

# Toxoplasmosis in the fetus and newborn: an update on prevalence, diagnosis and treatment

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*Toxoplasma gondii* is an unicellular coccidian parasite with worldwide distribution. It is estimated that more than a third of the world's population has been infected with the parasite, but seroprevalence is unevenly distributed across countries and different socioeconomic strata. The majority of newborns with congenital toxoplasmosis do not have any clinical signs of the disease at birth; however, 30–70% of those with clinical abnormalities were not detected initially, and are found to have new retinal lesions consistent with toxoplasmicchorioretinitis later in life. Congenital toxoplasmosis can also cause fetal death, stillbirths or long-term disabling sequelae, particularly among untreated infants. The disease appears to be more frequent and severe at certain latitudes. Congenital toxoplasmosis can be prevented and treated during gestation. Less severe disease is commonly reported in countries where prenatal screening and treatment have been systematically implemented. By contrast, severe disease appears to be observed primarily in infants born to untreated mothers. For definition purposes, it is best to use the term toxoplasma or *Toxoplasma gondii* infection when referring to asymptomatic patients with primary or chronic infection, and toxoplasmosis when referring to patients with symptoms or signs.

**KEYWORDS:** congenital toxoplasmosis • fetal infection • folinic acid • incidence • newborn infection • prenatal screening • prevalence • prevention • pyrimethamine • spiramycin • sulfadiazine • *Toxoplasma gondii* • treatment

## Organism, lifecycle & epidemiology

*Toxoplasma gondii* has the capacity to infect any warm-blooded animal. The parasite circulates in nature primarily in three infectious stages: the tachyzoite, the bradyzoite (with the capacity to form tissue cysts) and the sporozoite (which are formed inside oocysts). Tachyzoites, morphologically resemble a half-moon and have a specialized organ, the apical complex organelle, used to actively penetrate host cells. Tachyzoites are responsible for the symptoms and signs exhibited by patients during the primary infection or reactivation of a chronic infection. Transformation into tachyzoites (from the sporozoite or bradyzoite form) is required for the parasite to rapidly spread to cells and tissues, which results in parasitemia [1]. Most infections in nature occur by oral ingestion of the parasite, and thus, the initial site of infection is usually the GI tract. Following active penetration of the host's enterocytes, *T. gondii* spreads to the spleen, liver, lungs

and lymphoid tissue. However, it appears that the CNS, retina and cardiac and skeletal muscles are the main and ultimate target organs [2–4]. The bradyzoite, seeded in different tissues and grouped to form cysts, is responsible for chronic infection. The transition from the tachyzoite to bradyzoite stage requires the intervention of a normal immune system.

Members of the are the definitive host of the parasite, whereas humans and other warm-blooded animals are intermediate hosts. The sexual cycle of *T. gondii* takes place only in the small intestine of members of the felidae family, and it facilitates the formation of atypical strains. Unusual and virulent strains can be potentially generated when radically different strains meet in the GI tract of wild cats that are capable of moving through vast tropical areas [5,6]. The epithelial cells can be infected by tachyzoites, bradyzoites or sporozoites. Schizogony (endopolygeny) takes place in the intestinal

lumen, and schizonts are formed in the intestinal tract, in 3–15 days, to give rise to gametes. After fertilization, the female gamete becomes a zygote and then an immature oocyst. Oocysts are first expelled into the intestinal lumen and then to the outside in feces: sporogony and maturation of the oocysts can take place, in 1–5 days, only in the outside environment [7]. Felids can shed up to 10 million oocysts a day for 20–24 days following initial infection. Sporulation is required for oocysts to become infectious, a process facilitated by warm and moist soils; sporulated oocysts remain infective in the soil for up to 18 months [1]. An asexual cycle can also take place outside intestinal tissues in members of the felidae family.

In intermediate hosts, *T. gondii* undergoes asexual development alone, and it does it in two phases: in the first phase, tachyzoites multiply rapidly by repeated endodyogeny in any nucleated host cell; and in the second phase, tachyzoites of the last generation determine the formation of tissue cysts where bradyzoites multiply slowly by endodyogeny. Tissue cysts mark the terminal stage in the lifecycle in the intermediate host and are infectious. Tissue cysts can also be found in felids when some zoites (sporozoites, tachyzoites or bradyzoites) break up the intestinal lamina propria, get phagocytized and multiplied by endodyogeny.

The genome of *T. gondii* is available on the TOXO DB website [101]. The parasite appears to circulate in nature in three main clonal strains, primarily isolated from developed areas of the world. However, atypical and more complex strains appear to be increasingly reported from tropical areas in South America and North America. To date, the majority of isolates from North America and Western Europe belong to one of the three closely related clonal lineages (referred to as types 1, 2 and 3) [8,9]. Clonal propagation is probably favored by the ability of *T. gondii* to be transmitted between intermediate hosts via ingestion of tissue cysts, a feature that distinguishes it from related parasites [10]. The major clonal lineages in North America are thought to have resulted from a few natural genetic crosses between highly similar parental types, the progeny of which expanded to give rise to the clonal population structure during the past 10,000 years [10,11]. Recent studies have suggested that the ancestral type 2 lineage has been the common parental stock for at least three independent crosses that led to clonal expansion; in addition, this study also suggested that some of the North American strains possess unique alleles or uncommon combinations of alleles [12]. These atypical isolates, formerly designated as X, A and 2, were found to comprise an entirely new and unique lineage designated as 12, which appears to be abundant in North America. Further analysis of group 12 revealed that it also has a clonal population structure. To date, type 12 strains have not been reported in Europe, which otherwise share a preponderance for the three clonal types, with type 2 being especially common [8,9]. This suggests that type 12 may be endemic to North America, possibly as a result of genetic recombination with a native North American isolate and ancestral type 2. Despite the presence of a fourth major group in North America, the overall population structure remains highly clonal, suggesting that *T. gondii* primarily has four major clonal lineages. Group 12 includes a number of isolates from both wild animals

and several humans, which were formerly grouped as atypical isolates based on restriction fragment length polymorphism analysis [8]. This group also contains members of types X and A, which have previously been associated with severe encephalitis in sea otters in western USA [13,14].

