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Short Communication

Occurrence of antibodies against *Toxoplasma gondii* and its isolation and genotyping in donkeys, mules, and horses in Brazil

Solange M. Gennari[a,*], Patrícia de O. Esmerini[a], Marcos G. Lopes[a], Herbert S. Soares[a], Sérgio N. Vitaliano[a,b], Aline D. Cabral[a], Hilda F.J. Pena[a], Maurício C. Horta[c], Paulo H. Cavalcante[d], Kleber P. Fortes[e], Eliana M.C. Villalobos[f]

[a] *Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Orlando Marques de Paiva, 87, CEP 05508-270 São Paulo, SP, Brazil*
[b] *Curso de Medicina Veterinária, FMU, São Paulo, SP, Brazil*
[c] *Colegiado de Medicina Veterinária, Universidade Federal do Vale do São Francisco, Petrolina, PE, Brazil*
[d] *Instituto de Teologia Aplicada, Faculdades INTA, Sobral, CE, Brazil*
[e] *Instituto de Ciências Biológicas, UFMG, Belo Horizonte, MG, Brazil*
[f] *Instituto Biológico, São Paulo, SP, Brazil*

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ABSTRACT

The occurrence of antibodies against *Toxoplasma gondii* was determined in donkeys, mules, and horses from different regions of Brazil. Serum samples from 304 donkeys (67.11%), 118 horses (26.05%), and 31 mules (6.84%) were analyzed by means of the indirect fluorescent antibody test (cutoff = 64). Antibodies against *T. gondii* were detected in 129 equids (28.47%) (82 donkeys, 32 horses, and 15 mules). Tissue samples from 19 seropositive and 50 seronegative animals were obtained in order to isolate the parasite by means of mouse bioassay, and *T. gondii* was isolated from a donkey. Through genotypic characterization of the isolate, by means of polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) using 11 genotypic markers, the genotype #163 (TgCkBr220), which has already been described in chickens in Brazil, was identified.

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1. Introduction

Toxoplasma gondii is an obligate intracellular apicomplexan parasite distributed worldwide. It causes toxoplasmosis in humans and in domesticated and wild animals (Dubey, 2010). Infection occurs after ingestion of food and water contaminated with oocysts from the feces of infected cats or ingestion of undercooked meat containing tissue cysts from infected intermediate hosts.

The consumption of horsemeat is a common practice in some parts of the world. In Brazil, horses are mainly bred for working roles, sports, and recreation; however, Brazil is among the top ten exporters of horsemeat (Brasil, 2014).

Pomares et al. (2011) described three cases of severe toxoplasmosis in France, probably acquired through ingestion of raw horsemeat imported from Canada and Brazil.

In addition, in France, Elbez-Rubinstein et al. (2009) described a case of congenital toxoplasmosis in an infant born to an immunocompetent mother who was reinfected

* Corresponding author. Tel.: +55 11 30917654; fax: +55 11 30917928.
E-mail address: sgennari@usp.br (S.M. Gennari).

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during pregnancy, probably by ingesting raw horsemeat imported from South America.

In some parts of the world, donkey milk is widely used for human nutrition and, recently, Mancianti et al. (2014) detected the DNA of *T. gondii* in milk from seropositive jennies, thus suggesting that consumption of raw milk from these animals could be a potential source of human infection.

In Brazil, very few studies on *T. gondii* seroprevalence among equids have been conducted, with proportions ranging from 1.52% to 43.2% in donkeys, 0% to 23.8% in mules (Mendonça et al., 2001; Oliveira et al., 2013), and 1.33% to 32.8% in horses (reviewed by Dubey et al., 2012; Evers et al., 2013; Finger et al., 2013).

In the present study, the occurrence of *T. gondii* antibodies in donkeys, mules, and horses from different regions of Brazil was determined, and tissue samples from these animals were used for mouse bioassaying and genotyping.

2. Materials and methods

Blood samples from 453 equids were collected in order to detect antibodies against *T. gondii*. From this total, 304 (67.11%) were from donkeys (*Equus asinus*), 118 (26.05%) from horses (*Equus caballus*), and 31 (6.84%) from mules (crossbreed between male donkeys and mares). The equids were on farms located in the northeastern region of Brazil (392 samples), and a further 61 horses were sampled from a slaughterhouse in the state of Minas Gerais, which receives animals from different localities.

