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Serum occurrence of anti-*Toxoplasma gondii* antibodies in dairy cows slaughtered in an abattoir for human consume

Ocorrência sorológica de anticorpos contra *Toxoplasma gondii* em vacas leiteiras abatidas para consumo humano em abatedouro

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ABSTRACT

Toxoplasma gondii *is a worldwide parasite recognized as one of the main zoonosis in human beings. The present study aimed to evaluate serology of* ***T. gondii*** *from dairy cows slaughtered in an abattoir for human consume. Serum samples from 120 dairy cows (60 pregnant and 60 non-pregnant) were collected, and indirect fluorescent antibody test (IFAT) was performed to detect anti-****T. gondii*** *antibodies by considering positive animals with titers ≥ 50. Serologic results from cows showed 29.1% (35/120), which 29 (48.3%) e 6 (10,0%) were from pregnant and non-pregnant cows, respectively. This revealed a risk 8.4 times-higher of positively in pregnant than non-pregnant cows (OR=8.4, 2.91<OR<25.6, P<0.0001). There was a statistical difference in the anti-****T. gondii*** *antibody frequency between Jersey and Holstein breed cows, 38.3% (23/60) and 20% (12/60) of prevalence, respectively (OR=2.49, 1.02<OR<6.13, P=0.04). Titers for cows were 50 (n=23), 100 (n=10), 200 (n=1) and 400 (n=1). There was no difference among age of gestation and anti-****T. gondii*** *antibody occurrence (P=0.74) in pregnant cows. The potential role of beef in epidemiology of* ***T. gondii*** *for human beings is yet enigmatic, and more studies are necessary to elucidate the real risk of this food for consumers.*

Key words*:* ***Toxoplasma gondii****, dairy cattle, antibody occurrence.*

RESUMO

Toxoplasma gondii *é um parasito reconhecido mundialmente como o agente de uma das principais zoonoses em seres humanos. O presente estudo teve como objetivo avaliar a ocorrência de anticorpos contra* ***T. gondii*** *em vacas leiteiras abatidas em um matadouro para consumo humano. Amostras de soro de 120 vacas (60 prenhas e 60 não prenhas) foram coletadas e examinados pelo teste de imunofluorescência indireta (IFI). Os animais foram considerados positivos com títulos ≥50. Anticorpos contra* ***T. gondii*** *foram observados em 29.1% (35/120) dos animais, dos quais 29 (48,3%) e 6 (10,0%) eram, respectivamente, gestantes e não gestantes. As vacas gestantes apresentaram um risco 8.4 maior de soropositividade que os animais vazios (OR=8,4; 2,91<OR<25,6; P<0,0001). A comparação entre as raças também mostrou diferença significativa na ocorrência de anticorpos, sendo 38.3% (23/60) nas vacas Jersey e 20% (12/60) nas vacas Holandesa (OR=2.49, 1.02<OR<6.13, P=0.04). O principal título observado foi de 50 (n=23), seguido por 100 (n=10), 200 (n=1) e 400 (n=1). Nas 60 vacas gestantes não houve diferença na ocorrência de anticorpos com relação à idade gestacional (P=0,74). O potencial da carne bovina na epidemiologia do* ***T. gondii*** *para os seres humanos ainda é enigmático, e mais estudos são necessários para elucidar o real risco deste alimento para os consumidores.*

Palavras-chave: ***Toxoplasma gondii****, bovinos leiteiros, ocorrência de anticorpos.*

INTRODUCTION

Toxoplasma gondii is an intracellular parasite that infects a variety of cell types from a wide range of mammals and birds throughout the world, including humans. Human infection occurs by two main

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routes, ingestion of oocysts and undercooked or raw meat containing tissue cysts of the parasite (ANDREWS et al., 1997). Usually, ***T. gondii*** does not produce clinic signals, but the primary infection during pregnancy in women and a few animal species may result in abortion, fetal abnormalities or perinatal death (COOK et al., 2000). Despite of isolation in cattle fetuses, ***T. gondii*** is not an important cause of abortion in cattle (DUBEY, 1983; DUBEY, 1986; CANADA et al., 2002; COSTA et al., 2011).

Once that the proportion of human beings infected with oocysts is not know, the consumer animals assume an important role in the transmission of this parasite. In the United States (US) pork was considered as one of the most important sources of ***T. gondii*** (DUBEY et al., 1991; MEAD et al., 1999). Additionally, a prevalence study of pork, beef, and chicken from US (DUBEY et al., 2005) were performed and ***T. gondii*** tissue cysts were found just in pork, however, the prevalence in pork was low and calculated to be 0.38%.

The role of beef in the prevalence of ***T. gondii*** for humans needs to be studied. ***Toxoplasma gondii*** cysts can survive in tissues from experimentally infected cattle for more than three years after infection (DUBEY & THULLIEZ, 1993). However, the parasite has rarely been isolated from naturally infected cattle tissues (GILOT-FROMONT et al., 2009). ESTEBAN-REDONDO & INNES (1997) described that beef and milk cannot be ruled out as potential reservoirs of infection in the epidemiology of the disease. The present study aimed to evaluate anti-***T. gondii*** antibody occurrence from dairy cows slaughtered in an abattoir for human consume.

MATERIAL AND METHODS

Study area and sampling

The 120 samples were randomly obtained from pregnant and non-pregnant dairy cows (***Bos taurus***) at an abattoir located at Presidente Getúlio municipality, Santa Catarina state, southern Brazil. Sixty Jersey (41 pregnant, and 19 non-pregnant) and 60 Holstein (19 pregnant, and 41 non-pregnant) cows were obtained. The blood samples were collected weekly from April/2008 to June/2009, after bleeding and after obtaining the sera these were stored at -18ºC until be tested.