It appears that there is a predilection of certain strains for different geographical locations. In western Europe, type 2 strains appear to predominate and type 1-like or atypical strains are usually reported only in imported meat or patients infected abroad. By contrast, in South America, type 1 and 3 strains are widely distributed and type 2 strains are rarely found [15]. North America appears to have a mixed picture of 1/3 and 2 strains. Recent reports suggest a correlation between type 1, 3, 1/3 mixed genotypes or atypical strains, and unusual and more aggressive clinical manifestations in humans [16]. Children with congenital toxoplasmosis born in Brazil appear to have a more aggressive ocular disease than those born in Europe [17]. Primary infection in adults in South America have resulted in community-acquired pneumonia, acute hepatitis, fever of unknown origin and death in HIV-negative and immunocompetent individuals [18,19]. Regarding genetics of the host, susceptibility for the development of disease following infection in humans has also been suggested. For example, the *HLA DQ3* gene has been associated with cases of severe hydrocephalus in congenital toxoplasmosis and in toxoplasmic encephalitis in AIDS patients [20,21]. In addition, polymorphisms in *ABCA4* have been associated with ocular and brain disease, whereas polymorphisms in *COL2A1* encoding type 2 collagen have only been associated with ocular disease [22].

Transmission occurs when oocysts, present in food, water and tissue cysts (present in raw or undercooked meat) are ingested [2]. Other routes of transmission have been documented including organ transplantation (i.e., when seronegative patient receives an allograft from an infected donor) or laboratory accidents. Transfusion of blood products has rarely been reported. In a recent study, the following risk factors were associated with acute infection in the USA: eating raw ground beef; eating raw lamb; eating locally produced cured, dried or smoked meat; working with meat; drinking unpasteurized goat's milk; and having three or more kittens. Ingestion of raw oysters and clams was also reported to be a novel route of transmission [23]. Untreated water has been reported to be the source of major outbreaks of acute toxoplasmosis in Canada [24] and Brazil [25], and was found to be associated with acute infection in the USA (although it was not statistically significant due to sample size) [23]. Similarly, in a small case-control study from South America in pregnant women, drinking bottled water was reported to be protective [26] when compared with other sources of drinking water [26]. It appears that a significant proportion of infections in the USA is derived from ingestion of oocysts. Recently, Boyer *et al.*, reported that 59 out of 76 (78%) mothers of congenitally infected infants had probably been infected with oocysts [27]. The presence of antibodies specific to antigens only present in sporozoites was used as the marker for infection with oocysts.

It is important to emphasize that in all epidemiological studies, attempting to determine the risk factors associated with acute

**Table 1. Incidence of vertical transmission and prevalence of clinical signs in infants born to mothers who were infected with *Toxoplasma gondii* during gestation.**

Study	Variable measured	Gestational age at which the mother acquired <i>Toxoplasma gondii</i> infection, % (95% CI)			Ref.
		13 weeks	26 weeks	36 weeks	
Dunn <sup>†</sup> , n = 603	Incidence of vertical transmission	6% (3–9)	40% (33–47)	72% (60–81)	[29]
	Risk of clinical signs present or developed before 3 years of age	61% (34–85)	25% (18–33)	9% (4–17)	
SYROCOT <sup>‡</sup> ; n = 1438	Incidence of vertical transmission	15% (13–17)	44% (40–47)	71% (66–76)	[30]
Wallon <sup>§</sup> ; n = 377	Incidence of vertical transmission	1st trimester: 4.5% (2–9)	2nd trimester: 31.7% (24–41)	3rd trimester: 62.8% (52–73)	[36]

<sup>†</sup>More than 84% received treatment.  
<sup>‡</sup>83% received treatment.  
<sup>§</sup>92% received treatment.  
n: Number of women who seroconverted during gestation.

toxoplasma infection, a specific risk factor is usually found in up to 50% of the patients [28]. Thus, although education most probably plays a role in the prevention of primary infection, it has a limited value. Many individuals have been documented to have acquired their primary infection without having a specific known risk factor for acute toxoplasma infection. This observation explains why pregnant women have been infected with the parasite during gestation, despite the fact that they did not have any of the known dietetic or hygienic risk factors during gestation. It also justifies the need to implement systematic serological programs (i.e., routine serologic screening in seronegative pregnant woman) aimed at the prevention and early treatment of congenital toxoplasmosis.

Congenital toxoplasmosis primarily occurs in mothers infected with *T. gondii* for the first time during gestation and in whom the parasite crosses the placenta and infects the offspring [29,30]. However, although rare, three exceptions to this dictum have been described in the literature: women who acquire their primary infection shortly before conception (i.e., within 3 months of conception) [31], those chronically infected with *T. gondii* who are infected with a different but more virulent strain (e.g., with an RH-like strain) [32] and immunocompromised women whose immunodeficiency is significantly exacerbated during gestation (e.g., AIDS patients who develop toxoplasmic encephalitis during gestation) [33,34]. It appears that infection of the placenta is a *sine qua non* for fetal infection to take place. However, placental infection does not necessarily result in fetal infection. It is likely that duration and magnitude of the initial parasitemia in the mother influence the transmission of the parasite to the fetus [3]. During this initial parasitemic phase, before effective immune responses are developed, tissues including the placenta can be successfully infected. Tachyzoites can replicate in the placenta and reach the fetus through the placental circulation. Some observations suggest that the infection of the placenta can be a source of fetal infection even long after maternal parasitemia has subsided [35]. The delay in fetal infection from the placental stage to the fetal tissues is apparently inversely related to the gestational age; the older the gestational age at which the mother is infected, the

shorter the delay of infection of *T. gondii* from the placenta to the fetus [3]. The most important biological variable that determines the incidence and severity of infection in the fetus and newborn is the time of gestation when maternal infection was acquired. The incidence of mother-to-child transmission increases with gestational age; mothers infected at later stages of gestation have a higher incidence of fetal infection. Severity of congenital disease is inversely correlated with the time of gestation when maternal infection was acquired. The majority of stillbirths, abortions, substantial brain necrosis and hydrocephalus caused by *T. gondii* infection in the fetus are reported when the mother was infected by the parasite earlier in gestation, whereas a significant number of neonates without clinical signs of infections or mild infections are reported when the mother was infected later in gestation (TABLE 1) [29,30,36]. However, an exception to the latter dictum may occur when pregnant women become infected in the third trimester with atypical and highly virulent strains [37].

### Prevalence of toxoplasma infection & incidence of congenital toxoplasmosis

The seroprevalence of *T. gondii* varies according to age, geographical locale and socioeconomic strata of a specific population [38]. Seroprevalence is probably determined by the likelihood of a specific population or a group of individuals within a specific population to have been exposed to oocysts (e.g., in cat feces, soil, water, vegetables and plants) or tissue cysts (ingesting or handling meat). In northern European countries the seroprevalence can be as low as 10%, whereas in some areas of Brazil [39] and in Madagascar [40] it can be as high as 80%. In the USA, Jones *et al.* estimated that the overall age-adjusted seroprevalence of *T. gondii* infection is 11% [23]. In the majority of developed countries, including the USA, the seroprevalence has been declining throughout the past decade, reflecting improved sanitation and meat processing policies, whereas in some developing countries, the seroprevalence has reported to be stable or even increased [38]. Although hygienic and alimentary habits at the individual level within a specific population are likely to impact their toxoplasma seroprevalence, water

sanitation, meat processing and other measures, at the health policy and government level, are also important.

