Cell PRESS

The placenta: a main role in congenital toxoplasmosis?

Florence Robert-Gangneux[1,2], Jean-Benjamin Murat[3,4], Hélène Fricker-Hidalgo[3,4], Marie-Pierre Brenier-Pinchart[3,4], Jean-Pierre Gangneux[1,2] and Hervé Pelloux[3,4]

[1] Laboratoire de Parasitologie–Mycologie, Centre Hospitalier et Universitaire de Rennes, Rennes, France
[2] EA4427-SERAIC, IRSET (Institut de Recherche en Santé Environnement Travail), Université Rennes 1, Rennes, France
[3] Laboratoire de Parasitologie–Mycologie, Centre Hospitalier et Universitaire de Grenoble, Grenoble, France
[4] Laboratoire Adaptation et Pathogénie des Microorganismes, UMR 5163 CNRS-UJF, Université Joseph Fourier, Grenoble, France

Systemic infections, such as toxoplasmosis, acquired during pregnancy can lead to placental infection and have profound effects on the mother-to-child relationship and the success of pregnancy. Placental permeability to *Toxoplasma gondii* is a main parameter that determines parasite transmission to the foetus, and the use of antibiotics to decrease placental parasite load and prevent congenital toxoplasmosis has been suggested for decades. Although parasitological examination of the placenta at birth is commonly used to diagnose neonatal congenital toxoplasmosis, this approach can be controversial. Here we argue in favour of placental examination for both diagnostic and epidemiological purposes.

The consequences of *Toxoplasma* infection during pregnancy

Infection with the intracellular protozoan parasite *Toxoplasma gondii* is one of the most frequent worldwide parasitic infections. Its widespread distribution in warm-blooded animals, as an intermediate host for its asexual replicating stages, offers a range of opportunities for human infection through undercooked meat or raw vegetables contaminated with oocysts spread by definitive hosts, i.e. cats or other Felidae in some parts of the world. Infection is usually asymptomatic when it occurs in an immunocompetent subject. However, the pathophysiology of toxoplasmosis in humans is far more complex when primary infection is acquired during pregnancy; it then results in congenital infection of the foetus in ~30% of cases, which can result in brain or eye damage, with the degree of severity dependent on gestational age and the use, or not, of preventive protocols.

The placenta prevents the passage of infectious agents towards the foetal compartment more effectively at the beginning of pregnancy than at the end. It is a key tissue in the mother-to-foetus relationship, not only because of its trophic role but also because it provides the tolerant immune microenvironment necessary for gestation [1]. During primary infection, parasites cross the intestinal barrier and invade monocyte cells in contact with the lamina propria, which allow them to disseminate through the blood flow towards virtually all organs, including placenta [2]. Infection of the placental tissue can result in a placentitis and can lead to subsequent infection of trophoblast cells, which are at the interface with the foetal compartment and may let the parasites proceed [3]. This important process has two main consequences: (i) placental infection may adversely affect this tenuous equilibrium between maternal and foetal compartments; and (ii) the placenta is directly involved in parasite transmission to the foetus, making it a main therapeutic and diagnostic target. This opinion paper focuses on these different aspects and places emphasis on the recovery of the placenta to diagnose congenital toxoplasmosis.

What is the role of the placenta in *T. gondii* transmission and pathophysiology?

T. gondii can invade and multiply within trophoblast cells [3], but the mechanisms by which this otherwise effective barrier can fail to protect the foetus, allowing some pathogens to enter, remain unclear although some hypotheses have been suggested. Whereas an efficient immune response against *Toxoplasma* requires a T helper (Th)-1 cytokine pathway response involving interferon γ (IFN-γ) [4,5], the placental microenvironment is rich in interleukin 10 (IL-10) and promotes a Th-2 immune response to ensure maternal–foetal tolerance [1], which could facilitate infection of placental tissue [6]. The interplay between immune effectors of successful pregnancy and of anti-infectious response has been extensively described elsewhere [1,7]. The pivotal cytokine in this complex process is IFN-γ, as shown in a mouse model where IFN-γ synthesis following *T. gondii* infection led to abortion in pregnant wild type mice, but not in pregnant IFN-γ knockout (KO) mice [8]. Such a deleterious effect of IFN-γ is also described in pre-eclampsia in humans [9]. Thus, a delicate balance exists between the anti-*T. gondii* effector functions of IFN-γ and its abortogenic effects, and both maternal and foetal environments contribute to this complex equilibrium (Figure 1) [10]. However, the role of IFN-γ could be more ambiguous, as it was shown *in vitro* that it upregulates the expression of intercellular adhesion molecule (ICAM)-1 adhesin at the trophoblast cell surface and thereby contributes to enhanced adhesion of infected monocytes [11]. In addition, ICAM-1 is induced during placentitis [12] and could directly support transepithelial migration of the

Corresponding author: Robert-Gangneux, F. (florence.robert-gangneux@univ-rennes1.fr)

 1471-4922/$ – see front matter doi:10.1016/j.pt.2011.09.005 Trends in Parasitology, December 2011, Vol. 27, No. 12

