

## Diagnosis of congenital toxoplasmosis by polymerase chain reaction on neonatal peripheral blood

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### Abstract

In a cohort of 12 consecutive neonates, polymerase chain reaction (PCR) established the diagnosis of 5 of 6 cases of congenital toxoplasmosis and did so earlier than serologic methods. We validated that PCR using neonatal peripheral blood is a sensitive, rapid, and cost-effective method to affirm the diagnosis of previously undiagnosed congenital toxoplasmosis.

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Congenital toxoplasmosis (CT), a major cause of fetal pathology, may appear when the mother is contaminated by the protozoan parasite *Toxoplasma gondii* during pregnancy. In France, the incidence of CT was 2.9 (95% CI: 2.5 to 3.2) per 10,000 live births in 2007 (King et al., 2008). According to the French national prevention policy, all pregnant women who are seronegative for *Toxoplasma* are surveyed monthly to detect toxoplasmic seroconversion. In case of maternal contamination during pregnancy, in order to definitely assess or rule out the diagnosis of congenital toxoplasmosis, a whole set of recommendations applies: i) prenatal diagnosis based on molecular testing of amniotic fluid and ultrasound examinations; ii) molecular testing of placenta and cord blood, comparative mother–child serologic tests and a clinical examination at birth; iii) neurologic and ophthalmologic examinations and a serologic survey during the first year of life. However, some deficiency in the regular follow-up of a pregnant woman may occur, and it is not rare that at delivery there is no or little information on the prenatal monitoring for CT of the mother and the fetus. This situation is critical and frequent in countries in which women are not

routinely screened for toxoplasmosis. When polymerase chain reaction (PCR) of amniotic fluid, placenta, and/or cord blood is not available, the diagnosis of CT depends only on serologic follow-up after birth. The definite diagnosis is then difficult to assess and delayed. Here we validate the use of an unusual practice based on the molecular testing of the neonatal peripheral blood.

From March 2003 to December 2008, all successive neonates who had blood sampled for molecular diagnosis of CT before the age of 3 weeks were prospectively enrolled in the study. Recruitment covered the Languedoc-Roussillon region, south of France. No exclusion criteria were applied in the selection of the cohort. Information was collected and analyzed for each mother and child pair in order i) to estimate the gestational age at which the maternal infection occurred and ii) to establish the diagnosis of CT.

Diagnosis of CT was defined according to the classification system and case definitions developed by the European Research Network on Congenital Toxoplasmosis (Lebech et al., 1996). All biological samples were tested in our laboratory, which is proficient in this diagnosis at the regional and national level (Bastien, 2002; Bastien et al., 2007; Sterkers et al., 2010a, 2010b). A series of tests were performed — amniotic fluid, placenta, and cord blood examination — by a highly performing conventional PCR (Bretagne et al., 1993; Chabbert et al., 2004) that has shown excellent sensitivity as

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Table 1  
 Characteristics and results of the diagnostic tests in the cohort of 12 neonates

	Neonate	Contamination (WA)	AF (PCR)	Cord blood (PCR)	Serology at birth	Comparative mother-child Western blot	Neonate peripheral blood		Criteria of definitive diagnosis	Category of infection
							PCR	Day		
Group 1 (no cord blood)	1	34	nd	nd	Comparable mother-child IC IgM positive IgA negative	IgG negative IgM positive	Positive	4	Npb PCR positive IgM WB positive IgM positive at 1 month	Definite CT
	2	39	nd	nd	Negative	nd	Positive	15	Npb PCR positive IgM positive at 1 month	Definite CT
	3	T3	nd	nd	Comparable mother-child IC IgM positive IgA negative	IgG negative IgM positive	Positive	2	Npb PCR positive IgM WB positive IgM positive at 1 month	Definite CT
	4	T3	nd	nd	Comparable mother-child IC IgM positive IgA negative	IgG negative IgM positive	Positive	2	Npb PCR positive IgM WB positive IgM positive at 1 month	Definite CT
	5	36	nd	nd	Comparable mother-child IC IgM positive IgA negative	IgG positive IgM positive	Positive	10	Npb PCR positive IgM and IgG WB positive IgM positive at 1 month	Definite CT
	6	19	nd	nd	Comparable mother-child IC IgM negative IgA negative	nd	Negative	3	Negative serology at 1 year	Not infected
	7	40	nd	nd	Comparable mother-child IC IgM negative IgA negative	IgG negative IgM negative	Negative	3	Negative serology at 1 year	Not infected
	8	12	negative	nd	Comparable mother-child IC IgM negative IgA negative	nd	Negative	15		Lost to follow-up
	9	20	negative	nd	Comparable mother-child IC IgM negative IgA negative	nd	Negative	2	Negative serology at 6 months	Not infected
	10	<6	nd	nd	Comparable mother-child IC IgM negative IgA negative	IgG negative IgM negative	Negative	3	Limited value of IgG at 8 months	Unlikely CT
Group 2	11	27-34	nd	negative	Comparable mother-child IC IgM positive IgA positive	IgG negative IgM positive	Negative	2	IgM WB positive IgM positive at 1 month	Definite CT
	12	35	nd	negative	Comparable mother-child IC IgM negative IgA negative	IgG negative IgM negative	Negative	15	Negative serology at 4 months	Not infected