The incidence of maternal seroconversion during gestation in the USA has been estimated to be between 0.2 and 1%. This range is similar to that reported by other countries. The overall rate of mother-to-child transmission ranges from 50 to 60% in mothers who have not been treated during gestation, and 25–30% in those who are systematically screened and treated during pregnancy [3]. The incidence of congenital toxoplasmosis per 10,000 live births significantly varies across geographical locations. In the USA, it is estimated in one case per 10,000 live births [41]. Thus, of 4 million births per year in the USA, 400 cases of congenital toxoplasma infection are estimated to occur every year [42]. However, Roberts and Frenkel estimated that the annual incidence of congenital disease in the USA probably ranged from 500 to 5000 infants [43]. Recently, in France, it was reported in 2.9 per 10,000 live births [44]. In certain countries, such as Brazil, rates as high as nine per 10,000 live births have been reported [45], while in other areas, such as the UK, symptomatic congenital toxoplasmosis was estimated in 0.34 per 10,000 [46].

### Immune response

A well-orchestrated cellular, humoral and innate immune response must be triggered upon parasite invasion, in order to prevent the uncontrolled proliferation of tachyzoites. Immune responses have been reported to be responsible for controlling parasite replication, including the activation of the monocyte–macrophage system, dendritic cells, natural killer cells, *T. gondii*-specific and cytotoxic CD4<sup>+</sup> and CD8<sup>+</sup> T cells. In addition, costimulatory molecules (e.g., CD28 and CD40 ligand) and cytokines, including IFN- $\gamma$ , IL-12, TNF- $\alpha$ , IL-10 and TGF- $\beta$  have also been implicated [47–51]. The role of Toll-like receptors (TLRs) – TLR3, 7 and 9 – in the innate immunity response against *T. gondii* has recently been proposed in a mouse model [52]. It appears that TLR recognition, such as that of TLR11, can also be critical for the prevention of pathogen-induced immune destruction of self-tissue [53]. It also appears that MyD88, IL-12 and IFN- $\gamma$  have a primary role during the early stages of infection (in the site of parasite entry at the mucosa and other peripheral organs), whereas CD8<sup>+</sup> T cells would be critical for controlling parasite replication and cyst formation in the CNS [48,54].

In cases of congenital toxoplasma infection, a delayed or diminished antigen-specific CD4 T-cell response has been reported. Following an effective immune response that clears the majority of tachyzoites, very few tachyzoites can persist and convert to the metabolically slower bradyzoite. The bradyzoite form, within tissue cysts, successfully escapes the effector capacity of the immune system. It appears, however, that an intact immune system is required to prevent a reversal of the bradyzoite to tachyzoite stage. If a significant depletion of T-cell-mediated immune response ensues, reversal of bradyzoites into rapidly proliferating tachyzoites will result in reactivation of the parasite leading to disease; this is the case of toxoplasmic encephalitis or disseminated toxoplasmosis in AIDS patients or other immunocompromised patients [1].

### Clinical manifestations of congenital toxoplasmosis in the fetus & newborn

A wide spectrum of clinical presentations in the fetus and newborn has been described, ranging from death to complete absence of clinical signs [3]. Factors likely to impact the incidence and severity of clinical signs include gestational age, genetics of the host and parasite, size of the inoculum, infecting form of the parasite (oocysts tissue cyst) and maternal treatment. In some countries where serological screening and prenatal treatment is systematically offered to pregnant women, such as France, the majority of the cases of congenital *T. gondii* infection do not have overt clinical disease during gestation or the neonatal period [44]. By contrast, in other areas of the world where no serological prenatal screening is implemented, such as the USA and Latin America, higher mortality rates and more severe cases have been observed [3,17,30,55–57].

Clinical manifestations in the fetus range from ultrasounds not revealing any abnormality to fetal death. Fetal ultrasounds may also reveal hydrocephalus, brain or hepatic calcifications, splenomegaly, pericarditis and ascites.

Clinical manifestations in the infant include chorioretinitis, encephalitis, seizures, abnormal cephalic perimeter (microcephaly, macrocephaly and hydrocephalus), nystagmus, hypotonia, palsies, spasticity, brain or hepatic calcifications, psychomotor or intellectual disability, splenomegaly, hepatomegaly, ascites, pericarditis, pneumonitis, diarrhea, hypothermia, jaundice, petechiae, skin rash, hearing loss or intrauterine growth retardation. The classic triad of chorioretinitis, hydrocephalus and brain calcifications is highly suggestive, but not necessarily diagnostic of congenital toxoplasmosis. Neurological manifestations in the newborn can be present as the sole manifestation of the infection or associated with other symptoms of disseminated disease [58,59]. Long-term sequelae include psychomotor retardation, visual and hearing impairment (potentially leading to blindness and deafness) [3,60]. Visual impairment is the most common long-term sequelae, and can greatly impact the quality of life of congenitally infected children. Although most severe cases are diagnosed during the first month of life, severe disease can sometimes only become obvious in the second or third month of life. However, in a recent study, Peyron *et al.* reported that congenital toxoplasmosis, when treated, appears to have little effect on the quality of life and visual function of infected individuals [61].

Congenital toxoplasmosis can occur in HIV-infected pregnant women who are chronically infected with the parasite, particularly in those who reactivate their toxoplasma infection during gestation (e.g., toxoplasmic encephalitis). These children appear to have a more rapid and disseminated disease than those not infected with HIV, including failure to thrive, fever, hepatosplenomegaly and seizures [2].

### Differential diagnosis

The clinical manifestations of congenital toxoplasmosis can also be observed in various combinations in other infections, including cytomegalovirus, HSV, rubella, syphilis, parvovirus B19, listeriosis and lymphocytic choriomeningitis virus [2,3].

## Laboratory diagnosis

As many infected fetuses and newborns do not exhibit any clinical signs at birth, performing laboratory tests only in those who exhibit clinical manifestations will fail to identify the majority of infected infants at birth. Laboratory methods available for the accurate diagnosis of *T. gondii* infection and toxoplasmosis include serological tests, PCR, histological and cytological examination of tissue and bodily fluids and isolation of the parasite (Box 1).

