Tropical Biomedicine 28(3): 615–619 (2011)

Case of fatal congenital toxoplasmosis associated with I/III recombinant genotype

Boughattas, S., Ben-Abdallah, R., Siala, E., Souissi, O., Maatoug, R., Aoun, K. and Bouratbine, A.*
Laboratoire de Recherche 05SP03, Laboratoire de Parasitologie Clinique, Institut Pasteur Tunis,
13 Place Pasteur, BP74, 1002 Tunis Belvédère, Tunisia.
*Corresponding author email: aida.bouratbine@pasteur.rns.tn
Received 20 April 2011; received in revised form 30 May 2011; accepted 10 June 2011

Abstract. We report a case of fatal congenital toxoplasmosis case in Tunis (North of Tunisia) associated with I/III recombinant genotype. The *Toxoplasma gondii* strain was isolated from placenta and characterized molecularly by a multilocus typing (*3'SAG2*, *5'SAG2*, *SAG3*, *AK69*, *APICO*, and *UPRT1*) and a sulfadiazine resistance mutation analysis. The isolate was shown to be a wild drug sensitive I/III recombinant strain.

INTRODUCTION

Congenital toxoplasmosis is caused by transplacental contamination of the fetus with *Toxoplasma gondii* following maternal primary infection during pregnancy. The contamination can cause various degrees of damage ranging from subclinical forms to fetal death. The clinical manifestation depends mainly on the stage of pregnancy at the time of infection. Fetal disease is more severe when contamination occurs early in pregnancy and less severe in the later trimester (Cook *et al*., 2000).

Disease severity can also be influenced by the parasite strain. Most *T. gondii* strains belong to three clonal types i.e. I, II and III (Howe & Sibley, 1995). However, some mixed/recombinant types are isolated from African continent (Boughattas *et al*., 2010) and few atypical genotypes are isolated from exotic areas (Dubey & Su, 2009). These latter strains are reported to induce severe and fatal toxoplasmosis forms (Delhaes *et al*., 2010). In addition, drug resistance of the parasite could increase the severity of clinical forms (Aspinall *et al*., 2002). We report in this study the identification and the molecular characterization of recombinant toxoplasma strain isolated after the death of an infected newborn.

Case report

A 31-year-old female living in Tunis (North of Tunisia) pregnant with her second baby was tested for *T. gondii* infection status. The diagnosis of the first sample at 16 weeks of amenorrhea (WA) was perfomed by ELISA Kits for IgG and IgM antibodies (Platelia toxo IgG®; Platelia toxo IgM® -Biorad, France). Negative results were obtained showing a seronegative toxoplasma status of the patient and so, the need of her follow-up until delivery. Six weeks later, presence of IgG and IgM antibodies against *T. gondii* was revealed indicating a seroconversion of the mother at her 2nd trimester of pregnancy. Spiramycin treatment was then initiated. Fetal contamination was investigated by ultrasonongraphy and by amniotic fluid analysis using real time quantitative (qPCR) and mouse inoculation. Normal fetal ultrasound was obtained. Investigation by molecular method using qPCR, targeting repetitive genes *Rep529* and *B1*, and by classical method using mouse isolation also failed to detect the parasite. At delivery, placenta of the mother was treated with trypsin and

subjected to qPCR and mouse bioassay. Direct detection was negative; however positive reaction was obtained by the mouse culture. A preterm female baby was born, and a confirmation of the congenital toxoplasmosis case was performed by biological and clinical processes. Western Blot (LDBIO Diagnostics- France) analysis demonstrated specific IgM antibodies synthesis by the newborn (Figure 1) and ophthalmologic finding revealed a chorioretinitis with pigmentary changes. Cardiopathy was also diagnosed leading to the baby's death at day 30 before any treatment was given. No autopsy was investigated after the death. The different biological and clinical data are summarized in Table 1.

