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Seroprevalence and Risk Factors for *Toxoplasma gondii* Infection in Goats in Southern Brazil

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ABSTRACT

***Background*:** *Toxoplasma gondii* is a coccidium, obligate intracellular protozoan, with complex life cycle, affecting virtually all-animal species homoeothermics. Goats are considered susceptible to infection by *T. gondii*, alterations being reported as pathological fetal death (with subsequent reabsorption), abortion, mummification, and/or the birth of weak goats. Because of these consequences to the animal, the disease is investigated throughout the Brazilian territory. Therefore, the aim of this study was to estimate the seroprevalence of *Toxoplasma gondii* antibodies and identify risk factors in goats.
***Materials, Methods & Results*:** It were collected 654 blood samples from goats distributed on west and mountainous regions of Santa Catarina, Brazil. The number municipalities with the highest numbers of goats were used, with an expected prevalence of 25%, error of 3.2% and confidence level of 95%. All collected samples were tested by indirect immunofluorescence assay (IFA), and 216 (33.02%; 95% CI 29.43-36.77) had antibodies against *T. gondii* (IFA ≥ 1: 64). Titration 1:64 was observed more frequently (60.2%; 130/216) among seropositive animals. The region with the highest prevalence was the west, i.e., more prone to infections present, with 1.16 more chances than the mountainous region. Animals Boer were more likely to *T. gondii* infection, being race is a risk factor for disease. When the presence of cats on the property, and these animals had chances of direct contact with cats, the chance of infection increased significantly, being 1.04 times higher went compared with lack of this feature. Regarding age, the animals aged two to five years had higher seropositivity (77.8%). Statistical analysis of effect-cause studies reported a relationship between soropositive animals and neurological problems ($P \leq 0.05$), but no relation was found for *T. gondii* infection and reproductive problems ($P > 0.05$).
***Discussion*:** In the state of Santa Catarina, approximately 33% of the goats were seropositive for *T. gondii*, according to results of this study. Several other studies has been performed on *T. gondii* antibodies detection in Brazil, such as Paraná which was 44.68%, in Rio Grande do Norte was 17.1%, in Rio de Janeiro was 29.12%, in Maranhão was 36.95%, in Bahia was 17.4%, in Minas Gerais was 21.4%, in Alagoas was 39% and in Rio Grande do Sul was 30%. The type of food and water source given to the animals was not considered a risk factor for *T. gondii* infection, although some authors mention that cat feces contaminated with oocysts of the parasite can be more easily ingested in the diet with concentrated (cats have access) and natural water sources. This study showed that 53% of the animals studied had contact with cats, and this variable was considered a risk factor for disease. Statistically, unverified relationship between reproductive problems and goats seropositive for *T. gondii*, although of approximately 24% of the animals studied showed some reproductive problems (abortion, mummified or heat repetition). In the effect-cause analysis, neurological issues were related to infection by *T. gondii* in goats, the that can easily be explained because the parasitic cysts of *T. gondii* may persist in the central nervous system for a long period. The results suggest that infection by *T. gondii* occurs in goats in two regions of Santa Catarina, in addition, and the risk factors related to disease was between both studied regions, race and presence and contact with cats, just as there is a relation between disease and neurological disorders in goats.

Keywords: goat, *Toxoplasma gondii*, neurological disorders.

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INTRODUCTION

Toxoplasma gondii is a coccidium, obligate intracellular protozoan, with complex life cycle, affecting virtually all-animal species homoeothermics [10]. The life cycle of *T. gondii* is presented in three ways, that is, tachyzoites, bradyzoites and oocysts [14] as well as the felines are the definitive hosts [10,14]. According to the literature, the domestic animals (intermediate hosts) most commonly involved with toxoplasmosis are pigs, sheep and goats, followed by less frequently dogs, rabbits, horses, birds and, finally, to a lesser extent are cattle and buffalo [33].

The first description of goat's toxoplasmosis as important reproductive problems occurred in Australia [27]. According to literature, goats are considered susceptible to infection by *T. gondii* [9], alterations being reported as pathological fetal death (with subsequent reabsorption), abortion, mummification, and/or the birth of weak goats [12]. Furthermore, death may occur rarely in adult goats, disseminated acute toxoplasmosis has being described with most recurrent clinical signs of encephalitis, fever, diarrhea, dyspnea, lethargy, pulmonary congestion and edema, renal infarction, necrotic foci in the liver and spleen, enteritis, cystitis and abomasite [8,25].

Although some soroepidemiological studies of goat toxoplasmosis have been done in many states of Brazil, in Santa Catarina state this information still lacking. In consequence, the objective of this study was to evaluate the seroprevalence of *T. gondii* in goats, and identify risk factors related to the occurrence of the disease in Santa Catarina regions.

MATERIALS AND METHODS

Blood samples were obtained from 654 goats in 57 municipalities in the western and mountain regions of the state of Santa Catarina (SC), Brazil (Figure 1), between August 2012 and March 2013. The animals were randomly chosen in order to represent different stages of development and weight, as well as different productive purposes (meat and milk). The sample distribution was in accordance with the distribution of goat's flocks across SC. This study the number municipalities with the highest numbers of goats were used, with an expected prevalence of 25%, error of 3.2% and confidence level of 95%.

The animals were manually restrained and blood samples were collected by venipuncture (Vacutainer®) from the external jugular vein. Blood samples were placed in tubes without anticoagulant and kept under refrigeration (10°C). They were then centrifuged and the serum was frozen (-20°C) until analysis by Immunofluorescence Assay (IFA).

