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Anti-*Toxoplasma gondii* antibodies in beef cattle slaughtered in the metropolitan region of Belém, Brazilian Amazon

Anticorpos anti-*Toxoplasma gondii* em bovinos de corte abatidos na região metropolitana de Belém, Amazônia brasileira

Ediclei Lima do Carmo[1,2]*; Rafaela dos Anjos Pinheiro Bogoevich Morais[1,2]; Michele de Souza Lima[3]; Carla Cristina Guimarães de Moraes[3]; George Rêgo Albuquerque[4]; Aristeu Vieira da Silva[5]; Marinete Marins Póvoa[1,2]

[1] Seção de Parasitologia, Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Ministério da Saúde, Ananindeua, PA, Brasil

[2] Programa de Pós-graduação em Biologia de Agentes Infecciosos e Parasitários, Universidade Federal do Pará – UFPA, Belém, PA, Brasil

[3] Faculdade de Medicina Veterinária, Universidade Federal do Pará – UFPA, Castanhal, PA, Brasil

[4] Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz – UESC, Ilhéus, BA, Brasil

[5] Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana – UEFS, Feira de Santana, BA, Brasil

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Abstract

The relevance of consuming raw or undercooked beef in the transmission of toxoplasmosis is unclear due to the high resistance of cattle to infection. However, this possibility needs to be considered in endemic areas, such as the Amazon, where the consumption of beef is frequent. The objective of this study was to determine the frequency of anti-*Toxoplasma gondii* IgG antibodies in beef cattle slaughtered in the metropolitan region of Belem, Pará state, Brazil. Blood samples were collected from 500 animals of both genders in a licensed slaughterhouse in Belém. Anti-*T. gondii* IgG antibodies were detected by an indirect immunofluorescence assay (IFA) with a cut-off titer of 1:64. Anti-*T. gondii* antibodies were found in 203 animals (40.6%), with a titer of 64 in 112 animals (55.2%), 128 in 68 animals (33.5%), 256 in 15 animals (7.4%), 512 in 5 animals (2.5%), and 1,024 in 3 animals (1.4%). No significant difference was observed between males and females ($p > 0.05$). The high frequency of anti-*T. gondii* antibodies observed in beef cattle slaughtered in Belém indicates that the meat of these animals may be an important source of infection for humans and carnivorous domestic animals when inadequately cooked beef is consumed.

Keywords: *Toxoplasma gondii*, cattle, serology, epidemiology, Amazon.

Resumo

A importância do consumo de carne bovina crua ou mal passada na transmissão da toxoplasmose ainda é pouco definida, devido à alta resistência desses animais à infecção. Contudo, em áreas endêmicas, como da Amazônia, onde o consumo de carne bovina é frequente, essa possibilidade precisa ser considerada. O objetivo do presente estudo foi determinar a frequência de anticorpos IgG anti-*T. gondii* em bovinos de corte abatidos na região metropolitana de Belém, Estado do Pará. Foram coletadas amostras de sangue de 500 animais, de ambos os sexos, em um abatedouro oficial do município de Belém. A detecção de anticorpos IgG anti-*T. gondii* foi realizada pela reação de imunofluorescência indireta (RIFI), com ponto de corte de 1:64. Anticorpos anti-*T. gondii* foram encontrados em 203 animais (40,6%), com títulos de 64 em 112 animais (55,2%); 128 em 68 (33,5%); 256 em 15 (7,4%); 512 em 05 (2,5%); e 1.024 em 03 (1.4%). Não foi observada diferença estatisticamente significativa entre machos e fêmeas ($p > 0,05$). A alta frequência de anticorpos anti-*T. gondii,* observada nos bovinos de corte abatidos em Belém, indica que a carne desses animais pode ser importante fonte de infecção para humanos e animais domésticos carnívoros, caso venha a ser consumida de forma inadequada.

Palavras-chave: *Toxoplasma gondii*, bovinos, sorologia, epidemiologia, Amazônia.

***Corresponding author:** Ediclei Lima do Carmo. Seção de Parasitologia, Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Ministério da Saúde, Rodovia BR 316, Km 07, s/n, Levilândia, CEP 67030-000, Ananindeua, PA, Brasil. e-mail: edicleicarmo@iec.pa.gov.br

Introduction

Toxoplasmosis is an infection caused by the protozoan *Toxoplasma gondii* and is one of the most important and prevalent zoonoses worldwide. This protozoan is able to infect several species of homeothermic animals. In humans, it can cause serious complications, especially in congenitally infected or immunocompromised individuals (TENTER et al., 2000; WEISS & DUBEY, 2009).

