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Isolation and genotyping of *Toxoplasma gondii* from pregnant dairy cows (*Bos taurus)* slaughtered

Isolamento e genotipagem de *Toxoplasma gondii* em vacas de leite (*Bos taurus*) prenhas abatidas

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Abstract

The current study aimed to evaluate serology, and isolate and genotype *Toxoplasma gondii* strains from pregnant dairy cows, slaughtered in an abattoir for human consumption, and their fetuses. Blood from 60 pregnant dairy cows and blood and tissue samples (brain, lung, heart, and liver) from their fetuses were collected and analyzed in a mouse bioassay. Antibodies against *T. gondii* were observed in 48.3% of cows and 3.7% of fetuses (IFAT, titers ≥ 50 for cows and 25 for fetuses were considered positive). Fourteen fetuses (23.3%) and six cows (10.0%) were identified as positive in the bioassay. *T. gondii* was isolated from a blood sample of a cow older than 4 years old in the 6th month of pregnancy, and from a blood sample of a fetus in the 6th month of gestation. These isolates were identified by polymerase chain reaction (PCR) as being of *T. gondii* and both strains showed type II alleles for all PCR-restriction fragment length polymorphism (PCR-RFLP) markers tested. *T. gondii* type II strain from cattle was isolated for the first time in Brazil. The current study also showed that transplacental transmission of *T. gondii* naturally occurs in dairy cows (23.3%) from Southern Brazil.

Keywords: *Toxoplasma gondii*, cattle, isolation, genotype characterization.

Resumo

O presente estudo teve como objetivo avaliar a ocorrência de anticorpos, isolar e genotipar *Toxoplasma gondii* de vacas prenhas abatidas em um matadouro para consumo humano e de seus respectivos fetos. Sangue de 60 vacas gestantes e amostras de sangue e tecidos (cérebro, pulmão, coração e fígado) de seus fetos foram coletados e utilizados para bioensaio em camundongos. Anticorpos contra *T. gondii* foram observados em 48,3% das vacas e em 3,7% dos fetos (foram considerados positivos títulos ≥50 para as vacas e ≥25 para os fetos). Quatorze fetos (23,3%) e seis vacas (10,0%) apresentaram-se positivas para *T. gondii* ao bioensaio. *T. gondii* foi isolado de amostra de sangue de uma vaca com mais de quatro anos no 6º mês de gestação e de amostra de sangue de um feto no 6º mês de gestação. Por PCR esses isolados foram identificados como sendo de *T. gondii* e ambas as cepas apresentaram o alelo tipo II em todos os marcadores de PCR-RFLP testados. Esta é a primeira identificação de genótipo tipo II de *T. gondii* em bovinos do Brasil. Além disso, este estudo mostrou que a transmissão transplacentária de *T. gondii* ocorre naturalmente em bovinos de leite (23,3%).

Palavras-chave: *Toxoplasma gondii*, bovinos, isolamento, caracterização genotípica.

Toxoplasma gondii is an intracellular parasite that infects a variety of cell types from a wide range of birds and mammals throughout the world, including humans. Human infection occurs by two main routes, ingestion of oocysts and undercooked or raw meat with tissue cysts of the parasite (DUBEY, 1996). The current study aimed to isolate and genotype *T. gondii* from pregnant dairy cows slaughtered for human consumption and their fetuses.

A total of 60 samples were obtained from pregnant dairy cows (*Bos taurus*) and their fetuses at an abattoir located in the municipality of Presidente Getúlio, Santa Catarina state, southern Brazil. The current study was approved by the Animal Ethics Committee of Universidade Estadual de Londrina (Nº 018/2009). Blood samples (5 mL) with EDTA were collected from these

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cows and from their fetuses' heart. White blood cells were separated by centrifugation at 550 g for 10 minutes, diluted in 1 mL of antibiotic saline solution (1,000 U penicillin and 100 µL of streptomycin.mL^{-1} of saline solution) and inoculated subcutaneously into three mice (0.3 mL/mouse). Tissues from fetuses, approximately 10 g of each tissue (brain, lung, heart, and liver) for fetuses ≥3 months of age, and a pool of organs (10 g) for fetuses ≤3 months were obtained. For tissue digestion the protocol described by Dubey (1998) was used. The result of the mouse bioassay was considered positive when tachyzoites, tissue cysts or anti-*T. gondii* antibodies were detected.

Serum samples were tested by indirect fluorescent antibody test (IFAT), performed according to Garcia et al. (1999), to detect antibodies against *T. gondii*. Sera were considered positive for titers ≥1:50 for cows and ≥1:25 for fetuses.

