


Occurrence of anti-*Toxoplasma gondii* antibodies and risk factors associated with infection in equids slaughtered for human consumption in Brazil

Ocorrência de anticorpos anti-*Toxoplasma gondii* e fatores de risco associados a infecção em equídeos abatidos para consumo humano no Brasil

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Abstract

Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii*. Ingestion of raw/undercooked meat is considering an important route of infection. Consumption of meat from equids is common in European and Asian countries and an increase in Brazil has been observed. The aim of this study was to evaluate occurrences of anti-*T. gondii* antibodies and risk factors relating to infection in equids slaughtered for human consumption in Minas Gerais, Brazil. Blood samples from 192 horses and 208 donkeys were collected in the exsanguination area during the slaughter. Serum samples were subjected to the indirect fluorescent antibody test (IFAT). Association analysis was performed using Pearson's chi-square test (χ^2) or Fisher's exact test, to evaluate risk factors relating to the prevalence of seroreagents. Antibodies against *T. gondii* were found in 13.5% of the equids, with higher occurrence in horses (18.75%) than in donkeys (8.65%). Associations between seropositivity and the following variables were found ($p \leq 0.05$): species, animal origin, purpose of rearing and source of water for animal consumption and contact with cats. Farms need to implement preventive measures to control *T. gondii* infection in these species and avert transmission of the parasite to the human population that will consume their meat.

Keywords: Toxoplasmosis, horses, donkeys, serology, Minas Gerais.

Resumo

Toxoplasmose é uma zoonose cosmopolita causada pelo *Toxoplasma gondii*. A ingestão de carne crua/mal cozida é considerada uma importante forma de infecção. A ingestão de carne de equídeos é comum nos países europeus e asiáticos e, no Brasil, tem sido observado um aumento no consumo. O objetivo deste estudo foi avaliar a ocorrência de anticorpos anti-*T. gondii* e correlacionar com possíveis fatores de risco relacionados à infecção em equídeos que foram abatidos para consumo humano em Minas Gerais, Brasil. Amostras de sangue de 192 equinos e 208 asininos foram coletadas na área de sangria durante o abate. As amostras de soro foram analisadas pela Reação de Imunofluorescência Indireta (RIFI). A análise estatística foi realizada usando o teste qui-quadrado de Pearson (χ^2) ou o teste exato de Fisher, buscando relacionar possíveis fatores de risco com a prevalência de anticorpos. Anticorpos foram encontrados em 13,5% dos equídeos, com maior ocorrência em equinos (18,75%) do que em asininos (8,65%). Foram encontradas associações entre soropositividade e as seguintes variáveis ($p \leq 0,05$): espécie, origem animal, finalidade da criação e fonte de água para consumo animal e contato com gatos. Os produtores precisam implementar medidas para controlar a infecção nessas espécies, evitando assim, a transmissão do parasita à população humana que consumirá sua carne.

Palavras-chave: Toxoplasmose, equinos, asininos, sorologia, Minas Gerais.

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Introduction

Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii*, a coccidian that can infect all homeothermic animals (Tenter et al., 2000). Felids, including the domestic cat, are definitive hosts and responsible for environmental contamination by releasing oocysts in their feces (Bresciani et al., 2016). Mammals, including humans, and birds are intermediate hosts (Robert-Gangneux & Dardé, 2012). Infection occurs mainly through ingestion of raw or undercooked meat containing tissue cysts, or food or water contaminated with infective oocysts; or through congenital transmission (Amendoeira, 1995; Tenter et al., 2000; Shapiro et al., 2019).

