

## Seminar

# Toxoplasmosis

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***Toxoplasma gondii* is a protozoan parasite that infects up to a third of the world's population. Infection is mainly acquired by ingestion of food or water that is contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts. Primary infection is usually subclinical but in some patients cervical lymphadenopathy or ocular disease can be present. Infection acquired during pregnancy may cause severe damage to the fetus. In immunocompromised patients, reactivation of latent disease can cause life-threatening encephalitis. Diagnosis of toxoplasmosis can be established by direct detection of the parasite or by serological techniques. The most commonly used therapeutic regimen, and probably the most effective, is the combination of pyrimethamine with sulfadiazine and folinic acid. This Seminar provides an overview and update on management of patients with acute infection, pregnant women who acquire infection during gestation, fetuses or infants who are congenitally infected, those with ocular disease, and immunocompromised individuals. Controversy about the effectiveness of primary and secondary prevention in pregnant women is discussed. Important topics of current and future research are presented.**

## Introduction

### The organism

*Toxoplasma gondii* is an obligate intracellular protozoan that belongs to the phylum Apicomplexa, subclass coccidia. It can take several different forms: the oocyst; the tachyzoite; and the cyst. The *T gondii* genome is haploid, except during sexual division in cats, and contains about  $8 \times 10^7$  base pairs.<sup>1</sup>

### Oocysts

Members of the cat family are definitive hosts of *T gondii*; replication of the parasite happens in the intestine of the cat, resulting in production of oocysts (figure 1).<sup>2</sup> During acute infection, several million oocysts ( $10 \times 12 \mu\text{m}$ ) are shed in the faeces of cats for 7–21 days. After sporulation, which takes place between 1 and 21 days,<sup>3</sup> oocysts containing sporozoites are infective when ingested by mammals (including man) and give rise to the tachyzoite stage.

### Tachyzoites

Tachyzoites (2–4  $\mu\text{m}$  wide and 4–8  $\mu\text{m}$  long) are crescentic or oval and are the rapidly multiplying stages of the parasite (figure 1). They enter all nucleated cells by active penetration and form a cytoplasmic vacuole.<sup>4</sup> After repeated replication, host cells are disrupted and tachyzoites are disseminated via the bloodstream and infect many tissues, including the CNS, eye, skeletal and heart muscle, and placenta. Replication leads to cell death and rapid invasion of neighbouring cells. The tachyzoite form causes a strong inflammatory response and tissue destruction and, therefore, causes clinical manifestations

of disease. Tachyzoites are transformed into bradyzoites under the pressure of the immune response to form cysts.

### Cysts

Bradyzoites persist inside cysts for the life of the host (figure 1). They are morphologically identical to tachyzoites but multiply slowly, express stage-specific molecules, and are functionally different. Tissue cysts contain hundreds and thousands of bradyzoites and form within host cells in brain and skeletal and heart muscles. Bradyzoites can be released from cysts, transform back into tachyzoites, and cause recrudescence of infection in immunocompromised patients. Cysts are infective stages for intermediate and definitive hosts.

### Different strains of *T gondii*

*T gondii* consists of three clonal lineages designated type I, II, and III, which differ in virulence and epidemiological pattern of occurrence.<sup>5,6</sup> Most strains isolated from patients with AIDS are type II. Type I and II strains have been recorded in patients with congenital disease, whereas strains isolated from animals are mostly genotype III.<sup>6,7</sup> Strain-specific peptides<sup>8</sup> could allow typing of *T gondii* strains with serum from a patient.

Sexual recombination between two distinct and competing clonal lines of the parasite has driven natural evolution of virulence in *T gondii*.<sup>9</sup> Acquisition of direct oral transmission by the parasite seems to be a recent evolutionary change that has led to widespread expansion of *Toxoplasma*.<sup>10</sup> Generation of specific gene-deficient strains of *T gondii*<sup>11,12</sup> and sequencing of the *Toxoplasma* genome (<http://ToxoDB.org/>) will provide further insight into virulence factors of the parasite and specific host immune responses.

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## Search strategy and selection criteria

MEDLINE searches for recent new literature using a large number of keywords for both clinical and basic research topics were used as a primary source of references. Reference lists in recent book chapters and review articles written by the authors were also used; inclusion or exclusion of individual manuscripts was based on scientific value and clinical importance.

