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Contribution of molecular diagnosis to congenital toxoplasmosis

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ABSTRACT

We evaluated the performance of three real-time polymerase chain reaction (PCR) assays on 73 samples from mothers and children with congenital toxoplasmosis. PCR assays had significantly higher sensitivity in prenatal period than in birth period when targeting the 529-bp repeat element (81.3% versus 36.0%) or the B1 gene (64.6% versus 20.0%).

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A positive *Toxoplasma gondii* polymerase chain reaction (PCR) assay on an amniotic fluid (AF) sample entails radical changes in pre- and post-natal management. For this reason, mass screening for congenital toxoplasmosis (CT) performed currently in France and in other European countries, requires the most efficacious and cost-effective serological and molecular tools (Montoya and Remington, 2008). However, the follow-up of pregnant women may be irregular, or mothers infected during pregnancy may refuse amniocentesis. In these situations and when prenatal diagnosis was negative, biological neonatal diagnosis of CT could be performed by molecular testing of different samples (AF, placenta, cord blood, peripheral blood) as well as comparative mother-child serologic tests. While performance variations among samples used for *Toxoplasma* DNA detection have been reported (Filisetti et al., 2003; Robert-Gangneux et al., 2010; Sterkers et al., 2011), we aim to evaluate the usefulness of PCR for the diagnosis of CT in prenatal and neonatal periods. We compared three different real-time PCR assays for the diagnosis of CT in prenatal and neonatal samples.

Seventy-three samples were collected from 57 mother-child pairs followed-up for CT, either in Lille 2 University Hospital or in the Cochin-Port Royal-Saint Vincent de Paul University Hospital group. 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). Clinical monitoring for toxoplasmosis in these mother-child pairs showed 12% of termination of pregnancy, 11% of symptomatic CT and 77% of asymptomatic CT (Table 1). The symptomatic and asymptomatic CT cases were confirmed by toxoplasmosis serological monitoring with an increase of immunoglobulin G (IgG) content in the first 12 months of life or a persistence of IgG after the first year of life or a presence of IgM and/or IgA. CT occurred after maternal infection in the first (7%), second (58%) and third (35%) trimester of pregnancy. Forty eight prenatal AF samples and 25 neonatal samples (9 AF, 9 peripheral or 7 cord bloods) from these mothers and children were collected and tested for *T. gondii* by real-time PCR. AF samples (10 ml) were centrifuged at 1300×*g* for 10 min. Peripheral blood (1 ml) and cord blood (5 mL) were centrifuged at 1083×*g* for 10 min to collect the white blood cells. DNA extraction was performed from the pellet of AF or from white blood cells using QIAamp DNA Mini Kit (Qiagen S.A., Courtaboeuf, France). Three real-time PCR assays were used, one targeting B1 gene with hybridization probes on LightCycler® I instrument (Reischl et al., 2003) and 2 targeting the 529-bp repeat element (Homan et al., 2000) with hybridization probes on LightCycler® I instrument (Reischl et al., 2003) or hydrolysis probe on ABI Prism®7000 instrument (Talabani et al., 2009). PCR inhibition was checked in each DNA extract by amplifying a non-competitive internal control (Talabani et al., 2009, Yera et al., 2009). For each PCR assay, DNA samples were tested at least in duplicate, and results were qualitative results according to the presence or absence of *T. gondii* DNA. The specificity of each PCR assay has previously been tested and was 100% (Sterkers et al., 2010; Talabani et al., 2009). Mouse inoculation

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Table 1
Results of real-time PCR assays and mouse inoculation in 73 samples from 57 mother-child pairs followed-up for congenital toxoplasmosis.