Horses are resistant to this disease and rarely present clinical signs after *T. gondii* infection (Dubey & Desmonts, 1987; Dubey, 2010). Although clinical disease in horses is not proven, symptoms that have been correlated with the presence of antibodies include motor incoordination of the limbs, abortion and excessive irritability (Macruz et al., 1975; Coiro et al., 2012; Santos et al., 2014). Stelzer et al. (2019) have shown low frequencies of anti-*T. gondii* antibodies in these animals, nevertheless, recent reports have notified occurrences of cases of acquired and congenital toxoplasmosis in countries where horsemeat consumption is common (Elbez-Rubinstein et al., 2009; Pomares et al., 2011). In Brazil, the frequency of anti-*T. gondii* antibodies in equids ranges from 10% to 28%. It has been detected in all Brazilian regions and in different animal populations (Gennari et al., 2015; Ribeiro et al., 2016; Moura et al., 2016; Venturi et al., 2017; Guerra et al., 2018; Moreira et al., 2019).

Although there is still little information on the role of donkeys in the epidemiology of toxoplasmosis, findings of anti-*T. gondii* antibodies in this species have shown that the meat of these animals is a possible source of infection (Machacova et al., 2014). Compared with horses, few studies in Brazil have evaluated exposure of donkeys to *T. gondii* (Oliveira et al., 2012, 2013; Gennari et al., 2015; Guerra et al., 2018). Some studies have suggested that there is a possibility of transmission through consumption of raw asinine milk, but although DNA of the parasite was detected, the infective capacity of the samples was not proven (Mancianti et al., 2014, Martini et al., 2014).

Brazil is currently the country with the eighth largest volume of exports of equid meat. Some European Union countries (Belgium, Netherlands, Italy and France) and Japan are the main consumers of Brazilian horsemeat (Brasil, 2016). Only three Brazilian slaughterhouses are authorized to export equid meat. They are located in the states of Minas Gerais, Rio Grande do Sul and Paraná (Brasil, 2017). In addition to forming a source of infection for humans, this meat can form the means for transporting the parasite to cats and other captive carnivorous animals (Silva et al., 2001).

The aim of this study was to evaluate occurrences of anti-*Toxoplasma gondii* antibodies and risk factors relating to infection in equids (horses and donkeys) that were slaughtered for human consumption in a slaughterhouse located in the Triângulo Mineiro and Alto Parnaíba mesoregion, Minas Gerais, Brazil.

Materials and Methods

Population and region studied

This study was conducted on equids that were slaughtered for human consumption in a slaughterhouse that is licensed for exports by the Federal Sanitary Inspection Service, and which is located in the Triângulo Mineiro and Alto Parnaíba mesoregion, Minas Gerais, Brazil. In this study, the sample calculation took into account the total herd of equidae raised in Brazil and the expected frequency of 50% for infection by *T. gondii* in these animals. In order to achieve 95% confidence level, it was necessary the inclusion of 384 animals. As some sample losses could occur in the process, 400 animals were included. The serum samples collected for this study were obtained from equids in six Brazilian municipalities: two in the state of Bahia, three in the state of Goiás and one in the state of Piauí, as indicated in Figure 1. The animal's owners agreed to participate in the study after being informed of the research objectives, the confidentiality of their identities and their right to refuse to participate at any time. All of the slaughtered animals were destined for external consumer markets in European Union countries and China.

This study was approved by the Ethics Committee for Animal Use of the Laboratory Animal Center (CEUA/NAL) of the Federal Fluminense University, in February 2013 (registration no. 20/13).

Sample collection

Blood samples were collected in the exsanguination area, in 10 mL tubes without anticoagulant. The samples were left at room temperature until clot formation. The clot was then removed and the tubes were centrifuged (Excelsa Baby II centrifuge, Fanem®) at 1000 g for 10 minutes, for serum separation. The serum samples were then transferred to sterile vials and were stored at -20 °C until the time of laboratory analysis.

