CT independently whether antepartum treatment was received; and

c. infants born to mothers with confirmed recently acquired *T gondii* infection during pregnancy, particularly those who likely had acquired the infection in the second or third trimester (even if AF PCR and/or fetal ultrasonography were negative) and had not received any antepartum anti-*Toxoplasma* treatment.

- CSF *Toxoplasma* PCR could be deferred in infants with low suspicion of CT, such as asymptomatic infants at birth whose mothers were diagnosed in the first trimester of pregnancy, had received antepartum treatment (spiramycin), had negative *Toxoplasma* AF PCR, and had normal monthly fetal ultrasonography results and the infants were asymptomatic and had negative *Toxoplasma* IgM ISAGA and IgA ELISA results at birth.
- CSF analysis (CSF cell count, differential, protein, and glucose).
- Complete blood count and liver function tests

Clinical evaluation

- Detailed physical examination

If CT has been confirmed:

o at birth (along with detailed maternal clinical history); every 2–3 months afterward during the first year of life and every 4–6 months after the first year

If CT has not been confirmed but also has not been ruled out:

o detailed physical examination at birth and every 2–3 months afterward during the first year of life and every 4–6 months after the first year (while continuing serologic testing every 4–6 weeks to document appropriate decrease in *Toxoplasma* IgG titers, according to the half-life of IgG, ∼30 days, until complete disappearance)

- Pediatric neurologic evaluation

If CT has been confirmed:

- at birth and every 2–3 months afterward during the first year of life and every 4–6 months after the first year

If CT has not been confirmed but also has not been ruled out:

- neurologic evaluation at birth and every 2–3 months afterward during the first year of life and every 4–6 months after the first year of life (while continuing serologic testing every 4–6 weeks to document appropriate decrease in the *Toxoplasma* IgG titers, according to the half-life of IgG, ∼30 days, until complete disappearance)

- Pediatric ophthalmologic examination

If CT has been confirmed:

- during the first year of life if CT: ophthalmologic evaluation at birth and every 3–4 months (preferably by a retinal specialist)

TABLE 9 Continued

- during the second year of life: ophthalmologic evaluation every 4–6 months
- during the third year of life if CT: ophthalmologic evaluation every 6 months

Even if evidence of chorioretinitis has not been found in the initial ophthalmologic evaluation, close serial ophthalmologic follow-up is still indicated for infants with suspicion of CT.[b]

If evidence of active *Toxoplasma* chorioretinitis has been found, closer follow-up might be indicated.

If CT has not been confirmed but also has not been ruled out:

- ophthalmologic evaluation at birth and every 2–3 months afterward during the first year of life and every 4–6 months after the first year (while continuing serologic testing every 4–6 weeks to document appropriate decrease in *Toxoplasma* IgG titers, according to the half-life of IgG, ∼30 days, until complete disappearance)
- Auditory brainstem responses

If CT has been confirmed:

- shortly after birth and yearly audiologic evaluation afterward for the first 3 years of life

If CT has not been confirmed but also has not been ruled out:

- shortly after birth

Imaging evaluation

- Computed tomography of the head should be considered when there is suspicion of CT to evaluate for the presence of intracranial calcifications, ventriculomegaly, hydrocephalus, etc. Head ultrasonography has been used mainly in cohorts in Europe,[99,131,186] where the rate of symptomatic CT cases is very low compared with the United States. (Limited comparative data have shown that ultrasonography of the head had lower sensitivity than computed tomography of the head for intracranial calcifications[98]; published empirical data on the comparative performance of MRI, ultrasonography, or computed tomography of the head are lacking, but brain MRI could also be considered for the initial evaluation because it obviates the risk of radation associated with head CT.)
- Abdominal ultrasonography at birth for intrahepatic calcifications and/or hepatosplenomegaly

For assistance with the diagnosis and management of infants or children with CT, contact Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory, Palo Alto, CA; www.pamf.org/serology; http://www.pamf.org/serology/testinfo.html; telephone: (650) 853-4828; fax: (650) 614-3292; e-mail: toxolab@pamf.org; and/or Professor Rima McLeod from the Toxoplasmosis Center at the University of Chicago (Center of the National Collaborative Chicago-based Congenital Toxoplasmosis Study); telephone: (773) 834-4130. Additional information for the laboratory interpretation of serologic test results can be found in Press et al,[187] Montoya et al,[188] and Montoya et al.[154] IVIG, intravenous immunoglobulin.

a Neonatal CSF serology: On the basis of available data routinely collected at the National Reference Laboratory for Toxoplasmosis, there is no evidence suggesting CSF serologic testing (CSF *Toxoplasma* IgM and/or CSF *Toxoplasma* IgG) could offer any incremental diagnostic information compared with the information gained from the following: (1) CSF cell count/protein/glucose and (2) CSF *T gondii* PCR and (3) CNS imaging alone for the diagnosis of CT and particularly for the diagnosis of CNS involvement during CT. (In the PAMF-TSL database [1992–2013], among 125 infants who had both *Toxoplasma* CSF PCR and CSF serologies [*Toxoplasma* IgG and IgM] performed, only 3 infants [2 neonates and a 15-month-old child] had positive CSF *Toxoplasma* IgM without a positive CSF *Toxoplasma* PCR. However, all 3 infants already had an established diagnosis of CT on the basis of serologic test results from blood samples that were diagnostic of CT [unpublished data].)

b Age at diagnosis of chorioretinitis: Only 7% of children with CT (20 of 281 CT cases) had chorioretinitis diagnosed at their first examination and only 40% (20 of 50) of children who had an eventual diagnosis of eye disease had lesions detected at their first examination.[131,137] Wallon et al[139] found, in cohorts of prenatally treated infants in France, that the initial eye lesions in children with CT were detected after 7 months of age in 75% of children and after 3 years of age in 50% of children with CT.

situation has been reported to occur in <4% of cases (in 0.09% [1 CT case in 1058 HIV-infected women] of HIV-infected women followed as part of the European Collaborative Study and Research Network on CT[206]; and in 0.5% [95% CI: 0.24%–0.91%; 10 CT cases in 2007 HIV-exposed infants] of HIV-exposed infants from Brazil between 1998 and 2011[208]; and in 3.7% [3 CT cases in 82 HIV-infected pregnant women] of HIV-infected pregnant women with chronic *T gondii* infection in Brazil[207]).

In this setting, the risk of MTCT appeared to be greater when the pregnant woman's latent infection was reactivated with or without clinical manifestations (eg, *Toxoplasma* encephalitis, pneumonia, fever). Reactivation was more likely to occur if the maternal CD4+ T-lymphocyte count decreased below 200 cells/mm^3 in the absence of effective anti-*Toxoplasma* prophylaxis. However, in HIV-exposed infants, CT has been documented to occur even when mothers were not severely immunosuppressed (eg, 6 of 10 CT cases in HIV-exposed infants occurred with maternal CD4+ T-lymphocyte counts >200/mm^3, with 1 CT case occurring with a maternal CD4+ T-lymphocyte count >500/mm^3).[208] Cautious monitoring of HIV-infected pregnant women is indicated.

In the absence of clinical trial information, the following recommendations have been made by PAMF-TSL: (1) if the mother's CD4+ T-lymphocyte count decreases below 200 cells per mm^3 in the absence of clinical manifestations of toxoplasmosis, prophylaxis with agents known to be effective against *Toxoplasma* (eg, trimethoprim/sulfamethoxazole) should be instituted; (2) for mothers with clinical manifestations of toxoplasmosis, anti-*Toxoplasma* treatment with effective regimens (eg, P/S and folinic acid) should be initiated as soon as possible; and (3) if the mother's CD4+ T-lymphocyte count remains at ≥200 cells per mm^3, as a cautionary measure, spiramycin should be continued throughout pregnancy.

