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

Madlaine Frigo Silveira Barbosa de Macedo¹; César Augusto Barbosa de Macedo¹; Maria Paula de Carvalho Ewald¹; Guilherme Felippelli Martins¹; Dauton Luiz Zulpo¹; Ivo Alexandre Leme da Cunha¹; Alessandra Taroda¹; Sergio Tosi Cardim¹; Chunlei Su²; João Luis Garcia*¹

¹Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina – UEL
²Department of Microbiology, The University of Tennessee

Received April 27, 2011
Accepted August 11, 2011

**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. Antibodies against *T. gondii* foram observados em 48,3% das vacas e em 3,7% das fetos (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
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 (χ²) 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 (MARQUIES, 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.

<table>
<thead>
<tr>
<th>Cow breed</th>
<th>IFAT titer¹</th>
<th>Gestation age (months)</th>
<th>Bioassay of tissues²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow</td>
<td>Fetus</td>
<td></td>
</tr>
<tr>
<td>06- Jersey</td>
<td>N</td>
<td>N</td>
<td>6</td>
</tr>
<tr>
<td>25-Holstein</td>
<td>100</td>
<td>N</td>
<td>7</td>
</tr>
<tr>
<td>34-Jersey</td>
<td>100</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td>37-Jersey</td>
<td>50</td>
<td>N</td>
<td>6</td>
</tr>
<tr>
<td>39-Jersey</td>
<td>N</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td>40-Holstein</td>
<td>N</td>
<td>N</td>
<td>7</td>
</tr>
<tr>
<td>53-Holstein</td>
<td>N</td>
<td>N</td>
<td>6</td>
</tr>
<tr>
<td>74-Jersey</td>
<td>N</td>
<td>N</td>
<td>6</td>
</tr>
<tr>
<td>75-Jersey</td>
<td>50</td>
<td>N</td>
<td>7</td>
</tr>
<tr>
<td>103-Jersey</td>
<td>50</td>
<td>N</td>
<td>6</td>
</tr>
<tr>
<td>105-Jersey</td>
<td>50</td>
<td>N</td>
<td>7</td>
</tr>
<tr>
<td>119-Holstein</td>
<td>N</td>
<td>N</td>
<td>8</td>
</tr>
<tr>
<td>125-Jersey</td>
<td>25</td>
<td>N</td>
<td>7</td>
</tr>
<tr>
<td>126-Holstein</td>
<td>N</td>
<td>N</td>
<td>6</td>
</tr>
<tr>
<td>127-Jersey</td>
<td>N</td>
<td>N</td>
<td>5</td>
</tr>
<tr>
<td>131-Jersey</td>
<td>25</td>
<td>N</td>
<td>7</td>
</tr>
</tbody>
</table>

¹Indirect fluorescence antibody test; ²the mouse bioassay was performed with blood from cows and fetal tissue samples from blood (Bl), brain (Br), heart (H) and lung (Lu). N = negative. Isolation of *brain cysts and 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-REDONDON; 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.

Acknowledgements

J.L. Garcia is recipient of CNPq fellowship.

References


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