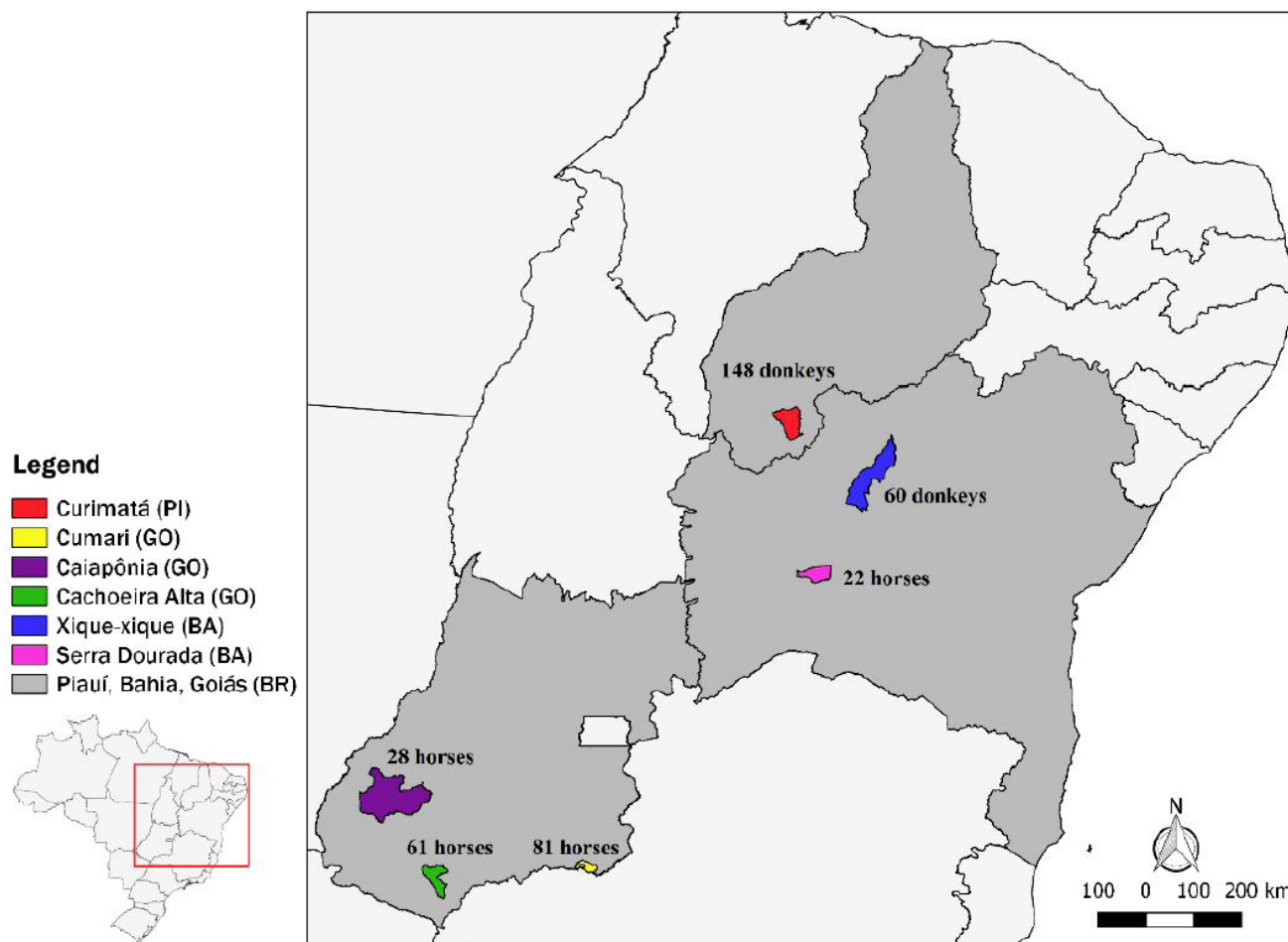


Figure 1. Distribution of equids subjected to detection of anti-*Toxoplasma gondii* IgG by IFAT from three Brazilian states.

Epidemiological questionnaire

The farm owners and/or abattoir technical manager answered an epidemiological questionnaire regarding variables such as sex, species, animal origin, diet, contact with cats and rodents, purpose of the animals and origin of water offered to the animals.