Patient no. (Pregnancy trimester at infection)	Clinical findings of CT	Sample[a]	Biological Data			
			PCR assay[b]			Mouse inoculation
			B1 LC®	RE LC®	RE ABI®	
1 (1)	Ventriculomegaly	PNAF	+	+	+	ND[c]
		NNAF	-	-	-	
2 (1)	Asymptomatic	PNAF	+	+	+	ND
		CB	-	-	-	
3 (1)	Asymptomatic	PNAF	-	-	-	-
4 (1)	Termination of pregnancy	PNAF	-	+	+	-
5 (2)	Asymptomatic	PNAF	+	+	+	ND
		PB	-	-	-	
6 (2)	Asymptomatic	PNAF	+	+	+	ND
		PB	-	-	-	
7 (2)	Asymptomatic	PNAF	+	+	+	ND
8 (2)	Abnormal neurodevelopment	PNAF	+	+	+	ND
9 (2)	Termination of pregnancy	PNAF	-	-	-	ND
		NNAF	+	+	+	
10 (2)	Asymptomatic	PNAF	+	+	+	ND
11 (2)	Asymptomatic	PNAF	+	+	+	ND
12 (2)	Asymptomatic	PNAF	+	+	+	ND
		PB	-	-	-	
13 (2)	Asymptomatic	PNAF	+	+	+	ND
14 (2)	Termination of pregnancy	PNAF	+	+	+	ND
		PB	-	-	-	
15 (2)	Asymptomatic	PB	-	-	-	ND
16 (2)	Termination of pregnancy	PNAF	+	+	+	ND
17 (2)	Termination of pregnancy	PNAF	+	+	+	ND
18 (2)	Asymptomatic	NNAF	-	+	+	ND
		CB	-	-	-	
19 (2)	Asymptomatic	NNAF	-	-	-	ND
		PB	-	+	+	
20 (2)	Asymptomatic	NNAF	-	-	-	ND
		CB	-	-	-	
21 (2)	Asymptomatic	PNAF	-	-	-	ND
22 (2)	Asymptomatic	PNAF	+	+	+	ND
23 (2)	Asymptomatic	PNAF	+	+	+	ND
24 (2)	Asymptomatic	PNAF	+	+	+	+
25 (2)	Asymptomatic	PNAF	-	-	-	ND
		NNAF	-	+	+	
26 (2)	Asymptomatic	PNAF	+	+	+	+
27 (2)	Asymptomatic	PNAF	+	+	+	+
28 (2)	Asymptomatic	PNAF	+	+	+	+
29 (2)	Termination of pregnancy	PNAF	+	+	+	-
30 (2)	Asymptomatic	PNAF	+	+	+	+
31 (2)	Termination of pregnancy	PNAF	+	+	+	+
32 (2)	Asymptomatic	PNAF	+	+	+	-
33 (2)	Asymptomatic	PNAF	-	+	+	-
34 (2)	Asymptomatic	PNAF	+	+	+	+
35 (2)	Asymptomatic	PNAF	+	+	+	-
36 (2)	Asymptomatic	PNAF	-	+	+	-
37 (2)	Asymptomatic	PNAF	+	+	+	-
38 (3)	Asymptomatic	PNAF	-	+	+	ND
39 (3)	Asymptomatic	PNAF	+	+	+	ND
40 (3)	Asymptomatic	PNAF	-	-	-	ND
41 (3)	Chorioretinitis	PNAF	-	-	-	ND
42 (3)	Chorioretinitis	PNAF	-	+	+	ND
43 (3)	Asymptomatic	PNAF	+	+	+	ND
		NNAF	-	-	-	
44 (3)	Asymptomatic	PNAF	-	+	+	ND
		CB	-	-	-	
45 (3)	Asymptomatic	NNAF	-	-	-	ND
		CB	-	-	-	
46 (3)	Ocular lesion	PB	+	+	+	ND
47 (3)	Asymptomatic	PNAF	-	-	-	ND
48 (3)	Asymptomatic	PNAF	+	+	+	ND
49 (3)	Asymptomatic	PB	+	+	+	ND
50 (3)	Asymptomatic	PNAF	+	+	+	ND
51 (3)	Asymptomatic	PNAF	-	-	-	ND
		PB	-	-	-	
52 (3)	Asymptomatic	CB	-	+	+	ND
53 (3)	Chorioretinitis	NNAF	+	+	+	ND
		CB	+	+	+	
54 (3)	Asymptomatic	PNAF	-	+	+	+

(continued on next page)

Table 1 (*continued*)