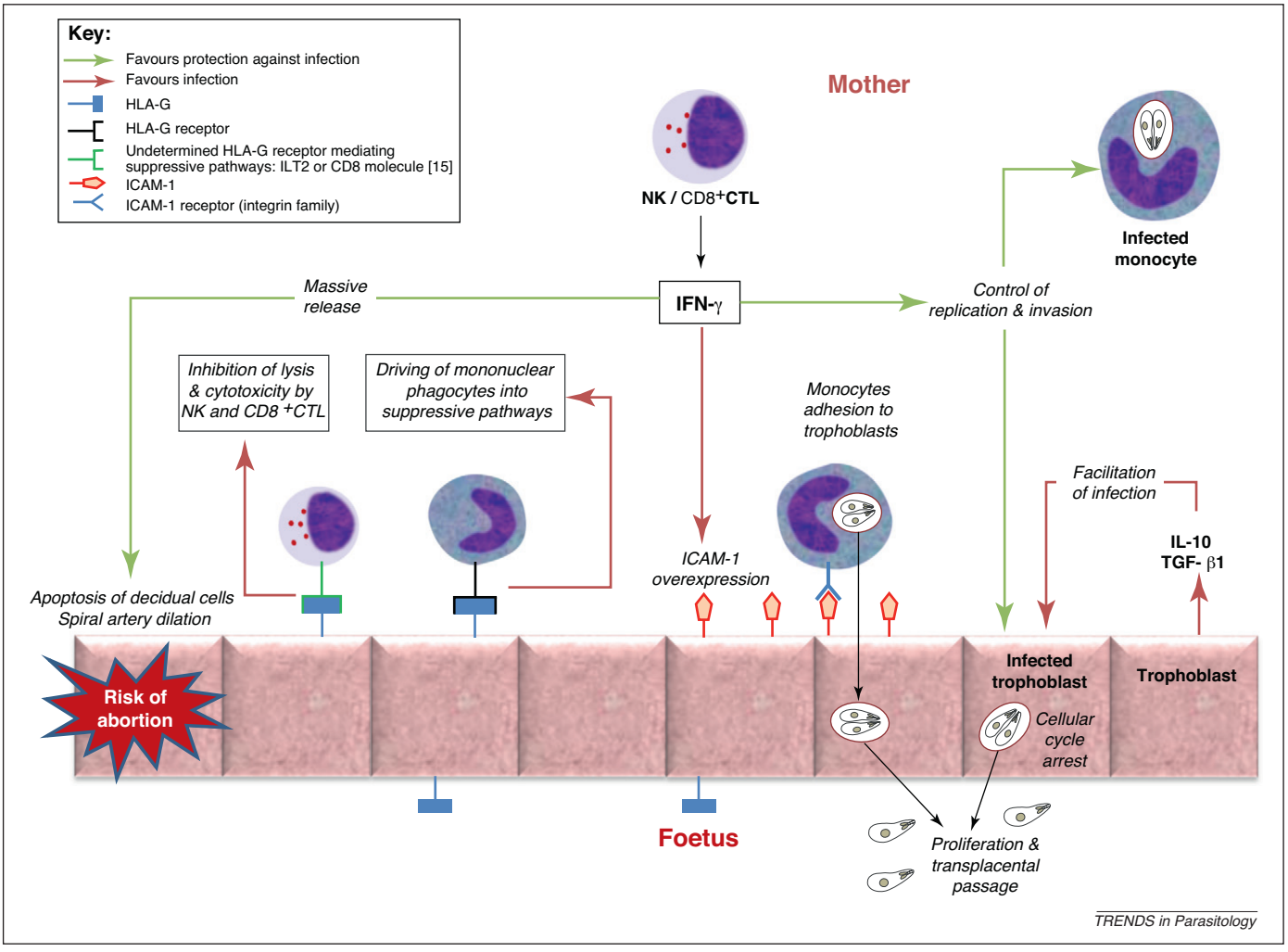

Figure 1. Tentative scheme of the pathophysiological hypotheses controlling trophoblast–cell infection and transplacental transfer of *Toxoplasma gondii*. Green arrows indicate mechanisms favouring protection against infection (even if they can be detrimental to pregnancy). Interferon γ (IFN-γ) produced by natural killer (NK) cells or CD8+ cytotoxic lymphocytes (CTL) directly controls both invasion of monocytes and trophoblasts by *T. gondii* and replication of the parasite in infected cells. Massive IFN-γ release has immunopathological effects, of which apoptosis of decidual cells and spiral artery dilation [62]. Red arrows indicate mechanisms favouring the progression of infection. Some of these are essential immunomodulatory mechanisms that compensate for the Th-1 inflammatory cytokines induced by *Toxoplasma*, and could avoid foetal loss, particularly when infection occurs in early pregnancy. Human trophoblast cells produce interleukin 10 (IL-10) and transforming growth factor β1 (TGF-β1) [1], which promote a Th-2 immune response to ensure maternal–foetal tolerance but induce a significant increase in both *T. gondii* intracellular replication and invasion [6]. IFN-γ secretion induces intercellular adhesion molecule (ICAM)-1 upregulation on the trophoblast surface, enhancing adhesion of infected monocytes to the trophoblast cell surface. Infected trophoblast cells then lose the ability for apoptosis, which results in parasite persistence in placental tissues [63], and this can be a reservoir for immediate or delayed congenital infection. The strong expression of human leukocyte antigen-G (HLA-G) on trophoblast cells can inhibit lysis by maternal NK cells and can mediate suppression of the allocytotoxic T cell response against the foetus. HLA-G expression also drives mononuclear phagocytes into suppressive pathways [15].