Laboratory analysis

Analyses to detect anti-*T. gondii* IgG antibodies were performed at the Laboratory for Toxoplasmosis and Other Protozoan Diseases (LabTOXO) of the Oswaldo Cruz Institute (IOC), Fiocruz. Samples were analyzed by means of the indirect immunofluorescent antibody test (IFAT), which was performed as described by Camargo (1964) and in accordance with the protocol established at LabTOXO. Tachyzoites of the RH strain of *T. gondii* that were maintained in Swiss Webster mice were used as the antigen. Positive and negative controls that had previously been tested, and which were stored at the serum bank of LabTOXO, were also used. The commercial conjugate anti-horse IgG FITC (Sigma-Aldrich®) was used in all reactions, at a dilution of 1:75 in Evan’s Blue. Samples were considered positive when formation of the antigen-antibody-fluorescein conjugate complex was observed at a dilution of 1:64 or greater, thus showing total fluorescence of the tachyzoite surface.

Statistical analysis

The serological results and epidemiological variables were analyzed using the GraphPad Prism 7 statistical software. Associations between two categorical variables were determined using Pearson’s chi-square test (χ^2) or Fisher’s exact test, with a significance level of 5%. In order to evaluate the impact among variables, odds ratio (OR) values were described with their respective 95% confidence interval (CI).

Results

A total of 400 animals (192 horses and 208 donkeys) with an average age of eight years, from different Brazilian states, were included in this study. Presence of anti-*T. gondii* antibodies was observed in 13.5% (54/400) of the samples. A positive result at a dilution of 1:64 was observed in 48 samples, while the remaining six were positive at a dilution of 1:256. A higher frequency of antibodies was observed in horses (18.75%) than in donkeys (8.65%), and this finding was statistically significant (Table 1). Horses were twice as likely to have anti-*T. gondii* antibodies as donkeys (OR: 2.4359; 95% CI: 1.3313-4.4569). The animals in the state of Goiás had the highest occurrences (Caiapônia, 28.57%; Cachoeira Alta, 26.23%; and Cumari, 14.81%), whereas no occurrence was observed in the animals in the state of Bahia ($p = 0.01$).

Table 1. Risk factor associated with seroprevalence of *Toxoplasma gondii* infection in equids slaughtered in Minas Gerais, Brazil.

Variable	N°	% IFAT	p Value	OR (CI 95%)
Species†				
Donkeys	208	8.65%	0.005	2.4359 (1.3313-4.4569)
Horses	192	18.75%		
Sex				
Female	209	15.79%	0.209	0.659 (0.367-1.184)
Male	191	10.99%		
Ration consumption				
No	383	12.79%	0.064	2.84 (0.959-8.409)
Yes	17	29.41%		
Presence of cats†				
No	111	21.62%	0.005	0.420 (0.233-0.757)
Yes	289	10.38%		
Presence of rodents				
No	22	0.00%	0.056	1.167 (1.120-1.216)
Yes	378	14.29%		
Municipality†				
Cachoeira Alta (GO)	61	26.23%	0.010	Undefined
Caiapônia (GO)	28	28.57%		
Cumari (GO)	81	14.81%		
Curimatá (PI)	148	12.16%		
Serra Dourada (BA)	22	0.00%		
Xique Xique (BA)	60	0.00%		
Water source†				
Well	251	9.56%	0.004	0.4194 (0.2346-0.7496)
Riverside	89	26.97%	0.0001	3.4585 (1.8966-6.3064)
Stream	169	11.83%	0.4928	0.7777 (0.4303-1.4057)
Weir	148	12.16%	0.6537	0.8308 (0.4531-1.5232)
Cistern	72	12.50%	0.9332	0.8984 (0.4176-1.9326)
Animal purpose†				
Meat	132	22.73%	0.0002	2.9902 (1.6669-5.3642)
Milk	245	8.98%	0.0010	0.3792 (0.2111-0.6812)
Sport	22	0.00%	0.0560	Undefined
Others	95	11.58%	0.6486	0.7979 (0.3937-1.6170)
Not informed	113	6.19%	0.0117	0.3372 (0.1476-0.7705)

OR: Odds ratio; CI: Confidence Interval; IFAT: indirect immunofluorescent antibody test; N°: animals' number. † $p < 0.05$.