The global seroprevalence of *T. gondii* infection is quite variable and is greater in tropical regions, such as Brazil, where it can vary from 50 to 80% among women of reproductive age and school-aged children (DUBEY et al., 2012). A serological survey performed in the metropolitan area of the City of Belém (Pará state), involving 2.740 individuals showed that the seroprevalence of toxoplasmosis in the general population was approximately 78% (CARMO, 2011). In studies conducted in other urban or rural areas in the Brazilian Amazon the seroprevalence was lower than the Belém one, but still high (CAVALCANTE et al., 2006; FERREIRA et al., 2009; CARMO et al., 2010a; VITALIANO et al., 2015).

Among the different *T. gondii* hosts, cattle are considered the most resistant to infection (DUBEY & THULLIEZ, 1993). However, studies have demonstrated that *T. gondii* cysts can remain viable in experimentally infected cattle tissue until the age of slaughter (approximately three years) (DUBEY & THULLIEZ, 1993; SCARPELLI et al., 2009).

The importance of beef in the transmission of toxoplasmosis is unknown (GARCIA et al., 2012). Surveys conducted in slaughterhouses in Brazil showed that seropositivitity in beef cattle ranged from 1.96 to 41.4% (DAGUER et al., 2004; LUCIANO et al., 2011). Thus, beef, which is the most consumed meat in the country, may be an important source of infection for humans, especially in areas where the frequency of human infection is high, such as the state of Pará in the Brazilian Amazon region (CARMO, 2011). This finding is indicative that beef can actually be important in the *T. gondii* chain of transmission in these areas (MEIRELES et al., 2003; MILLAR et al., 2008).

Due to the low availability of data on *T. gondii* infection in different livestock species in the Amazon region, the objective of the present study was to determine the frequency of anti-*T. gondii* IgG antibodies in beef cattle slaughtered in the metropolitan region of Belém, Pará state, Brazil.

Materials and Methods

Between June 2010 and January 2011, a group of beef cattle (*Bos taurus indicus*) extensively raised on farms located in different mesoregions of Pará state (Southeast, Northeast and Marajó) and sent to slaughter in a licensed slaughterhouse located in the city of Belém belonging to the Agricultural and Livestock Industry Cooperative of Pará State (Cooperativa da Indústria Agropecuária do Estado do Pará - SOCIPE) (01º 19' 43" S - 48º 28' 53" W) was investigated.

The study was approved by the Ethics Committee on Animal Research (Comitê de Ética em Pesquisa Animal - CEPAN) of the Instituto Evandro Chagas /SVS/MS (No. 0017/2006/CEPAN/IEC/SVS/MS).

After the obtainment of official consent from the slaughterhouse management and under the provision of a veterinarian, adult cattle of both genders were randomly selected during the slaughter procedure. Based on the number of animals slaughtered monthly (approximately 5,000), frequency of seropositivity obtained in a previous study (30%) (MORAES et al., 2008) and considering 95% confidence level, a minimum sample size of 331 animals was estimated for the study.

Blood samples from 500 animals were directly collected in tubes without anticoagulant after sectioning of the jugular vein. The obtained serum samples were sent to the Laboratory of Toxoplasmosis of the Evandro Chagas Institute/SVS/MS and stored at –20 °C prior to the serological tests.

All samples were tested via the indirect immunofluorescence assay (IFA) for the detection of anti-*Toxoplasma gondii* IgG according to the description of Camargo (1974). The sera were serially diluted (1:16 to 1,024) and deposited on slides previously sensitized with a *T. gondii* antigen prepared from tachyzoites obtained from mice experimentally infected with the RH strain of the parasite. FITC-rabbit anti-bovine IgG (Sigma-Aldrich®, St. Louis, MO, USA) was used in the specific secondary antibody reaction for cattle. Samples with titers ≥ 64 were considered positive (GARCIA et al., 1999; DAGUER et al., 2004). Positive and negative control sera, kindly provided by the Laboratory of Parasitology and Parasitic Diseases/Londrina State University (Universidade Estadual de Londrina - UEL), Paraná, were included in each test.