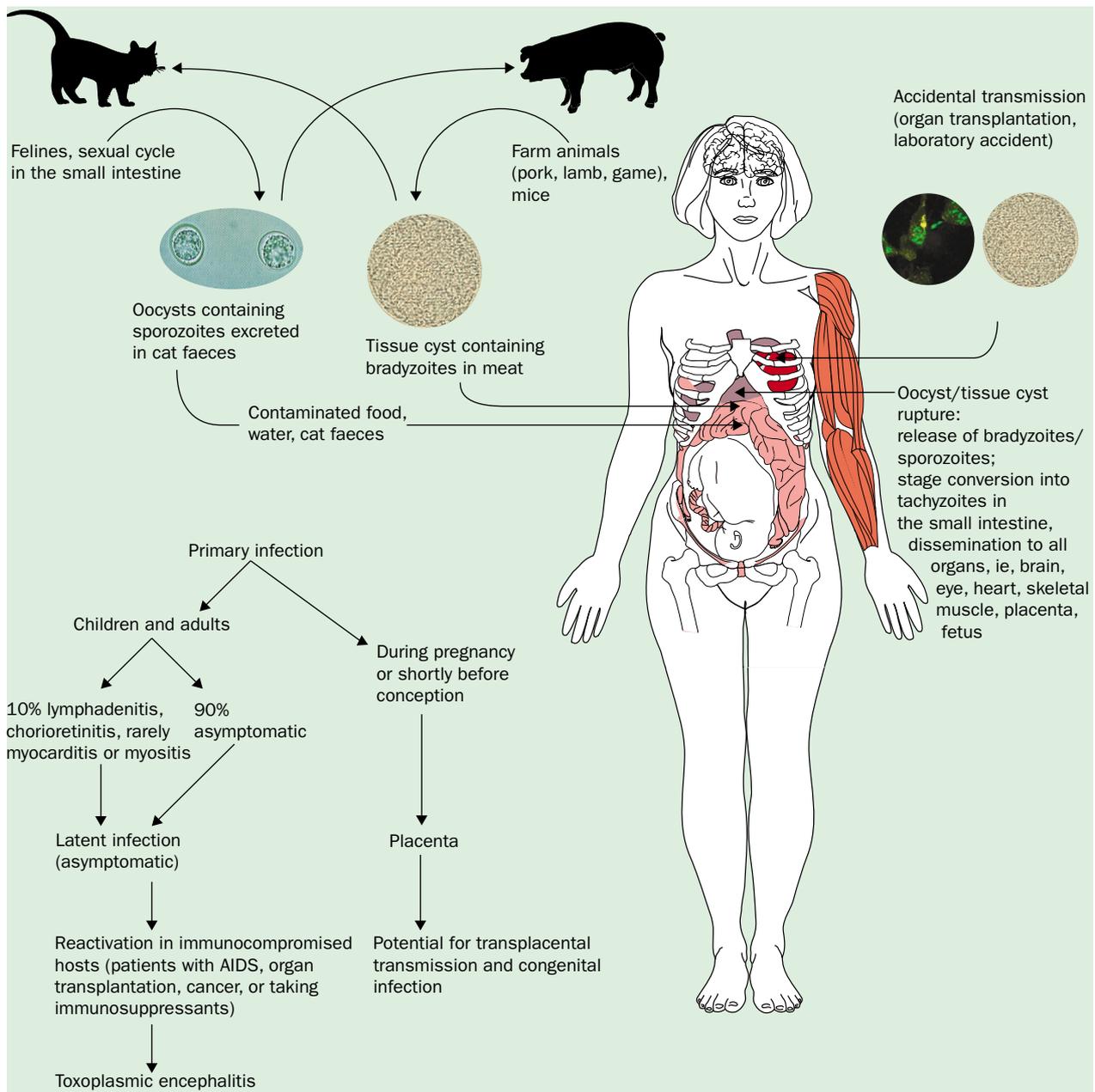


Figure 1: Life cycle of *T gondii* and clinical manifestations of toxoplasmosis

## Epidemiology Transmission

Human beings can be infected with *T gondii* by ingestion or handling of undercooked or raw meat (mainly pork and lamb) containing tissue cysts or water or food containing oocysts excreted in the faeces of infected cats (figure 1). Most individuals are infected inadvertently, thus the specific route of transmission cannot usually be established. Variations in seroprevalence of *T gondii* seem to correlate with eating and hygiene habits of a population. This finding lends support to the contention that the oral route is the major source of infection.<sup>13-15</sup> The seroprevalence of *T gondii* in market-weight pigs in the USA has been declining for the past 20 years, and it has been reported as low as 0.58%.<sup>16</sup> However, pigs from isolated small swine farms are still sold for human consumption and prevalence of the parasite in these animals can be as high as 93%.<sup>16</sup> Epidemics of toxoplasmosis in human beings and sheep attributed to

exposure to infected cats indicate an important role of oocyst excretion by cats in the propagation of infection in nature and man.<sup>17</sup> Several outbreaks of toxoplasmosis in human beings have been linked epidemiologically to drinking of unfiltered water.<sup>18,19</sup> Transmission during breastfeeding or direct human-to-human transmission other than from mother to fetus (see below) has not been recorded.

### Organ transplantation

Transmission of *T gondii* by organ transplantation from a seropositive donor to a seronegative recipient (donor [D] +/recipient [R] -) is an important potential cause of disease in heart, heart-lung, kidney, liver, and liver-pancreas transplant patients.<sup>20,21</sup> Reactivation of latent infection in the recipient (D -/R + or D +/R +) is the most usual mechanism for toxoplasmosis to arise in bone marrow, haematopoietic stem cell, and liver transplant patients, and in people with AIDS. Although rare,

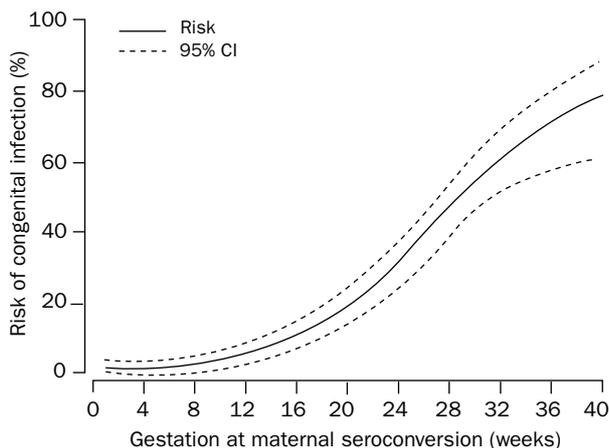


Figure 2: **Risk of congenital infection by duration of gestation at maternal seroconversion**

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*T gondii* can also be transmitted via blood or leucocytes from immunocompetent and immunocompromised donors.<sup>22,23</sup> Infections in laboratory personnel have arisen by contact with contaminated needles and glassware or infected animals.<sup>24,25</sup>

#### Congenital transmission

After maternal acquisition of *T gondii* for the first time during gestation, the parasite enters the fetal circulation by infection of the placenta. The birth prevalence of congenital toxoplasmosis ranges from one to ten per 10 000 livebirths.<sup>26–28</sup> Maternal infection acquired before gestation poses little or no risk to the fetus except in women who become infected a few months (at the most, 3) before conception.<sup>29,30</sup> Frequency of congenital transmission varies considerably according to the time during gestation that the mother became infected (figure 2). Infection acquired around the time of conception and within the first 2 weeks of gestation in women taking spiramycin does not result in vertical transmission, whereas rates of transmission are more than 60% in the last trimester.<sup>31–33</sup> Frequency of transmission and severity of disease are inversely related. Early maternal infection (first and second trimester) may result in severe congenital toxoplasmosis and can result in death of the fetus in utero and spontaneous abortion (table 1, figure 3). By contrast, late maternal infection (third trimester) usually results in normal appearing newborns. The overall frequency of subclinical infection in newborns with congenital toxoplasmosis is as high as 85%.<sup>31,33,34</sup> Infection initially goes unnoticed, but if it is not treated babies can later develop chorioretinitis or growth can be delayed in the second or third decade of life.<sup>15,35</sup>

Treatment of the mother during pregnancy is an attempt to reduce the frequency and severity of fetal infection. Spiramycin has been estimated to reduce the incidence of vertical transmission by about 60% (see Management and treatment).<sup>15,36</sup> Vertical transmission of *T gondii* in the setting of chronic infection is only recorded in immunocompromised women—ie, those with AIDS or receiving immunosuppressive drugs including corticosteroids. However, the rate of vertical transmission in this setting seems to be fairly low.<sup>37,38</sup>

#### Seroprevalence

In man, seroprevalence of *T gondii* infection rises with age, does not vary greatly between sexes, and is lower in cold regions, hot and arid areas, or at high elevations. In general,

incidence of the infection varies with the population group and geographic location—eg, seropositivity can be up to 75% by the fourth decade of life in El Salvador versus an overall seroprevalence of 22.5% in the USA.<sup>39</sup> The prevalence of *T gondii* antibodies has been steadily falling in various countries over the past few decades.<sup>40–42</sup>