Infected brains and peritoneal liquid from bioassayed mice (where tissue cysts and tachyzoites were observed) had DNA extracted to be tested by polymerase chain reaction (PCR) as described previously (GARCIA et al., 2006). The DNA amplification for *T. gondii* and *Neospora caninum* differentiation was performed using the method described by Homan et al. (2000) and Müller et al. (1996), respectively. Genotyping was performed through multilocus PCR-restriction fragment length polymorphism (PCR-RFLP) analysis at 13 markers: SAG2-3' and SAG2-5' (HOWE et al., 1997), SAG3, GRA6 and SAG1, BTUB, L358, c22-8, c29-2, PK1, Apico, SAG2-alt, and CS3 (SU et al., 2006; PENA et al., 2008).

The study variables were analyzed by the chi-square test (χ^2) with correction of Yates using the Epi Info program (CDC, 6.04b version). We considered as significant a p-value ≤ 0.05.

Pregnant cows showed a 48.3% (29/60) seroprevalence for *T. gondii*. The results of the bioassay are summarized in Table 1. Sixteen out of 60 (26.6%) slaughtered pregnant cows, 11 Jersey and five Holstein, were positive in the bioassay either by *T. gondii* detection in their blood or fetuses. Of these 16 cows, only six (37.5%) had antibody titers detected by IFAT, and all of them were ≥4 months of pregnancy. *T. gondii* was isolated from the blood of a cow (Nr. 53; brain cysts observed in bioassayed mice) older than 4 years old in the 6th month of pregnancy, and from the blood of a fetus (Nr.103; tachyzoites observed in peritoneal liquid of bioassayed mice) in the 6th month of gestation. The current study detected a *T. gondii* transplacental transmission rate in dairy cows of 23.3% (14/60).

The DNA extracted from brain cysts and tachyzoites isolated in mice were confirmed to be positive for *T. gondii* and negative for *N. caninum*. Genotyping of both strains (TgBoBr1and 2) identified type II lineage for all PCR-RFLP markers tested.

In the current study, two *T. gondii* strains were isolated, by bioassay in mice, from naturally infected animals, one of them from a pregnant cow and another one from a fetus. Dubey and Thulliez (1993) failed to isolate the parasite from the blood of calves and pregnant cows infected with high load of oocysts. Using the same protocols we were not able to isolate *T. gondii* strains (MARQUES, 2009) from zebu cows. Oliveira et al. (2001) infected *B. taurus*, *B. indicus*, and *Bubalus bubalis* with *T. gondii* oocysts orally, and described that *B. taurus* were more affected than the other species.

The current finding that *T. gondii* can be transplacentally transmitted in cattle was previously described (CANADA et al., 2002), but it has not been suggested that this parasite is an important bovine abortifacient.

Table 1. Positive results for *Toxoplasma gondii* by a mouse bioassay performed in slaughtered pregnant dairy cows and their fetuses in the municipality of Presidente Getúlio, Santa Catarina state, southern Brazil.

Cow breed	IFAT titer[1]		Gestation age (months)	Bioassay of tissues[2]				
	Cow	Fetus		Cows[3]	Fetus[4]			
					Bl	Br	H	Lu
06- Jersey	N	N	6	–	+	–	–	–
25-Holstein	100	N	7	+	–	–	–	–
34-Jersey	100	N	4	–	–	+	–	–
37-Jersey	50	N	6	–	+	–	+	–
39-Jersey	N	N	4	+	+	–	–	–
40-Holstein	N	N	7	+	–	+	–	–
53-Holstein	N	N	6	+*	–	–	–	–
74-Jersey	N	N	6	–	–	+	+	–
75-Jersey	50	N	7	–	+	+	–	–
103-Jersey	50	N	6	+	+T	+	+	+
105-Jersey	50	N	7	+	+	–	–	+
119-Holstein	N	N	8	–	+	–	–	–
125-Jersey	25	N	7	–	+	–	–	+
126-Holstein	N	N	6	–	+	–	–	–
127-Jersey	N	N	5	–	+	+	+	–
131-Jersey	25	N	7	–	–	–	–	+

[1]Indirect fluorescence antibody test; [2]the mouse bioassay was performed with blood from cows[3] and fetal tissue samples from blood (Bl), brain (Br), heart (H) and lung (Lu)[4]. N = negative. Isolation of *brain cysts and [T]tachyzoites in peritoneal liquid. + Positive; – negative.