parasites, as it was shown to coprecipitate with the microneme protein MIC-2 [13]; MIC-2 is secreted by the parasite and relocalised at the parasite surface during cell invasion [14].

Local mechanisms could compensate for infection-induced deleterious effects. Placental cells have the unique capacity to select specific genes within the major histocompatibility complex (MHC); amongst which is human leukocyte antigen (HLA)-G that may be responsible for the reprogramming of local maternal immune response to facilitate maternal–foetal tolerance [15]. During *Toxoplasma* infection, HLA-G could contribute to counterbalance the cytotoxic Th-1 immune response [15]. In a recent study, higher levels of soluble HLA-G were observed in amniotic fluid from foetus, whose mothers had a history of *Toxoplasma* infection during pregnancy than in those without infection [16]. Recent diverse hypotheses, which allowed progress in the understanding of trophoblast cell infection and immune regulation at the maternal–foetal interface are described in Figure 1.

What effect does maternal prenatal antiparasitic treatment have on transplacental transmission of *Toxoplasma*?

The macrolide antiparasitic spiramycin has been widely used for decades to decrease the frequency of vertical transmission of *Toxoplasma* in historical studies [17–19]. However, although the pharmacokinetics of spiramycin have been studied in rhesus monkeys, data on its transplacental transfer in humans are scarce. Dosages of

spiramycin in pregnant women resulted in highly variable concentrations within the amniotic fluid, but did not reach the concentration range reported to inhibit parasite growth *in vitro* [20]. These data are in agreement with the general notion that spiramycin does not cross the placenta but, rather, becomes concentrated within. By contrast, in monkey experiments, spiramycin accumulated in the liver and spleen of both mother and foetus tissues, suggesting transplacental passage of this drug. However, spiramycin was not detected in the foetus brain, a key target in toxoplasmosis, and the drug was given intravenously, which is not the case in humans [21,22]. It is of interest that the drug accumulated in the placenta at a 10- to 20-fold higher concentration than that obtained in foetal serum [22].

The association of pyrimethamine–sulphadiazine + folinic acid is an alternative commonly used at >18 weeks of gestation, particularly when amniocentesis has confirmed foetal infection or when the infection risk is high, i.e. when seroconversion occurs late in pregnancy [23]. A study on rhesus monkeys for congenital toxoplasmosis showed that early treatment with pyrimethamine–sulphadiazine effectively reduced parasite number in the infected foetus [24]. In this experimental model, pyrimethamine–sulphadiazine crossed the placenta easily, and pyrimethamine was found to accumulate in the brain tissue [25]. In the rodent *Calomys callosus*, a significant decrease in parasite load was also observed after treatment with pyrimethamine–sulphadiazine, even though vertical transmission of *T. gondii* still occurred [26]. In this model, the drug azithromycin was shown to inhibit vertical transmission of *T. gondii* [26] but, to date, no data support its use in humans.

Over the past decade, meta-analyses and prospective–cohort studies have reported conflicting conclusions on the effect of prenatal antiparasitic treatment [27]. Some studies report that treatment was efficacious and reduced the frequency of transplacental transmission [28,29] when given during the first month following infection; however, other studies have not demonstrated such an effect [30,31]. Independently of its capacity to inhibit transmission, pyrimethamine–sulphadiazine does seem more efficient at eradicating parasites from the placenta than spiramycin, as treatment with pyrimethamine–sulphadiazine often results in a reduced detection of *Toxoplasma* in the placenta [32].

There are divergent findings concerning the effect of prenatal treatment on the risk of clinical manifestations in infected infants. Two European studies showed that prenatal treatment, within the first 4 weeks of maternal infection, reduced the risk of intracranial lesions or serious neurological sequelae compared with no treatment [33,34]; still, another study reported no evidence that prenatal treatment significantly reduced the risk of clinical manifestations [30]. Although most studies suffer from a lack or a small number of adequate untreated controls, and thus a lack of evidence for treatment efficacy (or treatment inefficiency), the possible clinical benefits of such treatments cannot be excluded. In addition, some data, such as gestational age at seroconversion or delay for treatment, are diversely taken into consideration between studies, although they probably play a significant role in the evaluation of treatment efficacy [35,36]. Indeed, increasing gestational age at seroconversion is strongly associated with increasing mother-to-child transmission and with decreasing risk of intracranial lesions [23,28]. Evidence of the efficacy of prenatal treatment to prevent congenital toxoplasmosis would be needed from a large randomised clinical trial [37,38]. However, owing to ethical considerations, it is difficult to consider an untreated control group. Therefore, a national prospective survey was started in France in 2010 to compare the efficacy of spiramycin versus pyrimethamine–sulphadiazine (http://clinicaltrials.gov/ct2/show/NCT01189448). Although this trial is not designed to formally answer the question of treatment efficacy, it will bring potential information about the best possible treatment.