Toxoplasms gondii was isolated from the placenta from the mouse culture. The strain, called PL03/06, was virulent for mice at the time of inoculation: two of the five inoculated mice died 10 days post inoculation (D10pi). The three remaining mice were euthanized at the 4th week after inoculation by cervical dislocation. Anti-toxoplasma antibodies in mice sera samples was positive by indirect immune-fluorescent (IIF) serology, and presence of *Toxoplasma* cysts in mice brains was observed by microscopy. Molecular method allowed the detection of the parasitic DNA in mouse extracts by PCR amplification. Multilocus genotypic characterization of the strain was performed using multiplex PCR using the following markers *3'SAG2*, *5'SAG2*, *SAG3*, *Apico* (Dubey & Su, 2009), *AK69*, *UPRT1* (Khan *et al.*, 2005) and *DHPS* (Aspinall *et al.*, 2002). For the last marker, the amplified region encoding amino acid residue 407 was previously described as critical for sulfonamide resistance in *T. gondii*. Except for the *UPRT1* gene, the polymorphism within each locus was analyzed by RFLP patterns to distinguish each strain type. Isolate type were determined by comparison of its profiles to the profiles of the three clonal reference strains. Confirmation by sequencing, using Applied Biosystems 3130 Genetic Analyzer, was investigated for *DHPS* gene, for the polymorphic *UPRT1* intron and whenever there were unclear enzyme digestion results. All sequences were deposited in GenBank

The genetic characterization of our patient's isolate yielded a combination of type I and III alleles. No digestion was observed in the both extremities 3' and 5' of *SAG2* gene indicating type I allele. The same allele type I was revealed after double digestion of the *Apico* PCR product. The product of restriction of the *AK69* marker indicated type III allele. The profile obtained after RFLP of *SAG3* product showed concomitant presence of the two

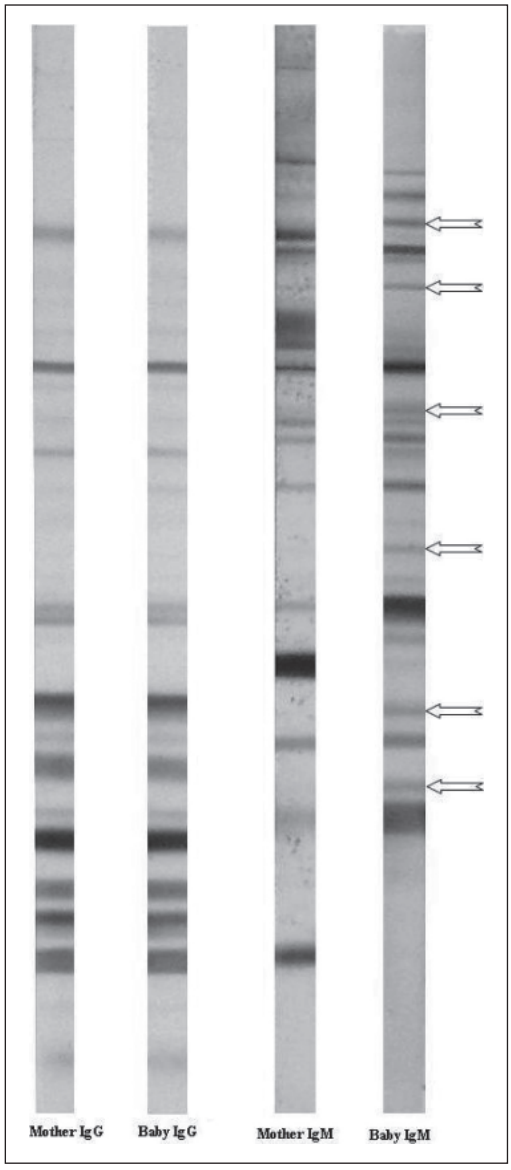

Figure 1. Mother-baby comparative immunologic profiles at day 12 after birth by Western blot IgG, IgM. Arrows indicate baby's new synthesized bands.

