Parasitology International 63 (2014) 470–472

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Short communication

First isolation and molecular characterization of *Toxoplasma gondii* from a human placenta in Argentina

Lais Pardini [a,b,*], Liliana A. Carral [c], Mariana Bernstein [a], María L. Gos [a,b], Patricia Olejnik [c], Juan M. Unzaga [a], Federico J. Kaufer [c], Cristina B. Freuler [c], Ricardo A. Durlach [c], María C. Venturini [a]

[a] *Laboratorio de Inmunoparasitología, FCV, UNLP, Argentina*
[b] *CONICET, Argentina*
[c] *Centro de toxoplasmosis y otras zoonosis, Hospital Alemán, Buenos Aires, Argentina*

ARTICLE INFO

Article history:
Received 13 September 2013
Received in revised form 11 October 2013
Accepted 17 October 2013
Available online 26 October 2013

Keywords:
Toxoplasma gondii
Human
Placenta
Isolation
Genotyping

ABSTRACT

Blood sample and placenta were taken from a 37-week pregnant woman; serologic results indicated acute toxoplasmosis. Placenta was inoculated into mice. Seropositive mice were sacrificed and tissue cysts from brain were inoculated into new mice. Specific DNA was detected by PCR, and the isolate was characterized as Type II by nPCR-RFLP for nSAG2, SAG3, BTUB, GRA6, c29-2, c22-8, L358, PK1 and Apico markers. This is the first isolation and molecular characterization of *Toxoplasma gondii* from humans in Argentina.

Introduction

Toxoplasmosis is a worldwide distributed zoonosis caused by *Toxoplasma gondii*, which can infect warm-blooded animals and humans [4]. In humans, the infection may occur vertically (congenital) or horizontally by eating raw or undercooked meat containing tissue cysts mainly from pigs, goats and sheep, and water or vegetables contaminated with oocysts [4], making toxoplasmosis a major foodborne-disease [12]. If infection occurs during pregnancy in a seronegative woman, it can cause severe damage to the fetus and a strict control of the newborn is recommended due to the possibility of clinical signs like encephalitis and ophthalmologic disease [8,22]. Strategies for prevention include serological screening of pregnant women for diagnosis and early treatment of the mother and the offspring. Treatment with anti-parasitic drug combinations is recommended to reduce the transmission rate and the risk of congenital damage [6]. A positive relationship between gestational age and prenatal transmission risk as well as a negative relationship between gestational age and the degree of severity of lesions in the fetus has been suggested by Ruiz Lopes et al. [19]. Toxoplasmosis is commonly subclinical in immunocompetent adults; however immunosuppressed individuals may develop encephalitis and retinochoroiditis [4].

More than one third of the population worldwide is infected with *T. gondii* [3]. Tenter et al. [21] reported a seroprevalence of 37–58% in women and newborns in Central Europe and of 54–77% in West African countries. Data from Latin America is scarce. A study conducted in Brazil reported detection of *T. gondii* antibodies in 67.3% of periparturient women [13]. In Argentina, *T. gondii* antibodies were detected in 47.3% of pregnant women [6]. Seroprevalence of *T. gondii* was 51.7%, 42.2% and 23.8% in the provinces of Buenos Aires, Santa Fe and Chaco, respectively [6]. Seroprevalence in blood donors at German Hospital (Buenos Aires, Argentina) was 67.4% in 1967, 42.5% in 1992, 39.4% in 1997 and 35% in 2002 [6]. Serological studies provide indirect evidence of *T. gondii* presence in a population; however, to the best of our knowledge, *T. gondii* isolation from human samples has not been described in Argentina.

In the past several years, molecular studies have improved and allowed better knowledge about the relationship between genotypic characteristics of *T. gondii* and biological behavior in the host. *T. gondii* isolates from North America and Europe have been characterized by molecular techniques in three typical genotypes known as types I, II and III [11]; however in South America atypical genotypes have been described and associated with high virulence in a mouse model. Isolates from tissues and human fluids in Europe and USA are 70% type II [1,11], frequently found in congenital symptomatic and asymptomatic cases of toxoplasmosis. In Brazil, studies have reported congenital cases of toxoplasmosis in Paraná and São Paulo states and emphasized the importance of the placenta as a control barrier for vertical transmission [7,19]. Isolates from Brazil were characterized as atypical genotypes

* Corresponding author at: Laboratorio de Inmunoparasitología, Universidad Nacional de La Plata, Facultad de Ciencias Veterinarias, calle 60 y 118, 1900 La Plata, Argentina. Tel.: +54 221 4249621; fax: +54 221 4257980.
E-mail address: laispardini@fcv.unlp.edu.ar (L. Pardini).