A Previously T gondii–Infected Pregnant Woman Possibly Reinfected by a Different T gondii Strain

There are very few case reports of previously immune pregnant women with serologic evidence of chronic *T gondii* infection who delivered an infant

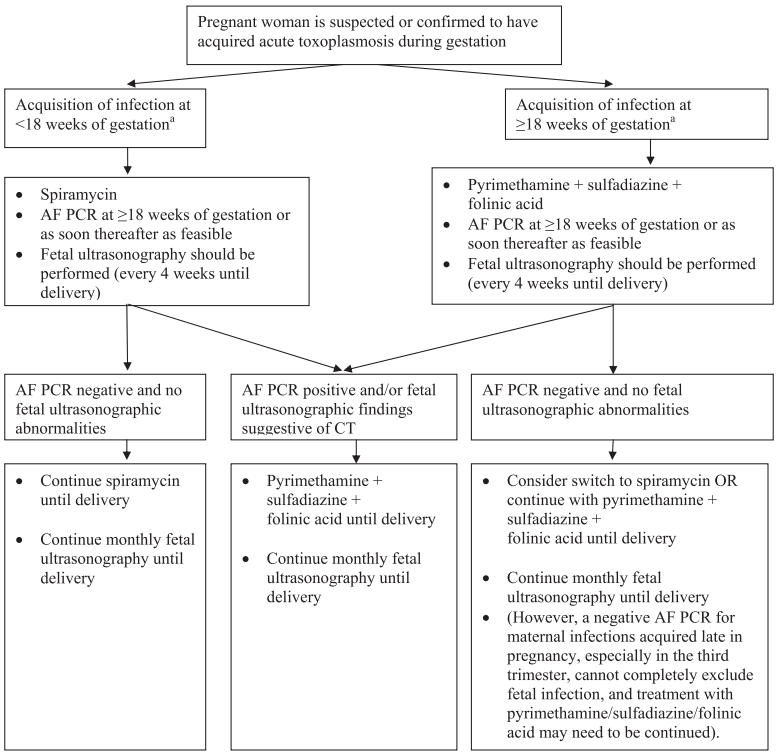


FIGURE 9

Therapeutic algorithm for the pregnant woman with suspected acute *T gondii* infection acquired during pregnancy. [a]This gestational age refers to the time at which the *T gondii* infection was most likely acquired and not the time the amniocentesis was performed. (Reprinted with permission from Montoya, JG, Remington JS. Management of Toxoplasma gondii infection during pregnancy. *Clin Infect Dis.* 2008;47:558.)

with CT[151,155]; this situation was presumably attributable to reinfection during pregnancy by a different *T gondii* strain (eg, in the case report from France by Elbez-Rubinstein et al,[144] the previously immune pregnant woman had ingested imported raw horse meat). This unusual situation might be considered when there is a strong clinical suspicion with recent international travel, but currently there are no routinely used laboratory tests that could help distinguish reinfections from a new *T gondii* strain from preexisting infection. If this clinical scenario is suspected, consultation with reference toxoplasmosis centers in the United States regarding treatment is encouraged.

Acute T gondii Infection Occurring in a Pregnant Woman After Travel

When acute *T gondii* infection is suspected in a pregnant woman after international travel, it is important to remember that more virulent *T gondii* strains circulating outside the United States and Europe have been reported to cause more severe clinical manifestations in infants with CT.[38,155]

Postnatal Management of the Infant With CT

There is a large variation between the different postnatal treatment protocols followed in different centers in the United States and Europe, as shown in Table 11. In the United States, the postnatal treatment protocol that is used is the one proposed by the NCCCT study (Tables 10 and 11).[112] Currently, there is no evidence on the comparative efficacy or effectiveness of the postnatal treatment strategies, and the selection of different strategies relies on the experience of the different centers.

Adverse Effects

Treatment of infants with P/S was frequently associated with serious adverse events, reported to occur in 20% to 50% of cases.[14] However, data from Denmark have shown that the medication was not well tolerated in only 14% (4 of 29) of treated infants.[209] The main adverse effect was neutropenia, reported to occur more often with higher doses of P/S and especially when folinic acid was not administered.[14] Moreover, seizures have been reported with cases of pyrimethamine overdose resulting from prescription dosing errors.[210]

Adverse effects with pyrimethamine/sulfadoxine (Fansidar, F. Hoffman-LaRoche Ltd, Basel, Switzerland) were much less frequent, mainly skin rashes and urticarial reactions, and reported to occur in approximately 1% to 2% of cases.[14] The disadvantage of the long half-life of sulfadoxine may need to be taken into account. Treatment discontinuations of pyrimethamine/sulfadoxine attributable to adverse events in adults with *Toxoplasma* chorioretinitis have been reported to occur in up to 26% of patients.[211] The frequency of serious adverse events with pyrimethamine/sulfadoxine has been reported to occur in 1 in 2100 prescriptions, skin reactions have been reported to occur in 1 in 4900 prescriptions, and death has been reported to occur in 1 in 11 100 prescriptions.[212]

Variations in Postnatal Treatment Regimens Across Centers in the Medications Used

In French centers before 2001, infants were treated with P/S for the first 3 weeks of life, changed to spiramycin until 2 months of age, and then changed

TABLE 10 Antepartum and Postnatal Treatment Protocols in the United States for CT, According to the PAMF-TSL and the Toxoplasmosis Center at the University of Chicago

During pregnancy

Spiramycin (oral)[a]

- Recommended for women suspected or confirmed of having acquired their infection at <18 weeks of pregnancy
- Spiramycin should be administered until delivery in women with negative AF PCR test results and negative follow-up fetal ultrasonographic results or low suspicion of fetal infection
  - o Spiramycin is not teratogenic and is available in the United States only through the Investigational New Drug process at the FDA (telephone: 301-796-1600)[b]
- Spiramycin dose = 1 g (3 million IU) PO TID
  - o Total daily dose = 3 g (9 million IU) per day
- Consultation with medical consultants familiar with toxoplasmosis is strongly recommended
- Spiramycin is not recommended during pregnancy if the fetus has been documented to be or suspected to have been infected
- For fetal infections, maternal treatment with pyrimethamine/sulfadiazine/folinic acid should be instituted (see below)

Pyrimethamine[c] + sulfadiazine + folinic acid (oral)[b]

- Recommended for women ≥18 weeks of pregnancy for whom (1) it is suspected or confirmed that they acquired acute infection at or after the 18th week of pregnancy, (2) a positive AF PCR test result is documented, or (3) an abnormal fetal ultrasonograph is suggestive of CT
- Pyrimethamine dose[c]: 100 mg/day PO divided BID for 2 days followed by 50 mg/day PO QD
- Sulfadiazine dose: 75 mg/kg per dose PO × 1, followed by 100 mg/kg per day PO divided BID (maximum sulfadiazine = 4 g/day)
- Folinic acid (leucovorin) dose: 10–20 mg/day PO QD (during and 1 week after pyrimethamine therapy)
  - o Folic acid should not be used as a substitute for folinic acid
  - o Pyrimethamine is potentially teratogenic and should not be used before the 18th week of pregnancy
  - o Sulfadiazine should not be used alone

Infants with CT (treatment regimen is usually recommended for 1 year)

Pyrimethamine[c]

- First 2 days: 2 mg/kg per day PO divided BID for 2 days
- Intensive initial therapy from day 3 to 2 months (or to 6 months)[d]: 1 mg/kg per day PO QD (although statistically significant difference was not found between the 2-month versus the 6-month intensive therapy regimen; equivalence between these 2 regimens was not established)
- Intensive initial therapy for 2 months might be considered in infants with CT who are asymptomatic at birth, whereas continuation of the initial intensive therapy for 6 months might be considered for infants with CT who are symptomatic
- After the first 2 months (or the first 6 months) of intensive therapy: 1 mg/kg per day PO TIW to complete a total therapy of 12 months (daily + TIW regimen)