### Diagnosis of *T. gondii* infection in the fetus

The diagnosis of fetal infection during gestation can be accomplished by the use of PCR in amniotic fluid (AF). This test is usually reserved for women in whom the diagnosis of acute toxoplasma infection acquired during gestation has been established or is highly suspected, women in whom fetal abnormalities suggestive of congenital toxoplasmosis have been found by ultrasound or women chronically infected with *T. gondii* in whom reactivation of the parasite might have occurred during gestation due to immunosuppression.

If safe and feasible, AF-PCR should be performed at 18 weeks of gestation or later. Sensitivity of the AF-PCR varies according to the trimester in which the mother acquired the infection. The sensitivity of the test for pregnant women, whose maternal infection was estimated to have been acquired during the first trimester, has been reported to be between 33 and 75%; during the second trimester, it was between 80 and 97%; and during the third trimester, it was between 68 and 88%. The specificity is likely to be 100% regardless of the trimester of maternal infection, assuming that laboratory contamination has been carefully ruled out. In general, AF-PCR is an excellent method to rule out infection in the fetus of an infected mother, with a negative predictive value of 96–100% during the first trimester; 93–100% for the second trimester and 48–98% for the third trimester [62–64]. It is possible that sensitivity of the test can be enhanced by the selection of specific primers and probes, DNA target and volume of the sample. Several European investigators have reported that the 529 gene target appears to be more sensitive than the most commonly used *BI* target [36,65].

Previously, performance of amniocentesis was considered contraindicated in HIV-positive women owing to the risk of infecting the fetus with HIV during the procedure. However, Mandelbrot *et al.* reported that the risk of transmission from HIV positive mother to the fetus was negligible if the mother was receiving antiretroviral therapy (ART). Current French guidelines recommend the initiation of ART prior to performing amniocentesis in HIV-infected women. The objective is to obtain an undetectable viral load before the procedure [66]. Thus, in cases of HIV-positive women with evidence of seroconversion for *T. gondii* infection or suspicion of reactivation of toxoplasmosis during pregnancy, amniocentesis should be ideally performed once the HIV viral load is undetectable after initiating ART.

Serial fetal ultrasounds should be performed in pregnant women suspected to have or diagnosed with acute *T. gondii* infection. Findings suggestive of congenital toxoplasmosis include CNS and hematopoietic system abnormalities [2,3].

## Box 1. Diagnosis of congenital toxoplasmosis in the fetus and newborn.

### Fetus<sup>†</sup>

- Obtain AF by amniocentesis at or after 18 weeks of gestation<sup>†</sup>
  - PCR aimed at the detection of *Toxoplasma gondii* DNA
- Obtain serial fetal ultrasounds
  - Every 4 weeks throughout gestation

### Newborn<sup>†</sup>

- Clinical history and physical examination
- Pediatric neurologic evaluation
- Pediatric ophthalmologic examination
- CBC with differential and platelet count
- Liver function tests (including direct bilirubin, GGTP)
- Peripheral blood (serum) for *Toxoplasma gondii*-specific immunoglobulins
  - IgG (the dye test is the gold standard for the detection of IgG)
  - IgM ISAGA (5 days after birth)
  - IgA ELISA (10 days after birth)
- PCR
  - Peripheral blood, urine or CSF if a lumbar puncture is indicated
- CSF analysis if lumbar puncture is indicated
  - Cell count with differential protein and glucose
- ABR or OAE before 3 months of age
  - Full audiologic evaluation before 24–30 months of age
- Head ultrasound or CT scan (without contrast)
- Serial ophthalmologic evaluation

<sup>†</sup>Inoculation of AF and newborns' tissue in mice or tissue culture at reference laboratories (e.g., Palo Alto Medical Foundation Toxoplasma Serology Laboratory, CA, USA [105]) can be performed in an attempt to isolate and genotype the parasite. See the main text for further explanation.

AF: Amniotic fluid; ABR: Auditory brainstem response; CBC: Complete blood cell count; CSF: Cerebral spinal fluid; GGTP:  $\gamma$ -glutamyl transpeptidase; ISAGA: Immunosorbent agglutination assay; OAE: Otoacoustic emissions.

Attempts to isolate the parasite from AF can be useful in an attempt to genotype the parasite strain and perform pathogenesis studies in animal models. However, in clinical practice, they are seldom used owing to the cost and long turnaround time [2,3,63].

When available, placental or fetal tissue (abortions and stillbirths) can be used in an attempt to perform PCR, isolation or histological analyses at reference laboratories.

### Diagnosis of *T. gondii* infection in the newborn

Physicians should be aware that infected newborns might appear completely normal at birth or only have subtle alterations in their physical examination. In addition to testing the clinical specimens from the newborns, newborns suspected to have congenital toxoplasmosis and whose mothers had no serological testing for toxoplasmosis during gestation, serological testing should be performed in the mother after birth in an attempt to determine whether the mother could have been infected during gestation. The authors recognize that serological test results of the mother after birth might be difficult to interpret, and that the assistance of a reference laboratory is indicated in this instance. In the authors' experience, several cases of acute infection acquired

during gestation have been diagnosed in the mother when testing her post-delivery serum. In addition to a complete clinical history and physical examination, complete neurological and ophthalmological evaluations should be performed. A retinal specialist with expertise in the examination of newborns is recommended, if available.

The definitive diagnosis of congenital toxoplasmosis in the newborn can be accomplished by the use serological tests and PCR. A positive toxoplasma IgG in an infant of 12 months of age is considered diagnostic of congenital toxoplasmosis, and is considered the 'gold standard' for ultimate and definite laboratory diagnosis. By contrast, a negative toxoplasma IgG, at 12 months of age or earlier in an infant capable of producing IgG antibodies and not receiving antitoxoplasma treatment, rules out the possibility of congenital toxoplasmosis. Infants born to chronically infected mothers will be born with their maternal toxoplasma IgG because of passive transfer of IgG across the placenta from the mother to the fetus. Thus, these infants can be recognized, as their IgG titer at birth should be similar to that of the mother and decreases according to the half-life of the IgG (4 weeks): titers should decrease by at least 50% every month until they disappear before 1 year of age. Caution should be exercised in infants treated with antitoxoplasma drugs [67]. During treatment, toxoplasma IgG in treated infants usually declines and can become negative; but once the treatment is discontinued, it rebounds and becomes positive again [68].

Serological diagnosis can also be made in newborns with positive toxoplasma IgM or IgA antibody titers, 5 or 10 days after birth, respectively (in order to exclude maternal blood contamination). The immunosorbent agglutination assay (ISAGA) method for IgM and the ELISA method for IgA have been found to have superior performance for the diagnosis of congenital toxoplasmosis in the infant. Low positive IgM ISAGA test results can be observed in newborns who have received transfusion of blood products, and these results are more often false positive [2]. The IgM ELISA and IgA ISAGA can also be performed in serum. However, it appears that the IgM ISAGA is a more sensitive method than the IgM ELISA for the diagnosis of congenital disease [69].