#### Pathogenesis

Inoculum size,<sup>43</sup> virulence of the organism,<sup>44</sup> genetic background,<sup>45</sup> sex,<sup>46</sup> and immunological status seem to affect the course of infection in human beings and animal models of toxoplasmosis. Once the parasite has been orally ingested, it actively invades intestinal epithelial cells or it gets phagocytosed by them.<sup>4,47</sup> Intracellularly, *T gondii* induces formation of a parasitophorous vacuole that contains secreted parasite proteins and excludes host proteins that would normally promote phagosome maturation, thereby preventing lysosome fusion. The molecular characterisation and function of several proteins from different parasite organelles, including rhoptries, micronemes, and dense granules, have been reported;<sup>48–50</sup> these molecules, and the immunodominant tachyzoite surface antigen SAG1, are among the most promising vaccine candidates (see Prevention).<sup>51,52</sup>

Infection with *T gondii* results in a strong and persistent T-helper-1 (Th1) response characterised by production of proinflammatory cytokines including interleukin 12, interferon  $\gamma$ , and tumour necrosis factor  $\alpha$ . The combined action of these cytokines and other immunological mechanisms protects the host against rapid replication of tachyzoites and subsequent pathological changes. After invasion of enterocytes, *T gondii* infects antigen-presenting cells in the intestinal lamina propria and induces a transient local Th1 response.<sup>43,53</sup>

Dendritic cells—with their ability to produce interleukin 12—are the main activators of the Th1 immune response after infection of mice with *T gondii*.<sup>54</sup> Granulocytes can also contribute to early production of interleukin 12.<sup>55</sup> The activated macrophage inhibits or kills intracellular *T gondii*.<sup>56</sup> However, the parasite can partly counteract these actions even at early stages of infection. *T gondii* can exploit antigen-presenting cells as so-called Trojan horses by downregulation of cell-surface molecules and interference with apoptosis pathways.<sup>57–59</sup> Sensitised CD4+ and CD8+ T lymphocytes are both cytotoxic for *T gondii*-infected cells.<sup>60</sup> Proinflammatory (eg, interferon  $\gamma$  and tumour necrosis factor  $\alpha$ )<sup>56,61</sup> and downregulatory (eg, interleukin 10, transforming growth factor  $\beta$ )<sup>56</sup> cytokines are both involved in balancing of this response. The proportion of  $\gamma\delta$  T cells is enhanced during acute infection.<sup>62,63</sup> Within 2 weeks after infection, IgG, IgM, IgA, and IgE antibodies against many *T gondii* proteins can be detected. Production of IgA antibodies on

	Infection acquired		
	First trimester	Second trimester	Third trimester
<b>Outcome in offspring</b>			
Congenital toxoplasmosis	9.0%	27.0%	59.0%
Subclinical	22.2%	74.4%	89.8%
Clinically apparent	77.8%	15.6%	10.2%
Perinatal death or stillbirth	5.0%	2.0%	0%

Table adapted and modified from Desmonts G, Couvreur J. Congenital toxoplasmosis: a prospective study of the offspring of 542 women who acquired toxoplasmosis during pregnancy. In: Thalhammer O, Baumgarten K, Pollak A, eds. Pathophysiology of congenital disease: perinatal medicine, 6th European congress. Stuttgart: Georg Thieme Verlag, 1979: 51–60. With permission of Georg Thieme Verlag.

Table 1: **Outcome in babies born to women who acquired *T gondii* infection during pregnancy**

	Antibody class/ test	Screening	Pregnancy	Newborns	Eye disease	Immunocompromised patients
<b>Indirect detection/ serology</b>	IgG	+	+ (identification of women at risk and those protected)	+ (maternal antibodies may persist until 12 months of age; differentiation of maternal and fetal IgG by western blot or ELIFA)	+ (low titres are usually seen in patients with reactivation of congenital disease; intraocular antibody production [ratio of ocular and blood antibody titres])	+ (identification of patients at risk of reactivation, ie, AIDS, bone marrow transplant patients)
	IgG avidity	-	+ (high avidity results rule out infection in recent 3–4 months; low avidity antibodies may persist)	-	+ (high avidity results rule out infection in recent 3–4 months; low avidity antibodies may persist)	-
	IgM*	-†	+ (IgM antibodies may persist for prolonged times, negative IgM rules out infection in pregnant women during the first two trimesters)	+ (ISAGA more sensitive than ELA; differentiation of maternal and fetal IgG by western blot)	+ (high titres usually in patients with acute acquired disease, negative results in patients with reactivation of congenital disease)	+ (IgM of little value; may or not be present with active or latent disease)
	IgA	-	+ (IgA antibodies may persist for prolonged times)	+ (increased value compared to IgM tests)	-	-
	IgE	-	+ (high specificity, low sensitivity)	-	-	-
<b>Direct detection</b>	PCR	-	+ (amniotic fluid)	+ (blood, urine)	+ (particularly useful in patients with atypical retinal lesions or suboptimum response to therapy [vitreous or aqueous fluid, vitreous fluid preferred])	+ (cerebrospinal fluid, bronchoalveolar lavage, ocular fluids, ascitic fluid, pleural fluid, peritoneal fluid, bone marrow aspirate, peripheral blood, and/or tissue)
	Histology (immunohistochemistry‡)/ cell culture or mouse inoculation	-	+ (placenta and fetal tissues in cases of fetal loss)	-	-	+ (any affected tissue)
<b>Comments/aims</b>		Determination of sero-prevalence/ epidemiological studies	Combined detection of IgG and IgM antibodies for screening in early pregnancy	Increased sensitivity of combined IgA and IgM antibody detection	Serological distinction between congenital and recently acquired infection	Direct detection more sensitive than indirect detection

ELIFA=enzyme-linked immune filtration assay. \*Value of commercially available tests varies considerably. †Detection of IgM may be used for neonatal screening. ‡With *T gondii*-specific antibodies.

Table 2: Value of serological tests for the diagnosis of infection with *T gondii*

mucosal surfaces seems to protect the host against reinfection.<sup>64,65</sup> Reinfection can happen but does not seem to result in disease or in congenital transmission of the parasite.

