# Risk factors associated with *Toxoplasma gondii* infection in dairy cattle, State of Rio de Janeiro[1]

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**ABSTRACT.-** Albuquerque G.R., Munhoz A.D., Teixeira M., Flausino W., Medeiros S.M. & Lopes C.W.G. 2011. **Risk factors associated with *Toxoplasma gondii* infection in dairy cattle, State of Rio de Janeiro.** *Pesquisa Veterinária Brasileira 31(4):287-290*. Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23890-000, Brazil. E-mail: lopescwg@ufrrj.br

Toxoplasmosis is one of the most common parasitic zoonoses throughout the world. Infection in man and animals varies in different geographical areas influenced by many environmental conditions. Seroprevalence of *Toxoplasma gondii* infection in cattle in Brazil ranges from 1.03 to 71%. A cross-sectional survey was carried out in 58 out of 453 farms in the South Fluminense Paraiba Valley, State of Rio de Janeiro, Brazil. Over 3-year-old cattle (n=589) from dairy herds were selected for blood collection and detection of anti-*T. gondii* antibodies by indirect immunofluorescence reaction (IFA) with initial titration of 1:16; titers $\geq$ 64 were considered positive. Univariate analysis of risk factors showed that cats in contact with cattle, cats in contact with drinking water, and number of cats were associated with *T. gondii* seroprevalence. Logistic regression revealed a two-fold increased risk for infection of cattle (*p*=0.0138) through larger number of cats (>3) compared with low numbers of cats (1-2) on the farm. In contrast, the presence of chickens was considered a protective factor (*p*=0.025).

INDEX TERMS. Dairy cattle, toxoplasmosis, antibodies, risk factors.

**RESUMO.-** [**Fatores de risco associados à infecção por *Toxoplasma gondii* em bovinos leiteiros no estado do Rio de Janeiro.**] Toxoplasmose é uma das mais comuns zoonoses parasitárias do mundo. Infecções em seres humanos e em animais variam nas diferentes áreas geográficas influenciadas pelas condições ambientais. A soroprevalência da infecção por *Toxoplasma gondii* em bovinos no Brasil varia de 1,03 a 71,0%. O estudo transversal foi realizado em 58 de um total de 453 propriedades na região Sul Fluminense do estado do Rio de Janeiro. Vacas leiteiras acima de 3 anos de idade (n=589) foram selecionadas para coleta de sangue e a detecção de anticorpos anti-*T. gondii* foi feita pelo teste de imunofluorescência indireta (IFI) com titulação inicial de 1:16 e títulos $\geq$ 64 foram considerados positivos. Após análise univariada dos fatores de risco, gatos em contato com bovinos, em contato com a água de beber dos animais e o número de gatos foram associados com a soroprevalência de *T. gondii*. A regressão logística demonstrou que o número maior de gatos (>3) teve um risco duas vezes maior (p=0,0138) que propriedades que tinham um número menor de gatos (1-2). Em contraste, a presença de galinhas foi considerada um fator de proteção (p=0,025).

TERMOS DE INDEXAÇÃO. Bovinos leiteiros, toxoplasmose, anticorpos, fatores de risco.

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## INTRODUCTION

Toxoplasmosis is one of the most common parasitic zoonoses throughout the world. Infection in man and animals

varies in different geographical areas influenced by many environment conditions. The infection is more prevalent in warm climates and in low lying areas than in cold climates and mountain regions and in humid areas than dry areas. This is probably related to conditions favoring sporulation and survivor of oocysts in the environment (Dubey & Beattie 1988). Cultural habits and hygiene may play a role because of food borne transmission. The tissue form of the parasite, a microscopic cyst consisting of bradyzoites, can be transmitted to humans by eating undercooked, contaminated meat (Tenter et al. 2000).

The importance of transmission from cattle to humans is still a controversy. Some studies show that consumption of undercooked meat is considered of great risk to humans (Baril et al., 1999; Cook et al. 2000). In an experimental infection in calves and pregnant cows, Dubey (1983) observed viable cysts of *Toxoplasma gondii* in animal tissues until the age of slaughter. Lord et al. (1975) and Kean et al. (1969) related outbreaks of human toxoplasmosis due to meat consumption, although Dubey & Jones (2008) found this information not valid. Also many attempts of parasite isolation from cattle tissues failed (Dubey & Streitel 1976, Dubey 1992, Dubey et al. 2005) even in experimental design (Dubey 1986). More recently Dubey & Jones (2008) and Kijlstra & Jongert (2008) considered transmission from cattle not important for human infection.