Analysis of cerebrospinal fluid (CSF) can be helpful in infants suspected of being infected with *T. gondii* and who have clinical signs and imaging studies suggestive of CNS involvement. CSF can be obtained and analyzed by serological, PCR and other routine tests, if the lumbar puncture is safe and feasible. A positive *T. gondii*-specific IgM in fluid CSF is diagnostic of congenital disease, whereas positive toxoplasma IgG titers probably reflect passive transfer of serum toxoplasma IgG [3]. A positive *T. gondii* PCR in the CSF, peripheral blood and urine of the newborn is considered diagnostic of congenital toxoplasmosis. These tests should be attempted in every newborn suspected to be infected with the parasite, and specimens should be ideally obtained before treatment is initiated [36,70,71]. Analyses of CSF for tests other than PCR can be helpful. Congenital toxoplasmosis is one of the rare entities that can produce CSF eosinophilia or extremely high levels of protein (up to 1000 g/dl) [72].

Brain imaging studies of the newborn may reveal calcifications or hydrocephalus; CT scan without contrast is superior to ultrasound examination in the detection of these CNS abnormalities [3,41]. An MRI scan of the brain, although useful, can be hazardous for the newborn owing to the need for sedation. Calcifications can also be detected in the liver ultrasound of infected infants.

A routine evaluation of the eyes performed by an experienced ophthalmologist is recommended every 3 months until 18 months of age followed by every 6–12 months until 18 years of age. However, the frequency of evaluations should be modified according to the severity of the disease. Any new visual symptom or sign should prompt immediate evaluation regardless of routine visits [61,73]. Screening for hearing loss with either auditory brainstem responses or otoacoustic emissions should be performed periodically during the first year in newborns in whom congenital toxoplasmosis is suspected or confirmed. Both methods appear to be reliable in infants younger than 3 months of age [74]. All infants with congenital *T. gondii* infection should have a complete auditory evaluation, if they fail the screening tests. In addition, all infected infants regardless of surveillance findings should be referred for an audiologic assessment at least once in the first 24 months of age [75].

Infected newborns with clinical or subclinical infection can present with leukopenia or leukocytosis. They may also have peripheral lymphocytosis, monocytosis and/or eosinophilia. Anemia and thrombocytopenia have also been frequently reported. Elevated liver enzymes and indirect bilirubin levels have also been described in disseminated disease [3].

## Treatment

### Prevention of fetal infection

An attempt to prevent maternal infection through education should be implemented in all obstetric practices. However, it should be recognized that up to 50% of women can become infected during gestation, even if they do not exhibit any of the conventional risk factors for acute infection or any illness suggestive of acute toxoplasmosis (TABLE 2). Thus, the attempt of suppressing the maternal behaviors that can lead to acute infection through education can be effective only in approximately 50% of the targeted population. In order to diagnose 100% of the acute maternal infections during gestation, systematic serological screening in all pregnant women should be carried out. Women initially found to be seronegative for both IgG and IgM can be followed during gestation in order to detect seroconversion. The early detection of acute maternal infection will empower parents with the information regarding their risk of congenital toxoplasmosis. In addition, it will also allow the initiation of early treatment with drugs, such as spiramycin, in an attempt to decrease mother-to-child transmission (TABLE 3). Spiramycin is a macrolide antibiotic that is active against *T. gondii* and has been reported to decrease the frequency of vertical transmission, especially if initiated earlier following seroconversion of the mother [3,76]. Spiramycin achieves very high tissue levels in the placenta. In studies using historical controls, the incidence of congenital toxoplasmosis has been reduced by 60% [58]. The authors recommend spiramycin for

**Table 2. Measures for primary prevention of congenital toxoplasmosis in seronegative pregnant women.**

Category	Preventive measures
<i>Infection with oocyst</i>	
Untreated water and edibles	Avoid drinking untreated water, including from wells or reservoirs that have not been secured from potential contamination by feces from wild or domestic cats Avoid consuming vegetables and fruits not washed or washed with untreated water
Contaminated soil	Avoid contact with materials potentially contaminated with cat feces, especially handling of cat litter or gardening Wearing gloves is recommended when these activities cannot be avoided
<i>Infection with tissue cyst</i>	
Infected meat and other food	Cook meat to 'well done' or thoroughly to 67°C Meat should not be 'pink' in the center Freeze meat to -20°C for at least 48 h Note that meat that is smoked, cured in brine or dried may still be infectious Avoid contact with mucous membrane when handling raw meat Wash hands carefully after contact with raw meat Kitchen surfaces and utensils that have come in contact with raw meat should be washed wearing gloves Refrain from skinning or butchering animals without gloves Avoid drinking unpasteurized goat's milk Avoid eating raw oysters, clams or mussels

Up to 50% of individuals can become infected with *Toxoplasma gondii*, even if they do not participate in behaviors associated with the acute infection [28].

pregnant women who are suspected to have or have been diagnosed with acute infection or toxoplasmosis during the first 18 weeks of gestation or shortly before conception until amniocentesis is performed and results of AF-PCR are available [77]. Spiramycin should be continued for the duration of the pregnancy if the AF-PCR is negative for the theoretical possibility that the placenta might have been infected and had not reached the fetus at the time of the amniocentesis, but do so later during gestation. Spiramycin is commercially available in Europe and Canada. The drug is not commercially available in the USA, but it may be obtained through the US FDA, following consultation with the Palo Alto Medical Foundation Toxoplasmosis Laboratory, or the US National Collaborative Treatment Trial Study (Chicago, IL, USA). For many years, Sanofi-Aventis has been providing spiramycin to pregnant women in the USA at no cost. Spiramycin is given at a dose of 1 g (3 million U) every 8 h (total dosage of 3 g or 9 million U per day) [77]. Allergic manifestations, GI tract intolerance and paresthesias have been reported during treatment [78]. Spiramycin is not efficacious for treatment of congenital toxoplasmosis, and should not be given in cases of documented fetal infection (e.g., positive AF-PCR) or in cases where women seroconvert after week 18 of gestation until fetal infection can be satisfactorily ruled out, as the rates of transmission later in gestation are higher and the negative predictive value of the AF-PCR is lower [77].