WA = Week of amenorrhea; AF = amniotic fluid; nd = not done; IC = immune charge; Npb = neonate peripheral blood; WB positive = positive Western blot (i.e., comparison of the immunoblot assays from mother and child led to the identification of specific bands in the child); CT = congenital toxoplasmosis; T3 = third trimester of pregnancy.

compared with other real-time PCR assays (Sterkers et al., 2010b) and mice inoculation (Desmonts & Couvreur, 1974); serologic follow-up during the first year of life for IgG, IgM, and IgA by ELISA, immunofluorescence, and/or immunosorbent agglutination assay (ISAGA) (Pratlong et al., 1996); and comparative immune charge (relating specific IgG to total IgG, according to Desmonts and Couvreur, 1974). This panel was completed by a comparative mother–child immunoblot assay (Robert-Gangneux et al., 1999; Tissot Dupont et al., 2003) i) in case of contamination during the second or third trimester and ii) in case of contamination during the first trimester associated with a positive result of one of the first-line tests.

Twelve consecutive neonates were tested for toxoplasmosis by PCR using peripheral blood, either in cases with an incomplete or deficient follow-up during pregnancy ( $n = 10$ ) or in cases with comprehensive medical records and a high risk for CT due to the belated date of contamination ( $n = 2$ ) (Table 1). When carried out, amniotic fluid, placenta, and cord blood examination (PCR and mice inoculation), as well as serologic screening at delivery were not contributive for the diagnosis of CT (Table 1).

Six (50%) of 12 infants developed CT; 4 were uninfected; in 1 case the diagnosis of CT was unlikely, and the remaining case was lost during follow-up. In all cases of CT, the maternal contamination occurred in the third trimester. The PCR tests were performed using neonatal peripheral blood on days 2 to 4 (8 cases), 10 (1 case), and 15 (3 cases). The PCR was positive in 5 (83%) of the 6 cases of CT and negative in the remaining cases. In 1 neonate, the positive PCR using peripheral blood was the first test which affirmed the diagnosis of CT.

Early diagnosis of CT allows the practitioner to start the antiparasitic treatment early, in order to minimize i) the clinical consequences of CT and ii) the risk of loss to follow-up before definite diagnosis. Therefore an early diagnosis of CT is a cost-effective strategy. Our prospective study demonstrates the interest of molecular diagnosis of CT using peripheral blood in the neonatal and early postnatal period. In 1998, Bergstrom et al. (1998) reported the feasibility of this approach in one case report. Here, we validate that this unusual practice can be performed in routine. Indeed, molecular detection of *T. gondii* in the neonatal peripheral blood may affirm the diagnosis of CT rapidly after birth, either as the first and only evidence for the disease or by definitely strengthening a positive immunoblot, without waiting for the serologic persistence of IgMs at 1 month of life. Thus, the diagnosis of CT was affirmed for neonate numbers 1–5 and 11. For neonate number 2, due to a very late contamination at 39 weeks of amenorrhea, serology and comparative immunoblot at birth were negative and the positive PCR on neonatal peripheral blood was the first argument for the diagnosis of CT. Without this result, the diagnosis of CT would have been delayed and the child might have been lost to follow-up.