Sulfadiazine

- 100 mg/kg per day PO divided BID

Folinic acid (leucovorin)[e]

- 10 mg PO TIW

Prednisone if CSF protein ≥1 g/dL or severe chorioretinitis in vision-threatening macular area (based on expert opinion)

- 1 mg/kg per day PO divided BID (until CSF protein <1 g/dL or until resolution of severe chorioretinitis) (based on expert opinion, if steroids are to be used, they should be initiated after 72 hours of anti-*Toxoplasma* therapy)

Older children (diagnosed beyond the neonatal period) with active disease (chorioretinitis)

Pyrimethamine/sulfadiazine[f,g,h] (for at least 1–2 weeks after resolution of all signs and symptoms and for ∼4–6 weeks total)

Pyrimethamine[c]:

- First 2 days: 2 mg/kg per day PO divided BID (maximum 50 mg/day)
- Then: 1 mg/kg per day PO QD (maximum 25 mg/day)

Sulfadiazine

- 75 mg/kg/dose PO × 1, followed by 100 mg/kg per day PO divided BID

Folinic acid (leucovorin)[e]

- 10–20 mg PO TIW

Prednisone (severe chorioretinitis)

- 1 mg/kg per day divided BID (maximum 40 mg/day; rapid taper) (based on expert opinion)

For assistance with the diagnosis and management of infants or children with CT, you can contact the following: Palo Alto Medical Foundation Toxoplasma Serology Laboratory (PAMF-TSL), Palo Alto, CA; www.pamf.org/serology; http://www.pamf.org/serology/testinfo.html; telephone: (650) 853-4828; fax: (650) 614-3292; e-mail; toxolab@pamf.org; and/or Professor Rima McLeod from the Toxoplasmosis Center at the University of Chicago (Center of the National Collaborative Chicago-based Congenital Toxoplasmosis Study [NCCCT]); telephone: (773) 834-4130). Pharmacokinetic data: (1) The pharmacokinetic data of spiramycin during pregnancy studied by Couvreur et al[189] in 20 pregnant women revealed that the placental concentration of spiramycin was 4 times the maternal blood concentration (2.3 vs 0.47 mg/L) and the fetal blood concentration was 47% of the maternal blood concentration (0.29 vs 0.68 mg/L); (2) only pharmacokinetic studies in mice were conducted to estimate the pharmacokinetic data for maternal treatment with P/S; and (3) the pharmacokinetic data for maternal treatment with pyrimethamine/sulfadoxine (Fansidar) studied by Dorangeon et al[190] showed that the levels of pyrimethamine in the newborn infant at the time of delivery were 50% to 100% of maternal blood levels and the levels of sulfadoxine were 100% of the maternal blood level. The pharmacokinetic data for infants' treatment with pyrimethamine/sulfadoxine (Fansidar) studied by Corvaisier et al,[191] did not establish the concentration of the pyrimethamine/sulfadoxine that is most efficacious in treating children with CT. They studied 32 infants with CT receiving pyrimethamine/sulfadoxine every 10 days for 1 year, and 101 measurements were collected. Even when the dose was standardized for body weight, the difference of the pyrimethamine minimum and maximum concentrations ranged between 8- and 25-fold and for sulfadoxine between fourfold and fivefold. There is a need for future studies to address the association between long-term clinical outcomes in children with CT and the pharmacokinetic parameters of these drugs. BID, twice per day; PO, by mouth; QD, daily; TID, 3 times per day; TIW, 3 times per week.

TABLE 10 Continued

a Acquisition of spiramycin in the United States: Spiramycin is not commercially available in the United States. It can be obtained at no cost and after consultation (with PAMF-TSL, telephone 650-853-4828, or the NCCCT, telephone 773-834-4152, through the US FDA, telephone 301-796-1600). Through this program, Sanofi-Aventis, for many years, has been providing spiramycin at no cost.

b Experience from the antepartum treatment strategies used in Germany: In Germany, where a different antepartum treatment approach is used,[5] a very low overall transmission rate of 4.8% has been documented, compared with an overall transmission risk in other Western European cohort studies of ∼30% (for *T gondii* infections across all trimesters).[3,96] The reported transmission risks in Germany according to the time of maternal primary infection during pregnancy were 1.3% (6 of 479 children) for the first trimester, 10.6% (17 of 160 children) for the second trimester, and 21.7% (10 of 46 children) for the third trimester.[5] Prenatal treatment approach in Germany: Once acute *T gondii* infection is diagnosed during pregnancy, spiramycin is administered until up to the 16th week of pregnancy. However, after the 16th week, treatment is changed to P/S plus folinic acid in all women and for at least 4 weeks until performance of *T gondii* AF PCR assay and independently of fetal infection status. Subsequently, if fetal ultrasonography indicates congenital infection or if the AF PCR result is positive for *T gondii*, treatment with P/S is continued until delivery (otherwise, it is subsequently changed back to spiramycin prophylaxis). Therefore, all women with acute *T gondii* infection during pregnancy will be treated also with at least 4 weeks of combination therapy. Screening during the second and third trimester occurs less frequently than screening during the first trimester, because there is no mandatory routine antepartum screening program during pregnancy.

c Acquisition of pyrimethamine: As of June 2015, pyrimethamine is no longer available in retail pharmacies in the United States. It is only available through a special pharmacy program at Walgreens (http://www.daraprimdirect.com/how-to-prescribe). After a physician prescribes pyrimethamine, he or she will need to fill out a patient Prescription & Enrollment form (http://www.daraprimdirect.com/forms/Daraprim-Prescription-Form.pdf) and fax it to Walgreens Specialty Pharmacy. From there, a dedicated representative for this drug will contact the patient to arrange for the prescription to be delivered. For questions about the pyrimethamine prescription, call this toll-free number: 1-800-222-4991. Medication will be delivered to the patient on the same day if marked in the enrollment form as "STAT/URGENT." The medication should be provided/sent directly to the patient or his/her physician without any delays for insurance clearance. In this form you may add: "Request for no delay in the delivery of medication for insurance clearance." For patients without insurance, the medicine will be made available without charge by the company (after completion of the relevant form at the link above).

d Comparative efficacy of the initial daily treatment of 2 months versus the 6-month initial regimen: This was tested in the context of an RCT by the NCCCT study group.[112] Although this RCT did not show a statistically significant difference between the 2 treatment arms, the study was not powered to detect a small to modest difference in relative reduction in outcomes (eg, a difference <60%); thus, the absence of a detected statistically significant difference does not also mean that the 2 arms are equivalent in efficacy. Children in this study who had begun treatment at 2.5 months of age had completed the 12-month therapy course at 14.5 months of age. In the recently published data from the Lyon cohort,[139] there were also children with CT who were treated with pyrimethamine and sulfonamides for >12 months; the median duration of treatment was 15 months, but the interquartile range was 12 to 19 months. In the Toulouse cohort, children were treated until 24 months of age.[131]

e Folic acid should not be used as a substitute for folinic acid (leucovorin).

f Treatment options of acute toxoplasmic chorioretinitis in children: There are no comparative pediatric trials on the relative efficacy of different treatment regimens for acute toxoplasmic chorioretinitis. In the adult literature there are also only a few comparative trials (eg, comparison of intravitreal clindamycin plus dexamethasone versus PO P/S,[192] azithromycin versus P/S,[193] or trimethoprim/sulfamethoxazole versus P/S[194]) and there is lack of high-quality evidence to determine the best treatment option.[195] In immunocompetent adults, many observational studies suggest a benefit of short-term antimicrobial therapy.[196] Some experts in the United States, as an alternative to the classic treatment option of P/S, have also tried short-term antimicrobial therapy in children with toxoplasmic chorioretinal lesions that are not in a vision-threatening area, alternative combinations such as pyrimethamine plus clindamycin or azithromycin, and then continuation with clindamycin or azithromycin alone.[109] However, there is no systematic published experience with those alternative treatment options.