Of note, several investigators have cast doubt over the efficacy of spiramycin in impacting vertical transmission of *T. gondii* [30,79,80]. In a study of 1438 infected mothers who were treated during pregnancy (from 18 prenatal screening cohorts), the authors reported that the sooner the prenatal treatment was started after seroconversion, the lower the adjusted odds of mother-to-child transmission (odds ratio [OR]: 0.94 per week; 95% CI: 0.90–0.98), and that compared with mothers treated after 8 weeks of seroconversion (upper quartile of delay from seroconversion), mothers

treated earlier tended to have a lower odds of mother-to-child transmission, particularly if prenatal treatment was initiated within 3 weeks after seroconversion [30]. In a second analysis, the authors obtained data on clinical manifestations in 691 infected infants (from 26 cohorts, 19 prenatal screening and seven neonatal screening cohorts). Their analysis revealed that the adjusted odds of any clinical manifestations did not significantly differ between infants of treated mothers and those of untreated mothers (OR: 1.11;  $p = 0.74$ ). From this study, the authors concluded that their results constituted weak evidence for an association between early treatment and reduced risk of congenital toxoplasmosis, and advocated for a large randomized controlled clinical trial that could provide clinicians and patients with valid evidence of the potential benefit of prenatal treatment. Several major biases in study design and interpretation have revealed a greater strength in favor of prenatal treatment. It is an assumption that the efficacy of spiramycin can be simply determined by estimating the frequency of infected versus uninfected newborns in the treated versus untreated mothers. In this model, an infected newborn is counted as a 'failure' and an uninfected newborn as a 'success' of the drug effect. However, the antiparasitic effect and pharmacokinetics of the drug favor a different conceptual frame. It is understood that spiramycin partially, but not completely, decreases the frequency of vertical transmission; in addition, it is also likely that in some instances, spiramycin will only decrease the toxoplasma parasitic load in the fetus, without necessarily avoiding fetal infection. Thus, it is biologically possible that spiramycin prevents transmission in some cases and decreases severity in others. Thus, offspring with a lower parasitic burden, who were spared early from severe disease, will later contribute to an increase in the number of infected infants at the end of gestation (most probably with milder forms of the disease). The net effect of such intervention would be an overall decrease of severe cases and death, but only a 'marginal' effect on the frequency of

**Table 3. Drug regimens to prevent and treat congenital toxoplasmosis.**

Indication for initiation of drug regimen	Objective	Medication and per oral dosage	Indications for continuation or changing drug regimen	Comments
Maternal infection is highly suspected or documented to have occurred 3 months before conception or during pregnancy but before 18 weeks of gestation, or immunocompromised women suspected of having reactivated latent <i>Toxoplasma gondii</i> infection	Prevention of fetal infection	Spiramycin; 1 g (3 million U) every 8 h (total of 3 g or 9 million U per day)	If serial fetal ultrasounds are normal and AF-PCR is negative continue spiramycin until the end of gestation. If fetal ultrasound is abnormal (suggestive of congenital toxoplasmosis) or AF-PCR is positive switch spiramycin to pyrimethamine/sulfadiazine/folinic acid and treat until the end of gestation	Does not treat the infected fetus; should be administered with food; can cause allergic manifestations and GI tract intolerance; only available in the USA through the investigational new drug process at the US FDA and prior evaluation by a medical consultant is required <sup>†‡</sup>
Maternal infection is highly suspected or documented to have occurred during pregnancy but after 18 weeks of gestation (for some European countries the cutoff for this indication is 14 weeks)	Treatment of fetal infection	Pyrimethamine: 50 mg every 12 h for 2 days followed by 50 mg daily; sulfadiazine: initial dose of 75 mg/kg, followed by 50 mg/kg every 12 h (maximum, 4 g/day); folinic acid* (leucovorin): 10–20 mg daily should be administered until 1 week following cessation of pyrimethamine treatment	Congenital infection is highly suspected or documented by abnormal ultrasound and/or positive AF-PCR. In this setting, treatment is recommended until the end of gestation. If congenital infection is not likely (e.g., negative AF-PCR and normal follow-up fetal ultrasounds) consider switching to spiramycin. Consultation with reference laboratory or group is recommended	Pyrimethamine is teratogenic, should not be used before 14 weeks of gestation; reversible neutropenia is the most frequent toxic effect, thrombocytopenia and anemia may also occur; require CBC weekly and sulfadiazine can cause hemolysis in patients with G6PD deficiency, bone marrow suppression, allergic reactions and renal failure
Congenital infection in the newborn is highly suspected (e.g., clinical signs suggestive of congenital toxoplasmosis are present and the infant was born to woman likely to have been infected during gestation) or congenital toxoplasmosis has been confirmed in the infant (e.g., positive PCR in peripheral blood, urine or CSF, or detection of <i>Toxoplasma gondii</i> -specific IgM or IgA in peripheral blood) <sup>†</sup>	Treatment of newborn infection	Pyrimethamine: 1 mg/kg every 12 h for 2 days followed by 1 mg/kg per day for 6 months followed by the same dose three-times a week; sulfadiazine: 50 mg/kg every 12 h and folinic acid (10 mg three-times a week) should be administered until 1 week following cessation of pyrimethamine treatment	Treatment duration should be 1 year. Current studies are attempting to determine whether regimens of shorter duration are also effective in infants without clinical signs	Medications are not available in suspension <sup>§</sup> ; pyrimethamine: reversible neutropenia is the most frequent toxic effect, thrombocytopenia and anemia may also occur; require CBC weekly; sulfadiazine can cause hemolysis in newborns with G6PD deficiency, bone marrow suppression, allergic reactions and renal failure; newborns should be weighed every week and doses adjusted; referral to US National Collaborative Treatment Trial Study is suggested in the USA

<sup>†</sup>Folic acid should not be used as a substitute for folinic acid.

<sup>‡</sup>Palo Alto Medical Foundation Toxoplasma Serology Laboratory (CA, USA).

<sup>§</sup>Refer to [3] for instructions on administration of the medication to the newborn.