### Pathology

Histopathological changes in toxoplasmic lymphadenitis in immunocompetent individuals are frequently distinctive and sometimes diagnostic<sup>66</sup> and consist of reactive follicular hyperplasia, irregular clusters of epithelioid histiocytes encroaching on and blurring the margins of the germinal centres, and focal distension of sinuses with monocytoid cells. Langhans giant cells, granulomas, microabscesses, foci of necrosis, and parasites (or their DNA)<sup>67</sup> are not typically seen or detected. Eye infection in immunocompetent patients produces acute chorioretinitis characterised by severe inflammation and necrosis.<sup>68</sup> Granulomatous inflammation of the choroid is secondary to necrotising retinitis. Exudation into the vitreous or invasion of the vitreous by a budding mass of capillaries might happen. Although rare, tachyzoites and cysts can be seen in the retina. The pathogenesis of recurrent chorioretinitis is controversial. Rupture of cysts can release viable organisms that induce necrosis and inflammation; alternatively, chorioretinitis can

result from a hypersensitivity reaction triggered by unknown causes.<sup>68</sup> Biopsy-proven toxoplasmic myocarditis and polymyositis in the setting of acute toxoplasmosis have been reported in otherwise immunocompetent individuals and in patients on corticosteroids.<sup>69</sup>

Damage to the CNS by *T gondii* is characterised by many foci of enlarging necrosis and microglia nodules.<sup>70</sup> In infants, periaqueductal and periventricular vasculitis and necrosis are distinctive of toxoplasmosis.<sup>71</sup> Necrotic areas can calcify and lead to striking radiographic findings suggestive—but not pathognomonic—of the disease. Hydrocephalus can result from obstruction of the aqueduct of Sylvius or foramen of Monro. Tachyzoites and cysts are seen in and adjacent to necrotic foci near or in glial nodules, perivascular regions, and cerebral tissue uninvolved by inflammatory change. Presence of many brain abscesses is the most characteristic feature of toxoplasmic encephalitis in severely immunodeficient patients and is especially characteristic in people with AIDS.<sup>70</sup> Identification of tachyzoites is pathognomonic of active infection (see Management). At autopsy in AIDS patients with toxoplasmic encephalitis, almost universal involvement of the cerebral hemispheres is noted, as is a remarkable predilection for the basal ganglia.<sup>72</sup> In cases of

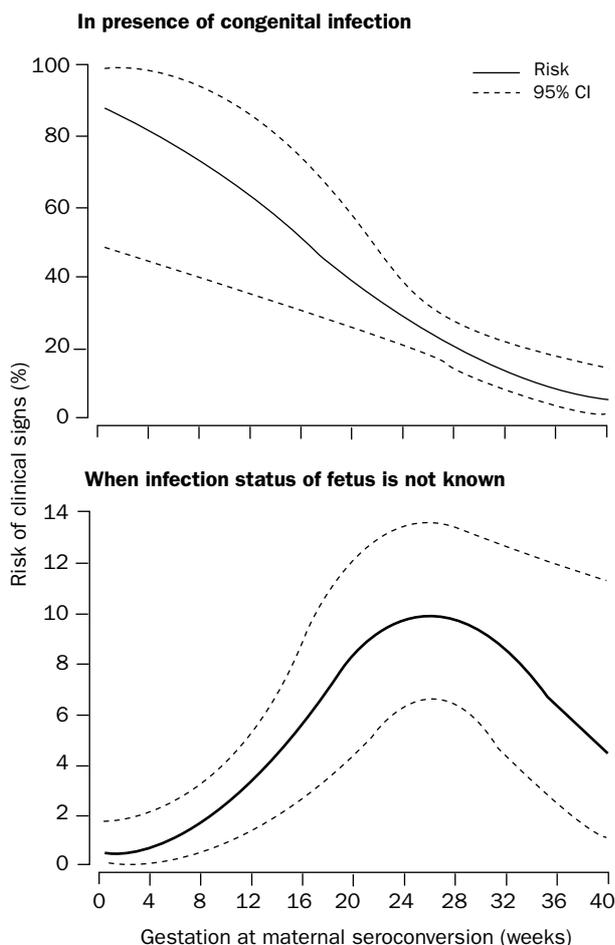


Figure 3: Risk of developing clinical signs (not necessarily symptomatic) before age 3 years according to gestational age. Reprinted from reference 33, with permission of Elsevier.

congenital toxoplasmosis, necrosis of the brain is most intense in the cortex and basal ganglia and at times in the periventricular areas.<sup>71</sup> Pulmonary toxoplasmosis in the immunodeficient patient can arise as interstitial pneumonitis, necrotising pneumonia, consolidation, and pleural effusion.<sup>73</sup>

By PCR, *T gondii* DNA can be shown in amniotic, cerebrospinal, bronchoalveolar lavage, ocular, pleural, or ascitic fluids and in peripheral blood or urine.<sup>74</sup>

### Clinical presentation

Clinically, infection with *T gondii* can go unnoticed or could cause signs and symptoms that vary depending on the immune status of the patient and the clinical setting—eg, immunocompetent, ocular disease, immunocompromised, or congenital toxoplasmosis.

### Immunocompetent adults and children

*T gondii* primary infection in children and adults (including pregnant women) is asymptomatic in most patients. In about 10%, it causes a self-limited and non-specific illness that rarely needs treatment. The most typical clinical manifestation is isolated cervical or occipital lymphadenopathy. Lymph nodes are not tender, do not suppurate, are usually discrete, and stay enlarged for less than 4–6 weeks. A form of the disease characterised by chronic lymphadenopathy has been described, and lymph-node enlargement can fluctuate for months. Very infrequently, myocarditis, polymyositis, pneumonitis, hepatitis, or encephalitis can arise in otherwise healthy

individuals. Acute toxoplasma infection during pregnancy is asymptomatic in most women.

### Ocular toxoplasmosis

Toxoplasmic chorioretinitis can be seen in the setting of congenital or postnatally acquired disease as a result of acute infection or reactivation.<sup>75,76</sup> Chorioretinitis in individuals with acute acquired toxoplasmosis can arise sporadically or in the context of an outbreak of acute disease.<sup>77</sup> Typical findings of toxoplasmic chorioretinitis include noticeably white focal lesions with an overlying and intense vitreal inflammatory reaction. The classic “headlight in the fog” appearance is attributable to the presence of active retinal lesions with severe inflammatory reaction. Recurrent lesions are usually recorded at the borders of chorioretinal scars, which are typically found in clusters. Chorioretinitis in adults has been traditionally deemed a late manifestation and reactivation of congenital disease; however, it has been reported with increasing frequency in association with acute infection.<sup>75</sup> To establish whether the original infection was congenital or acquired in patients who have recurrences of chorioretinitis is difficult.