Patient no. (Pregnancy trimester at infection)	Clinical findings of CT	Sample[a]	Biological Data			
			PCR assay[b]			Mouse inoculation
			B1 LC®	RE LC®	RE ABI®	
55 (3)	Asymptomatic	PNAF	+	+	+	ND
56 (3)	Asymptomatic	PNAF	-	-	-	-
57 (3)	Asymptomatic	PNAF	-	+	+	-

[a] PNAF = prenatal AF; NNAF = neonatal AF; PB = peripheral blood taken at birth; CB = cord blood taken at birth.
[b] One protocol of real-time PCR targeting B1 gene with hybridization probes on LightCycler® I instrument (Reischl et al., 2003) and two targeting the 529-bp repeat element with hybridization probes on LightCycler® I instrument (Reischl et al., 2003) or hydrolysis probe on ABI Prism®7000 instrument (Talabani et al., 2009).
[c] ND = not done.

was performed on prenatal AF as previously described (Robert-Gangneux et al., 1999). Nevertheless if the volume of samples was insufficient, priority was given to PCR testing.

No degree of inhibition was found, in agreement with published data and the extraction method used (Yera et al., 2009). In each PCR assay, *T. gondii* DNA was detected in both tested reactions of all positive samples. The results of PCR targeting the 529-bp repeat element (PCR-RE) were similar whatever the technology used, either hybridization probes on LightCycler® or hydrolysis probe on ABI Prism®7000 (Table 1) (Cycle threshold Ct data not shown, but available at request). When comparing the sensitivity of PCR according to the target used, the 529-bp repeat element was found better than B1 gene for the diagnosis of CT in both prenatal (81.3% versus 64.6%) and birth (36.0% versus 20.0%) periods. The differences were not statistically significant using Fisher's exact test, probably due to limited sampling. PCR-RE was significantly more sensitive than mouse inoculation for prenatal diagnosis (88.9% versus 44.4%, $P < 0.05$ using Fisher's exact test). When comparing the sensitivity of PCR according to the period of diagnosis, both PCR-RE and PCR-B1 had significantly better sensitivity in the prenatal period than in the birth period (81.3% and 64.6% versus 36.0% and 20.0%, $P < 0.05$ using Fisher's exact test respectively). When comparing the sensitivity of PCR according to the sample tested, AF had significantly better sensitivity than blood using PCR-RE or PCR-B1 (75.4% or 57.9% versus 31.3% or 18.8%, $P < 0.05$ using Fisher's exact test respectively). PCR-RE allowed the confirmation of infection at birth period in 8 children with a negative prenatal diagnosis (2 children) or without prenatal diagnosis (6) (Table 1). The maternal infection of these children occurred in second (4) and third (4) trimester of pregnancy. The detection rates of PCR-RE were evaluated according to the different neonatal samples in the 12 children whose mothers had a negative prenatal diagnosis (3) or had no prenatal diagnosis (9); as 4/7 in neonatal AF, 3/5 in peripheral blood and 2/5 in cord blood samples.

In this study, we observed the better detection rate of PCR targeting the 529-bp repeat element than PCR targeting the B1 gene in the context of CT diagnosis, as it has been reported with a statistical significance from artificial AF samples (Sterkers et al., 2010). These results could be in contradiction with those of Cassaing et al. (2006) showing no discordances between the two targets in prenatal diagnosis. However, these authors analyzed only 8 AF samples from fetuses with CT. We showed a higher sensitivity of PCR in this diagnosis during the prenatal period than the birth period when testing AF and blood samples. In France when prenatal AF is positive for *T. gondii* and no lesion is found in CT, women are offered sulfadiazine and pyrimethamine to treat the fetus. This prenatal therapy is parasiticide on the tachyzoite stages and may lead to the reduction then the disappearance of the parasitical load in the biological fluids. This could account for the lower detection rate of PCR in children born to treated mothers and to a better performance of PCR on prenatal AF. It could be also that AF are more suited for diagnosing CT than neonatal blood samples, since most of maternal-fetal contamination appeared during pregnancy too far from the delivery to still observe a parasitemia in the children.