AF: Amniotic fluid; CBC: Complete blood cell count; CSF: Cerebral spinal fluid; G6PD: Glucose-6-phosphate dehydrogenase.



vertical transmission. This new conceptual framework appears to shed light on the results observed in the systematic review on congenital toxoplasmosis [30]. In this study, a good number of severe cases may have been initially excluded from their analysis in order to avoid 'referral bias'. In addition, in their analysis of clinical manifestations, four cohorts from outside Europe (two in Brazil, one in Colombia and one in MA, USA) containing significant numbers of severe cases, which were mainly based on neonatal screening, were excluded. Furthermore, in a recent study, Cortina-Borja *et al.* reported that prenatal treatment (spiramycin ± pyrimethamine/sulfadiazine) reduced the risk of severe neurological sequelae or death (SNSD) [81]. In this study, the primary outcome was SNSD, a composite outcome, comprising a pediatric report at any age of microcephaly, insertion of 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 treatment, 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 OR for prenatal treatment, adjusted for gestational age at maternal seroconversion, was 0.24 (95% Bayesian credible interval: 0.07–0.71). This effect was robust to most sensitivity analyses. The number of infected fetuses that must be treated to prevent one case of SNSD was three (95% Bayesian credible interval: 2–15) after maternal seroconversion at 10 weeks and 18 (9–75) at 30 weeks of gestation. Despite these results, the authors concluded that the finding that prenatal treatment reduced the risk of SNSD in infected fetuses should be interpreted with caution because of the low number of SNSD cases and uncertainty about the timing of maternal seroconversion. In addition, as these were observational data, the authors stated that policy decisions about screening required further evidence from a randomized trial of prenatal screening and from analyses of cost-effectiveness that take into account the incidence and prevalence of maternal infection.

The studies supporting both positions (for and against the recommendation of spiramycin treatment) primarily suffer from the same methodological pitfalls: a lack of randomization or small sample sizes in their control groups [30,78,82–86]. It has been suggested that only a large, randomized, controlled clinical trial would provide clinicians and patients with valid evidence of the potential benefit of prenatal treatment with spiramycin [30]. However, the data provided, to date, have not ruled out a potential benefit from spiramycin [87], and in the authors' opinion, these data are highly consistent with a drug benefit. Until there is further clarification on this subject, the authors continue to recommend spiramycin treatment for women with suspected or confirmed acute *T. gondii* infection acquired during the first 18 weeks of gestation, and view performance of randomized clinical trials that include placebo treatment in this setting as unethical.

#### **Treatment of the infected fetus**

Once fetal involvement is highly suspected (e.g., presence of abnormal fetal ultrasound findings suggestive of congenital disease or maternal infection acquired after 18 weeks of gestation)

or documented (e.g., positive AF-PCR), the combination of pyrimethamine, sulfadiazine and folinic acid should be administered to the mother in an attempt to initiate early treatment of the fetus (TABLE 3). Pyrimethamine should be avoided during the first trimester or first half of gestation, as teratogenic effects in animals have been reported when administered during the organogenesis period [3].

As stated previously, the effectiveness of treatment *in utero* to prevent SNSD in the fetus was suggested in an observational cohort study recently published in Europe. In addition, the benefits of prenatal treatment appears to be further supported by several studies from the USA, revealing that severe clinical signs of congenital toxoplasmosis, including hydrocephalus, eye disease or intracranial calcifications, are very common, whereas western European investigators rarely observe these severe clinical signs in infected infants [55]. Although there are several intercontinental differences related to parasite and host genetics, treatment strategies and referral bias exist between the two populations, it is likely that prenatal treatment significantly contributes to the lower rates of severity observed in the infected newborns in western Europe. Pregnant women in some European countries (e.g., France, Austria and Slovenia) undergo universal screening and treatment, whereas women in the USA do not.

The usefulness of additional antitoxoplasma drugs, such as trimethoprim (TMP)–sulfamethoxazole (SMZ) or clindamycin, for the treatment of fetal infection during gestation has not been determined. In a recent retrospective study, TMP–SMX in combination with spiramycin was used in an attempt to prevent vertical transmission of the parasite in 76 pregnant women [88]. The investigators resorted to the use of a TMP–SMX/spiramycin/folinic acid combination because the pyrimethamine/sulfadiazine/folinic acid combination was not readily available in Italy. Spiramycin was administered immediately after the diagnosis of seroconversion and TMP–SMX after week 14; both medications were given throughout gestation, but TMP–SMX was suspended 2 weeks before delivery. Transmission was reported in two out of 73 (2.6%) cases. No serious neurological sequelae or death was observed. Therapy was well tolerated in the mother. Results from this study could serve as an impetus to consider TMP–SMX in future randomized clinical trials in the setting of pregnancy, as shortages of pyrimethamine/sulfadiazine are not uncommon in several countries. Of note, the usefulness of TMP–SMX in other clinical settings such as toxoplasmicchorioretinitis and toxoplasmic encephalitis has been suggested in several studies, and it is now frequently considered by clinicians worldwide when the use of pyrimethamine/sulfadiazine is not feasible or the intravenous route is required. TMP–SMX is available in oral and intravenous form, whereas pyrimethamine/sulfadiazine is only available in the oral form [89–91].

#### **Treatment of the newborn suspected or confirmed to be infected**

Most clinicians recommend treatment of all infected newborns, regardless of their clinical presentation [3,73]. Poor outcomes have been reported in infected children who do not receive treatment

and even in those who only receive short courses of treatment (e.g., 1 month) [59,92]. Until new data become available, the authors recommend that infected children are treated for 1 year (TABLE 3). Infected newborns who are not treated are at particular risk for the development of new chorioretinal lesions later in life and other long-term sequelae.

Treatment should be given to newborns when the diagnosis of fetal infection was made during gestation, regardless of whether the mother received treatment, and should also be given to newborns with clinical signs suggestive of congenital toxoplasmosis pending results of confirmatory methods. For newborns without clinical signs and with equivocal serology results, treatment can be withheld pending a definitive diagnosis.

The optimal duration of treatment has not been determined. Currently, it is recommended to treat newborns for 1 year (TABLE 3) [2,3,93]. There are insufficient data on the effectiveness of other regimens such as TMP-SMX or pyrimethamine/clindamycin/folinic acid. Despite great advances in the authors' understanding of the biology and epidemiology of the parasite and development of new diagnostic methods for the past 100 years, options for treatment remain limited and scattered research efforts are devoted to the search of new therapeutic agents.

Steroids may be necessary, if CSF protein exceeds 1 g/dl in CSF or when retinal lesions are very close to the macula. Treatment of severe hydrocephalus, with ventricular shunt placement is recommended when necessary with the hope to mitigate long-term sequelae.

