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Sero-occurrence of anti-*Toxoplasma gondii* antibodies and vertical transmission in slaughtered beef cows (*Bos indicus*)

Soro ocorrência de anticorpos contra *Toxoplasma gondii* e transmissão vertical em vacas de corte (*Bos indicus*) abatidas

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Abstract

Toxoplasma gondii is a protozoan parasite recognized as an important public health problem. The objective of the present study was to evaluate the occurrence of anti-*T. gondii* antibodies in pregnant and non pregnant zebus' breed beef cows (*Bos indicus*), their fetuses, killed at an abattoir in northern of Paraná state. In the present study 169 cows were evaluated, 92 pregnant (in different stages of gestation) and 77 non pregnant. Sero-occurrence of anti-*T. gondii* antibodies was performed by Indirect fluorescent antibody test (IFAT) considering positive animals with titers ≥ 50 for cows and ≥ 25 for fetuses. Blood (with EDTA) from pregnant cows and blood and tissue samples (brain, lung, heart, and liver) from their fetuses were collected and used for mouse bioassay. Antibodies against *T. gondii* were observed in 26.0% of cows and 2.5% of fetuses. There was no statistical difference when prevalence of toxoplasmosis was compared between pregnant (23.9%), and non-pregnant (28.6%) animals, and age of gestation ($p > 0.59$). However, the occurrence of anti-*T.gondii* antibodies increased with age of animals ($p=0.004$). Mouse bioassay showed three fetuses positives (3.2%), however, none *T. gondii* strain was isolated. The present study showed that transplacental transmission of *T. gondii* naturally occurs in zebu beef cows from Brazil, however, in low rate (5.4%). The anti-*T. gondii* antibodies occurrence increase with the age of animals, which could be related to the fact that main transmission in cattle *T. gondii* is horizontal.
Key words: Toxoplasmosis, zebu, vertical transmission

Resumo

Toxoplasma gondii é um protozoário reconhecido como um dos mais importantes parasitas em saúde pública. O objetivo do presente estudo foi avaliar a ocorrência de anticorpos contra *T. gondii* em vacas de corte zebuínas (*Bos indicus*) gestantes, e seus fetos, bem como, em vacas não gestantes abatidas em um matadouro no norte do Paraná. No presente estudo foram avaliadas 169 vacas, 92 prenhas (em diferentes fases de gestação) e 77 não prenhas. A ocorrência de anticorpos contra *T. gondii* foi realizada por meio da reação de imunofluorescência indireta (IFI) considerando animais positivos aqueles com títulos ≥ 50 para as vacas e ≥ 25 para os fetos. Sangue (EDTA) de vacas prenhas e amostras de sangue e tecidos (cérebro, pulmão, coração, e fígado) de seus fetos foram coletadas e utilizadas para o bioensaio

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em camundongos. Anticorpos contra *T. gondii* foram observados em 26,0% das vacas e em 2,5% dos fetos Não houve diferença quando a soropositividade de anticorpos foi comparado entre vacas gestantes (23,9%), e não gestantes (28,6%), bem como, a idade da gestação (p > 0,59). No entanto, a ocorrência de anticorpos aumentou com a idade dos animais (p = 0,004). O bioensaio mostrou três fetos positivos (3,2%), porém, nenhuma cepa foi isolada. O presente estudo mostrou que a transmissão transplacentária de *T. gondii* ocorre naturalmente em vacas de corte zebuínas do Brasil, no entanto, esta ocorrência foi baixa (5,4%). A maior ocorrência de anticorpos associada com a idade dos animais poderia estar relacionada a transmissão horizontal do *T. gondii* nestes animais.
Palavras-chave: Toxoplasmose, zebu, transmissão vertical

Introduction

Toxoplasma gondii is an Apicomplexa protozoa parasite world wide distributed. Usually this parasite does not cause clinical signs, however, it is an important abortifacient in some species, including human beings (COOK et al., 2000). However, this is not an important bovine abortifacient (CANADA et al., 2002). Humans can become infected either by ingestion of oocysts or tissue cysts or by transplacental transmission of tachyzoites (GARCIA; NAVARRO, 1995). Tissue cysts are consumed in both raw and undercooked meat. Thus production animals, such as, pigs and sheep are important infection source for humans (DUBEY et al., 2005). However, the real role of beef in infection of human toxoplasmosis remains unknown.

In spite of many studies have been done about *T. gondii*, little is known about beef cattle. Thus, the present study aimed to evaluate the sero-occurrence of the *T. gondii* in pregnant, their fetuses, and non pregnant zebus breed beef cows slaughtered in an abattoir from northern Paraná state, Brazil. Additionally, we evaluated the transplacental transmission of *T. gondii* in this host.

Materials and Methods

Study area and sampling

The survey was carried out in an abattoir located at Londrina municipality, Southern Brazil. The 169 samples were randomly obtained from breeding beef cows (*Bos indicus)* weekly from January to July of 2007. The pregnant animals were selected in the slaughter line and for each pregnant animal a non pregnant cow was collected keeping the same characteristics of those (origin and age), when it was possible. The present work was approved by Animal Ethic Committee from Universidade Estadual de Londrina (N. 088/2007).