### Immunocompromised patients with or without AIDS

By contrast with the favourable course of toxoplasmosis in almost all immunocompetent individuals, the disease can be life-threatening in those who are immunocompromised.<sup>78</sup> In these individuals, toxoplasmosis almost always happens as a result of reactivation of chronic infection.<sup>79</sup>

The CNS is the site most typically affected by infection. Clinical presentation of toxoplasmic encephalitis varies from a subacute gradual process evolving over weeks to an acute confusional state, with or without focal neurological deficits, evolving over days. Clinical manifestations include mental status changes, seizures, focal motor deficits, cranial nerve disturbances, sensory abnormalities, cerebellar signs, movement disorders, and neuropsychiatric findings. Meningeal signs are rare. Constitutional symptoms and signs such as fever and malaise can vary. The most typical focal neurological findings are hemiparesis and speech abnormalities.<sup>80</sup> The differential diagnosis of toxoplasmic encephalitis lesions includes CNS lymphoma, progressive multifocal leukoencephalopathy, cytomegalovirus ventriculitis and encephalitis, focal lesions caused by other organisms including *Cryptococcus neoformans*, *Aspergillus* spp, *Mycobacterium tuberculosis*, and *Nocardia* spp, or bacterial brain abscess. Toxoplasmosis in immunocompromised patients can also present as chorioretinitis, pneumonitis, or multiorgan involvement presenting with acute respiratory failure and haemodynamic abnormalities similar to septic shock.<sup>78</sup> Toxoplasma pneumoniae seems to be more frequent in recipients of bone-marrow transplants and in patients with AIDS.

### Congenital toxoplasmosis

Fetuses with congenital toxoplasmosis usually look normal on prenatal ultrasound. If present, ultrasonographic findings suggestive of congenital disease include intracranial calcifications, ventricular dilatation, hepatic enlargement, ascites, and increased placental thickness.<sup>81</sup> Neonatal clinical manifestations of congenital toxoplasmosis vary widely and include hydrocephalus, microcephaly, intracranial calcifications, chorioretinitis, strabismus, blindness, epilepsy, psychomotor or mental retardation, petechia due to thrombocytopenia, and anaemia.<sup>82,83</sup> The classic triad of chorioretinitis, hydrocephalus, and cerebral calcifications is rather rare. None of the signs described in newborns with congenital disease is pathognomonic for toxoplasmosis and

	Drug	Dosage	Duration
<b>Acute asymptomatic acquired infection</b>	Treatment not recommended*	..	..
<b>Acute toxoplasmosis in pregnant women†</b>	Spiramycin	3 g qd in three divided doses without food	Until term‡ or until fetal infection is documented
<b>Documented fetal infection (after 12 or 18 weeks of gestation)§</b>	Pyrimethamine	Loading dose: 100 mg qd in two divided doses for 2 days, then 50 mg qd	Until term
	<i>plus</i> Sulfadiazine	Loading dose: 75 mg/kg qd in two divided doses (max 4 g qd) for 2 days, then 100 mg/kg qd in two divided doses (max 4 g qd)	Until term
	<i>plus</i> Leucovorin (folinic acid)	5–20 mg qd	During and for 1 week after pyrimethamine treatment
<b>Congenital toxoplasma infection in the infant¶</b>	Pyrimethamine	Loading dose 2 mg/kg qd for 2 days, then 1 mg/kg qd for 2–6 months, then this dose every Monday, Wednesday, and Friday	1 year
	<i>plus</i> Sulfadiazine	100 mg/kg qd in two divided doses	1 year
	<i>plus</i> Leucovorin	10 mg three times a week	During and for 1 week after pyrimethamine treatment
	Corticosteroids   (prednisone)	1 mg/kg qd in two divided doses and symptoms	Until resolution of signs
<b>Toxoplasmic chorioretinitis in adults</b>	Pyrimethamine	Loading dose: 200 mg qd, then 50–75 mg qd	Usually 1–2 weeks after resolution of symptoms
	<i>plus</i> Sulfadiazine	Oral 1–1.5 g qd	Usually 1–2 weeks after resolution of symptoms
	<i>plus</i> Leucovorine	5–20 mg three times a week	During and for 1 week after pyrimethamine treatment
	Corticosteroids	1 mg/kg qd in two divided doses	Until resolution of signs and symptoms
<b>Acute/primary treatment of toxoplasma encephalitis in patients with AIDS</b>	Standard regimens		
	Pyrimethamine	Oral 200 mg loading dose, then 50–75 mg qd	At least 4–6 weeks after resolution of signs and symptoms
	Leucovorin	Oral, intravenous, or intramuscular 10–20 mg qd (up to 50 mg qd)	During and for 1 week after pyrimethamine treatment
	<i>plus</i> Sulfadiazine	Oral 1–1.5 g q6h	**
	<i>or</i> Clindamycin	Oral or intravenous 600 mg q6h (up to intravenous 1200 mg q6h)	**
	Possible alternative regimens		
	Trimethoprim	Oral or intravenous 5 mg (trimethoprim sulfamethoxazole component)/kg q12h (daily doses as high as 15–20 mg/kg of the trimethoprim component have been used)	**
	Pyrimethamine plus leucovorin <i>plus one of the following</i>	As in standard regimens	**
	Clarithromycin	Oral 1g q12h	**
	Atovaquone	Oral 750 mg q6h	**
Azithromycin	Oral 1200–1500 mg qd	**	
Dapsone	Oral 100 mg qd	**	

Adapted from reference 143, with permission. qd=once a day. \*Acute acquired infection in immunocompetent patients does not need specific treatment unless severe or persistent symptoms or evidence of damage to vital organs are present. If such signs or symptoms arise, treatment with pyrimethamine/sulfadiazine, and leucovorin should be initiated (for doses, see Toxoplasmic chorioretinitis in adults). †Practices vary widely between centres. ‡German and Austrian guidelines recommend spiramycin prophylaxis until 17 weeks of pregnancy followed by a 4-week course of pyrimethamine plus sulfadiazine plus leucovorin. §Practices vary widely between centres. Pyrimethamine plus sulfadoxine is used in some centres, monthly alternating cycles of pyrimethamine plus sulfadiazine and spiramycin. ¶Practices vary widely between centres. Monthly alternating cycles of pyrimethamine plus sulfadiazine and spiramycin. ||When cerebrospinal protein is  $\geq 1$  g/dL and when active chorioretinitis threatens vision. \*\*Duration of treatment as for pyrimethamine in patient with toxoplasma encephalitis.

Table 3: Guidelines for treatment of *T gondii* infection

can be mimicked by congenital infection with other pathogens, including cytomegalovirus, herpes simplex virus, rubella, and syphilis.