In fact, the placenta plays a major role in the efficacy of any prenatal treatment, which depends on the delay in transplacental passage, i.e. the time interval between placental infection and progression of the parasites into the foetal compartment, a concept little known in humans. Indeed, if transmission occurs early after maternal infection and placental colonisation, treatment has little chance in preventing foetal infection, as it is started after serologic evidence of maternal infection, i.e. at least 2 or 3 weeks after blood flow dissemination. However, the observation that some women, infected early in pregnancy, have a negative prenatal diagnosis, yet deliver an infected asymptomatic newborn, strongly suggests that parasite release from the placenta may be delayed [39], thus possibly justifying continuous antiparasitic treatment through pregnancy.

Toxoplasma detection in placental tissue at delivery: a mirage or a predictive factor for congenital infection?

Consistent with the fact that antiparasitic treatment may help prevent congenital transmission, parasites are rarely detected in the placenta at birth when neonates are free of infection, thereby suggesting that early parasite clearance from the placenta protects the foetus. Consequently, parasitological examination of placental tissue is one of the biological tools used for years to diagnose *Toxoplasma* congenital infection at birth, along with serological screening of the newborn (Box 1) [40]. Nevertheless, its usefulness is debated by some researchers because of improvements in prenatal diagnosis and serological techniques. The search for parasites in the placenta relies on two technical approaches: mouse inoculation with placental homogenates and PCR detection of parasite DNA. Retrospective studies analysing the performance of placenta examination report 45–71% sensitivity, depending on the technique used [32,41–45]. Combining both methods yields the best sensitivity [43,44] (Table 1). Sensitivity is defined as the ratio of the number of positive results/number of infected infants (Box 2), with the understanding that such evaluations are meaningful only if they are performed on cohorts of children benefiting from a 9- to12-month follow-up to monitor the disappearance of maternal antibodies.

Recently, a value of 25% sensitivity in diagnosis was reported, which prompted a request that this methodology be abandoned for the diagnosis of congenital toxoplasmosis [46]. However, care should be taken so that results

Box 1. How to diagnose a congenital *Toxoplasma* infection

Period of diagnosis	Sample	Techniques	Criterion of positivity
Prenatal diagnosis	Amniotic fluid	PCR/mouse inoculation	Positive DNA detection[a]/at least one positive mouse with positive serology and brain cyst detection
Neonatal diagnosis	Placenta/cord blood	PCR/mouse inoculation	Positive DNA detection/at least one positive mouse with positive serology and brain cyst detection
	Cord blood serum	Serology (IgG, IgM, IgA)	IgM or IgA detection[b]
	Cord blood serum + mother serum (at delivery)	Western blot on mother–cord blood paired samples	Specific IgG or IgM pattern in the newborn
Postnatal follow-up	Infant serum	Serology (IgG, IgM, IgA)	IgM or IgA detection or absence of decrease of IgG titres over 6 months or persistence of IgG over 12 months of age
	Infant serum + mother serum (at delivery)	Western blot on mother–child paired samples[c]	Specific IgG or IgM pattern in the newborn

[a]In rare instances, a positive result can be obtained in the placenta in the absence of congenital infection.

[b]Attention must be focused on the possible contamination of cord blood by maternal serum at delivery. A positive result needs to be checked at 1 week of life.

[c]Repeated twice prior to 2–3 months of life.

obtained from routine tests are not misinterpreted, particularly when the techniques used changed or evolved over the study period. Indeed, various explanations could account for poor sensitivity, such as the amount of placental tissue analysed, the number of mice inoculated with placental homogenate, the homogenisation technique used and the PCR technique itself (conventional or RT-PCR, gene target). The number of inoculated mice can vary widely from one to six for placental samples. As the serologic screening of mice which are examined 5–6 weeks after inoculation can vary from one out of six to six out of six positive mice, this may have considerable impact on the global sensitivity of diagnosis.