Indirect fluorescent antibody test (IFAT)

IFAT to detect antibodies against ***T. gondii*** was performed according to CAMARGO (1973). Cow sera were diluted twofold starting at a dilution of 1:25. 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 were fluorescent in titers ≥50.

Statistical analysis

Variables were analyzed by the Chi-square test (χ^2) corrected by Yates and using the Epi Info program (CDC, 6.04b version). We have considered as significant a *P*-value of ≤ 0.05.

RESULTS AND DISCUSSION

Serologic results from these animals are showed in table 1. Cows showed 29.1% (35/120) of prevalence for ***T. gondii***. Seroepidemiological surveys for toxoplasmosis in cattle showed anti-***T. gondii*** antibody occurrence ranged from 1.03 to 71% in Brazil (MARANA et al.,1995; GONDIM et al., 1999; GARCIA et al., 1999; 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, 1996; MORE et al., 2008; OPSTEEGH et al., 2011). Therefore, 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.

When gestational condition was compared we observed 8.4 times-higher anti-***T. gondii*** antibodies risk in pregnant (48.3%) than non-pregnant (10.0%) cows (OR=8.4, 2.91<OR<25.6, P<0.0001). The cell-mediated immune response changes in pregnant cows what may influence disease pathogenesis. There is a down regulation of Th-1-type of immune responses of dam during pregnancy. Considering that these pro-inflammatory responses are important in protection against intracellular parasites, pregnant cows could be more sensitive than non pregnant cows. WIENGCHAROEN et al. (2011) suggested 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 ($3x10^8$) of RH strain tachyzoites subcutaneously. However, it is not mimic the natural route of infection in herbivorous such as ruminants. 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. Differently, from the present study, in another study (GARCIA et al., 2012) we did not observe statistical difference when prevalence of toxoplasmosis was compared between pregnant (23.9%) and non-

Table 1 Outcome of association between variables and presence of antibodies for ***Toxoplasma gondii*** (Indirect Fluorescent Antibody Test-IgG) in slaughtered pregnant dairy cows

Variables	Positives (%)	Negatives (%)	Total (%)	OR(CI-95%)	P
Cows					
Pregnant	29 (48.3)	31 (51.6)	60 (50.0)	8.4(2.91<OR<25.6)	<0.001
Non-pregnant	6 (10.0)	54 (90.0)	60 (50.0)		
Total	35(29.1)	85(70.9)	120(100)		
Breed					
Jersey	23(38.3)	37(61.7)	60(50.0)	2.49(1.02<OR<6.13)	<0.05
Holstein	12(20.0)	48(80.0)	60(50.0)		
Total	35(29.1)	85(70.9)	120(100)		
Age of gestation (trimester)					
First	6 (42.8)	8(57.2)	14(26.1)	NC	0.74
Second	13(46.4)	15(53.6)	28(59.8)		
Third	10(55.5)	8(44.5)	18(14.1)		
Total	29 (48.3)	31(51.7)	60(100)		

OR= Odds ratio, CI=Confidence Interval, NC=not calculated, P values

pregnant (28.6%) cows, however, this study used ***Bos indicus*** beef cows, and herein we used ***B. taurus*** dairy cows. OLIVEIRA et al. (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. Herein, there was statistical difference when the breed of cattle was considered (Table 1, OR=2.49, 1.02<OR<6.13, P<0.05). Jersey and Holstein cows showed 38.3% (23/60) and 20% (12/60) of prevalence.

The main titer observed in the present study was 50 (n=23), following by 100 (n=10), 200 (n=1) and 400 (n=1). The correlation between antibodies anti-***T. gondii*** and the presence of tissue cysts is know in sheep and pigs (OPSTEEGH et al., 2010), however, this is not know in cattle (OPSTEEGH et al., 2011). These authors described that the risk of human infection is higher from seronegative than seropositive cattle, by the fact that they detected DNA from ***T. gondii*** from negative animals and did not detect from positive animals. Moreover, infectivity and pathogenicity of ***T. gondii*** in cattle vary with the strain, and pathogenicity is generally only slightly to moderate (FAYER & FRENKEL, 1979). After infection, cattle may eradicate the parasite from their tissues, and this is often followed by disappearance of antibodies in some cattle (DUBEY & THULLIEZ, 1993). The factors that confer this natural resistance are not yet known (ESTEBAN-REDONDO & INNES, 1997). 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. Even when they conducted a study with nine cows infected experimentally with oocysts of ***T. gondii*** the predominant titer was 64.

When the period of gestation was evaluated, there were no differences in the prevalence of ***T. gondii*** relative to the first (42.8%), second (46.4%), and third (55.5%) trimesters in the 60 pregnant cows studied. Additionally, considering that almost all of the cows were more than four years old (116/120), the prevalence and proportionality with the age comparison were not calculated. Usually, serum prevalence 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 than vertical transmission (BAÑALES et al., 2006).

In conclusion, we observed in the present work an anti-***T. gondii*** antibody occurrence of 29.1% in dairy cows slaughtered to human consume. When gestational condition and breed of animals were considered the risk of seropositively was higher in pregnant (48.3% against 10.0% in non-pregnant), and Jersey (38.3% against 20.0% in Holstein). Further investigations need to be performed to elucidate whether either breed or other conditions could be correlated with seropositively, and the potential role of beef in epidemiology of ***T. gondii*** for human beings. Including the risk for butchers, and meat workers.

ESTEBAN-REDONDO & INNES (1997) described that beef and milk cannot be ruled out as potential reservoirs of infection in the epidemiology of the disease.

BIOETHICS AND BIOSSECURITY COMMITTE APPROVAL

The present work was approved by Animal Ethic Committee from Universidade Estadual de Londrina (n.018/2009).

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