g Duration of anti-*Toxoplasma* therapy for acute toxoplasmic chorioretinitis: There are no comparative trials in children or in adults for the relative efficacy of different treatment durations for ocular toxoplasmosis. On the basis of experience in adult patients, anti-*Toxoplasma* treatment may be considered to be continued for at least 1 to 2 weeks after resolution of clinical signs and symptoms of acute chorioretinitis (with sharpening of the lesion borders and/or scarring of the lesion) and for ∼4 to 6 weeks total.[197,198] (Although often the acute eye disease resolves within 10 to 14 days after initiation of treatment, there are cases that take a longer time to resolve). Longer treatment durations have also been proposed with courses up to 3 months in children with P/S, on the basis of French experience,[139,186] and up to 4 months in adults.[196] Close ophthalmologic follow-up every 2 to 3 weeks is necessary in all cases of active *Toxoplasma* chorioretinitis to determine optimal duration of treatment.

h Anti-*Toxoplasma* prophylaxis for the prevention of recurrent eye disease in infants with CT: There are no randomized clinical trials addressing the efficacy of postnatal prophylaxis to prevent recurrent eye disease in children with CT before their first recurrence. The reported rates of recurrence of eye disease vary according to the presence or absence of antepartum treatment and the presence or absence of postnatal treatment. A placebo-controlled, open-label RCT in Brazil by Silveira et al[199] on prophylactic trimethoprim/sulfamethoxazole (TMP/SMX) in 124 patients with a history of recurrent *Toxoplasma* chorioretinitis found that prophylactic TMP/SMX (TIW) for 20 months was associated with a 75% decrease in the risk of recurrence of eye disease (HR: 0.25; 95% CI: 0.08–0.75). The median age of the enrolled subjects in this trial was 26 years, and no infants were enrolled. Although children as young as 7 years of age were included, the exact number of such subjects was not reported.[199] Another RCT also found that prophylactic TMP/SMX in adults from Brazil (N = 95) decreased the risk of recurrence by 100% within 12 months of prophylaxis[200] (0 of 46 [0%] and 6 of 47 [12.80%] in the TMP/SMX and placebo groups, respectively; P = .026). For young adolescents with recurrences of eye disease during puberty, some experts in the United States have also tried the use of azithromycin as a suppressive therapy for several months. Nevertheless, data on the use of azithromycin for *Toxoplasma* chorioretinitis are very sparse (both from human studies[193,201] and from studies in animal models[202]). Alternative approach to anti-*Toxoplasma* prophylaxis: home/self-monitoring for any signs and symptoms of decreased visual acuity, daily monitoring of visual acuity using home vision test methods (eg, Allen card pictures, print-outs of newspapers, etc), and prompt referral to an ophthalmologist if new symptoms occur are suggested so that treatment can be promptly initiated for any recurrence.[109] As previously discussed, the risk of recurrence of eye disease in the United States: Where the majority of the infants with CT had mothers who were not treated during pregnancy, recurrent eye disease developed in 72% of children with CT who were diagnosed after their first year of life and thus were not postnatally treated (18 of 25; 95% CI: 51%–89%).[109] For comparison, recurrent eye disease developed in only 31% (34 of 108; 95% CI: 23%–41%) of US infants with CT who had received 12 months of postnatal treatment during their first year of life.[110] The risk of recurrence of eye disease in Brazil: In a cohort of 30 infants from Brazil with CT diagnosed by neonatal screening, the risk of recurrence of eye disease was 43%[38] (vs 29% in 281 children with CT from European cohorts in that study). The risk of recurrence of eye disease in the Lyon cohort: The risk of recurrence of eye disease and within 12 years after the diagnosis of the first eye lesion was ∼34%.[139] Of note, infants in the Lyon cohort had mothers who were treated during pregnancy and the infants were also postnatally treated.

to pyrimethamine/sulfadoxine to complete 12 months of total therapy. However, more recently published protocols from several European centers did not include spiramycin in their postnatal treatment regimens, because spiramycin does not have CNS penetration and is a parasitostatic medication. In contrast, P/S penetrates the CNS and is parasitocidal.

In the Dosing Schemes

Some centers have used a daily dosing scheme for P/S, whereas other centers have used pyrimethamine/sulfadoxine dosed every 10 to 15 days (because of the long half-life of sulfadoxine). In the latter regimen, the pyrimethamine dose was higher. In the United States, the initial daily dosing scheme was kept during the

TABLE 11 Proposed Postnatal Treatment Protocols, According to the Toxoplasmosis Center at the University of Chicago and European Toxoplasmosis Centers

Author and Regimen	Dosing Frequency	Duration
McLeod et al[112,a] (US cohort)		
P/S	Every day for the first 2 (or 6) months and 3 times per week after the first 2 months (or 6 months)	12 months (total)
Pyrimethamine		
First 2 days: 1 mg/kg per dose BID for 2 days		
From day 3 of life and for the first 2 months (or 6 months)[a]: 1mg/kg per day for 2–6 months		
After the first 2 months (or 6 months): 1 mg/kg per day TIW for up to 12 months of therapy total		
Sulfadiazine: 100 mg/kg per day divided BID for 12 months		
Folinic acid: 10 mg TIW for 12 months		
Wallon et al[3] (Lyon cohort)		
P/S: for the first 2 months	Q day	12 months (total)
Pyrimethamine/sulfadoxine: for the next 10 months	Q 15 days	
Berrebi et al[131] (Toulouse cohort)		
Pyrimethamine/sulfadoxine[b]	Q 15 days	24 months (total)
• ≤10 kg: pyrimethamine: 6.25 mg + sulfadoxine: 125 mg Q 15 days		
• >10 kg: pyrimethamine: 12.5 mg + sulfadoxine: 250 mg Q 15 days		
Folinic acid: 50 mg Q 15 days		
Kieffer et al[99] (French experience: Paris/Lyon/Marseille)		
Paris cohort: P/S	Q day	12 months (total)
Pyrimethamine:		
1 mg/kg per day for the first 2 months		
0.5 mg/kg per day for the following 10 months		
Sulfadiazine: 80–100 mg/kg per day Q days for 12 months		
Folinic acid: 50 mg Q 7 days for 12 months		
Lyon cohort: P/S for the first 2 months followed by pyrimethamine/sulfadoxine for the following 10 months		
P/S for the first 2 months	Q day (P/S) (protocol after 2002)	12 months (total) (but also 12–14 months reported)[3]
Pyrimethamine: 0–2 months: 1 mg/kg per day (protocol after 2002)		
Sulfadiazine: 0-2 mo: 100 mg/kg per day divided TID Q day (protocol after 2002)		
Pyrimethamine/sulfadoxine for the following 10 months	Q 10 days (pyrimethamine/sulfadoxine)	
Pyrimethamine: 1.25 mg/kg Q 10 d for the following 10 months		
Sulfadoxine: 25 mg/kg Q 10 days for the following 10 months		
Folinic acid: 50 mg every 7 days for 12 months		
Marseille cohort: pyrimethamine/sulfadoxine		
Pyrimethamine/sulfadoxine (as in the Lyon cohort)		
Pyrimethamine: 1.25 mg/kg Q 10 days for 12 months	Q 10 days (pyrimethamine/sulfadoxine)	12 months (total)
Sulfadoxine: 25 mg/kg Q 10 days for 12 months		
Folinic acid: 50 mg every 7 days for 12 months		
Kieffer and Wallon[186] (recent expert literature review)		
Kieffer et al proposed the following 2 postnatal treatment protocols for a total duration of 12 months:		
A. P/S for 12 months	Q day (P/S)	12 months (total)
Pyrimethamine:		