In addition, the sensitivity of PCR techniques has improved over the years. Currently, it is acknowledged that quantitative PCR targeting the 200- to 300-fold repeated sequence REP529 (Genbank AF487550) gives more sensitive results, compared with the 35-fold repeated B1 gene which has been largely used since the 1990s [47]. Recent data show that it allowed a significant gain in prenatal diagnosis, which is now ~90% [48]. A retrospective study of 102 placentas evaluated this PCR target, and pointed to a sensitivity of 71% [45]. However, it must be kept in mind that sensitivity is not the main issue, of greater importance are the high positive and negative predictive values (PPV and NPV) from placental analysis (Box 2). In particular, PPV is >93% in most studies [32,42,45,46] (Table 1), making it a valuable diagnostic tool despite isolated positive PCR results occurring in rare instances. This may be related to residual DNA from dead parasites or, in rare instances, to the persistence of parasites with no foetal transmission [49]. From this standpoint, mouse inoculation has a higher specificity [32,42,45]. However, it requires, at least in France, an animal facility and a special

Table 1. Data of the literature concerning sensitivity, specificity, positive (PPV) and negative (NPV) predictive values of the biological techniques used alone or in combination for *Toxoplasma* detection in the placenta

Technique	Sensitivity *n*/*N* (%)	Specificity *n*/*N* (%)	PPV *n*/*N* (%)	NPV *n*/*N* (%)	Ref.
Mouse assay	13/29 (45)	58/58 (100)	13/13 (100)	58/74 (78)	[42]
	28/54 (52)	188/189 (99)	27/29 (93)	188/214 (88)	[54]
	8/19 (42)	105/105 (100)	105/116 (91)[b]	8/8 (100)[b]	[43]
	33/55 (60)	ud[c](99)	ud (97)	ud (79)	[32]
	nc[d]	ud (100)[b]	8/8 (100)[b]	nc	[46]
	18/27 (67)	69/69 (100)	18/18 (100)	69/78 (88)	[45]
	17/33 (52)	51/52 (98)	17/18 (94)	51/67 (76)	[41]
PCR	28/54 (52)	187/189 (99)	28/30 (93)	187/213 (88)	[54]
	5/20 (25)	107/113 (95)	5/11 (45)[b]	107/122 (88)[b]	[43]
	nc	nc	5/6 (83)[b]	nc	[46]
	20/28 (71)	71/73 (97)	20/22 (90)	71/79 (90)	[45]
	14/23 (61)	48/52 (92)	14/18 (78)	48/57 (84)	[41]
Mouse or PCR[a]	31/54 (57)	188/189 (99)	31/32 (97)	188/211 (89)	[54]
	10/20 (50)	107/113 (95)	10/16 (63)	107/117 (91)	[43]
	13/51 (25)	733/734 (99)	13/14 (93)	733/771 (95)	[46]
	20/27 (71)	72/74 (97)	20/22 (90)	72/80 (90)	[45]
	17/23 (74)	38/43 (88)	17/22 (77)	38/44 (86)	[41]

[a]At least one positive test when both are performed.

[b]Calculated from the authors' data.

[c]ud, unknown data

[d]nc, not computable.

Box 2. Definition of the concepts of sensitivity, specificity, positive predictive value and negative predictive value

Sensitivity = a/a + b
a = true positive results (= positive results obtained with the technique among infants with proven infection)
b = false negative results (= negative results obtained with the technique among infants with proven infection)
a + b = total number of infected infants

Specificity = c/c + d
c = true negative results (= negative results obtained with the technique among infants in whom the infection could be ruled out[a])
d = false positive results (= positive results obtained with the technique among infants in whom the infection could be ruled out[a])
c + d = total number of noninfected infants

Positive predictive value (PPV) = a/a + d
a = true positive results
d = false positive results

Negative predictive value (NPV) = c/c + b
c = true negative results
b = false negative results

[a]After a 9- to12-month follow-up attesting the disappearance of maternal IgG.

ministerial agreement, which can restrain some laboratories from using this assay.

Apart from technical considerations, other factors could account for methodologies utilised by practitioners in examination of placentas. Currently, it is thought that detection of parasites in a placenta is rarely the only positive test at birth and is usually associated with other positive tests [46], whether prenatal diagnosis, or another neonatal test such as detection of specific IgM or IgA in the neonate serum. The detection of specific neosynthesised IgG in the infant, by comparative analysis of mother–newborn paired sera using immunoblotting or enzyme-linked immunofiltration assay [50] is also a current method for reaching diagnosis (Box 1) [44,51,52]. Nevertheless, detection of parasites in the placenta has been the only early positive test to show congenital infection in several infants from two studies, in whom other tests did not yield any positive result [43,53]. In addition, in countries where serologic screening of pregnant women allows early diagnosis and treatment of seroconversion, it has been shown that prenatal treatment can decrease the sensitivity of IgM detection at birth [42] as well as isolation of viable parasites from the placenta by mouse inoculation [32], thereby justifying the combination of several diagnostic approaches. In view of this and of the high PPV of placental examination, we consider that placenta recovery for parasite search should be made in situations where prenatal diagnosis is negative or is not performed, in particular when infection occurs in late pregnancy (Table 1) [42,45,46,54].

