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ORIGINAL ARTICLE

Detection of a high prevalence of antibodies against *Toxoplasma gondii* in cattle in Northern and Midwestern Brazil

Jenevaldo Barbosa da Silva[I], Gustavo Nunes de Santana Castro[II], Priscilla Nunes dos Santos[II], Adivaldo Henrique da Fonseca[II], Danillo Henrique da Silva Lima[III], Henrique dos Anjos Bomjardim[III], Alessandra dos Santos Belo Reis[III], Susiane de Oliveira Soares[III], José Diomedes Barbosa[III]

[I]Laboratório de Imunoparasitologia, Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias FCAV-UNESP, Via de Acesso Prof. Paulo Donato Castellane s/n, 14884-900, Jaboticabal, SP, Brazil. E-mail: jenevaldo@hotmail.com. [II]Laboratório de Doenças Parasitárias, Departamento de Epidemiologia e Saúde Pública, Universidade Federal Rural de Rio de Janeiro (UFRRJ), Br 465, Km 7, 23890-000, Seropédica, RJ, Brazil. E-mail: adivaldo@ufrrj.br. [III]Instituto de Medicina Veterinária, Universidade Federal do Pará, Rodovia BR 316 Km 61, Bairro Saudade, 68740-970, Castanhal, PA, Brazil. E-mail: diomedes@ufpa.br.

ABSTRACT: The aim of this study was to determine the prevalence of *Toxoplasma gondii* in cattle from the Northern and Midwestern regions of Brazil. Serum samples were collected from 1789 animals and tested by indirect enzyme-linked immunosorbent assay (ELISA). The overall prevalence of *T. gondii* was 83.40% (1492/1789). The prevalence rates of *T. gondii*-seropositive animals observed in the states of Pará, Tocantins and, Mato Grosso were 87.45%, 87.79% and 73.06%, respectively. The detection of high prevalence rates of *T. gondii* in cattle deserves special attention because they are the main source of high biological value protein for humans. This finding indicates the need for further studies on the risk that these animals may pose to public health.

Key words: cattle, ELISA, IFAT, *Toxoplasma gondii*.

Detección de alta prevalencia de anticuerpos contra *Toxoplasma gondii* en bovinos del Norte y Medio Oeste de Brasil

RESUMEN: El objetivo del presente estudio fue determinar la prevalencia de *Toxoplasma gondii* en bovinos de las regiones del Norte y Medio Oeste de Brasil. Las muestras de suero se colectaron de 1789 animales y se testaron por ELISA. La prevalencia total de *T. gondii* fue de 83.40% (1492/1789). La proporción de animales seropositivos a *T. gondii*, observados en los estados de Pará, Tocantins y Mato Grosso, fue de 87.45%, 87.79% y 73.06%, respectivamente. La detección de alta prevalencia a *T. gondii* en bovinos merece atención especial, porque ellos son la principal fuente biológica de valor proteico para los humanos. Estos hallazgos indican la necesidad de estudios posteriores sobre el riesgo que estos animales pueden significar para la salud pública.

Palabras clave: bovino, ELISA, IFAT, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasma gondii, the etiological agent of toxoplasmosis (1), is a protozoan with a heteroxenous life cycle that includes an asexual stage in omnivorous and herbivorous hosts and a sexual stage in carnivorous hosts (2). The infection is normally asymptomatic (3); however, clinical signs such as fever, adenopathy and apathy are observed in immunosuppressed animals (4), and spontaneous abortion is an important detrimental effect in both humans and ruminants.

Toxoplasma gondii is transmitted by the ingestion of oocytes shed in the feces of definitive hosts, or by the ingestion of meat from intermediate hosts containing bradyzoites (4). Serological studies have shown *T.*

gondii infection in several animal species around the world (5-7). Brazil is the largest meat exporter in the world (8) and serological studies for this important zoonosis must be pursued because of the frequent reports on *T. gondii* circulation in livestock in many regions of the country (9-11). However, there is still a shortage of epidemiological data regarding *T. gondii* circulation in Brazilian beef cattle.

Beef cattle production in Brazil is distributed across all the states, and the Midwestern and Northern states alone hold approximately 115 million cattle, representing 55% of the entire Brazilian cattle herd (12). Thus, this study was significant given that its goal was to evaluate the seroprevalence of *T. gondii* in cattle from the states in Northern and Midwestern Brazil.

MATERIALS AND METHODS

Animals and areas studied

The evaluated animals were selected in Midwestern and Northern Brazil. All the animals were Nelore cattle (*Bos indicus*) and of approximately three years of age. They were raised predominantly in an extensive farming system, grazing pasture and receiving supplementation in the feeding trough. The sample size was determined using the Epi-Info software considering a 5% error margin and an infection frequency of 50% in the cattle population of Brazil.

Sampling

For this study, 1879 animals were selected. The samples were collected between January and March 2013. The farms selected were authorized for cattle export. These cattle routinely undergo a rigorous health protocol involving screening for brucellosis (*Brucella abortus*), tuberculosis (*Mycobacterium bovis*), leptospirosis (*Leptospira* spp.) and bovine viral diarrhea (*Pestivirus* sp).

Blood samples were collected from cattle from 15 towns in the state of Mato Grosso (Tangará, Denise, Nova Marilândia, São Felix do Araguaia, Santa Cruz do Xingu, Castanheira, Primavera do Leste, Brasnorte, Canarana, Espigão do Norte, Campo Novo, Vila Rica, Juína, Luciara and São José do Xingu), 8 towns in the state of Pará (Marabá, Itupiranga, Xinguara, Rio Maria, Água Azul do Norte, Curionópolis, Santa Maria das Barreiras and Bannach) and 14 towns in the state of Tocantins (Miranorte, Bernardo Sayão, Porto Nacional, Santa Fé do Araguaia, Alvorada, Talismã, Bandeirantes do Tocantins, Colinas, Divinópolis do Tocantins, Araguanã, Pium, Miracema, Couto Magalhães and Gurupi). At least three different farms were selected in each town, and at least 20 samples were taken from each farm.