TABLE 11 Continued

Author and Regimen	Dosing Frequency	Duration
1 mg/kg per day for 2 months 0.5 mg/kg per day for the following 10 months Sulfadiazine: 100 mg/kg per day divided BID for 12 months		
or		
B. Pyrimethamine/sulfadoxine for 12 months	Q 10 days (pyrimethamine/sulfadoxine)	12 months (total)
Pyrimethamine: 1.25 mg/kg every 10 days for 12 months Sulfadoxine: 25 mg/kg every 10 days for 12 months Folinic acid: 50 mg every 7 days for 12 months		
Hotop et al[5] (German cohort)		
For asymptomatic infants:		
P/S for 3 months	Q day	3 or 6 or 12 months total (according to the severity of the disease)
Pyrimethamine: 1 mg/kg per day for 3 months Sulfadiazine: 50 mg/kg per day for 3 months Folinic acid for 3 months		
For symptomatic infants (mild ventriculomegaly or intracranial calcifications but with normal neurologic examination or retinal scars without active retinal inflammation)		
P/S for 6 months		
Pyrimethamine: 1 mg/kg per day for 6 months Sulfadiazine: 100 mg/kg per day for 6 months		
For severely symptomatic infants (seizures, abnormal neurologic examination, or active chorioretinitis)		
P/S for 12 months		
Pyrimethamine: 1 mg/kg per day for 12 months Sulfadiazine: 100 mg/kg per day for 12 months		
Röser et al[116] (Danish cohort)		
P/S + folinic acid for 3 months	—	3 months

Ongoing clinical trials for postnatal treatment of CT: There is an ongoing RCT that has been recently launched in France (TOSCANE trial[203]) to compare the efficacy of P/S for 3 months versus 12 months in asymptomatic children with CT with regard to the development of chorioretinitis within a 2-year follow-up (estimated study completion date: September 2016). BID, twice per day; Q, ; TIW, 3 times per week.

a See footnote in Table 10 on the comparative efficacy of 2 months versus 6 months of intensive therapy.

b The sulfadoxine half-life of 120–195 hours allows for a more simple administration scheme. However, should an allergic reaction occur, the clinical implications may be more significant because, due to the long half-life, there would be a continued drug exposure, even after medication discontinuation.

first 2 months (or 6 months [considered for symptomatic CT]) and was then transitioned to a 3-times/week dosing scheme in the following months.

In the Duration of Treatment

The majority of centers treated infants with CT for 12 months, but some centers used longer treatment courses of up 24 months.[131] Some centers in Germany individualized the duration of the postnatal treatment according to the severity of CT (eg, 3 months of total treatment of asymptomatic infants, 6 months for mildly symptomatic infants [ie, minor ventricular dilation or intracranial calcifications but with a normal neurologic examination and the presence of retinal scars but without active inflammation], and 12 months for severely symptomatic infants [seizures, abnormal neurologic examination, or chorioretinitis]).

Protocols for Postnatal Monitoring

The different postnatal monitoring protocols, according to the PAMF-TSL, the Toxoplasmosis Center at the University of Chicago, and European toxoplasmosis centers, are outlined in Table 12. There is significant variation across centers of the frequency of recommended clinical and serologic testing follow-up of infants with CT and the duration of follow-up.

Special Considerations in the United States With Different Clinical Scenarios, According to the Infant's Infection Status

Infants With Confirmed or Suspected CT

The management of infants with confirmed or suspected CT, according to the Toxoplasmosis Center at the University of Chicago and the PAMF-TSL, is outlined in Tables 10–12.

Infants in Whom CT Is Unlikely

There is no clear consensus on the management of asymptomatic

TABLE 12 Proposed Postnatal Monitoring Protocols for Infants With CT in the United States and Different European Centers, According to the PAMF-TSL and European Toxoplasmosis Centers

PAMF-TSL

Infants with confirmed CT:

- See Table 9

Infants unlikely to be infected:

- Complete clinical, radiologic, and laboratory evaluation (as discussed in Table 9) is needed at birth even for those infants (head ultrasonography may be used)
- Decision to defer postnatal treatment should be cautiously made (for reasons previously discussed in the text)
- Serologic follow-up is needed every 4–6 weeks until *Toxoplasma* IgG antibodies are undetectable
  - If the IgG assay result becomes negative, confirmation of this negative result, with another testing in the following 4–6 weeks, may be considered
  - However, if subsequent serologic testing indicates CT, then the child should be treated

Kieffer and Wallon[186] (expert opinion from Paris and Lyon cohorts)

Infants with confirmed CT:

After completion of treatment of 1 year, the following monitoring is suggested:

- Clinical + serologic follow-up every 3 months for the second year of life
- Clinical + serologic follow-up every 6 months for the third year of life
- Clinical + serologic follow-up yearly thereafter, indefinitely
- If recurrence of eye disease is documented (beyond the neonatal period), treatment should be resumed for 3 months with pyrimethamine/sulfadoxine (with documentation of scarring of the lesions at the end of therapy)[a]
- If serologic rebound is documented, but without associated symptoms of recurrence (eg, without recurrence of eye disease), treatment is not indicated

Infants unlikely to be infected:

- There is no need for treatment of these infants
- However, serologic follow-up is needed every 2 months until *Toxoplasma* IgG antibodies are undetectable
- If subsequent serologic testing indicates CT, then the child should be treated

Wallon et al[3] (Lyon cohort)

Evaluation at birth includes:

- Head ultrasonography
- Ophthalmologic examination
- Neonatal blood testing for *Toxoplasma* IgM, IgA, and IgG

Individuals with proven infection were treated for 12 months (pyrimethamine plus sulfadiazine for 2 months, and then pyrimethamine plus sulfadoxine for 10 months)

- All children underwent a pediatric check-up and an assessment of neurologic development, every 3 months for at least 1 year
- All children underwent serologic testing for *Toxoplasma* IgG and IgM, every 3 months for at least 1 year
- Neurologic, ophthalmologic, and serologic testing were repeated every 3 months for the first 2 years of life
- Neurologic, ophthalmologic, and serologic testing were repeated every 6 months during the third year of life
- Neurologic, ophthalmologic, and serologic testing were repeated every year thereafter (without age limit)

Berrebi et al[131] (Toulouse cohort)

Evaluation at birth includes:

- Clinical evaluation
- Head ultrasonography
- Ophthalmologic examination
- Placenta/cord blood for parasitologic studies

Infants likely to be infected:

- Clinical and ophthalmologic follow-up every 1 month for the first year
- Clinical and ophthalmologic follow-up every 2 months in the second year
- Clinical and ophthalmologic follow-up every 3 months in the third year
- Clinical and ophthalmologic follow-up every 3–6 months afterward

Infants unlikely to be infected:

- Clinical plus ophthalmologic plus serologic follow-up every 3 months, until disappearance of *Toxoplasma* IgG antibodies; up to 12–18 months of age

[a]Timing of diagnosis of first chorioretinal lesion in infants/children with CT: In 75% of cases, the initial chorioretinal lesions were detected for the first time after the first 7 months of age, in 50% of cases after the first 3 years of age, in 25% of cases after 8 years of age, in 20% of cases after 10 years of age, and in 10% of cases after 12.5 years of age.[139] (Of note, the majority of these infants were treated once their mothers were diagnosed with acute *T gondii* infection during routine antepartum screening.)

infants in whom CT cannot be confirmed or ruled out. Some experts in France would elect not to treat infants who have no proof of infection at birth: cases with documented maternal seroconversion during pregnancy but with a negative evaluation (negative AF PCR assay result, normal fetal ultrasonogram, normal clinical and ophthalmologic evaluation at birth; normal imaging evaluation at birth; and no detection of *Toxoplasma* IgM and IgA at birth). Instead, they elect to simply follow those infants serologically every 2 months until complete disappearance of the *Toxoplasma*

IgG antibodies (that were presumably transplacentally transferred maternal *Toxoplasma* IgG antibodies). However, if follow-up serologic testing indicates congenital infection, these infants generally require treatment. A rationale for deferring treatment in those infants is that those infants had already received the benefit of antepartum treatment.