### Diagnosis

*T gondii* infection can be diagnosed indirectly with serological methods and directly by PCR, hybridisation, isolation, and histology. Whereas indirect serological methods are widely used in immunocompetent patients, definitive diagnosis in immunocompromised people is mostly undertaken by direct detection of the parasite (table 2). Direct demonstration of the organism (mouse

inoculation, cell culture, or PCR for *T gondii* DNA) from cerebrospinal fluid, blood, and urine,<sup>84,85</sup> and ophthalmologic testing, radiological studies, and examination of cerebrospinal fluid could assist diagnosis of congenital disease.

### Indirect detection

Detection of IgG antibodies to *T gondii* should be done in pregnant women and immunocompromised patients. First, absence of IgG antibodies before or early in pregnancy allows identification of women at risk of acquiring the infection. Second, presence of IgG antibodies allows

identification of immunocompromised patients—ie, bone marrow transplant recipients or people with AIDS—at risk for reactivation of latent infection. The Sabin-Feldman dye test,<sup>86</sup> immunofluorescent antibody test,<sup>87</sup> ELISA,<sup>88</sup> IgG avidity test,<sup>89–91</sup> and agglutination and differential agglutination test<sup>92</sup> can be used for detection of IgG antibodies. These arise within 1–2 weeks after infection and persist for the individual's lifetime.

Tests for the avidity (functional affinity) of IgG antibodies have become standard to discriminate between recently acquired infection and those obtained in the more distant past.<sup>89</sup> Presence of high avidity antibodies essentially rules out infection acquired in the recent 3–4 months; by contrast, low avidity antibodies can persist beyond 3 months of infection.<sup>89–91,93,94</sup> The differential agglutination (AC/HS) test has also proven helpful in differentiation between a probable acute or chronic infection in pregnant women<sup>92</sup> in combination with a panel of other assays.<sup>74,95</sup> The double-sandwich IgM ELISA and IgM immunosorbent agglutination assay (ISAGA) can be used for detection of IgM antibodies that arise within the first week of infection, rapidly increase, and thereafter decline and disappear at highly variable rates.<sup>96,97</sup> False-positive results and persistence of positive titres even years after initial infection hamper correct interpretation of results obtained in IgM antibody tests.<sup>95,98</sup> The greatest value of testing for IgM lies in the fact that a negative test essentially rules out recently acquired infection. However, results of commercial kits used to detect IgM antibodies in non-reference laboratories are sometimes unreliable with false positive rates as high as 60%.<sup>95</sup> The strength of combinations of serological tests in assessment of the stage of infection has been shown by different researchers.<sup>95,99,100</sup>

The IgM ISAGA<sup>101</sup> is highly sensitive and specific and frequently used for diagnosis of congenital infection in newborns. Tests for the detection of IgA antibodies were more sensitive than those for detection of IgM antibodies in the fetus and newborn.<sup>102,103</sup> Presence of IgG antibodies in the newborn's serum could be their own or their mother's antibodies. Testing for IgM and IgA antibodies will identify up to 75% of infected babies.<sup>15,82,103</sup> In babies with suspected congenital toxoplasmosis with positive IgG but negative IgM and IgA tests results, use of IgG/IgM western blots of mother-infant pairs can prove useful.<sup>104</sup> Maternally transferred IgG antibodies usually decline and disappear within 6–12 months.

In adults, IgA antibodies can remain positive for a year or longer, and therefore are of minor value for diagnosis of recent infection. Tests for the detection of IgE antibodies should only be used in combination with other serological methods.<sup>105,106</sup> Local antibody production in the eye has been used successfully for diagnosis of ocular toxoplasmosis.<sup>107</sup>

### Direct detection

PCR amplification of the 35-fold repetitive B1 gene for detection of *T gondii* DNA in body fluids and tissues has successfully been used to diagnose congenital,<sup>32,108</sup> ocular,<sup>109</sup> cerebral, and disseminated<sup>110–112</sup> toxoplasmosis. Real-time PCR and use of other genes—ie, 300-fold repetitive AF146527—will probably be more generally used in the future.<sup>113,114</sup>

Sensitivity of PCR results can be affected by the appropriateness of sample handling, shipping and storage conditions, the particular technique used for amplification and detection of PCR products, and by previous use of anti-*T gondii* specific drugs. If contamination is not an issue, specificity and positive predictive value of PCR results approach 100%. In an initial study,<sup>31</sup> sensitivity of

amniotic fluid PCR was close to 100%; however, Romand and colleagues<sup>32</sup> estimated sensitivity to be 64%, negative predictive value 87.8%, and specificity and positive predictive value 100%. Sensitivity varied greatly according to gestational age and was significantly higher for maternal infections that arose between 17 and 21 weeks of gestation.<sup>32</sup> Lack of homogeneity in methods used for evaluation and in patients' selection are the most probable explanations for the noted differences. Amniotic fluid PCR undertaken before week 18 is probably less reliable than tests done after this time and has not been systematically studied.

PCR has revolutionised prenatal diagnosis of congenital toxoplasmosis by enabling early diagnosis, thereby avoiding use of more invasive procedures on the fetus.<sup>31,74</sup> Peripheral blood, cerebrospinal fluid, and urine should be considered for PCR examination in any newborn suspected to have congenital disease. PCR of vitreous or aqueous fluid is helpful to establish diagnosis in patients who present with atypical retinal lesions, who show a suboptimum response to appropriate antitoxoplasma treatment, or who are immunocompromised.<sup>109,115</sup> In immunocompromised patients suspected to have localised or disseminated toxoplasmosis, PCR of blood (buffy coat), affected body fluids (including bronchoalveolar lavage or cerebrospinal, pleural, ascitic, peritoneal, or ocular fluids), bone-marrow aspirate, or tissues should be regarded as an important diagnostic aid.<sup>74</sup> A positive result of brain tissue PCR may not differentiate between a patient with toxoplasmic encephalitis and an individual with different brain pathology but who is chronically infected (dormant infection) with *T gondii*.

Isolation of *T gondii* from blood or body fluids shows that infection is acute. Isolation techniques need live parasites and thus are not sensitive; however, they are highly accurate for typing of strains. Attempts to isolate the parasite can be undertaken by inoculation of mice or of cell cultures of virtually any human tissue or body fluid.<sup>74,116</sup>

Demonstration of tachyzoites in tissue sections or smears of body fluid—eg, bronchoalveolar lavage or cerebrospinal fluid—shows that *T gondii* causes the pathological changes seen in that system or patient. Tachyzoites can be recorded in primary acute infection or in reactivation of previously acquired latent infection.