Indirect fluorescent antibody test (IFAT)

The serum samples were collected after bleeding and stored at –18ºC until be tested. IFAT to detect antibodies against *T.* gondii was performed according to Camargo (1973). Cow and fetus sera were diluted twofold starting at a dilution of 1:25, incubated on antigen slides for 30 min at 37 °C, and washed in PBS (pH 9.0). Cattle IgG antibodies were detected with flurescein isothiocyanate-conjugate rabbit anti-bovine IgG (Whole molecule – SIGMA®). Sera were considered positive if the entire surface of the tachyzoites was fluorescent in titers ≥ 50 for cows and ≥ 25 for fetuses.

Mouse bioassay for T. gondii

Blood samples with EDTA were collected from the pregnant cows after they were bled along the inspection line and from their fetuses by cardiac puncture. The white blood cells, from each pregnant cow, were separated by centrifugation (550xg – 10min), and it was diluted in 1 ml of antibiotic saline solution (1,000 U penicillin and 100 µl of streptomycin/ ml of saline solution) and inoculated subcutaneously into 3 mice (0.3 ml/ mouse)/each sample. Approximately 10 g of tissue fragments

(brain, lung, heart, and liver) were collected for fetuses ≥3 months of age, while similar tissue fragments (10 g) for fetuses ≤3 months were pooled for bioassay. For tissue digestions the protocol described by Dubey (1998) was used. Briefly, each sample was homogenized in a blender for 30 seconds in 250 ml of saline solution (0.14M NaCl). After homogenization 250 ml of pepsin solution (this proportion was added for equivalent to 50g of tissues) was added and incubated at 37 °C for 1 hr. The homogenate was filtered through 2 layers gauze and centrifuged at 1180 x *g* for 10 min. The supernatant was discarded and the sediment was resuspended in 20 ml PBS (pH 7.2) and 15 ml 1.2% sodium bicarbonate (pH 8.3) was added and centrifuged at 1180 x *g* for 10 min. The supernatant was discarded and the sediment was resuspended in 5 ml of antibiotic saline solution (1,000 U penicillin and 100 µl of streptomycin/ ml of saline solution) and inoculated intraperitoneally either into 2 mice (1ml/ mouse) for each organ or into 3 mice for pool of tissues. All mice were Swiss Webster and treated in the drink water with dexamethazone (10µg/ml) per 10 days after inoculation.

Examination of mice

Impression smears of lung from the mice that died were fixed in methanol, stained with Giemsa, and examined microscopically. Blood samples were drawn from the mice that survived 60 days after post-inoculation, and the brain of each mouse was examined microscopically for tissue cysts by squashing a portion of brain between a coverslip and a glass slide. Serum from each mouse was diluted at 1:16 and 1:64 and examined for anti-*T. gondii* and *N. caninum* antibodies, using IFAT. Titres ≥16 were considered as positive.

Statistical Analysis

Variables such as: pregnant and non pregnant, trimester of gestation, and age were analyzed by the Chi-square test (χ^2) corrected by Yates and the Fisher Exact Test, using the Epi Info program (CDC, 6.04b version). Association among these variables and occurrence of seropositives were estimated from values obtained by the odds ratio (OR), a confidence interval at 95%. We have considered as significant a *P*-value of ≤ 0.05.

Results

Seroprevalence of T. gondii

Cows showed 26.0% (44/169) of sero-occurrence for *T. gondii* (Table 1). Seropositive pregnant females (23.9%, 22/92) were not different from non pregnant females (28.6%, 22/77, OR=0.79, IC 0.37-1.67, p=0,61). There were no differences between the time of gestation and presence of antibodies (p=0.84). Titers obtained were 50 (n=17), 100 (n=19), 200 (n=2), and 400 (n=6). The prevalence for *T. gondii* increased with age (p=0.004).

The seroprevalence of fetuses was 2.5% (2/81) for *T. gondii*. The fetuses had titers of 25 and were ≥ 4 months of age. During this study, 92 fetuses were collected for evaluation; 24 were in the first trimester of gestation, 55 in the second, and 13 were in the third trimester of gestation. Additionally, 11 fetuses were less than 2 months of age and sera were not obtained from these animals. Thus, 81 sera from fetuses were evaluated, and two (2.4%) of these was serologically positive.

Mouse bioassay evaluation

Considering *T. gondii* bioassay, all blood samples from cows were negative, and three fetuses had tissue samples detected as positive (IFAT titer =16), however, neither brain cysts nor tachyzoites were isolated in mice.

Table 1. Outcome of the association between variables studied in zebu cows and presence of antibodies against *Toxoplasma gondii* (IFI-IgG).