Table 1. Summary of biological and clinical data of mother and baby

Patient	Date	Target	Used techniques	Observations
Mother	16WA	Serum	ELISA IgG	–
			ELISA IgM	–
	22WA	Serum	ELISA IgG	+ : 70 IU/ml
			ELISA IgM	+
	34WA	Serum	ELISA IgG	+ : ≥240 IU/ml
			ELISA IgM	+
	35WA	Amniotic Fluid	qPCR	–
			Mouse bioassay	–
		Foetus	Ultrasonongraphies	Normal
	Delivery	Placenta	qPCR	–
			Mouse bioassay	+ : Death 2/5 mice at D10pi
Newborn	Birth	Cord blood	ELISA IgG	+ : ≥ 240 IU/ml
			ELISA IgM	–
			ISAGA IgM	–
			Western Blot IgG	–
			Western Blot IgM	–
		Eyes	Ophthalmologic fundi	Pigmentary chorioretinitis
	Day 12	Serum	ELISA IgG	+ : ≥ 240 IU/ml
			ELISA IgM	–
			ISAGA IgM	–
			Western Blot IgG	– : No new bands
			Western Blot IgM	+ : New synthetized bands
	Day 30		CARDIOPATHY / DEATH	

alleles I and III. Sequencing confirmed this result by revealing double pick at the polymorphic sites [GenBank: HM133637, HM133638]. The presence of superposed pick was also observed by *UPRT1* gene [GenBank: HM133635, HM133636] indicating existence of more than one allele in the *Toxoplasma* strain. The sequence analysis of the *DHPS* gene concurred with RFLP result obtained after restriction by Cfr13I enzyme (Zakimi *et al.*, 2006). In fact DNA sequence analysis of these *DHPS* fragment revealed that the sample was similar to the wild-type *DHPS* in respect to amino acid residue 407 [GenBank: HM536197].

DISCUSSION

Congenital toxoplasmosis is acquired mainly after the contamination of the mother during pregnancy, and rarely before or due to a reactivation of an old infection (Elbez-Rubinstein *et al.*, 2009). The majority of *T. gondii* strains isolated in Europe and the United States are within the three main clonal genotypes i.e. I, II, and III with a predominance of type II. However South American isolates have been shown to be highly divergent (Dubey & Su, 2009) and African isolates frequently show mixed/recombinant genotypes (Boughattas *et al.*, 2010). Several studies suggested a correlation between *T. gondii* genotype and virulence severity (Darde, 2008). Type I strains have been associated with high level of virulence in mice. Types II strains are avirulent in mice and Type III strains show an intermediate virulence. Atypical strains have been shown to be virulent because the majority of mice died 09–30 days after inoculation (Delhaes *et al.*, 2010) instead of 6th week when they should be euthanized.

Mixed genotypes have already been identified especially from African immuno-compromised patients. Several forms were

associated with such genotypes mainly of I/III type (Genot *et al.*, 2007). However, with regard to congenital toxoplasmosis, only two studies have been investigated in African continent (Hameed & Hassanein 2008; Boughattas *et al.*, 2010), and none of them explored virulence study in mice or correlation with clinical observations. Our current study is the first one in this continent which used these approaches. According to the literature, I/III genotype has never been associated with fatal congenital toxoplasmosis. Even in mice, the new combinations of alleles led to a dramatic increase in virulence, this reinforces the strong argument of a higher virulence of this recombinant/mixed strain, perhaps at the same level of virulence as the atypical strains.

We also studied the sulfadiazine resistance of our isolate. This drug is the major one administrated in confirmed congenital toxoplasmosis cases. It was reported that the alteration in codon 407 of *DHPS* gene confers substantial resistance to a range of related inhibitors and this is due entirely to the Asparagine - Aspartate change at position 407 (Aspinall *et al.*, 2002). However no mutation at nucleotide sequence of the coding 407 amino acid was observed in our isolate.