The immunoperoxidase technique, which uses antisera to *T gondii*, has proven both sensitive and specific and is superior to conventionally stained tissue sections. It has been used successfully to show the presence of the parasite in the CNS of AIDS patients.<sup>117</sup>

## Management and treatment

### Infection in the immunocompetent host

Immunocompetent adults and children with toxoplasmic lymphadenitis are usually not treated unless symptoms are severe or persistent. Characteristic histological criteria and findings of a panel of serological tests that accord with recently acquired infection are diagnostic for toxoplasmic lymphadenitis in older children and adults.<sup>99</sup> If needed, treatment is usually administered for 2–4 weeks followed by reassessment of the patient's condition. The combination of pyrimethamine, sulfadiazine, and folinic acid for 4–6 weeks is the most typical drug combination (table 3). Infections acquired by laboratory accident or transfusion of blood products are potentially most severe, and these patients should always be treated.

### Maternal and fetal infection

Management of maternal and fetal infection varies considerably between different countries and centres within

the same country.<sup>36</sup> The antibody status of a pregnant woman should be obtained before or early in pregnancy. One in five pregnant women in the USA request termination of their pregnancy if they are told they have a recently acquired *T gondii* infection (based on positive tests for IgM antibodies) and that their offspring might be at risk for congenital infection.<sup>95,118</sup> However, 60% of these women are found to be chronically infected when tested at a reference laboratory.<sup>118</sup> Thus, confirmatory serological testing done at a reference laboratory, with correct interpretation by an expert, diminished the rate of unnecessary abortions by about 50% in women with positive IgM toxoplasma test results reported by outside laboratories.<sup>95,118</sup> Thus, positive IgM test results should always undergo confirmatory tests in a reference laboratory.<sup>74,95</sup> Serological tests for measurement of IgG (dye test, AC/HS), IgM, IgA, and IgE antibodies have been successfully used as a panel of confirmatory tests.<sup>74,118</sup>

Negative tests for IgM antibodies during the first two trimesters essentially rule out recently acquired infection, unless serum samples are obtained so early that an IgM antibody response is not yet detectable (very rare) or too late that the IgM antibodies have already become undetectable. Definitive diagnosis of acute infection or toxoplasmosis requires demonstration of a rise in titres in serial specimens (either conversion from a negative to a positive titre or a significant rise from a low to a higher titre), but this change is rarely shown in countries where systematic screening during pregnancy is not available.<sup>119</sup>

Treatment with spiramycin should be initiated as immediately as feasible after diagnosis of recently acquired maternal infection (table 3). Findings of European studies have suggested that the incidence of congenital toxoplasmosis does not seem to be lower in women who took spiramycin during pregnancy when compared with those who did not.<sup>120–123</sup> However, these data should not prompt any change in current policies of spiramycin administration to pregnant women suspected to have or diagnosed with recently acquired *T gondii* infection.<sup>124</sup> The studies included very few untreated women in their analysis and most untreated women were infected during the third trimester. The design of studies undertaken to date has not permitted a definitive conclusion about use of spiramycin. Until appropriately designed studies are done, authorities continue to recommend spiramycin (for the first and early second trimester) or pyrimethamine/sulfadiazine (for late second and third trimester) for women with suspected or confirmed acute *T gondii* infection acquired during gestation.<sup>36,120,125</sup> Since maternal infection does not necessarily result in fetal infection, suspected or established maternal infection acquired during gestation (based on ultrasonography or serology) must be confirmed by prenatal diagnosis by PCR of amniotic fluid. This test has an overall reported sensitivity of 64–98.8%.<sup>31,32,34,126</sup> In case of a negative PCR result, pregnant women should receive spiramycin prophylaxis until the 17th week of pregnancy and have monthly ultrasound examinations for the entire pregnancy.<sup>81</sup>

Spiramycin is continued throughout pregnancy in the USA and France. In Austria and Germany, spiramycin prophylaxis is followed by a 4-week course of pyrimethamine plus sulfadiazine at 17 weeks of gestation; this approach seems to reduce the rate of clinical signs in the fetus (Prusa A-R, Universitätsklinik für Kinder- und Jugendheilkunde, Wien, Austria, personal communication). In case of a positive PCR result or very highly probable infection of the fetus (ie, acquisition of maternal infection in late second or third trimesters), treatment

consists of pyrimethamine/ sulfadiazine—in some countries this regimen is alternated with spiramycin.<sup>15</sup> Prenatal treatment with pyrimethamine/ sulfadiazine of women suspected or confirmed to have fetal infection reduces sequelae of the disease in the newborn.<sup>122</sup>

Antitoxoplasma treatment should be continued throughout pregnancy (table 3). Folic acid is added to regimens to reduce bone-marrow suppression; careful monitoring for haematotoxicity is mandatory. Ultrasound should be done at least monthly until term if the initial examination revealed no abnormalities; the presence of hydrocephalus has been used as an indication for termination of the pregnancy.

In most countries, treatment of the fetus is followed by treatment of the newborn throughout the first year of life.<sup>15,82</sup> However, lengths of treatment protocols vary greatly between centres in European countries.<sup>123</sup>

#### Chorioretinitis

The decision to treat active toxoplasmic chorioretinitis should be made based on results of an examination done by an ophthalmologist familiar with the disease. Low titres of IgG antibody are usual in patients with active chorioretinitis because of reactivation of congenital *T gondii* infection; IgM antibodies generally are not detected. Patients with chorioretinitis due to postnatally acquired disease usually have serological findings consistent with infection acquired in the recent past.<sup>75</sup> Most ophthalmologists would recommend treatment if they record severe inflammatory responses, proximity of retinal lesions to the fovea or optic disk, or both.<sup>127</sup>

Nine drugs (or commercially available combinations) have been used in 24 different regimens as treatments for typical cases of recurrent toxoplasmic chorioretinitis.<sup>127</sup> The combination of pyrimethamine, sulfadiazine, and prednisone is the most typically used regimen (table 3). Clindamycin or trimethoprim/sulfamethoxazole for a minimum of 3 weeks has also been used with favourable clinical results. Because toxoplasmic chorioretinitis can be self-limited in immunocompetent individuals, many clinicians may not treat small peripheral retinal lesions that are not immediately vision-threatening. The rate of recurrent toxoplasmic chorioretinitis can be greatly reduced with a long-term intermittent regimen of trimethoprim/sulfamethoxazole.<sup>128</sup> In some patients, the morphology of retinal lesions can be non-diagnostic, response to treatment can be suboptimum, or both. In such cases, detection of an abnormal *T gondii*-specific antibody response in ocular fluids (Goldman-Witmer coefficient) and demonstration of the parasite by PCR have been used successfully to establish diagnosis.