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http://dx.doi.org/10.1016/j.parint.2013.10.011

recorded in ToxoDB (http://toxodb.org/toxo/) as #6, 65 and 71 [14]. Isolate #6 is the most commonly found isolate in Brazil, while isolate #65 is the most commonly found isolate in humans [14]. This isolate was described previously in cats and chickens [5], which may indicate the relevance of animals in the epidemiology of human infections [5,14]. In Chile, three isolates from human patients were described as type I, showing different virulence in a mouse model; however, only 5′ SAG2 and 3′ SAG2 markers were used for characterization [20]. Isolates of *T. gondii* from domestic and wild animals from Argentina were characterized by molecular techniques as types II, III, and atypical [4,15–17], but until now *T. gondii* has not been isolated from humans.

The aims of this study were to isolate *T. gondii* from the placenta of a seropositive pregnant woman in Argentina and characterize the isolate by molecular techniques.

Samples

Blood and placenta from a *T. gondii* seropositive pregnant woman, at 37 weeks of gestational age (wga) were taken. The patient was serologically diagnosed of an acute infection and treated with spiramycin since 36 wga. Samples were analyzed at German Hospital, according to recommendations of the Argentine Consensus of Congenital Toxoplasmosis [6].

Bioassay in mice

Human placenta was treated with trypsin 1% (Difco) for 2 h at 37 °C, washed 3 times with saline solution, filtered through gauze and resuspended in saline solution with streptomycin (Richet) and penicillin (Roche) 500 IU/ml. Two Swiss NMRI (Naval Medical Research Institute) mice were inoculated intraperitoneally (IP) according to the methodology described by Robert-Gangneux et al. [18] and identified as MA and MB. Mouse MA was sacrificed at 4 weeks post-inoculation (wpi) and mouse MB died 3 wpi and was discarded. Brain of MA was smashed in saline solution with antibiotics and inoculated IP into 2 NMRI mice identified as M1a and M1b. Sera and brain from mouse M1a were obtained at 4 wpi. At Immunoparasitology Laboratory, Faculty of Veterinary Science (FCV-UNLP), the brain was homogenized in saline solution with antibiotics and antimycotic (1000 IU penicillin/ml, streptomycin 1 mg/ml and amphotericin B 2.5 mg/ml, PAA) and microscopic examination was performed to determine presence of tissue cysts in the fresh sample. Two Swiss (N:NIH) female mice identified as M2 and M3 and two, identified as M4 and M5 were inoculated IP and subcutaneously (SC), respectively. At 6 days post inoculation (dpi) peritoneal fluid from each mice was analyzed to determine the presence of tachyzoites. Animals used in this study were maintained in accordance with standards established by the Institutional Committee for the Care and Use of Laboratory Animals-FCV-UNLP.

Serology in mice

Indirect fluorescent antibody test (IFAT) to detect specific antibodies to *T. gondii* in mice sera (M1a, M2, M3, M4 and M5) was performed as described previously by Moré et al. [16]. Briefly, RH strain tachyzoites were used as antigen, sera were diluted in phosphate buffer solution (PBS) pH 7.2 starting at 1:25 to 1:6400 dilution and incubated at 37 °C for 30 min. Goat anti-mouse IgG-FITC (Sigma) conjugate diluted 1:100 was used [16]. Positive and negative sera were used as controls. Mouse MA and M1a serum was also analyzed by Sabin Feldman test (SF) [6].