"Defer Treatment" Strategy for Newborn Infants in the United States

Cautious use of this "defer treatment" strategy for newborn infants in the United States is advised, because the majority of these infants' mothers did not receive antepartum treatment. According to the PAMF-TSL, all of the following criteria should be fulfilled before deciding to defer postnatal treatment of an infant in the United States: (1) acute primary maternal infection that was most likely acquired in the first or second trimester of pregnancy, (2) negative AF PCR assay result at ≥18 weeks, (3) no laboratory evidence of CT in the newborn (positive neonatal *Toxoplasma* IgG test result, at lower titers than maternal IgG titers, but negative *Toxoplasma* IgM and IgA test results), and (4) no clinical signs or symptoms of CT at birth after a complete clinical and radiologic evaluation (head ultrasonography may be used for CNS imaging in such infants).

The NPV of a negative AF PCR assay result for infections acquired in the first and second trimester is nearly 100% and, when all the aforementioned criteria are fulfilled, the risk of fetal *T gondii* infection is nearly zero. However, when maternal primary infection has occurred in the third trimester, even a negative AF PCR assay result cannot completely exclude the possibility of fetal infection. The risk of MTCT and fetal infection increases steeply with advancing gestational age; therefore, the decision to defer postnatal treatment in those settings should be carefully discussed with the parents.

Infants in Whom Toxoplasma IgG Titers Decrease Below Detection Levels During Follow-up

A decrease in neonatal *Toxoplasma* IgG titers below the detection level (during serial follow-up after birth every 4 to 6 weeks) can exclude CT in infants who have not received postnatal treatment. However, a decrease in titers below detection during postnatal therapy cannot be used as an indication that the infant was not congenitally infected, because antibody titers may become negative during therapy but can rebound after therapy discontinuation in infants with CT.[98]

PREVENTION

Identification of Women at Risk of Primary *T gondii* Infection During Pregnancy

In a study by Boyer et al,[25] only 48% of mothers of infants with CT had clinical symptoms suggestive of acute toxoplasmosis during pregnancy or had reported risk factors for *T gondii* exposure (eg, exposure to undercooked meat or to cat feces). Only routine serologic screening during pregnancy would have identified the rest of the women, who were nevertheless at risk of delivering infected infants.

In addition, only 49% of mothers of infants with CT who had serologically documented evidence of acute *T gondii* infection acquired from oocysts had also reported that they had significant risk factors for such exposures.[24] (Of note, laboratory tests to detect antibodies to sporozoite-specific antigens are performed only in research laboratories.[55])

Antepartum Maternal Screening and Neonatal Screening Programs

Among Western European countries, only France[3] and Austria[213] have implemented long-standing free national routine antepartum screening programs (monthly screening until the end of pregnancy in France since 1978[113] and once per trimester in Austria since 1975,[214,215] although shorter intervals [maximum every 8 weeks] were later advocated by experts). Screening once per trimester leaves a "blind period" during maternal infections late in pregnancy.[216]

Their approach was followed by other countries, including Italy and Slovenia. In 1998, Italy passed legislation that requires screening for toxoplasmosis of all pregnant women. This program was implemented mostly in the northern parts of Italy, and according to the latest Italian recommendations, screening should begin by 13 weeks of gestation and continue monthly until the end of pregnancy.[217] In Switzerland, screening was applied only regionally in the Basel region and only for surveillance purposes.[214,218] In many other European countries, antepartum screening was widely adopted by experts and physicians despite the absence of national mandatory antepartum screening programs; this phenomenon has been termed "wild screening."[214]

Neither France nor Austria complemented their antepartum screening programs with additional postnatal surveillance programs for CT for approximately 3 decades. A recent European survey of 28 European countries in 2007 identified only 4 countries that had surveillance screening programs

specifically for CT (France, Germany, Italy, and Denmark).[10] Twelve other countries had surveillance systems only for symptomatic toxoplasmosis (CT or noncongenital toxoplasmosis), and another 12 did not have any surveillance systems for toxoplasmosis. The neonatal screening program for CT in France started only in 2007. In Italy, neonatal screening for CT is only regionally applied to the Campania region (since 1997). Denmark[116] had a national neonatal screening program since 1997, but this program was discontinued in August of 2007 because it was thought that there was no benefit from postnatal treatment, because some infants with CT developed new eye lesions despite postnatal treatment (3 new eye lesions developed during a 3-year follow-up period among 32 infants evaluated[219]). However, children with CT in this program were treated for a very short period of time (3 months) as opposed to the longer 12-month postnatal treatment courses proposed in other European countries (Table 11). In 2007, a dedicated surveillance network for CT also was instituted in Greece.[220]

Cost-effectiveness of Antepartum and Postnatal Screening Programs for Toxoplasmosis in the United States

The preventive effect of toxoplasmosis screening of pregnant women depends on both the magnitude of disease caused by CT (the incidence of maternal infection during pregnancy multiplied by the risk of MTCT multiplied by the proportion of symptomatic infected children) and the preventable proportion of disease (the sensitivity of the screening strategy multiplied by the efficacy of the preventive treatment multiplied by the adherence to therapy).

Early studies evaluating the cost-effectiveness of antepartum toxoplasmosis screening programs yielded conflicting results.[8,221] The 1999 study by Mittendorf et al[221] concluded that antepartum screening was not cost-effective, whereas the most recent 2011 decision analysis by Stillwaggon et al[8] concluded that implementation in the United States of a universal monthly screening of pregnant women, following the French protocol, is cost-effective and leads to a savings of $620 per screened infant if the incidence of CT in the United States is greater than 1 per 10 000 live births. This decision analysis made a number of assumptions, including a cost of $12 per test, an estimated cost of fetal death of over $6 million, and an incidence of acute primary maternal infection during pregnancy of 1 in 1000 (including also sensitivity analysis for an incidence of acute maternal infections as low as 0.2 cases/1000 pregnant women). It also assumed that treatment was highly efficacious and inexpensive. Although the study concluded that screening in the United States would be cost-effective, it remains unclear whether these conclusions would be reached if data were used assuming higher costs of screening, lower costs of loss, and less efficacy of treatment. Previous decision analyses have not arrived at the same conclusions.[9] However, in this previous decision analysis, among the strategies analyzed was pregnancy termination for documented fetal infections. The early Cochrane systematic review on treatments for toxoplasmosis during pregnancy published in 2000 suggested that, in countries where screening or treatment is not routine, these technologies should not be introduced outside the context of a carefully controlled trial.[6]

Several assumptions made in the earlier decision analysis by Mittendorf et al[221] differ from current evidence. Mittendorf et al[221] assumed that antepartum screening tests had a very low specificity, which translated to a very low PPV for a relatively rare disease like CT. This assumption was incorrect for samples sent for further confirmatory testing at the PAMF-TSL,[164] because confirmatory testing at the PAMF-TSL has been shown to have a specificity of 100% for the differentiation of acute from chronic *T gondii* infections.[162,222,223] The diagnostic tests used at the PAMF-TSL have been extensively cross-validated against national reference laboratories in France by using sera from pregnant women with complete ascertainment of their *T gondii* infection status. Furthermore, AF PCR assay has a specificity and a PPV of nearly 100%,[114,172] which means that a positive AF PCR assay result is diagnostic of fetal infection. In addition, in the Mittendorf et al study, pregnancy termination rates were assumed to be very high (they assumed that 12.1 fetuses without CT would be aborted for every fetus truly diagnosed with CT).[221] According to recent evidence, pregnancy termination would not be routinely performed once the diagnosis of CT is made.[133] Confirmatory serologic testing in a reference laboratory and communication and interpretation of the results by an expert decreased the rate of unnecessary abortions by approximately 50%.[169] AF PCR assay and fetal ultrasonography to confirm or exclude fetal infection and symptomatic CT may further decrease the number of elective abortions. Further support against pregnancy terminations for CT is provided by the analysis of Berrebi

et al[133]; even among fetuses infected in the first trimester and with normal fetal ultrasonograms, 97% (35 of 36) would be either completely asymptomatic (78% [28 of 36]) or only slightly affected at birth (19% [7 of 36] had chorioretinitis and/or moderate ventricular dilatation). Of note, all of these fetuses had mothers who received antepartum treatment. The updated review of the French experience over the past 2 decades (1987–2008) described a fetal loss rate of 0.9% (N = 18) and a pregnancy termination rate of 1% (N = 21).[3] Moreover, in the SYROCOT international consortium of CT, the reported fetal death rate was similar (~2%; 22 terminations of pregnancy [1%] and 13 fetal deaths [0.7%] among the 1745 pregnant women with primary *T gondii* infections from the 26 international cohorts).[1]