We found that only six out of 16 dams detected as positive in the bioassay had antibody titers to *T. gondii* detected by IFAT. This finding is not yet clear and needs to be further explored. There is an established correlation between anti-*T. gondii* antibodies and tissue cysts in sheep and pigs (OPSTEEGH et al., 2010), however, this has not been found in cattle (OPSTEEGH et al., 2011). These authors described that the risk of human infection is higher from seronegative than seropositive cattle based on the fact that they detected DNA from *T. gondii* from negative but not from positive animals. Moreover, infectivity and pathogenicity of *T. gondii* in cattle vary with the strain, and pathogenicity is generally only mild to moderate (FAYER; FRENKEL, 1979). After infection, cattle may eliminate the parasite from their tissues, which is often followed by disappearance of antibodies in some cattle (DUBEY; THULLIEZ, 1993). The factors involved in this natural resistance are not yet known (ESTEBAN-REDONDO; INNES, 1997). Costa et al. (2011) studied pregnant cows and their fetuses from a slaughterhouse in Jaboticabal, southeastern Brazil. They showed an 18% seroprevalence of anti-*T. gondii* antibodies in cows, however, only low titers of 1:64 were observed. Even when they studied nine cows experimentally infected with oocysts of *T. gondii* the predominant titer was the same.

In the current study a *T. gondii* type II strain was isolated for the first time from cattle in Brazil. Studies using a large number of *T. gondii* isolates from chickens, cats, and capybaras were performed in Brazil (PENA et al., 2008; DUBEY; SU, 2009; YAI et al., 2009), and similar conclusions were drawn: a) there is no *T. gondii* type II lineage in Brazil; b) *T. gondii* type I strains are rare; and c) most strains are not clonal, suggesting recombination and effective transmission of oocysts. It is interesting the fact that multilocus PCR-RFLP genotyping of *T. gondii* strains in our study revealed type II alleles in all genetic markers tested. Dubey et al. (2010) and da Silva et al. (2011), also using multilocus PCR-RFLP genotyping, described type II genotype in chickens from Fernando de Noronha island, and in sheep slaughtered in São Paulo, southeastern Brazil, respectively. Thus, considering these results, the first conclusion is not valid in Brazil. On the other hand, a recent study (FRAZÃO-TEIXEIRA et al., 2011) demonstrated that multilocus DNA sequencing is more accurate than multilocus PCR-RFLP to identify the actual genotypes in *T. gondii* isolates in regions of the world where genetic diversity is substantial such as Brazil.

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References

Canada, N, Meireles CS, Rocha A, Da Costa JM, Erickson MW, Dubey JP. Isolation of viable *Toxoplasma gondii* from naturally infected aborted bovine fetuses. *J Parasitol* 2002; 88(6): 1274-1248.

Costa, GHN, Costa AJ, Lopes WDZ, Bresciani KDS, Santos TR, Esper CR, Santana ÁE. *Toxoplasma gondii*: Infection natural congenital in cattle and an experimental inoculation of gestating cows with oocysts. *Exp Parasitol* 2011; 127(1): 277-281. PMid:20736009. http://dx.doi.org/10.1016/j.exppara.2010.08.005

Da Silva RC, Langoni H, Su C, Da Silva AV. Genotypic characterization of *Toxoplasma gondii* in sheep from Brazilian slaughterhouses: new atypical genotypes and the clonal type II strain identified. *Vet Parasitol* 2011; 175(1-2): 173-177. PMid:20970257. http://dx.doi.org/10.1016/j.vetpar.2010.09.021

Dubey JP, Thulliez P. Persistence of tissue cysts in edible tissues of cattle fed *Toxoplasma gondii* oocysts. *Am J Vet Res* 1993; 54(2): 270-273.

Dubey JP. Strategies to reduce transmission of *Toxoplasma gondii* to animals and humans. *Vet Parasitol* 1996; 64(1-2): 65-70. http://dx.doi.org/10.1016/0304-4017(96)00961-2

Dubey JP. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. *Vet Parasitol* 1998; 74(1): 75-77. http://dx.doi.org/10.1016/S0304-4017(97)00135-0

Dubey JP, Su C. Population biology of *Toxoplasma gondii*: what's out and where did they come from. *Mem Inst Oswaldo Cruz* 2009; 104(2): 190-195. http://dx.doi.org/10.1590/S0074-02762009000200011

Dubey JP, Rajendran C, Costa DGC, Ferreira LR, Kwok OCH, Qu D, Su C, Marvulo MFV, Alves LC, Mota RA, Silva JCR. New *Toxoplasma gondii* genotypes isolated from free-range chickens from the Fernando de Noronha, Brazil: Unexpected Findings. *J Parasitol* 2010; 96: 709-712. PMid:20486738. http://dx.doi.org/10.1645/GE-2425.1