Regarding the purpose of rearing, independent analysis of the categories revealed that animals raised for meat production were almost three times more likely to have anti-*T. gondii* antibodies (OR: 2.9902; 95% CI: 1.6669-5.3642). These animals had higher occurrence of anti-*T. gondii* antibodies (22.73%). Consumption of water from running sources, such as at riversides, increased the likelihood of presenting antibodies against *T. gondii* threefold among equids (OR: 3.4585; 95% CI: 1.8966-6.3064). Animals that consumed this type of water presented higher occurrence of infection (26.97%) than animals that consumed water from other sources ($p = 0.00005$).

Unexpectedly, the frequency of anti-*T. gondii* antibodies was proportionally higher in horses without contact with cats ($p = 0.005$). However, 55.6% (30/54) of the seropositive equids lived in establishment with cats. On the other hand, equids on farms with absence of rodents did not show any exposure to this protozoon (0%). In contrast, 14.29% of the animals that had contact with rodents were seropositive ($p = 0.056$).

There was no statistically significant association between serological positivity and sex and diet ($p > 0.05$) (Table 1).

Discussion

The present study revealed that equids slaughtered for human consumption in the Triângulo Mineiro region had earlier been exposed to *T. gondii*. There are no data about the seroprevalence of *T. gondii* infection in equids slaughtered in the Triângulo Mineiro and Alto Paranaíba mesoregion, although there are some records of infection in horses in this region (Naves et al., 2005).

The prevalence of 13.5% of anti-*Toxoplasma gondii* IgG antibodies observed in our study can be considered low, however low frequencies have also been detected in horse and donkey populations slaughtered for human consumption in other Brazilian regions. Horses slaughtered in the state of Paraná were found with seropositivity of 11.6% for *T. gondii*, these animals are coming from the states of Paraná, Minas Gerais, Rio de Janeiro, Goiás, Mato Grosso and Mato Grosso do Sul (Evers et al., 2013). Also, the prevalence of 14.4% of antibodies against *T. gondii* in animals from the state of Alagoas was reported by Valença et al. (2015). Otherwise, in the state of Pernambuco detected anti-*T. gondii* antibodies in 12.5% of the analyzed animals (Guerra et al., 2018). In Romania and China higher prevalences have been reported (Pastiu et al., 2015; Yang et al., 2013). Despite the low frequency of seropositive animals detected in this study, the occurrence of animals exposed to the protozoon suggests the possibility of parasite transmission to humans through the consumption of its meat. The diagnosis of equids with infection by *T. gondii* is difficult, since most are asymptomatic and naturally resistant to clinical toxoplasmosis. In addition, parasitism by *T. gondii* is not detected in the slaughter line. Thus, the management of these animals should be more appropriate for meat production.

Analyzing risk factors associated with infection in equids slaughtered for human consumption it is noted that, in our study, seropositivity to *T. gondii* was not associated with sex or diet. The animal gender did not affect the rate of positive. This data is similar to other study that observed that equids of both genders were exposed to the same risk factors (Alvarado-Esquivel et al., 2015). All the animals had been grazing on pasture and, among those that had received supplementary feed, consumption of feed doubled the likelihood of presenting antibodies against *T. gondii*, although this was not a statistically significant association, it is possible that cats or other mechanical carriers of oocysts, like rodents, were able to access the places where animal feed was stored, thereby contaminating it. Although ration consumption was not evaluated as possible risk factors in the present study, these have already been identified as factors associated with infection among horses (Alvarado-Esquivel et al., 2015).