An important tool for epidemiological studies

In congenital toxoplasmosis, the placenta not only allows the detection of *Toxoplasma*, thus improving diagnosis, but is also a source of potential *Toxoplasma* strains, or at least of *Toxoplasma* DNA, which can be typed for epidemiological studies. Indeed, the population structure of *T. gondii* comprises three major genotypes (types I, II and III) as well as recombinant genotypes which have a preferential distribution in some parts of the world [55,56]. In addition, highly diverse genotypes resulting from frequent genetic exchanges are circulating in South America. These atypical genotypes are responsible for severe forms of acute or congenital toxoplasmosis [57,58]. Although isolation of *T. gondii* strains responsible for congenital toxoplasmosis is complicated, the process is important. Parasites cannot be isolated from the mother's blood; they are rarely isolated from the amniotic fluid (apart from where amniocentesis is routinely performed, but the number of annual cases remains few) and almost never from cord blood. Thus, the placenta, which is easily recovered at delivery, is a safe and easy source of parasites, which can be used for *in vitro* susceptibility testing [59] or clinical epidemiologic studies [49,57]. Although handling this tissue is rather difficult if a laboratory is not skilled in the necessary techniques, procedures from reference laboratories can be disseminated to other labs or placental samples can be addressed to reference labs. The collaborative network of the Centre National de Référence de la Toxoplasmose in France gathers and types *Toxoplasma* isolates responsible for congenital infection or other severe infections and displays an annual cartography of the genotypes (https://www.chu-reims.fr/professionnels/cnr-toxoplasmose-1/rapports-dactivite/rapport-dactivites-2009/view). In 2009, 67 of 101 genotyped strains from congenital cases were isolated from placenta. Strain collection for typing should be maintained in the future, considering the need for a better understanding of the role of strain genotype in the pathophysiology of congenital toxoplasmosis [60] and the importance of evaluating the usefulness of potential new therapeutic tools [59,61]. Experience gained from routine neonatal diagnosis suggests that congenital infection is often associated with long-term placental infection, as parasites are isolated at delivery in 25–71% of cases (Table 1), despite treatment during pregnancy. Thus, the main issue at hand is to understand why these parasites persist within the placental tissue; is this because of a long delay until the mother is treated, which could cause sustained placental colonisation, or is it because of intrinsic virulence or resistance to antiparasitic therapies? These questions need to be addressed in larger studies highlight the major importance of placenta in *Toxoplasma* congenital infection.

Acknowledgements

We thank Newmed Publishing Services for editing the manuscript.

References

1 Entrican, G. (2002) Immune regulation during pregnancy and host–pathogen interactions in infectious abortion. *J. Comp. Pathol.* 126, 79–94
2 Lambert, H. and Barragan, A. (2010) Modelling parasite dissemination: host cell subversion and immune evasion by *Toxoplasma gondii*. *Cell Microbiol.* 12, 292–300
3 Abbasi, M. *et al.* (2003) Infection of placental trophoblasts by *Toxoplasma gondii*. *J. Infect. Dis.* 188, 608–616
4 Yap, G. *et al.* (2000) Cutting edge: IL-12 is required for the maintenance of IFN-gamma production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. *J. Immunol.* 165, 628–631
5 Denkers, E.Y. (1999) T lymphocyte-dependent effector mechanisms of immunity to *Toxoplasma gondii*. *Microbes Infect.* 1, 699–708
6 Barbosa, B.F. *et al.* (2008) BeWo trophoblast cell susceptibility to *Toxoplasma gondii* is increased by interferon-gamma, interleukin-10 and transforming growth factor-beta1. *Clin. Exp. Immunol.* 151, 536–545