Association between sex and origin of animals and IFA results were analyzed using the Chi-square test with Yates correction, and Williams G Test, respectively, using BioEstat 5.0 (AYRES et al., 2007), considering a 5% significance level. For the variable origin are calculed *odds ratio* with respective 95% confidence interval. A regression logistic model with sex and origin were run using the backward *LR* entry and calculation of Hosmer-Lemeshow goodness of fit.

Results and Discussion

Five hundred animals (139 males and 361 females) were selected. The frequency of seropositivity was 40.6% (203/500) (Table 1). Table 2 illustrates the distribution of *T. gondii*-positive animals according to the anti-*T. gondii* IgG antibody titers.

Regarding sex, the seropositivity was 47.5% (66/139) among males and 38.0% (137/361) among females; however, this difference was not significant (p=0.065) (Table 3), even when held the interaction of Monte Carlo to set error rate (χ^2=3.78; P Value=0.054), and by logistic regression using sex and municipality of origin (Wald Statistics=1.00; P Value=0.32). This result was expected because all animals were raised in an extensive system and equally exposed to the same risk factors for infection. Similar results were observed in other Brazilian states and in other parts of the world (DAGUER et al., 2004; KLUN et al., 2006; MOURA et al., 2010). Logistic regression analysis showed that IFA results among cattle from Bom Jesus do Tocantins, Novo Repartimento, Paragominas, Santa Luzia do

Table 1. Frequency of anti-*Toxoplasma gondii* IgG antibodies by IFA test in cattle slaughtered according to origin of the animals, in Belém/PA, Brazil.

Mesoregion	Municipality of origin	N	Reagent (%)	Non Reagent (%)
Southeast	Bom Jesus do Tocantins	22	06 (27.3)	16 (72.7)
	Jacundá	16	04 (25.0)	12 (75.0)
	Marabá	21	12 (57.1)	09 (42.9)
	Novo Repartimento	24	05 (20.8)	19 (79.2)
	Paragominas	68	36 (52.9)	32 (47.1)
	Piçarra	07	04 (57.1)	03 (42.9)
	São Domingos do Araguaia	08	05 (62.5)	03 (37.5)
	São Geraldo do Araguaia	19	09 (47.4)	10 (52.6)
Northeast	Ulianópolis	66	11 (16.7)	55 (83.3)
	Garrafão do Norte	41	16 (39.0)	25 (61.0)
	Igarapé Açú	30	11 (36.7)	19 (63.3)
	Santa Luzia do Pará	79	24 (30.4)	55 (69.6)
	Tailândia	29	12 (41.4)	17 (58.6)
	Viseu	43	27 (62.8)	16 (37.2)
Marajó	Ponta de Pedras	27	21 (77.8)	06 (22.2)
Total		500	203 (40.6)	297 (59.4)

N: number of samples (absolute frequency); Univariate Statistics: G=72.84; P-Value <0.0001.

Table 2. Distribution of seropositive cattle slaughtered according to the anti- T*oxoplasma gondii* IgG antibodies titers by IFA test, in Belém/PA, Brazil.

IgG Titer	N	Fr (%) (95% CI)
64	112	55.2 (48.3-62.0)
128	68	33.5 (27.0-40.0)
256	15	7.4 (3.8-11.0)
512	5	2.5 (1.1-5.6)
1,024	3	1.4 (0.5-4.2)
Total	203	40.6 (36.3-44.9)

N: number of samples (absolute frequency); *Fr*: relative frequency; CI: confidence interval.

Pará e Viseu are significantly different from other origins, then on farm researches must be made to set epidemiological differences that contribute with these results.

The production of beef cattle plays an important role in Brazil's economy because the country has the second largest beef cattle herd and is one of the leaders in beef exports worldwide. Pará state has the fifth largest herd in Brazil and is considered the main meat producer in the North region (MINERVINO et al., 2008; PEREIRA, 2012). Because beef consumption is frequent, there is a suspicion that this consumption contributes to the high prevalence of human infection observed in the region.