#### Infection in the immunocompromised host

##### *Toxoplasma encephalitis, generalised infection*

Transplant recipients who are most likely to acquire *T gondii* infection via the allograft (ie, heart, lung, heart-lung, and kidney) need to be tested—as well as the donor—for baseline toxoplasma IgG antibodies. A seropositive donor (D+) and seronegative recipient (R-) represent the highest risk for disease in these patients; trimethoprim/sulfamethoxazole prophylaxis is highly effective in this setting. Recipients from D-/R-, D-/R+, or D+/R+ pairs rarely develop toxoplasmosis. Serological results indicating apparent reactivation (rising IgG and IgM titres) in the absence of clinically apparent infection, and results consistent with chronic infection in the presence of toxoplasmosis, can be seen and could be misleading.<sup>129</sup> Thus, for immunocompromised patients in

## Future areas of work in *T gondii* infection and toxoplasmosis

### Clinical management

#### Diagnosis

- Avidity testing using recombinant antigens
- Amniocentesis and PCR techniques
- Congenital disease in newborns with negative IgM and IgA

#### Treatment, prophylaxis, screening

- Clinical trials comparing different drug regimens and strategies in different clinical settings—eg, eye disease and congenital toxoplasmosis or prevention of multiple episodes of recurrent episodes of chorioretinitis
- Prophylaxis and treatment of disease in bone-marrow transplant recipients
- Effectiveness of prevention strategies in pregnancy
- Cost-effectiveness of routine serological screening programmes during pregnancy to prevent congenital disease
- Susceptibility of the host to infection—eg, HLA types

### Basic research

#### Strains of *T gondii*

- Phylogeny
- Sequencing
- Mutants

#### Sources of infection

- Relative importance of different sources of transmission—eg, meat vs cats vs water
- Genotyping of strains in serum samples with peptides

#### Interaction of *T gondii* with immune cells—eg, antigen-presenting cells

- Dendritic cells
- Immune activation vs evasion
- Immune response in specific compartments—eg, eye and brain

#### Animal models of eye disease

- Vaccination

#### Proteins

- Strategies (DNA, adjuvants, mucosal)

whom toxoplasmosis is suspected, additional diagnostic methods—including PCR amplification of *T gondii* DNA or isolation of the parasite from blood or body fluids that could contain the parasite, and histological examination of tissues—are strongly recommended.

Pre-emptive antiparasitic treatment should be considered for all symptomatic seropositive immunocompromised patients suspected to have toxoplasmosis. When clinical manifestations suggest involvement of the brain, spinal cord, or both, neuroimaging studies such as CT or MRI are mandatory. These studies should be considered even if neurological examination does not indicate focal deficits. Empiric anti-*T gondii* treatment is an accepted practice for patients with multiple ring enhancing brain lesions (usually established by MRI), positive IgG antibody titres against *T gondii*, and advanced immunodeficiency; a clinical and radiological response to specific anti-*T gondii* treatment is judged supportive of the diagnosis of CNS toxoplasmosis. Patients with cerebral toxoplasmosis usually improve by more than 50% of their baseline neurological examination by 7–10 days.<sup>80</sup>

Monotherapy has no role in treatment of toxoplasmosis in immunocompromised patients. The most typically used and successful regimen continues to be the combination of pyrimethamine/sulfadiazine and folinic acid (table 3).<sup>130</sup> Clindamycin can be used instead of sulfadiazine in patients

intolerant to sulfonamides. Treatment is recommended for 4–6 weeks after resolution of all signs and symptoms (sometimes for several months or longer). Trimethoprim/sulfamethoxazole appears to be equivalent to pyrimethamine/sulfadiazine in patients with AIDS.<sup>131</sup> Atovaquone in combination with either pyrimethamine or sulfadiazine has sufficient activity to be considered for treatment of acute toxoplasmic encephalitis in patients with a Karnofsky performance status score—a combined measure of the ability to work, undertake normal activities without assistance, and to care for personal needs—of more than 30.<sup>132</sup> The role of other drugs in the treatment of toxoplasmosis in immunocompromised patients, including clarithromycin, azithromycin, or dapsone, has not been well established.<sup>78</sup> If these drugs have to be used as a last resort, they should always be given in combination with other drugs (preferably pyrimethamine).

After treatment of the acute phase (primary or induction treatment) in immunocompromised patients, maintenance therapy (secondary prophylaxis) should be started, usually with the same regimen that was used in the acute phase but at half doses. Currently, maintenance treatment should be continued for the life of the patient or until underlying immunosuppression has ceased. In patients with AIDS, primary and secondary prophylaxis are generally discontinued when the patient's CD4 count has returned to more than 200 cells per  $\mu\text{L}$  and HIV PCR peripheral blood viral load has been reasonably controlled for at least 6 months.<sup>133</sup>

### Prevention

Public-health measures to prevent *T gondii* infection are a possible approach to diminish burden of disease in human beings and animals. Wide differences exist in public-health policies to prevent congenital infection; however, data for the efficacy of such policies are scarce.<sup>134</sup> Systematic serological screening of all pregnant women is undertaken only in France and Austria.<sup>42,135</sup> Uncertainty about incidence of congenital infection, cost-effectiveness, difficulties with sensitivity and specificity of serological tests, and findings suggesting absence of spiramycin effectiveness have hampered attempts to implement screening programmes in several countries.<sup>134,136</sup> Neonatal screening has been implemented in several countries (eg, Denmark) or areas such as Massachusetts, USA,<sup>26,28,137,138</sup> through these programmes as many as 80% of infected newborns have been identified.

An effective vaccine against human *T gondii* infection is a desirable but elusive target. Only the attenuated live S48 strain of the parasite has been licensed for use in sheep in Europe and New Zealand.<sup>139</sup> Most research is now focused on vaccine candidates that can induce protective Th1 and humoral (including IgA) responses—both systemic and at the intestinal mucosa level—with the hope to mimic the lifelong immunity conferred by natural infection. Vaccine approaches have included use of purified or recombinant *T gondii* surface antigens,<sup>140,141</sup> live attenuated or mutant strains of the parasite,<sup>11</sup> or DNA with plasmids encoding colony-stimulating factors.<sup>142</sup>

### Outlook

Despite great progress in clinical and basic science research, many unresolved issues in toxoplasmosis remain to be addressed. These topics encompass important clinical issues such as epidemiology, diagnosis and treatment, and prevention (screening) strategies (panel).<sup>143</sup>

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None declared.

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