Esteban-Redondo I, Innes EA. *Toxoplasma gondii* infection in sheep and cattle. *Comp Immunol Microbiol Infect Dis* 1997; 20(2): 191-196. http://dx.doi.org/10.1016/S0147-9571(96)00039-2

Fayer R, Frenkel JK. Comparative infectivity for calves of oocysts of feline. coccidia: *Besnoitia, Hammondia, Cystoisospora, Sarcocystis,* and *Toxoplasma*. *J Parasitol* 1979; 65(5): 756-762. PMid:117090. http://dx.doi.org/10.2307/3280357

Frazão-Teixeira E, Sundar N, Dubey JP, Grigg ME, De Oliveira FCR. Multi-locus DNA sequencing of *Toxoplasma gondii* isolated from Brazilian pigs identifies genetically divergent strains. *Vet Parasitol* 2011; 175(1-2): 33-39. PMid:21051148. PMCid:3141217. http://dx.doi.org/10.1016/j.vetpar.2010.09.030

Garcia JL, Navarro IT, Ogawa L, Oliveira RC. Soroprevalência do *Toxoplasma gondii* em suínos, bovinos, ovinos e eqüinos, e sua correlação com humanos, felinos e caninos, oriundos de propriedades rurais do norte do Paraná, Brasil. *Cienc Rural* 1999; 29(1): 91-97. http://dx.doi.org/10.1590/S0103-84781999000100017

Garcia JL, Gennari SM, Machado RZ, Navarro IT. *Toxoplasma gondii*: Detection by mouse bioassay, histopathology, and polymerase chain reaction in tissues from experimentally infected pigs. *Exp Parasitol* 2006; 113(4): 267-271. PMid:16545804. http://dx.doi.org/10.1016/j.exppara.2006.02.001

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(1): 69-75. http://dx.doi.org/10.1016/S0020-7519(99)00170-8

Howe DK, Honoré S, Derouin F, Sibley LD. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. *J Clin Microbiol* 1997; 35(6): 1411-1414. PMid:9163454. PMCid:229759.

Marques FAC. Transmissão vertical do *Neospora caninum* em fêmeas de corte (*Bos indicus)* abatidas em frigorífico / Francisco Augusto Coelho

Marques. - Londrina. 2009. 81f. Tese (Doutorado) - Universidade Estadual de Londrina, Londrina.

Müller N, Zimmermann V, Hentrich B, Gottstein B. Diagnosis of *Neospora caninum* and *Toxoplasma gondii* infection by PCR and DNA hybridization immunoassay. *J Clin Microbiol* 1996; 34(11): 2850-2852. PMid:8897199 . PMCid:229420.

Oliveira FCR, Da Costa AJ, Sabatini GA. Clínica e hematologia de *Bos indicus, Bos taurus* e *Bubalus bubalis* inoculados com oocistos de *Toxoplasma gondii* (Apicomplexa:Toxoplasmatinae). *Cienc Rural* 2001; 31(4): 621-626. http://dx.doi.org/10.1590/S0103-84782001000400010

Opsteegh M, Teunisa P, Mensink M, Züchner L, Titilincu A, Langelaar M, Van Der Giessen J. Evaluation of ELISA test characteristics and estimation of *Toxoplasma gondii* seroprevalence in Dutch sheep using mixture models. *Prev Vet Med* 2010; 96(3-4): 232-240. PMid:20637514. http://dx.doi.org/10.1016/j.prevetmed.2010.06.009

Opsteegh M, Teunisa P, Züchner L, Koetse A, Langelaar M, Van Der Giessen J. Low predictive value of seroprevalence of *Toxoplasma gondii* in cattle for detection of parasite DNA. *Int J Parasitol* 2011; 41(3-4): 343-354. PMid:21145321. http://dx.doi.org/10.1016/j.ijpara.2010.10.006

Pena HFJ, Gennari SM, Dubey JP, Su C. Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. *Int J Parasitol* 2008; 38(5): 561-569. PMid:17963770. http://dx.doi.org/10.1016/j.ijpara.2007.09.004

Su C, Zhang X, Dubey JP. Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: A high resolution and simple method for identification of parasites. *Int J Parasitol* 2006; 36(7): 841-848. PMid:16643922. http://dx.doi.org/10.1016/j.ijpara.2006.03.003

Yai LEO, Ragozo AMA, Soares RM, Pena HFJ, Su C, Gennari SM. Genetic diversity among capybara (*Hydrochaeris hydrochaeris*) isolates of *Toxoplasma gondii* from Brazil. *Vet Parasitol* 2009; 162(3-4): 332-337. PMid:19375864. http://dx.doi.org/10.1016/j.vetpar.2009.03.007