Therefore, we recommend performing CT diagnosis at prenatal period on AF by PCR targeting the 529-bp repeat element. Nevertheless, we could observe the usefulness of PCR at birth in AF and blood samples when prenatal diagnosis was negative or absent, in agreement with recent results that report a PCR assay on neonatal peripheral blood as a sensitive and rapid method to affirm the diagnosis of previously undiagnosed CT in 5 of 6 children infected during the third trimester of pregnancy (Sterkers et al., 2011). PCR on placenta has been also reported as another sensitive method for the diagnosis of CT (Robert-Gangneux et al., 2010), but its pre-analytic treatment is still delicate compared to AF and blood samples.

In addition, loop-mediated isothermal amplification (LAMP) represents a new emerging, simple, and sensitive method (Lin et al., 2012). But, its specificity should be tested in context of CT diagnosis.

The sensitivity and specificity of molecular methods (PCR or LAMP) on neonatal AF should be evaluated on more samples for the diagnosis of CT.

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References

Cassaing S, Bessières MH, Berry A, Berrebi A, Fabre R, Magnaval JF. Comparison between two amplification sets for molecular diagnosis of toxoplasmosis by real-time PCR. J Clin Microbiol 2006;44:720–4.
Filisetti D, Gorcii M, Pernot-Marino E, Villard O, Candolfi E. Diagnosis of congenital toxoplasmosis: comparison of targets for detection of *Toxoplasma gondii* by PCR. J Clin Microbiol 2003;41:4826–8.
Homan WL, Vercammen M, De Braekeleer J, Verschueren H. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. Int J Parasitol 2000;30:69–75.
Lebech M, Joynson DH, Seitz HM, Thulliez P, Gilbert RE, Dutton GN, et al. 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 1996;15: 799–805.
Lin Z, Zhang Y, Zhang H, Zhou Y, Cao J, Zhou J. Comparison of loop-mediated isothermal amplification (LAMP) and real-time PCR method targeting a 529-bp repeat element for diagnosis of toxoplasmosis. Vet Parasitol 2012;185:296–300.
Montoya JG, Remington JS. Management of *Toxoplasma gondii* infection during pregnancy. Clin Infect Dis 2008;47:554–66.
Reischl U, Bretagne S, Krüger D, Ernault P, Costa JM. Comparison of two DNA targets for the diagnosis of toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. BMC Infect Dis 2003;3:7.
Robert-Gangneux F, Gavinet MF, Ancelle T, Raymond J, Tourte-Schaefer C, Dupouy-Camet J. Value of prenatal diagnosis and early postnatal diagnosis of congenital toxoplasmosis: retrospective study of 110 cases. J Clin Microbiol 1999;37:2893–8.
Robert-Gangneux F, Dupretz P, Yvenou C, Quinio D, Poulain P, Guiguen C, et al. Clinical relevance of placenta examination for the diagnosis of congenital toxoplasmosis. Pediatr Infect Dis J 2010;29:33–8.

Sterkers Y, Varlet-Marie E, Cassaing S, Brenier-Pinchart MP, Brun S, Dalle F, et al. Multicentric comparative analytical performance study for molecular detection of low amounts of *Toxoplasma gondii* from simulated specimens. J Clin Microbiol 2010;48:3216–22.

Sterkers Y, Ribot J, Albaba S, Issert E, Bastien P, Pratlong F. Diagnosis of congenital toxoplasmosis by polymerase chain reaction on neonatal peripheral blood. Diagn Microbiol Infect Dis 2011;71:174–6.

Talabani H, Asseraf M, Yera H, Delair E, Ancelle T, Thulliez P, et al. Contributions of immunoblotting, real-time PCR, and the Goldmann-Witmer coefficient to diagnosis of atypical toxoplasmic retinochoroiditis. J Clin Microbiol 2009;47: 2131–5.

Yera H, Filisetti D, Bastien P, Ancelle T, Thulliez P, Delhaes L. Multicenter comparative evaluation of five commercial methods for toxoplasma DNA extraction from amniotic fluid. J Clin Microbiol 2009;47:3881–6.