Enzyme-linked immunosorbent assay (ELISA)

The blood of the animals was collected by jugular venipuncture and later centrifuged to obtain the serum. IgG antibodies against *T. gondii* were detected by ELISA as described by Voller et al. (13).

The anti-*Toxoplasma gondii* antibodies were searched by ELISA. The "RH" strain was used to obtain the antigens as described by Camargo (14). The ELISA test was established using an optimal concentration of the *T. gondii* antigen (10 µg/ml) in a 0.05 M carbonate/bicarbonate buffer, pH 9.6. The single dilution of the ELISA test was 1: 200 in PBS-Tween (phosphate buffered saline, pH 7.2, and 0.05% Tween 20) for the positive- and negative-reference sera and test sera, and the conjugate serum was used at a 1:25,000 dilution in PBS-Tween, according to the manufacturer's guidelines (Sigma-Aldrich Chemical Company, St. Louis, Missouri, U.S.A.). The plates were read by using an ELISA reader (Dynex-Technologies®, Virginia, U.S.A.) with a 405-nm filter. Under these conditions, the lowest mean optical density (OD) of the negative sera was 0.90±0.013. The highest mean reactivity observed for the positive-reference sera was 1.13±0.065.

Thirty positive and 30 negative controls were used to determine the cut-off, which was calculated based on the sera of negative animals and analyzed using the statistic software MedCalc (version 11.4, http://www.medcalc.be). Serological detection based on ELISA and IFAT test has been proven to provide reliable results with high sensitivity and specificity in detection of *T. gondii*, particularly when the parasitemia is very low. The tests showed no cross-reaction with any other microorganism.

Statistical analysis

The data were analyzed using the chi-square and Kruskal-Wallis tests with a 95% confidence interval using the R software, version 2.2.1 (R Development Core Team, 2005).

RESULTS AND DISCUSSION

Of the 1789 samples evaluated by the ELISA test for *T. gondii*, 1492 were seropositive, which corresponded to a prevalence of 83.40%. The prevalence rates for *T. gondii* observed in Pará, Tocantins and Mato Grosso were 87.45%, 87.79% and 73.06%, respectively (Table 1).

TABLE 1. Serological prevalence of *T. gondii* determined by ELISA in Nelore cattle from the states of Mato Grosso, Pará and Tocantins./ *Prevalencia serológica de T. gondii por ELISA en bovinos Nelore de los estados de Mato Grosso, Pará y Tocantins.*

Province	Positive	Negative	Prevalence	Total
Mato Grosso	377	139	73.06%	**516**
Pará	669	96	87.45%	**765**
Tocantins	446	62	87.79%	**508**
Total	**1492**	**297**	**83.40%**	**1789**

All of the farms evaluated had at least one positive animal, and on some farms, 100% of the animals were positive for *T. gondii.* Thus, considering the farm as an epidemiological unit, the prevalence of *T. gondii* was 100%. The prevalence of *T. gondii* was significantly higher ($p<0.05$) in the states of Pará and Tocantins compared with Mato Grosso.

Several studies have been performed to investigate the serological prevalence of *T. gondii* in cattle because they are one of the main sources of animal protein consumed by humans around the world (9). A wide variation in the frequency of this agent has been observed in cattle in Brazil (9, 10, 15, 16) and other parts of the world (6, 17).

Recent studies in Brazil have shown both a low (9) and a high (18) prevalence of *T. gondii* in cattle. However, few studies have been performed in Midwestern and Northern Brazil, where the largest cattle herd for export in the world is found. Because these regions have a herd of 115 million cattle, studies in this area are more than justifiable. The high prevalence of *T. gondii* in cattle observed in the studied region constitutes a public health issue because this protozoan is capable of causing infection through the ingestion of meat products containing cysts. Our results showed a prevalence value higher than all those other values reported for Brazil.

Garcia *et al.* (19), Daguer *et al.* (20) and Santos *et al.* (18) reported *T. gondii* prevalences of 25.8%, 41.4% and 17.4%, respectively, for Brazilian cattle. In Southeastern Brazil, Fajardo et al. (9) observed that only 2.68% of animals were positive, while Gondin et al. (21) reported that 1.03% of animals were positive in the Northeastern region of Brazil. However, few studies have been performed in Midwestern and Northern Brazil. A recent study performed in Northern Brazil showed a prevalence of 41.6% for *T. gondii*-seropositive buffalo (22). Other studies indicated a *T. gondii* prevalence of 71.0% in cattle from Midwestern Brazil (23).

The prevalence of *T. gondii* also varies greatly in other parts of the world. A total of 83.3% of the cattle in Spain were diagnosed as positive (6), and 44.8% were positive in Sudan (24); conversely, only 5.7% of the animals in China were seropositive for *T. gondii* (25). These results demonstrate that the presence of this agent is highly variable, and comprehensive health safety measures are necessary in areas where the prevalence is higher because we still do not know the connection between *T. gondii* seroprevalence in cattle and the potential infection of other hosts such as humans. Cattle showed a significant serological prevalence of *T. gondii* using both of the techniques evaluated. These results suggest that cattle, when exposed to the same risks for *T. gondii* infection, have a high antibody mediated response.

CONCLUSIONS

The detection of high prevalence rates of *T. gondii* in livestock deserves special attention because cattle are the most important source of animal protein for humans. This finding indicates the need for further studies on the risk that such animals may pose to public health.

CONFLICT OF INTEREST STATEMENT

None of the authors of this work has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the contents of this paper.

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