The frequency of anti-*T. gondii* IgG antibodies observed among the cattle slaughtered in Belém (40.6%) was much larger than the frequencies found in other parts of the world, such as 2.4% and 4.8% in India and Iran, respectively (SHARMA et al., 2008; HAMZAVI et al., 2007), 3.3% in Tanzania (SCHOONMAN et al., 2010) and 7.3% in Spain (PANADERO et al., 2010). In comparison to studies conducted in other Brazilian locations, the seroprevalence was similar to that observed in Paraná of 41.4% (DAGUER et al., 2004) and greater than the seroprevalence observed in Bahia (11.8%) (SPAGNOL et al., 2009), Rio de Janeiro (1.96%) (LUCIANO et al., 2011), Santa Catarina (29.1%) (MACEDO et al., 2012) and Pernambuco (16.6%) (GUERRA et al., 2014).

These results demonstrate that the ecoepidemiological characteristics of the Amazon region, such as adequate temperature and humidity and the high density of felids close to grazing areas, favor the development and maintenance of *T. gondii* oocysts and consequently the transmission of toxoplasmosis between cattle herds. This finding includes the possibility of infection by atypical and virulent strains in circulation in the Amazon region, which have been associated with severe cases and outbreaks of disease in humans and have been isolated from wild animal samples (DEMAR et al., 2007; CARMO et al., 2010b; VITALIANO et al., 2014).

These animals are raised in an extensive system in the region, which may influence toxoplasmosis transmission routes (MILLAR et al., 2008). Although cattle are considered to be naturally resistant to *T. gondii* infection, they may be more exposed and susceptible to the parasite when raised extensively. As a result, the consumption of beef may be an important route of infection for humans and other carnivorous animals, including felines, which generally are present in the areas where herds are raised. Corroborating this possibility, an epidemiological study conducted in the metropolitan region of Belém demonstrated a significant association between a high consumption of beef, including its raw or undercooked form, and a high seroprevalence of infection in the population in this area (CARMO, 2011).

Most animals had low anti-*T. gondii* IgG antibody titers (64 and 256). Similar results were obtained by Daguer et al. (2004) and Spagnol et al. (2009). Cattle with low antibody titers may be in the chronic phase of toxoplasmosis and thus may contain viable cysts of the parasite in their tissues, which can initiate infection when consumed (GARCIA et al., 1999; DAGUER et al., 2004).

The survey in the present study was conducted in animals sent for slaughter. Therefore, it was not possible to identify potential risk factors for infection in the farms where these animals were raised. However, the presence of felids in grazing areas is the most

Table 3. Distribution of seropositive and seronegative cattle slaughtered according to sex of the animals, by IFA test, in Belém/PA, Brazil.

Sex	N	Reagent (%)	Non Reagent (%)	*p* value	OR (CI 95%)
Male	139	66 (47.5)	73 (52.5)	0.065*	1.48 (0.99-2.19)
Female	361	137 (38.0)	224 (62.0)		
Total	500	203	297		

N: number of samples (absolute frequency); *OR: Odds ratio*; CI: Confidence Interval; X^2(*Yates*) 3.396. * *p* value = 0.054; χ^2=3.78 (Interaction of Monte Carlo).

plausible factor to explain the infection of these animals. In the Amazon region, most farms are located near forested areas, and thus wild cats eventually approach these areas for hunting. These cats can prey on herd animals or share the same water sources used by the cattle (MICHALSKI et al., 2006). Additionally, the presence of cats wandering in these raising locations is common. Thus, the possibility is real that herds raised in extensive systems, such as most herds in Pará state, are continuously exposed to oocysts excreted by these felids, thereby increasing the risk of infection during grazing (TENTER et al., 2000; SANTOS et al., 2013).

Although this possibility is plausible among cattle herds raised extensively in the Amazon region, other researchers support the hypothesis that *T. gondii* infection tends to be more likely among animals raised in intensive or semi-intensive systems due to the closer contact between cattle and cats in the raising locations, with a consequent increased chance for exposure to *T. gondii* oocysts (ALBUQUERQUE et al., 2011). A study conducted in Minas Gerais showed that the main risk factor for infection in a group of cattle raised in a semi-intensive system was access by cats to locations where the food destined for the cattle was stored (FAJARDO et al., 2013).

With the results obtained here, we can conclude that the high frequency of seropositivity to anti-*T. gondii* antibodies in beef cattle slaughtered in the metropolitan region of Belém indicates that there is effective transmission of infection in farms where these animals are raised and that their meat and/or its derivatives can represent an important source of infection for the human and animal populations in this area if consumed raw or undercooked.

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