### Expert commentary

*T. gondii* is one of the most successful parasites to infect humans and has the capacity to cross the placenta and infect the fetus. Congenital toxoplasmosis can result in a wide spectrum of clinical manifestations from the absence of clinical signs to severe brain damage, potentially leading to major sequelae or death in offspring. Advances in our understanding of the biology of the parasite and epidemiology, and development of diagnostic tests and treatment regimens have served as an impetus for several nations to implement national programs for the prevention and treatment of congenital toxoplasmosis during gestation and at birth. In our opinion, these programs have been largely responsible for the significant decrease in death and major neurological sequelae that have been observed in countries where these prenatal programs have been applied. Parents are entitled to know whether their offspring is at risk of congenital infection, including toxoplasmosis, and to implement the measures that are likely to result in decreased frequency and severity of infection. In this regard, the biggest misconception in the care of pregnant women is to believe that obtaining a negative epidemiological history and the absence of clinical symptoms in the mother can successfully exclude women at risk of congenital toxoplasmosis. Up to 50% of pregnant women who have been infected with *T. gondii* do not have a history of exposure to the known risk factors for acute infection nor they have had any symptoms during gestation. Only systematic and universal serological screening during pregnancy can successfully identify those at risk for congenital infection. Available clinical data from large European cohorts suggest that early prenatal treatment

with spiramycin, in an attempt to prevent mother-to-child transmission and pyrimethamine/sulfadiazine/folinic acid to treat the infected fetus, are effective. As in many other infectious diseases, scientific data and clinical evidence have not necessarily been followed by adequate and widespread implementation. In the authors' opinion, each pregnant woman should at least be offered serological screening and, if acutely infected with the parasite, treatment. In addition, the infected fetus, newborn and child should be treated for at least 1 year with pyrimethamine/sulfadiazine/folinic acid.

### Five-year view

Recent studies on the effectiveness of prenatal treatment to prevent and treat congenital toxoplasmosis have been published over the past 5 years. Studies addressing this issue can be divided in three eras. Before 1999, several investigators reported on the apparent efficacy of spiramycin to prevent mother-to-child transmission and of pyrimethamine/sulfadiazine/folinic acid to treat the infected fetus. None of these studies were randomized and they only used historical controls as the untreated group. During that time, most clinicians acted based on this information and women acutely infected during gestation would often be offered treatment. In fact, several countries implemented universal serological screening programs and treatment during gestation. Between 1999 and 2006, several European investigators reported that in their analysis of their prenatal and postnatal programs, no significant evidence for effectiveness of prenatal treatment had been detected, and they suggested that large randomized clinical trials were required in order to settle this question. Of note, none of these studies were randomized, their untreated groups were characterized by small sample sizes or derived from their neonatal programs, and severe cases including those who died as a result of their infection were excluded from their analysis. In the third era, since 2007, two major studies were published which suggest that prenatal treatment is effective in decreasing mother-to-child transmission and preventing major neurological sequelae and death in the offspring [30,81]. The authors of these studies interpreted this evidence as 'weak' and still suggested the need for randomized clinical trials. In our opinion, these recent studies, and those before 1999, provide sufficient evidence for the efficacy of spiramycin as an attempt to prevent transmission and of pyrimethamine/sulfadiazine/folinic acid to decrease the frequency of death and major neurological sequelae in the infected fetus and infants. It has been well known for several years that spiramycin only partially prevents transmission (efficacy estimated at ~60%). For this reason, it is not surprising that in epidemiological studies attempting to estimate rates of transmission in the treated group, a 'weak effect' is observed as a result of a modest increase in infected infants at the end of gestation that otherwise would have died or been born with severe sequelae. However, in this model, a drug-related decrease of severe cases and death should be observed because of the decrease in parasitic load even in the infected offspring. These two outcomes are exactly what these recent studies from European colleagues revealed [30,81]. The authors consider it important that for the next 5 years clinical trials using drugs that might be more effective than spiramycin (e.g., other macrolides such as azithromycin followed by TMP-SMX) or that address the issue of length of treatment in the

congenitally infected infant, be performed. They believe that ongoing clinical trials such as TOXOGEST [102], TOSCANE [103] and Pyrimethamine, Sulfadiazine, and Leucovorin in Treating Patients With Congenital Toxoplasmosis [104] are heading in the right direction and results from these trials are eagerly waited and likely to be available within the next 5 years.

### Literature search

The authors searched MEDLINE using the following medical subject headings: 'fetal toxoplasmosis', 'prenatal toxoplasmosis', 'toxoplasmosis' and 'congenital', limited to English literature from 1995 to February 2012. A total of 667 references were summoned and the most important and methodologically appropriate references were

analyzed. References before 1995 that were considered pertinent by the senior author were also included. In addition, the authors also cited chapters published by recognized authorities in the field.

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JG Montoya is the Director of the Palo Alto Medical Foundation Toxoplasma Serology Laboratory, a not-for-profit institution and a National Reference Center for the Study and Diagnosis of Toxoplasmosis based in CA, USA. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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## Key issues

- Congenital toxoplasmosis appears to be more severe in areas where universal screening is not mandatory, atypical strains of the parasite circulate or epidemic outbreaks occur.
- When mothers seroconvert in the first trimester, the risk of fetal infection is low and increases during gestation; however, the risk of severe congenital toxoplasmosis is high but diminishes throughout gestation. It appears that in areas where atypical or more virulent strains are seen, severe cases might be observed when patients are infected with those strains during the second half of gestation.
- Universal screening and treatment during pregnancy is the most effective method to prevent *Toxoplasma gondii* congenital infection and may prove to be cost effective even in low prevalence areas.
- Performing serological screening only in pregnant women with known risk factors for *T. gondii* infection or with symptoms will fail to identify up to 50% of women at risk for congenital toxoplasmosis.
- Prenatal diagnosis of congenital *T. gondii* infection primarily relies on amniotic fluid (AF)-PCR, performed at week 18 of gestation or onwards.
- Serial fetal ultrasounds can be helpful in assessing the presence of cerebral calcifications, hydrocephalus and hepatosplenomegaly and should prompt initiation of antiparasitic medication.
- Spiramycin should only be administered to the mother when seroconversion has occurred before 18 weeks of gestation, and there is no evidence of fetal infection either by negative AF-PCR or normal serial fetal ultrasounds. Due to lack of efficacy, spiramycin should not be recommended when congenital infection in the fetus is highly suspected or confirmed.
- Pyrimethamine, sulfadiazine and folinic acid should be administered to mothers whose seroconversion is suspected or confirmed to have occurred after 18 weeks of gestation, mothers with positive AF-PCR or those with ultrasound abnormalities suggestive of congenital disease.
- The majority of newborns with congenital *T. gondii* infection are asymptomatic at birth.
- Chorioretinitis, cerebral calcifications and hydrocephalus are common in newborns with congenital toxoplasmosis and appear to be more frequent in infants born to untreated mothers.
- A thorough diagnostic evaluation is mandatory for a newborn with suspected congenital *T. gondii* infection and includes toxoplasma-specific IgG, IgM and IgA in serum and examination by PCR in peripheral blood, urine and cerebrospinal fluid.
- Consultation with a reference laboratory is recommended when congenital toxoplasmosis is suspected.

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