There was also no statistical association between serological positivity and the presence of rodents on the farms. However, no seropositive animals were detected on farms without rodents. According to Dubey & Frenkel (1998), rodents may act as an important source of *T. gondii* tissue cysts for dogs, pigs and, especially, cats. In this regard, the presence of rodents on farms where equids were being reared may have been attractive for felids. In turn, after becoming infected through predatory action, these felids may have contributed towards dispersion of *T. gondii* in the environment through their contaminated feces. Actions and practices aimed towards controlling these small mammals on farms should be encouraged, in order to reduce the risk of parasite transmission.

When we analyzed the species, we can see that the frequency of antibodies observed in this study was higher in horses than in donkeys, which is statistically significant. García-Bocanegra et al. (2012) reported similar results, however, it did not occur in other locations, where mules have statistically greater seropositivity than horses (Gennari et al., 2015). There is no evidence of difference in sensitivity to *T. gondii* infection among equid species. Thus, the difference between the species that was observed in the present study may be related to their municipality of origin. The seropositive horses were all from Cachoeira Alta (Goiás), Caiapônia (Goiás) and Cumari (Goiás), while

the seropositive donkeys were all from Curimatá (Piauí). In this study, no occurrences of *T. gondii* infection were detected in equids in municipalities in the state of Bahia, whereas the animals in the state of Goiás presented high frequencies of specific antibodies to the parasite. This difference can be explained, for example, in terms of differences in climatic conditions, since the climate of certain regions can often favor or hinder sporulation of *T. gondii* oocysts and consequently contamination of food, water and soil (Guerra et al., 2018).

The disparity of results found in the literature is probably due to differences in the following: serological techniques, interpretations, cutoff points, animal populations, geographical conditions, contact with cats and management conditions. These differences have made it difficult to compare studies (Camossi et al., 2010; García-Bocanegra et al., 2012; Papini et al., 2015). Moreover, Oliveira et al. (2012) and Papini et al. (2015) pointed out that there is no gold-standard technique for making the diagnosis of infection in equids.

Another risk factor for *T. gondii* infection in horses that was studied was the presence of cats in the farms. The contact with felids, especially domestic cats, is one of the main risk factors for acquisition of *T. gondii* infection among herbivorous animals. The importance of close contact between young cats and equids is consistent with the biology of the disease, as young cats are primarily responsible for the shedding of oocysts and the feces of felids can ensure contamination of pasture grass and water that are intended for animal consumption. Equids and other herbivore intermediate hosts of *T. gondii*, such as ruminants, can only become infected by ingestion of food and water contaminated with sporulated *T. gondii* oocysts from felids or by congenital transmission. Thus, the presence of felids on the properties is a fundamental link between these animals and the protozoan. However, in the present study, the greatest occurrence of *T. gondii* infection was among animals that, according to their owners and/or the abattoir technical manager, had not come into contact with cats. This probably constitutes a source of bias in our study, since the difficulty of assessing the real presence of cats in shelters or even fields where equids are kept.

Alike the the present research, Oliveira et al. (2012) also studied animals in Bahia and found that the sources of running water destined for consumption by equids were a risk factor for infection. In fact, water consumption on the riverside, a place also frequented by cats, is an ideal environment for sporulation of oocysts. It is important to note that felids are dispersal agents for *T. gondii* in the environment. Farming is considered to be an important risk factor for *T. gondii* infection in horses, because the activity keeps these animals close to a potentially contaminated environment.

In our study the age was not evaluated. Age was not a factor associated with infection in horses. In other species studies have shown that age could be is a risk factor, but this generally does not occur with equids (Munhoz et al., 2019).

The prevalence of anti-*T. gondii* antibodies described in this study (13.5%) shows the circulation of the parasite in the equine population in Brazil. This result is important in the scope of public health, because, although Brazilians are in the habit of eating equid meat, the country stands out in the export of this product. Moreover, since slaughtering of equids in Brazil mainly occurs as a way of discarding animals previously used for other purposes, their management may not be as appropriate as that applied to other production animals, as pointed out by Evers et al. (2012). Therefore, farms with a more technological approach to horse management, which tend to have better hygiene and sanitary protection practices, will be more able to prevent the spread of certain diseases in horses, including *T. gondii* infection.

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