In the more recent 2011 Stillwaggon et al[8] study, universal antepartum screening in the United States following the French protocol of monthly serologic screening during pregnancy (started at 11 weeks of gestation), antepartum treatment, fetal ultrasonography/AF PCR assay, and postnatal serologic and clinical follow-up of infants and treatment of infants as indicated was cost-saving. The findings from this analysis were robust to changes in the incidence of acute maternal primary infections, value of life, and test costs. Specifically, the screening approach included initial screening with commercial tests for IgG and IgM (at a cost of $12 per test) and subsequent confirmatory testing in reference laboratories of any positive results by using a panel of tests with high diagnostic accuracy and a specificity of 100%[163] (at a cost of $385 per confirmatory panel of tests). The cost of $12 per test included the costs for phlebotomy and reagents in a community hospital. Universal screening strategy remained a cost-saving approach, even for an incidence of maternal primary infections as low as 0.2 acute infections per 1000 women. This incidence would translate to approximately 0.5 CT cases per 10 000 children if a 25% MTCT risk is assumed. Moreover, the estimated cost of $12 per test is also in the cost range of several recently developed (already commercially available) tests for toxoplasmosis with novel technologies.

According to Calderaro et al,[162] who validated the diagnostic performance of 4 commercially available IgG and IgM tests for toxoplasmosis, there are commercially available IgG and IgM tests that have 100% analytical sensitivity and specificity. Moreover, according to the Wilson et al[163] validation published in 1997, of 6 commercial IgM tests, there are commercially available IgM tests with a specificity up to 98.6% (eg, the Vidas-Biomerieux IgM). In more detail, Calderaro et al showed that the sensitivity (point estimate) of all 4 commercial IgG assays tested was 100% and the specificity (point estimates) of the different IgG tests ranged between 98.5% and 100%. In the same study, the sensitivity (point estimates) of 4 commercial IgM tests ranged between 82.4% and 100% and the specificity ranged between 99.7% and 100%. Moreover, according to Wilson et al,[163] in an FDA-sponsored validation study of 6 commercial IgM tests, the sensitivity (point estimates) of these 6 IgM tests ranged from 93.3% to 100% and the specificity (point estimates) ranged from 77.5% to 98.6%. In reference laboratories, the specificity of confirmatory testing (for the diagnosis of a recently acquired acute infection) is 100%.[163]

Assumptions used in the decision analysis by Stillwaggon et al[8] were as follows:

1. a rate of acute maternal primary infections during pregnancy of 1.1 in 1000 (this is likely an overestimate, because it was based on US data from 1959–1966 and might not represent the incidence of acute *T gondii* infection during pregnancy in the current era with the decreasing overall seroprevalence rates; however, sensitivity analyses in this article revealed that the screening strategy would remain cost saving even at a rate of acute maternal infections as low as 0.2 per 1000 women);
2. a probability of fetal infection after acute maternal primary infection of 50%[98,224–228];
3. a probability of fetal death attributable to CT of 5%;
4. an estimated cost for fetal death attributable to CT of $6 million;
5. a probability of visual impairment from undiagnosed CT and no antepartum treatment of 48%;
6. a probability of visual and cognitive impairment of 45%; and
7. varying probabilities of visual impairment and visual/cognitive impairment for fetal infections by trimester.

These high rates of unfavorable outcomes from CT used in their model come from the older literature of infants with CT whose mothers were not treated during pregnancy.[8,98,133,224–228]

A study limitation that was acknowledged by Stillwaggon et al[8] was that this decision analysis model assumed that all mothers in the United States would receive care

by 12 weeks of gestation and would adhere to monthly follow-ups. However, this assumption might not represent a reality in the United States. Experience from France and Austria, where antepartum screening has been conducted for many years, indicated that late initial testing was common (25% of French pregnant women had their first test performed late) and there was also poor adherence to screening (only 30%–40% of pregnant women in France and Austria had all their mandatory antepartum screening tests completed).[97,214,216] It remains unclear how much a low adherence rate in pregnant women in the United States will affect the conclusions of this decision analysis. A follow-up decision analysis study should try to explore how changes in adherence rates with regard to the time of initiation of screening and the frequency of screening will affect the conclusions of this analysis.

Low-cost tests in state laboratories, combining screening for toxoplasmosis with screening for other congenital infections, may further reduce the screening cost (test cost and shipping cost) while keeping a centralized quality control for those screening tests (in the state laboratories) and maintaining high-quality confirmatory testing (at reference laboratories). Moreover, novel technologies are already widely used by several companies around the globe that provide low-cost serologic tests (eg, using microfluidics technologies in lateral flow devices, the recently introduced plasmonic gold technology).[229] Some of these technologies are already commercially available in the United States. Of course, previous rigorous validation of these novel tests is required to better understand their role in universal screening programs.

Moreover, the following changes in the future may make the universal screening strategy even more cost-effective: (1) "point-of-care test" technologies for serologic screening, (2) screening with the use of saliva specimens, and (3) multiplex screening[229] (multiplex IgG/IgM, which already are under evaluation and will soon provide further cost-saving alternatives for screening). In addition, screening for >1 congenital disease at the same time will provide further cost savings. Assessments of the feasibility of any universal antepartum screening strategy should take into account several strict criteria that should be fulfilled before any such strategy is considered feasible but should also take into account the rapidly changing picture in the field of novel diagnostic technologies.

In summary, the decision analysis article by Stillwaggon et al[8] indicated that prenatal screening and treatment of toxoplasmosis in the United States could be a cost-saving approach for congenital toxoplasmosis but only under the assumptions included in the model. Although several sensitivity analyses in their model (including variations for the main assumptions such as incidence of acute maternal infections, cost of tests and life-value equivalent) showed that the results were robust, the implementation of such a screening program would, nevertheless, require a significant reduction in the current cost of screening laboratory tests and significant changes in the structure of the US prenatal care system.

Aside from universal antepartum screening for toxoplasmosis, additional approaches to consider include the following: (1) antepartum education of women to avoid *T gondii*–related exposures during pregnancy and (2) ascertainment of adherence to antepartum screening, at least in high-risk women (eg, those with reported exposures through the oral route [oocyst/sporozoite-related exposures to cat feces and/or tissue cysts/bradyzoite-related exposures via ingestion of undercooked meat]; occupational exposures; immigrants from high-incidence areas; women traveling internationally during pregnancy; women with immunosuppressive diseases). However, physicians should be aware that selective screening of pregnant women on the basis of self-reported exposure risk factors has the potential to miss more than approximately 50% of women who give birth to infants with CT and thus probably a much larger number of acutely infected pregnant women.