In addition, it is noteworthy that the PCR may be positive even when peripheral blood is sampled as late as days 10 and 15 (neonate numbers 5 and 2, respectively). Nevertheless, in our cohort, 1 false-negative molecular test was recorded,

recalling that the diagnosis of CT is difficult and often needs a combination of biological tests.

In conclusion, with a low false-negative rate and no false-positive result, we validated that PCR on neonatal peripheral blood i) can be performed routinely, ii) is able to affirm the diagnosis of CT, and iii) may be the first positive examination. Therefore, this unusual practice appears to be a useful alternative diagnostic approach.

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## References

- Bastien P (2002) Molecular diagnosis of toxoplasmosis. *Trans R Soc Trop Med Hyg* 96(Suppl 1):S205–S215.
- Bastien P, Jumas-Bilak E, Varlet-Marie E, Marty P (2007) Three years of multi-laboratory external quality control for the molecular detection of *Toxoplasma gondii* in amniotic fluid in France. *Clin Microbiol Infect* 13:430–433.
- Bergstrom T, Ricksten A, Nenonen N, Lichtenstein M, Olofsson S (1998) Congenital *Toxoplasma gondii* infection diagnosed by PCR amplification of peripheral mononuclear blood cells from a child and mother. *Scand J Infect Dis* 30:202–204.
- Bretagne S, Costa JM, Vidaud M, Tran J, Nhieu V, Fleury-Feith J (1993) Detection of *Toxoplasma gondii* by competitive DNA amplification of bronchoalveolar lavage samples. *J Infect Dis* 168:1585–1588.
- Chabbert E, Lachaud L, Crobu L, Bastien P (2004) Comparison of two widely used PCR primer systems for detection of toxoplasma in amniotic fluid, blood, and tissues. *J Clin Microbiol* 42:1719–1722.
- Desmonts G, Couvreur J (1974) Toxoplasmosis in pregnancy and its transmission to the fetus. *Bull N Y Acad Med* 50:146–159.
- King L, Villena I, Ancelle T, Wallon M, Garcia P, Thulliez P, Goulet V (2008) Congenital toxoplasmosis: implementation of a surveillance system in France. *Bull Epidémiologique Hebdomadaire* 14-15:122–123.
- Lebech M, Joynson DH, Seitz HM, Thulliez P, Gilbert RE, Dutton GN, Ovlisen B, Petersen E (1996) Classification system and case definitions of *Toxoplasma gondii* infection in immunocompetent pregnant women and their congenitally infected offspring. European Research Network on Congenital Toxoplasmosis. *Eur J Clin Microbiol Infect Dis* 15:799–805.
- Pratlong F, Boulot P, Villena I, Issert E, Tamby I, Cazenave J, Dedet JP (1996) Antenatal diagnosis of congenital toxoplasmosis: evaluation of the biological parameters in a cohort of 286 patients. *Br J Obstet Gynaecol* 103:552–557.
- Robert-Gangneux F, Commerce V, Tourte-Schaefer C, Dupouy-Camet J (1999) Performance of a Western blot assay to compare mother and newborn anti-Toxoplasma antibodies for the early neonatal diagnosis of congenital toxoplasmosis. *Eur J Clin Microbiol Infect Dis* 18:648–654.
- Sterkers Y, Varlet-Marie E, Cassaing S, Brenier-Pinchart MP, Brun S, Dalle F, Delhaes L, Filisetti D, Pelloux H, Yera H, Bastien P (2010a) Multicentric comparative analytical performance study for molecular detection of low amounts of *Toxoplasma gondii* from simulated specimens. *J Clin Microbiol* 48:3216–3222.
- Sterkers Y, Varlet-Marie E, Marty P, Bastien P; ANOFEL Toxoplasma-PCR Quality Control Group (2010b) Diversity and evolution of methods and practices for the molecular diagnosis of congenital toxoplasmosis in France: a 4-year survey. *Clin Microbiol Infect* 16:1594–1602.
- Tissot Dupont D, Fricker-Hidalgo H, Brenier-Pinchart MP, Bost-Bru C, Ambroise-Thomas P, Pelloux H (2003) Usefulness of Western blot in serological follow-up of newborns suspected of congenital toxoplasmosis. *Eur J Clin Microbiol Infect Dis* 22:122–125.