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Characterization of the zoonotic potential of *Toxoplasma gondii* in horses from Rio de Janeiro State

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ABSTRACT

The aim of this study was to perform a survey on the prevalence of anti-*Toxoplasma gondii* antibodies in horses from Rio de Janeiro State, Brazil. From 2012 to 2013, a total of 624 blood samples were collected from horses from the eight regions comprising Rio de Janeiro State (Baixadas Litorâneas, Serrana, Norte Fluminense, Noroeste Fluminense, Centro-Sul, Metropolitana, Médio Paraíba, and Costa Verde). All sera samples were tested for anti-*T. gondii* antibodies by performing the modified agglutination test with a cut-off of 1:25. Positive serology for *T. gondii* was detected in 22.8% (142/624) of the horses studied. Seropositivity was detected in all regions sampled; furthermore, statistical significance was observed when all locations were compared at once. The Médio Paraíba region had the highest number of positive animals 54.76% (23/42) in the Bonferroni correction among Costa Verde, Centro Sul, Metropolitana, and Serrana ($p < 0.001$). Seropositivity was 39.58% (38/96) in Norte Fluminense, which was the second most prevalent region. The results indicated that the *T. gondii* parasite is widely distributed in horses in Rio de Janeiro State and represents a risk to public and animal health. These findings emphasize the need to increase control and prevention of this disease in horses.

1. Introduction

Toxoplasma gondii was first described by Nicolle and Manceaux in 1908. It is an obligate intracellular parasite and is a zoonotic pathogen with a worldwide distribution. Members of the Felidae family act as definitive host, and all warm-blooded animals, including horses, can be intermediate hosts. The pathogen can cause miscarriages and congenital diseases (Dubey, 2010; Moura et al., 2016; Netto et al., 2003).

Horses are infected by *T. gondii* when they ingest food or water contaminated with oocysts released by cats (Dubey, 2010; Masatani et al., 2016). Among domestic animals, horses exhibit high resistance to *T. gondii* infection (Dubey and Jones, 2008; Moura et al., 2016), but clinical signs, such as hyperirritability, incoordination, ocular disorders, and abortions, have been observed (Moura et al., 2016; Turner and Savva, 1992).

The presence of *T. gondii* in livestock animals is an important aspect in the epidemiology of toxoplasmosis because the consumption of raw or undercooked meat of an infected animal is a source of infection for humans (Alvarado-Esquivel et al., 2015; Bartova et al., 2015). In Brazil, the consumption of equine meat is uncommon. However, the elevated domestic production of this meat, mainly designated for export, has made Brazil the eighth largest exporter worldwide (MAPA, 2016). This fact highlights the importance of research aiming to evaluate the seroprevalence of *T. gondii* in horses in Brazil, particularly with respect to zoonosis.

Rio de Janeiro State is located in the southeastern region of Brazil, where the largest production of horses is concentrated. However, few studies have investigated the seroprevalence of *T. gondii* in these animals. Therefore, the aim of this study was to conduct a survey on the prevalence of anti-*T. gondii* antibodies in horses from Rio de Janeiro State to identify the most prevalent regions of the parasite and to serve as an important database regarding the seroprevalence of this coccidia in Brazil.

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2. Material and methods

This study was approved by the ethics committee of animal use (CEUA) of Universidade Federal Fluminense, Rio de Janeiro, Brazil, under protocol number 652.

2.1. Blood samples

The total number of samples required for this study in horses was calculated on the basis of an expected prevalence of 31.5% according to the findings of Vidotto et al. (1997), and the total population of 113.203 horses in Rio de Janeiro State according to data from the Brazilian Institute of Geography and Statistics (IBGE, 2013). A confidence interval (CI) of 95% was used and determined using Epi Info version 3.5.3® software (CDC 2010). Thus, the minimum number of samples required to make this study representative was 336.

A cross-sectional study was conducted using 624 serum samples from horses collected between 2012 and 2013. The samples were from male and female horses aged 3–22 years from the eight regions comprising Rio de Janeiro State (Baixadas Litorâneas, Serrana, Norte Fluminense, Noroeste Fluminense, Centro Sul, Metropolitana, Médio Paraíba, and Costa Verde), as shown in Fig. 1. The selection of animals was randomized to avoid bias.

The animals' blood samples were collected by venipuncture of the jugular vein and placed in 8-mL vacuum tubes without anticoagulant but with a gel clotting activator to obtain the serum.

After collection, the samples were placed in a vacuum flask, restored at 5 °C, and then taken to the laboratory for processing. The tubes were centrifuged at 5000 rpm for 20 min to separate the serum. Once the sera were obtained, they were stored in microtubes and frozen at −20 °C to perform the serological tests.