Variables	Positives(%) (%)	Negatives (%)	Total (%)	*P*	OR	IC (95%)
Cows						
Pregnant	22 (23.9)	70 (76.1)	92 (54.4)	0.61[1]	0.79	0.37-1.67
Nonpregnant	22 (28.6)	55 (71.4)	77 (45.6)			
	44 (26.1)	125 (73.9)	169 (100)			
Trimester of Gestation						
First	4 (16.7)	20 (83.3)	24 (26.1)			
Second	15 (27.3)	40 (72.7)	55 (59.8)	0.59[2]		
Third	3 (23.1)	10 (76.9)	13 (14.1)			
	22 (23.9)	70 (76.1)	92 (100)			
Age (years)						
2.0 – 3.0	16 (16.8)	79 (83.2)	95 (56.2)			
>3.0– 4.5	08 (29.6)	19 (70.3)	27 (16.0)	0.004[1]		
>4.5	20 (42.5)	27 (57.5)	47 (27.8)			
	44 (26.1)	125 (73.9)	169 (100)			

[1]Chi-square by Yates corrected; [2]Chi-square by Fisher Exact; OR = odds ratio
Source: Elaboration of the authors.

Transplacental transmission

Considering that two fetuses were positive in serology and three others were positive in mouse bioassay, the rate of transplacental transmission observed in the present study was 5.4% (5/92).

Discussion

The host parasite relations between cattle and *Toxoplasma gondii*, even today, after more than 100 years of it discovery, remain enigmatic. The present study showed that transplacental transmission of *T. gondii* naturally occurs in pregnant zebu beef cows, however, in low rate (5.4%). This result was described previously (CANADA et al., 2002), and the authors did not suggest that this parasite is an important bovine abortifacient. Wiengcharoen et al. (2011) described that *T. gondii* could be a cause of abortion in cows. This was based on an experimental study where they infected heifers with high dose (3 x 10^8) of RH strain tachyzoites subcutaneously. However, it is not mimic the natural route of infection. Differently, using sporulated oocysts (high dose, 10^5) to infect pregnant cows in mid gestation, Costa et al. (2011) did not observed either abortion or *T. gondii* from fetuses.

In the present study, the sero occurrence became higher when the animals get older, this is in agreement with the fact that infection with *T. gondii* in bovine mainly occurs by oocysts, what mean that horizontal infection is more important. However, this result was not observed in *N. caninum*, where most important is vertical transmission (BAÑALES et al., 2006).

Sero epidemiological surveys for toxoplasmosis in cattle observed anti-*T. gondii* antibody occurrence ranged from 1.03 to 71% in Brazil (MARANA et al., 1994; GONDIM et al., 1999; GARCIA et al., 1999; OGAWA et al., 2005; SANTOS et al., 2008; MOURA et al., 2010; FRAZÃO-TEIXEIRA; OLIVEIRA, 2011), and 0 to 91% in some parts of the world (DUBEY; STREITEL, 1976; HASHEMI-FESHARKI, 1994; MORÉ et al., 2008; SCHOONMAN; WILSMORE; SWAI, 2010; OPSTEEGH et al., 2011). Caution should be taken

when the results of prevalence studies are being evaluated, since the differences in results might be directly related to the serological techniques employed, the cut-off values, sample size, and the type (breed and/or species) of animal that is being investigated. Additionally, ELISA tests can demonstrate cross-reactivity with *Sarcocystis cruzi* when *T. gondii* soluble antigens are used in indirect ELISA (UGGLA; HILALI; LÖVGREN, 1987). However, this does not occur with the dye test and IFAT (UGGLA; HILALI; LÖVGREN, 1987).

Interesting the fact that Garcia et al. (1999), Ogawa et al. (2005), that worked with animals from northern Paraná state, found 25.8% and 26%, and Santos et al. (2010) that studied cattle from Bahia found 26% of serum prevalence of antibodies, which were similar than observed for us in the present study.

Using the same protocol for isolation of *T. gondii* strains we previously (MACEDO et al., 2012.) isolated two strains from taurine pregnant cows, however, in the present study none strain were isolated from zebu pregnant cows. Oliveira, Da Costa and Sabatini (2001) infected *B. taurus*, *B. indicus*, and *Bubalus bubalis*, with *T. gondii* oocysts by oral route, and described that *B. taurus* were more affected than the others, this could partially explain the absence of isolating in the present study.

Considering titers of antibodies observed in the present study higher percentage of them (81%) remained lower than 200. Costa et al. (2011) studied pregnant cows and their fetuses from a slaughterhouse in Jaboticabal, Brazil. The authors showed a positivity of 18% for toxoplasmosis, however, just low titers of 64 were observed. Nevertheless, nine cows experimentally infected with oocysts of *T. gondii* did not have titers higher than 64.

Some works were not able to detect *T. gondii* from naturally infected bovine tissues (JAMRA; DEANE; GUIMARÃES, 1969; DUBEY; STREITEL, 1976; PASSOS; LIMA; FIGUEIREDO, 1984), what may be related to small number of animals investigated 98, 352, and 99, respectively. Additionally, Dubey et al. (2005) evaluated the prevalence of *T. gondii* by cat and mouse bioassay in 2,094 beef from meat stores in US, and none isolation was made in these samples.

Herein, we demonstrated that transplacental transmission of *T. gondii* naturally occurs in pregnant zebu beef cows from Brazil, however, in low rate (5.4%, 5/92). The overall sero occurrence of *T. gondii* in cows from the present study was 26.0%.

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