Cattle are commonly resistant to the infection, but signs of fever, anorexia, diarrhea, nasal discharge, and cough could be present (Sanger et al. 1953, Dubey 1983, Oliveira et al. 2001). *Toxoplasma gondii* was first isolated from bovine tissue by Sanger et al. (1953) and since then has been isolated from many organs (Dubey 1983, Dubey & Thulliez 1993). In Brazil, Jamra et al. (1969) and Spósito Filha et al. (1988) succeeded in the isolation of *T. gondii* from human food of animal origin and from retina and diaphragm of slaughtered cattle respectively. More recently, Scarpelli et al. (2009) performing bioassays revealed the presence of the parasite in many tissue and semen samples of animals infected with oocysts and tachyzoites on several experimental days between 7 and 84 suggesting the possibility of sexual transmission of *T. gondii.* In cattle the seroprevalence of antibodies anti-*T. gondii* ranges from 1.03 to 71% (Gondim et al. 1999, Albuquerque et al. 2005, Santos et al. 2009, Spagnol et al. 2009). Risk factors associated with bovine infection are scarce in the literature, but herd size and farming system has been identified by Klun et al. (2006) and Schoonman et al. (2009).

The aim of this paper was to study possible risk factors for *T. gondii* infection in cattle from Rio de Janeiro, Brazil.

## MATERIALS AND METHODS

A cross-sectional survey was carried out in the municipalities of Resende and Rio Claro in the South Fluminense region of the State of Rio de Janeiro, Brazil. Livestock information, number of farms and animals were obtained from records of the regional Company for Technical Rural Assistance (Emater) from both municipalities. We randomly selected 58 farms among

the 453 in the region. Sample sizes were estimated with an expected prevalence of 21.30% and a 5% sample error. Only mature dairy cows over three years of age were used for blood collection. In Resende, 314 blood samples out of 1.556 cows and in Rio Claro 275 blood samples out of 1079 cows were collected, a total of 589 samples.

**Table 1. Anti-*Toxoplasma gondii* antibodies in cattle in Brazil, environmental and farm management characteristics**

| Factor | Animals | | $\chi^2$ | P-value | OR | 95% CI |
|---|---|---|---|---|---|---|
| | Positive | Negative | | | | |
| **Presence of cats on the farm** | | | | | | |
| Yes | 60 | 306 | 1.70 | 0.192 | 1.42 | 0.85-2.39 |
| No | 27 | 196 | | | | |
| **Cats in contact with cattle** | | | | | | |
| Yes | 50 | 200 | 6.67 | 0.009 | 2.65 | 1.2-6.0 |
| No | 10 | 106 | | | | |
| **Cats in contact with drinking water** | | | | | | |
| Yes | 45 | 178 | 5.28 | 0.021 | 2.15 | 1.1-4.3 |
| No | 15 | 128 | | | | |
| **Number of cats** | | | | | | |
| 1-2 | 29 | 216 | 5.78 | 0.016 | 2.41 | 1.16-4.99 |
| >3 | 31 | 90 | | | | |
| **Farm size** | | | | | | |
| >100 hectares | 36 | 179 | 0.82 | 0.36 | 1.27 | 0.78-2.08 |
| <100 hectares | 51 | 323 | | | | |
| **Type of management** | | | | | | |
| Company | 33 | 155 | 1.39 | 0.23 | 1.37 | 0.83-2.25 |
| Familiar | 54 | 347 | | | | |
| **System** | | | | | | |
| Extensive | 30 | 211 | 1.45 | 0.22 | 0.73 | 0.44-1.20 |
| Semi-Intensive | 57 | 291 | | | | |
| **Cattle age** | | | | | | |
| 3<5 | 29 | 123 | 2.58 | 0.11 | 1.54 | 0.92-2.58 |
| >5 | 58 | 379 | | | | |
| **Presence of rats** | | | | | | |
| Yes | 70 | 417 | 0.19 | 0.65 | 0.84 | 0.46-1.56 |
| No | 17 | 85 | | | | |
| **Herd size** | | | | | | |
| <50 cow | 50 | 272 | 0.20 | 0.65 | 1.14 | 0.70-1.86 |
| >50 cow | 37 | 230 | | | | |
| **Abortion** | | | | | | |
| Yes | 11 | 76 | 0.67 | 0.41 | 1.43 | 0.70-2.80 |
| No | 46 | 456 | | | | |
| **Water source** | | | | | | |
| Treated | 8 | 79 | 0.81 | 0.36 | 0.66 | 0.28-1.49 |
| Others[a] | 67 | 435 | | | | |
| **Contact with other animals[b]** | | | | | | |
| Yes | 22 | 65 | 0.66 | 0.41 | 0.77 | 0.45-1.29 |
| No | 152 | 350 | | | | |
| **Breeding method used** | | | | | | |
| Artificial insemination | 11 | 76 | 0.00 | 0.96 | 1.05 | 0.50-2.16 |
| Natural | 61 | 441 | | | | |
| **Chickens** | | | | | | |
| Yes | 16 | 145 | 3.6 | 0.058 | 0.55 | 0.30-1.02 |
| No | 71 | 357 | | | | |

Results of univariate analysis presented as odds ratio (OR) and 95% confidence intervals (CI).
[a] River, dam.
[b] Equines, dogs, sheep, goat, poultry.

Blood samples were centrifuged and stored at -20ºC. Detection of anti-*T. gondii* antibodies were performed using indirect immunofluorescence reaction (IFA) following Camargo (1964). Initial dilution was 1:16 until negative reaction and titers ≥1:64 were considered positive.