7 Pfaff, A.W. *et al.* (2007) Cellular and molecular physiopathology of congenital toxoplasmosis: the dual role of IFN-gamma. *Parasitology* 134, 1895–1902
8 Shiono, Y. *et al.* (2007) Maternal-fetal transmission of *Toxoplasma gondii* in interferon-gamma deficient pregnant mice. *Parasitol. Int.* 56, 141–148
9 Hackmon, R. *et al.* (2007) Reduced third-trimester levels of soluble human leukocyte antigen G protein in severe preeclampsia. *Am. J. Obstet. Gynecol.* 197, 255
10 Pfaff, A.W. *et al.* (2008) Impact of foetus and mother on IFN-gamma-induced indoleamine 2,3-dioxygenase and inducible nitric oxide synthase expression in murine placenta following *Toxoplasma gondii* infection. *Int. J. Parasitol.* 38, e1–5, 249–258
11 Pfaff, A.W. *et al.* (2005) *Toxoplasma gondii* regulates ICAM-1 mediated monocyte adhesion to trophoblasts. *Immunol. Cell Biol.* 83, 483–489
12 Juliano, P.B. *et al.* (2006) ICAM-1 is overexpressed by villous trophoblasts in placentitis. *Placenta* 27, 750–757
13 Barragan, A. *et al.* (2005) Transepithelial migration of *Toxoplasma gondii* involves an interaction of intercellular adhesion molecule 1 (ICAM-1) with the parasite adhesin MIC2. *Cell Microbiol.* 7, 561–568
14 Carruthers, V. and Boothroyd, J.C. (2007) Pulling together: an integrated model of *Toxoplasma* cell invasion. *Curr. Opin. Microbiol.* 10, 83–89
15 Hunt, J.S. *et al.* (2005) HLA-G and immune tolerance in pregnancy. *FASEB J.* 19, 681–693
16 Robert-Gangneux, F. *et al.* (2011) High level of soluble HLA-G in amniotic fluid is correlated with congenital transmission of *Toxoplasma gondii*. *Clin. Immunol.* 138, 129–134
17 Couvreur, J. *et al.* (1988) Prophylaxis of congenital toxoplasmosis. Effects of spiramycin on placental infection. *J. Antimicrob. Chemother.* 22 (Suppl. B), 193–200
18 Desmonts, G. and Couvreur, J. (1974) Congenital toxoplasmosis. A prospective study of 378 pregnancies. *N. Engl. J. Med.* 290, 1110–1116
19 Couvreur, J. *et al.* (1993) In utero treatment of toxoplasmic fetopathy with the combination pyrimethamine-sulfadiazine. *Fetal Diagn. Ther.* 8, 45–50
20 Gratzl, R. *et al.* (2002) Treatment of toxoplasmosis in pregnancy: concentrations of spiramycin and neospiramycin in maternal serum and amniotic fluid. *Eur. J. Clin. Microbiol. Infect. Dis.* 21, 12–16
21 Schoondermark-Van de Ven, E. *et al.* (1994) Effectiveness of spiramycin for treatment of congenital *Toxoplasma gondii* infection in rhesus monkeys. *Antimicrob. Agents Chemother.* 38, 1930–1936
22 Schoondermark-Van de Ven, E. *et al.* (1994) Pharmacokinetics of spiramycin in the rhesus monkey: transplacental passage and distribution in tissue in the fetus. *Antimicrob. Agents Chemother.* 38, 1922–1929
23 Montoya, J.G. and Remington, J.S. (2008) Management of *Toxoplasma gondii* infection during pregnancy. *Clin. Infect. Dis.* 47, 554–566
24 Schoondermark-van de Ven, E.M. *et al.* (1997) Prenatal diagnosis and treatment of congenital *Toxoplasma gondii* infections: an experimental study in rhesus monkeys. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 74, 183–188
25 Schoondermark-van de Ven, E. *et al.* (1995) Study of treatment of congenital *Toxoplasma gondii* infection in rhesus monkeys with pyrimethamine and sulfadiazine. *Antimicrob. Agents Chemother.* 39, 137–144
26 Costa, I.N. *et al.* (2009) Azithromycin inhibits vertical transmission of *Toxoplasma gondii* in *Calomys callosus* (Rodentia: Cricetidae). *Placenta* 30, 884–890
27 Wallon, M. *et al.* (1999) Congenital toxoplasmosis: systematic review of evidence of efficacy of treatment in pregnancy. *BMJ* 318, 1511–1514
28 Thiebaut, R. *et al.* (2007) Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. *Lancet* 369, 115–122
29 Foulon, W. *et al.* (1999) Treatment of toxoplasmosis during pregnancy: a multicenter study of impact on fetal transmission and children's sequelae at age 1 year. *Am. J. Obstet. Gynecol.* 180, 410–415
30 Gilbert, R.E. *et al.* (2001) Effect of prenatal treatment on mother to child transmission of *Toxoplasma gondii*: retrospective cohort study of 554 mother-child pairs in Lyon, France. *Int. J. Epidemiol.* 30, 1303–1308
31 Gilbert, R. and Gras, L. (2003) Effect of timing and type of treatment on the risk of mother to child transmission of *Toxoplasma gondii*. *BJOG* 110, 112–120
32 Bessieres, M.H. *et al.* (2001) Neonatal screening for congenital toxoplasmosis in a cohort of 165 women infected during pregnancy and influence of in utero treatment on the results of neonatal tests. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 94, 37–45
33 Gras, L. *et al.* (2005) Association between prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: a cohort study in 13 European centres. *Acta Paediatr.* 94, 1721–1731
34 Cortina-Borja, M. *et al.* (2010) Prenatal treatment for serious neurological sequelae of congenital toxoplasmosis: an observational prospective cohort study. *PLoS Med.* 7 pii: e1000351
35 Thulliez, P. (2001) Commentary: Efficacy of prenatal treatment for toxoplasmosis: a possibility that cannot be ruled out. *Int. J. Epidemiol.* 30, 1315–1316
36 McLeod, R. *et al.* (2009) Why prevent, diagnose and treat congenital toxoplasmosis? *Mem. Inst. Oswaldo Cruz* 104, 320–344
37 Chene, G. and Thiebaut, R. (2009) Options for clinical trials of pre and post-natal treatments for congenital toxoplasmosis. *Mem. Inst. Oswaldo Cruz* 104, 299–304
38 Gilbert, R. (2009) Treatment for congenital toxoplasmosis: finding out what works. *Mem. Inst. Oswaldo Cruz* 104, 305–311
39 Romand, S. *et al.* (2001) Prenatal diagnosis using polymerase chain reaction on amniotic fluid for congenital toxoplasmosis. *Obstet. Gynecol.* 97, 296–300
40 Fricker-Hidalgo, H. *et al.* (1996) Congenital toxoplasmosis: specific IgM in fetal blood, cord blood and in the newborn. *Ann. Biol. Clin. (Paris)* 54, 165–168
41 Fricker-Hidalgo, H. *et al.* (1998) Detection of *Toxoplasma gondii* in 94 placentae from infected women by polymerase chain reaction, *in vivo*, and *in vitro* cultures. *Placenta* 19, 545–549
42 Naessens, A. *et al.* (1999) Diagnosis of congenital toxoplasmosis in the neonatal period: a multicenter evaluation. *J. Pediatr.* 135, 714–719
43 Fricker-Hidalgo, H. *et al.* (2007) Value of *Toxoplasma gondii* detection in one hundred thirty-three placentas for the diagnosis of congenital toxoplasmosis. *Pediatr. Infect. Dis. J.* 26, 845–846
44 Robert-Gangneux, F. *et al.* (1999) Value of prenatal diagnosis and early postnatal diagnosis of congenital toxoplasmosis: retrospective study of 110 cases. *J. Clin. Microbiol.* 37, 2893–2898
45 Robert-Gangneux, F. *et al.* (2010) Clinical relevance of placenta examination for the diagnosis of congenital toxoplasmosis. *Pediatr. Infect. Dis. J.* 29, 33–38
46 Filisetti, D. *et al.* (2010) Placental testing for *Toxoplasma gondii* is not useful to diagnose congenital toxoplasmosis. *Pediatr. Infect. Dis. J.* 29, 665–667
47 Burg, J.L. *et al.* (1989) Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *J. Clin. Microbiol.* 27, 1787–1792
48 Wallon, M. *et al.* (2010) Accuracy of real-time polymerase chain reaction for *Toxoplasma gondii* in amniotic fluid. *Obstet. Gynecol.* 115, 727–733
49 Ajzenberg, D. *et al.* (2002) Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. *J. Infect. Dis.* 186, 684–689
50 Pinon, J.M. *et al.* (1996) Early neonatal diagnosis of congenital toxoplasmosis: value of comparative enzyme-linked immunofiltration assay immunological profiles and anti-*Toxoplasma gondii* immunoglobulin M (IgM) or IgA immunocapture and implications for postnatal therapeutic strategies. *J. Clin. Microbiol.* 34, 579–583
51 Pinon, J.M. *et al.* (2001) Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M, and A antibodies. *J. Clin. Microbiol.* 39, 2267–2271
52 Tissot Dupont, D. *et al.* (2003) Usefulness of Western blot in serological follow-up of newborns suspected of congenital toxoplasmosis. *Eur. J. Clin. Microbiol. Infect. Dis.* 22, 122–125
53 Robert-Gangneux, F. *et al.* (2009) Congenital toxoplasmosis after a preconceptional or periconceptional maternal infection. *Pediatr. Infect. Dis. J.* 28, 660–661
54 Bessieres, M.H. *et al.* (2009) Diagnosis of congenital toxoplasmosis: prenatal and neonatal evaluation of methods used in Toulouse