If adherence to antepartum screening is poor, neonatal screening programs can at least capture infants with CT who were not detected in utero who would benefit from postnatal treatment. Nevertheless, the risk of adverse long-term sequelae in children in the United States who were only postnatally treated (and whose mothers did not receive antepartum treatment) is significant (85% with vision impairment, 36% with recurrences in eye disease, 27% with abnormal cognition at or after 3.5 years of age, and 16% with a decrease in IQ of >15 points).[112]

Primary Prevention Strategies

Primary prevention strategies in seronegative individuals are shown in Table 13. A survey of pregnant women in the United States indicated that the majority were not aware that acquisition of *T gondii* infection was associated with the

TABLE 13 Measures for Primary Prevention of *T gondii* Infection in Seronegative Pregnant Women, According to the PAMF-TSL Experience and CDC Recommendations[62]

Transmission	Recommendations/Considerations
Meat and other edibles	• Meat should be cooked up to at least 63°C (145°F) for whole cut meat (excluding poultry), up to at least 71°C (160°F) for ground meat (excluding poultry), and up to at least 74°C (165°F) for all poultry (whole cuts and ground) (a food thermometer should be used) • Meat should be frozen at −20°C (−4°F) for at least 48 hours • Freezing and thawing at specific temperatures for specific time can kill *T gondii* tissue cysts • Infected meat that has been smoked, cured in brine, or dried may still be infectious • Contact with mucous membranes should be avoided when handling raw meat • Gloves should be worn when handling raw meat and hands should be thoroughly washed after handling raw meat • Kitchen surfaces and utensils should be thoroughly washed after contact with raw meat • Drinking unpasteurized goat milk should be avoided • Eating raw oysters, clams, or mussels should be avoided • Skinning or butchering animals without gloves should be avoided
Untreated water	• Drinking untreated water, including that from wells, or water with potential contamination by feces from domestic or wild cats should be avoided
Cat feces and soil	• Contact with material/soil potentially contaminated with cat feces, especially handling of cat litters or gardening, should be avoided. However, if not possible to be avoided, disposable gloves should be worn when gardening and during any contact with soil or sand and hands should be washed with soap and warm water afterward. • Cats should be kept indoors. Stray cats should not be handled or adopted while the woman is pregnant. • Cat litter box should be changed daily, because *T gondii* does not become infectious until 1 to 5 days after it is shed in a cat's feces. • Cats should be fed canned or dried commercial food, not raw or undercooked meats.

CDC, Centers for Disease Control and Prevention.

consumption of undercooked meat.[230]

Effect of Antepartum Education on the Prevention of CT

The efficacy of preventive educational interventions targeting pregnant women has been studied by 2 cluster-randomized controlled trials with a total of 5455 women,[231] but both of them were of low methodologic quality and did not target any objective clinical outcomes. Studies in Belgium from 1979–1982 and 1983–1990 in which antepartum education started around the tenth week of pregnancy, and therefore could not significantly affect transmission rates in the first trimester, have shown that educational tools targeting pregnant women reduced seroconversion rates by 63% to 92%.[232] However, other epidemiologic studies, summarized in the systematic review by Gollub et al,[13] failed to replicate those findings.

A survey in 2006 of a random sample of 1200 US obstetrician/gynecologists revealed that although all respondents routinely counseled their pregnant women about risks associated with handling cat litter, fewer had counseled them about risks associated with eating undercooked meat (78%), handling raw meat (67%), gardening (65%), or the need to wash fruit and vegetables (34%). Of note, 73% of the respondents were not aware that *Toxoplasma* IgM tests from nonreference laboratories could have a high false-positive rate, and most (91%) were not aware of the *Toxoplasma* IgG-avidity test that could be used to determine the most likely time at which *T gondii* infection was acquired during pregnancy. The identified educational knowledge gaps were subsequently addressed in a practice bulletin from the American College of Obstetricians and Gynecologists. In 2012, an updated survey was circulated to members as well as nonmembers of the Collaborative Ambulatory Research Network to follow up on any accomplished changes.[233] Eighty percent of providers in that survey had diagnosed a nonacute primary *T gondii* infection in their patient population over the previous 5 years, 43% had performed serologic screening for at least some asymptomatic pregnant women (62% of those had used appropriate serologic tests), and 13% had correctly identified the role of *Toxoplasma* IgG avidity. Providers from the northeastern United States were 2 times more likely to routinely screen for *T gondii* than those in the West, and female providers were 1.5 times more likely to screen than male providers.[233]

It is important that physicians, general practitioners, and specialists caring for pregnant women and/or pediatric patients become more familiar with *Toxoplasma* serologic tests. For samples sent to the toxoplasmosis reference laboratories, clinical interpretation of the serologic test results and estimation of the most likely time at which acute primary maternal

infection with *T gondii* might have been acquired are provided to the referring physicians. (Additional practical information for the interpretation of the test results can be found on the PAMF-TSL Web site [http://www.pamf.org/serology/testinfo.html], and in the "Treatment" section of this report.)

KEY CONSIDERATIONS

1. Further studies understanding the benefits and limits of routine antepartum screening of all pregnant women in the United States for toxoplasmosis should be conducted. The cost estimates for universal CT prenatal screening per the French model appear to be unrealistic in the current American health care system. The implementation of such a screening program would require a significant change to the structure of American prenatal care.
2. Performing serologic screening only in pregnant women with known risk factors for *T gondii* infection or only in women with symptoms will fail to identify up to 50% of primary *T gondii*–infected women who are at risk of transmitting *T gondii* infection to their fetuses.
3. Large-scale observational studies support the benefit of early diagnosis and treatment of primary *T gondii* infection during pregnancy.
4. Observational studies support the benefit of postnatal treatment of infants with CT.
5. The time of initiation of antepartum treatment after acute maternal primary infection is critical and supports iterative screening.
6. According to the literature, the Centers for Disease Control and Prevention, FDA, and PAMF-TSL maternal serologic test results suggestive of acute *T gondii* infection in a pregnant woman (eg, positive IgM test result) should be confirmed in reference laboratories for toxoplasmosis, because false-positive test results in nonreference laboratories are not uncommon. Serologic testing of women with clinical suspicion of acute toxoplasmosis during pregnancy should be performed at a reference laboratory for toxoplasmosis to avoid unnecessary delays in the establishment of diagnosis and initiation of treatment that can affect the risk of MTCT and the risk of severe clinical manifestations of CT.
    1. Optimally, newborn infants with suspected CT should be evaluated by specialists, including experienced neonatologists, retinal specialists, neurologists, and pediatric infectious disease specialists.
    2. According to the PAMF-TSL, serologic testing of infants with suspected congenital toxoplasmosis should be performed at a reference laboratory for toxoplasmosis because of the availability of the highly sensitive IgM-ISAGA test as well as PCR testing for body fluids such as blood, urine, and CSF.

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ABBREVIATIONS

AC/HS: differential agglutination (test)
AF: amniotic fluid
CI: confidence interval
CNS: central nervous system
CSF: cerebrospinal fluid
CT: congenital toxoplasmosis
ELISA: enzyme-linked immunosorbent assay
EMSCOT: European Multicenter Study of Congenital Toxoplasmosis
EUROTOXO: European Toxoprevention Study
FDA: US Food and Drug Administration
GRADE: Grading of Recommendations Assessment, Development, and Evaluation
HR: hazard ratio
Ig: immunoglobulin
ISAGA: immunosorbent agglutination assay
MTCT: mother-to-child transmission
NCCCT: National Collaborative Chicago-Based Congenital Toxoplasmosis Study
NPV: negative predictive value
OR: odds ratio
PAMF-TSL: Palo Alto Medical Foundation Toxoplasma Serology Laboratory
PCR: polymerase chain reaction
PPV: positive predictive value
P/S: pyrimethamine/sulfadiazine
RCT: randomized controlled trial
SNHL: sensorineural hearing loss
SNSD: serious neurologic sequelae or death
SYROCOT: Systematic Review on Congenital Toxoplasmosis

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