2.2. Modified agglutination test

The modified agglutination test (MAT) was used to screen anti-*T. gondii* antibodies of the immunoglobulin G class from 624 equine serum samples from Rio de Janeiro State. The sera tested were diluted 1:25 in 0.01 M phosphate-buffered saline (PBS), pH 7.2, according to the protocol previously established by Dubey and Desmonts (1987). The antigen was provided by the Animal Parasitic Diseases Laboratory of the United States Department of Agriculture, EUA.

In each well of the 96-well plates, 25 μL of antigen solution was added with 25 μL of a previously diluted serum. Positive and negative controls were also added. The plates were incubated at 37 °C for 12 h. MAT reading was based on the sedimentation profile of the tachyzoites suspension in which formation of a web indicated the presence of antibodies and formation of a blue dot in the bottom indicated the absence of antibodies.

2.3. Statistical analysis

Descriptive statistical analysis was used to calculate the relative and absolute frequencies of results in serology. The Chi-square test was adopted to assess differences in cases positive for *T. gondii* among the eight regions of Rio de Janeiro State, at a 5% significance level. The Bonferroni correction was used for multiple comparisons of intersections of two-by-two regions in an attempt to identify the occurrence of such differences. The Statistical Package for Social Sciences (SPSS) version 18.0® software was used.

3. Results

Anti-*T. gondii* antibodies were detected in 22.8% (142/624) of the horses studied (Table 1). The presence of anti-*T. gondii* antibodies in horses was detected in all regions sampled. Furthermore, statistical differences in the presence of anti-*T. gondii* antibodies were found by the Chi-square test ($p < 0.05$) when the regions were compared.

The Medio Paraíba region had the highest rate of seropositivity (54.76% or 23/42) for *T. gondii* in horses in Rio de Janeiro State. Compared with other regions, the Medio Paraíba region seropositivity rate was statistically different by Bonferroni correction ($p < 0.001$) from the rates in the following regions: Baixada litorânea, Centro-Sul, and Metropolitana, as shown in Table 2. The second most prevalent region for anti-*T. gondii* in horses was the Norte Fluminense region, where 39.58% (38/96) of the animals were positive.

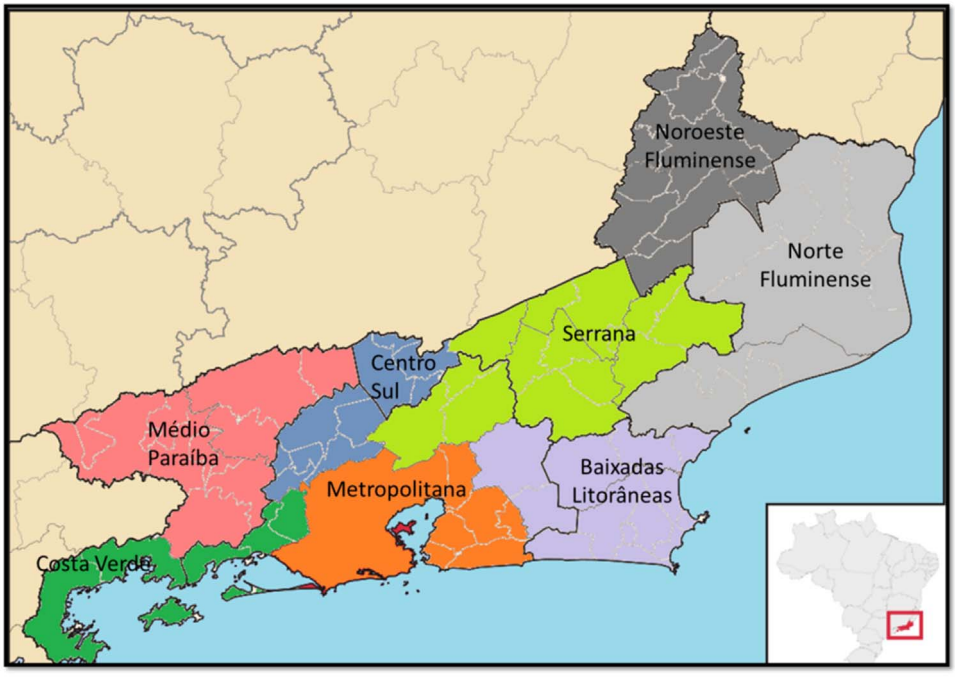

Fig. 1. Map of Rio de Janeiro State showing the regions from where the horse blood samples were collected.