University Hospital and incidence of congenital toxoplasmosis. *Mem. Inst. Oswaldo Cruz* 104, 389–392
55 Sibley, L.D. and Ajioka, J.W. (2008) Population structure of *Toxoplasma gondii*: clonal expansion driven by infrequent recombination and selective sweeps. *Annu. Rev. Microbiol.* 62, 329–351
56 Darde, M.L. (2008) *Toxoplasma gondii,* "new" genotypes and virulence. *Parasite* 15, 366–371
57 Delhaes, L. *et al.* (2010) Severe congenital toxoplasmosis due to a *Toxoplasma gondii* strain with an atypical genotype: case report and review. *Prenat. Diagn.* 30, 902–905
58 Demar, M. *et al.* (2007) Fatal outbreak of human toxoplasmosis along the Maroni River: epidemiological, clinical, and parasitological aspects. *Clin. Infect. Dis.* 45, e88–e95
59 Meneceur, P. *et al.* (2008) *In vitro* susceptibility of various genotypic strains of *Toxoplasma gondii* to pyrimethamine, sulfadiazine, and atovaquone. *Antimicrob. Agents Chemother.* 52, 1269–1277
60 Brenier-Pinchart, M.P. *et al.* (2010) *In vitro* differential phenotypic characteristics among type-II *Toxoplasma gondii* strains from congenital toxoplasmosis in humans. *J. Parasitol.* 96, 798–799
61 Bougdour, A. *et al.* (2009) Drug inhibition of HDAC3 and epigenetic control of differentiation in Apicomplexa parasites. *J. Exp. Med.* 206, 953–966
62 Senegas, A. *et al.* (2009) *Toxoplasma gondii*-induced foetal resorption in mice involves interferon-gamma-induced apoptosis and spiral artery dilation at the maternofoetal interface. *Int. J. Parasitol.* 39, 481–487
63 Pfaff, A.W. and Candolfi, E. (2008) New insights in toxoplasmosis immunology during pregnancy. Perspective for vaccine prevention. *Parassitologia* 50, 55–58