Serum samples were tested for *T. gondii* immunoglobulin G (IgG) antibodies by means of the indirect fluorescent antibody test (IFAT), as previously described (Camargo, 1974), with a cutoff of 1:64 (Garcia et al., 1999; Mendonça et al., 2001). Positive and negative control horse serum was used on each slide, and the positive serum was retested using twofold serial dilutions.

Tissue samples (brain, tongue, diaphragm, and heart; 10 g of each tissue) from nine donkeys and 10 horses that were *T. gondii*-seropositive and from 50 seronegative horses were used for isolation by means of mouse bioassaying (Dubey, 1998). Due to difficulties in obtaining tissue samples from equids, seronegative animals were also used in the bioassay to increase the possibility of parasite isolation. Ten mice were inoculated with a pool of tissue obtained from each seropositive animal and digested in acid pepsin (approximately 1.0 mL per mouse). Samples from 50 seronegative horses, from the slaughterhouse, were divided into 10 groups of five horses each, and tissues from the brain, tongue, and heart of each group were pooled (treated as one sample) and inoculated into four mice per group.

Dead mice or mice that were sacrificed when ill were examined for the presence of *T. gondii* cysts or tachyzoites using impression smears from the cerebrum and lungs. Survivors were bled on day 60 post inoculation (p.i.) after sedation (100 mg/kg ketamine and 10 mg/kg xylazine) by means of an intraperitoneal injection. Serum from each mouse was tested for antibodies against *T. gondii* (IFAT $\geq$ 16). The positive mice were sacrificed and their brains were examined for tissue cysts (Dubey, 2010).

Genomic DNA was extracted from the brains and lungs of the *T. gondii*-positive mice using the Wizard® DNA Clean-Up System (Promega, Madison, WI, USA).

The genotypes of *T. gondii* were determined by means of polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) using 11 markers, as previously described: SAG1, SAG2 (5′–3′ SAG2 and alt.SAG2), SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico (Su et al., 2006; Dubey et al., 2007), and CS3 (Pena et al., 2008). Six *T. gondii* reference strains were included: RH88 (human, USA), type I lineage; PTG (sheep, USA), type II lineage; CTG (cat, USA); type III lineage and MAS (human, France); TgCgCa1 (cougar, Canada); and TgCatBr5 (cat, Brazil), as atypical isolates (Su et al., 2006). Ultrapure water and DNA of HFF (human foreskin fibroblast cells) were included as negative controls.

Possible associations between the presence of antibodies against *T. gondii* and the animal species were ascertained using the chi-squared test. Associations between the locality of sampling and occurrence were also analyzed for the donkey samples, using the same method. A p-value $\leq$0.05 was considered significant.

All procedures were conducted in accordance with the animal protocols approved by the Ethics Committee of the Faculty of Veterinary Medicine, USP, Brazil.

3. Results

Antibodies against *T. gondii* were found in 129 (28.47%) of the 453 equids, comprising 82 (26.97%) of the 304 donkeys, 32 (27.11%) of the 118 horses, and 15 (48.38%) of the 31 mules. The antibody titer ranged from 64 to 264 (Table 1).

An association between the host species and *T. gondii* infection was observed, wherein mules presented higher occurrence ($p = 0.039$).

T. gondii-positive animals were found in all the municipalities studied. The occurrence was highest among donkeys in Campo Maior, Piauí ($p < 0.05$). Only in Petrolina, Pernambuco, were all three species of equids sampled, and the occurrence of *T. gondii* antibodies was highest among the mules ($p = 0.019$).

T. gondii was isolated by means of mouse bioassaying, in a sample from a donkey (IFAT = 64) in Mossoró, Rio Grande do Norte. Only two of the 10 mice inoculated were infected (brain cysts detected), and they died on days 16 and 17 p.i.

Genotyping of the *T. gondii* isolate showed the presence of the alleles I, III, III, III, III, III, II, I, III, III, III, and III for the genetic markers SAG1, 5′–3′ SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, Apico, and CS3, respectively. This genotype was previously reported in the chicken isolate TgCkBr220 and was designated as ToxoDB-PCR-RFLP genotype #163 (Dubey et al., 2010).

4. Discussion

This study confirms the presence of antibodies against *T. gondii* in equids in Brazil, with a total occurrence of 28.47% and proportions of 26.97%, 27.11%, and 48.38%, respectively, for donkeys, horses, and mules.

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