PCR diagnosis and genotyping of *T. gondii*

DNA from brain of mouse M1a was extracted using a commercial kit (Promega Wizard Genomic DNA Purification Kit) according to manufacturer's instructions. Polymerase chain reaction (PCR) for *T. gondii* was performed in a thermocycler (PCR Sprint Thermo Electron Corporation) using specific primers TOX5–TOX8 [16] with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation (94 °C; 30 s), annealing (60 °C, 30 s) and extension (72 °C, 60 s) and a final extension at 72 °C for 7 min. DNA from cell cultured RH *T. gondii* tachyzoites and nuclease free water (Gibco) were used as positive and negative controls, respectively. Amplicons were revealed on 1.5% agarose gels and stained with SYBR Safe (Invitrogen). Genotyping was performed using nested-PCR (n-PCR) followed by restriction fragment length polymorphism (RFLP) analysis. Extracted DNA was amplified by n-PCR for nSAG2, SAG3, BTUB, GRA6, c29-2, c22-8, L358, PK1 and Apico markers [16]. DNA from *in vitro* cultured *T. gondii* strains RH, ME49 and NED were used as positive control for clonal types I, II and III respectively. Digestion products were visualized after electrophoresis with 3% agarose gels for all markers except for Apico where a 2.5% gel was used, and stained with SYBR safe using a 100 bp standard (Cien Marker, Biodynamics).

Acute toxoplasmosis was diagnosed in a woman by serological tests [1:16,000 (SF), 1:160 (IFAT-IgM), 1:4000 (IFAT-IgG), 1:1000 (ISAGA-IgM and ISAGA-IgA), negative (ISAGA-IgE) and 6% low (avidity test)] at 37 weeks of pregnancy. In the newborn, specific *T. gondii* antibodies were detected by SF (1:16,000); however IFAT-IgM, ISAGA-IgM and ISAGA-IgA were negative.

Mice MA and M1a developed antibodies to *T. gondii* detected at 4 wpi by SF (1:16,000). Specific *T. gondii* antibodies were found in mice M1a, M4 and M5 (1:6400), M2 and M3 (1:3200) by IFAT. Tissue cysts were observed in brain of mouse M1a and eight cysts per mouse were inoculated. Mice M1b and M2, M3, M4, M5 were used to maintain the isolate by mice bioassay at German Hospital and FCV-UNLP. Tachyzoites were obtained from peritoneal exudate (M3) at 6 dpi and grown in Vero cell cultures, with RPMI (PAA) and 3% fetal calf serum (PAA) at FCV-UNLP. Parasites were stored in liquid nitrogen at −196 °C. The isolate was also maintained by mice passages.

Specific *T. gondii* DNA was detected by PCR and the isolate was characterized as type II for all markers used in this study.

According to our knowledge this is the first isolation and genotyping of *T. gondii* from human tissues in Argentina. The isolate characterized in our study was classified as genotype II, which differs from atypical genotypes described in animals and humans in different countries from South America, such as Brazil, Chile and French Guiana [2, 5, 14, 20]. Type II isolates have been reported previously in pigs and zoo kangaroos in Argentina [15, 17].

Unfortunately, it was not possible to determine the source of infection in the pregnant woman in our study. The infection could be related to consumption of raw or undercooked meat or derivatives [4]. Even though pig, sheep and goat meat consumption is not as frequent as beef or chicken meat consumption in Argentina, antibodies to *T. gondii* have been detected in these species [4, 9, 10]; therefore, they could be considered a possible source of infection in this country. Based on serological results from mother and child and according to Argentine Consensus of Congenital Toxoplasmosis and the World Health Organization, we can conclude that the isolate described in this study was from an acute infection [6].

Placenta examination was critical for diagnosis of congenital toxoplasmosis, considering that IFAT-IgM, ISAGA-IgM and IgA in the newborn were negative. Unfortunately, it was not possible to perform serological studies or follow clinical evolution of the newborn to determine if the baby was infected during pregnancy. In Argentina, it is not usual to collect placenta samples at birth as performed in our study, which could be a potential additional tool for the correct diagnosis of congenital toxoplasmosis. Although the isolate was characterized as type II, further studies are needed to determine the relevance of different *T. gondii* genotypes in the human population of Argentina to improve the knowledge and epidemiology of toxoplasmosis.

Acknowledgments

We would like to thank Mr. I. Ercoli and Mrs. S. Peñaloza for their technical assistance and Lic. Lucía Campero and Dr. Magdalena Rambeaud for language revision of this paper.

Financial support for this study was provided by SeCyT through BID *PICT-2007-01204, UNLP 2008–2011: V179*, German Hospital, Buenos Aires, Argentina.

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