In conclusion, there is increasing evidence that severe toxoplasmosis cases are often associated with atypical strains. These stains are often isolated from exotics areas, rarely from Africa. In this continent mixed/recombinant toxoplasmic strains are more frequent. The reports in the literature regarding the correlation between such strains and clinical observations from congenital cases are still not enough to make recommendations for clinical practice. Our study revealed that it may be necessary for physicians to inquire about genotype of *T. gondii* strains infecting patients. A special awareness is needed when these strains are isolated from congenital toxoplasmosis cases. Their damage may also be increased when a drug resistance is noted.

Acknowledgements. This study was supported by the Ministry of Higher Education, Research and Technology in Tunisia and carried out within the framework of the Research Lab "Parasitoses emergentes" LR 05SP03.

REFERENCES

Aspinall, T.V., Joynson, D.H., Guy, E., Hyde, J.E. & Sims, P.F. (2002). The molecular basis of sulfonamide resistance in Toxoplasma gondii and implications for the clinical management of toxoplasmosis. *Journal of Infectious Diseases* **185**: 1637-1643.

Boughattas, S., Ben-Abdallah, R., Siala, E., Souissi, O., Aoun, K. & Bouratbine, A. (2010). Direct genotypic characterization of *Toxoplasma gondii* strains associated with congenital toxoplasmosis in Tunisia (North Africa). *American Journal of Tropical Medicine and Hygiene* **82**: 1041-1046.

Cook, A.J., Gilbert, R.E., Buffolano, W., Zufferey, J., Peterson, E., Jenum, P.A., Foulon, W., Semprini, A.E. & Dunn, D.T. (2000). Sources of Toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. *British Medical Journal* **321**: 142-147.

Darde, M.L. (2008). *Toxoplasma gondii*, "new" genotypes and virulence. *Parasite* **15**: 366-371.

Delhaes, L., Ajzenberg, D., Sicot, B., Bourgeot, P., Dardé, M.L., Dei-Cas, E. & Houfflin-Debarge, V. (2010). Severe congenital toxoplasmosis due to a Toxoplasma gondii strain with an atypical genotype: case report and review. *Prenatal Diagnosis* **24**.

Dubey, J.P. & Su, C. (2009). Population biology of *Toxoplasma gondii*: what's out and where did they come from. *Memorias do Instituto Oswaldo Cruz* **104**: 190-195.

Elbez-Rubinstein, A., Ajzenberg, D., Darde, M.L., Cohen, R., Dumètre, A., Yera, H., Gondon, E., Janaud, J.C. & Thulliez, P. (2009). Congenital toxoplasmosis and reinfection during pregnancy: case report, strain characterization, experimental model of reinfection, and review. *Journal of Infectious Diseases* **199**: 280-285.

Genot, S., Franck, J., Forel, J.M., Rebaudet, S., Ajzenberg, D., de Paula, A.M., Dardé, M.L., Stein, A. & Ranque, S. (2007). Severe *Toxoplasma gondii* I/III recombinant-genotype encephalitis in a human immunodeficiency virus patient. *Journal of Clinical Microbiology* **45**: 3138-3140.

Hameed, D.M. & Hassanein, O.M. (2008). Genotyping of *Toxoplasma gondii* strains from female patients with toxoplasmosis. *Journal of the Egyptian Society of Parasitology* **38**: 511-520.

Howe, D.K. & Sibley, L.D. (1995). *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *Journal of Infectious Diseases* **172**: 1561-1566.

Khan, A., Su, C., German, M., Storech, G.A., Clifford, D.B. & Sibley, L.D. (2005). Genotyping of *Toxoplasma gondii* strains from immunocompromised patients reveals high prevalence of type I strains. *Journal of Clinical Microbiology* **43**: 5881-5887.

Zakimi, S., Kyan, H., Oshiro, M., Sugimoto, C. & Fujisaki, K. (2006). PCR-based discrimination of Toxoplasma gondii from pigs at an abattoir in Okinawa, Japan. *The Journal of Veterinary Medical Science* **68**: 401-404.