Information about each farm and the animals kept was collected by means of a structured questionnaire, which was administered on all the selected farms through a single visit. The questionnaire was designed to comprise mostly close-ended (categorical) questions to ease data processing, minimize variation, and improve precision of responses (Thrusfield 2004). Important farm and animal level data included type of management, system, breeding method used, water source, contact with other animals, farm size, herd size, presence of cats on the farm, cats in contact with cattle, cats in contact with drinking water, number of cats, presence of rats, abortion.

Differences in the seroprevalence between animals were analyzed by the Chi-square test. The same test was used to analyze the influence of all examined factors as independent categorical variables of *T. gondii* seroprevalence. Variables significant at $p \leq 0.1$ with 95% confidence level were tested for colinearity and were selected for inclusion in the multivariate logistic regression model. Overall analyses and fit of the logistic regression models was assessed with the software Epi Info 2000 (Dean & Arnet 2002).

## RESULTS

Environmental and farm management characteristics of the examined cattle population are presented in Table 1. The overall seroprevalence in both municipalities was 14.77% (87 from 589), with titers ranging from 1:64 to 1:1024 (Albuquerque et al. 2005).

Univariate analysis of risk factors showed that cats in contact with cattle, cats in contact with drinking water, and number of cats were associated with *Toxoplasma gondii* seroprevalence (Table 1). These factors were simultaneously analyzed in a logistic regression model to determine their relative contribution to *T. gondii* seropositivity (Table 2). A two-fold increased risk of infection was shown for dairy cattle ($p$=0.0138) through a large number of cats (>3) compared with a low number (1-2). In contrast, the presence of chickens was considered a protective factor ($p$=0.025) compared with the absence of chickens on the farms from Resende.

**Table 2. Risk factors for *Toxoplasma gondii* infection in dairy cattle in Brazil. Final logistic regression model. Results presented as odds ratio (OR) and 95% confidence interval (CI)**

| Factor | OR | 95% CI | *P*-value |
|---|---|---|---|
| Number of cats | | 1.16 - 3.67 | 0.0138 |
| 1-2 | 1.00 | | |
| >3 | 2.06 | | |
| Cats in contact with cattle | | | |
| No | 1.00 | 1.05 - 4.61 | 0.0356 |
| Yes | 2.20 | | |

## DISCUSSION

According to the data analyzed there are significant differences (p<0.05) in the association between *Toxoplasma gondii* specific antibodies and some factors studied. Cats were strongly associated with increased risk of infection. However, the more presence of cats on the farms does not lead to a real risk if they are not in direct contact with cattle, or drinking water, as much as their number on the farms increased the risk of infection. Likewise, Schoonman et al. (2009) did not consider presence of cats without direct contact with cattle as risk factor. This is understandable since the cat is the only definitive host of *T. gondii,* and hence, oocysts are numerous in the environment. In Brazil, cats are commonly used to avoid rodent infestation on farms, but increased risk is seen with their waste contaminated feed, grain, silage and water sources. Also, cats use to bury their feces away, and *T. gondii* oocysts can survive two years in the soil and may remain confined to the area for a long time (Dubey & Beattie 1988). In the first infection, cats can shed a high number of oocysts, but only for a short time, then the shedding stops for a long period. Nevertheless, a higher number of cats on the farm raise the chance for a contaminated environment and these results in a high level of positive cattle.

There was no significant association between *T. gondii* seroprevalence and cattle age, but similar to Arias et al. (1994), there were more positive cases in 3 to 5-year-old cows. Dubey (1986) reported that calves had higher titers than adults and inoculated animals had high titers very soon after the infection. Therefore, within a short time, anti-*T. gondii* antibodies decreased to negative reaction according to the sensibility of the test. In cattle, results were different from other animal species, including humans, where there is a positive relationship between age and a seropositive population (Weigel et al. 1995, Jones et al. 2001, Ali et al. 2003).

In this study, the herd size was not related to seropositive cattle, similar to the findings of Schoonman et al (2009), but different of Klun et al. (2006). An explanation for this is the point that farms with a higher number of animals used a semi-intensive system for cattle, what means more risk for contact with oocysts. Thus, despite not statistically significant, as intensive was the system of production, higher positive rates were encountered. This may be explained by the more direct contact with cats and oocysts confined cows have. Also, in semi-intensive systems the feed for cattle are much more exposed to environmental contamination (Penkert 1973).

The presence of chickens on the farms seems to be a factor of protection for the cattle. In Resende, farms without chickens had 2.6 times more positive animals (p=0.02). In Rio Claro, although not statistically significant, farms with chickens had 12.9% seropositive cattle, while farms without chickens had 14.6%. Recent investigations have been concerned with behavioral aspects that influence toxoplasmosis. Since it can affect the spread of *T. gondii* oocysts, food habits of others animals have epidemiological and ecological implicitness. Thus, the protection provided by chicken presence is probably due to forage as chickweed and other plants, seeds, and insects that partially clean

the environment from contaminating oocysts. Pastured chickens are often found positive for *T. gondii* infection (Dubey et al. 2008).

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