The identification of immunoglobulin G (IgG) anti-*T. gondii* in blood serum was performed by IFA using microscope slides containing tachyzoites of *T. gondii* RH strain. Sera samples were previously diluted in PBS (pH 7.2) up to 1:64 were used. Samples were incubated for 40 min at 37°C in a humidified chamber. A secondary antibody, an anti-goat-IgG conjugated to fluorescein[1], was added and incubated for 40 min at 37°C in a humidified chambre [4]. Sera samples from positive and negative sheep were used as controls. All sera samples positives at dilutions of 1:64 were subjected to maximum titration [29]. IFA reactions, with peripheral or diffuse fluorescence of tachyzoites were considered positive, as well as fluorescence with apical or polar reactions were considered negative.

To study risk factors and cause-effect relation of toxoplasmosis in goats an interview with farmer was performed. The following questions were asked regarding each animal: region (west or mountain), breed (non-breed, Boer or Anglo), sex (male or female), age (between 6 months and 1 year, or more than 1 year), diet (with concentrate or without concentrate), water source (natural, river, well water or piped water), reproductive issues (yes or no), neurological issues (yes or no), type of production (milk, meat or mixed) and contact with cats (yes or no).

All variables collected by the questionnaire were tested for its frequency distribution; continuous variables were tested by histogram, mean, standard deviation and range. For categorical variables were performed frequency distribution and bar charts. The whole statistical process was carried out with R-language, v.3.1.1 (R Development Core Team 2009). Variables with large amounts of missing data (> 10%) and limited variability (< 20%) were not included in the univariable analysis. A univariate analysis was performed with the Pearson chi-squared test ($\chi 2$), while a chi-square test for linear trends or Fisher's exact test was initially used to examine the association between positivity in BTM samples and the remaining eight independent variables. A crude prevalence ratio (PR) was applied to assess the impact of individual factors on outcomes [24]. A multivariable Poisson regression with robust variance was used to estimate the adjusted

prevalence ratio (PR) and 95% confidence interval (95% CI) of the estimates [7]. Poisson approach was chosen as recommended by Barros and Hirakata [3]. Univariate analysis was first conducted using all the eight pre-selected variables. Subsequently, all variables with $P \leq 0.20$ were selected for inclusion in the multivariable analysis. Variance inflation factor (VIF) were estimated to verify relationship between all selected independent variables to check for potential collinearity, in which coefficient > 2.50 was considered as high, if any high VIF is found variable with lowers p-value was considered for the multivariable model.

A second univariate model was built in order to test for a effect-cause presence, it was used here as outcome variable the presence either reproductive issue or neural issue and as predicted variable the diagnostic test result for *T. gondii* infection, for this model a $P \leq 0.05$ was considered significant associated.

Multivariate models were built in a manual forward method; each remaining variable was added to the best previous model, selected by the Akaike Information Criterion (AIC). A backwards elimination step was used, resulting in a final model in which only variables with $P \leq 0.05$ were retained. Confounding effects were investigated by checking changes in the point estimates of the variables that remained in the model. Changes in parameter estimates > 25% were considered as a confounder and kept in the model until the final model, and finally two-way interaction terms between variables with biological plausibility were investigated. We used deviance perform as a goodness of fit test for overall model.

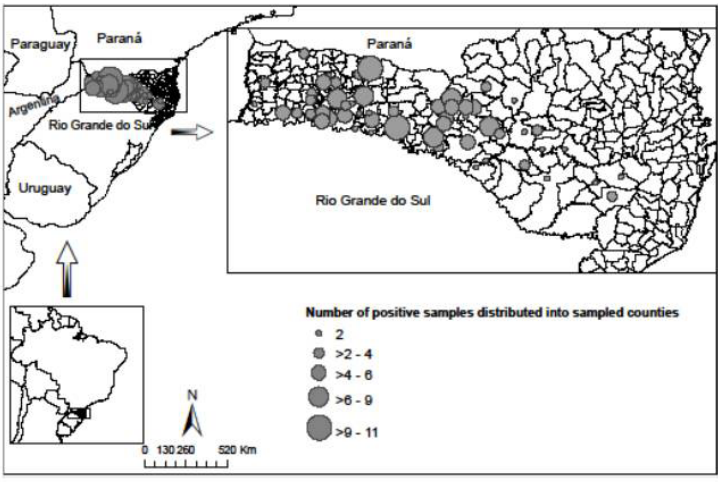

Figure 1. Map of the studied area showing farm location in Santa Catarina State, state borders, and neighboor countries. Sampled farms are indicated by grey circles and the size of each circle represents the amount of positive samples.

RESULTS

Among the 654 samples analyzed, 216 (33.02%; IC95% 29.43-36.77) of the samples were seropositive for *T. gondii* distributed in both regions studied. In west region it was found the highest concentration of seropositive samples, i.e. 82.9% (Table 1). Among the 216 seropositive samples, 60.2% (n = 130) had titration 1:64, 30.5% (n = 66) had title 1:256, 6.1% (n = 13) had titration 1:1024 and 3.2% (n = 7) had a titer of 1:4096.

In the univariate analysis, the region, sex, age, water source, breed, and contact with cats were significantly associated with *T. gondii* infection ($P < 0.20$) [Table 1]. Nevertheless, for the multivariate model analyses on the region, breed and contact with cats were considered to be risk factor (Table 2). The region were the samples came from was considered risk factor, been the west region more likely to have the infectious 1.16 in comparison to the mountain; breed was also significant associated with *T. gondii* infection, and one can notice that when animal breed was Boer in comparison to mixed breed the chances of seropositive was 1.64 greater; and finally the presence of contact with cats on the flock represented more chances of *T. gondii* infection been identified 1.04. None of the two-way interaction terms were significant at 5% and none potential confounding variables were identified. The model goodness-of-fit was tested by deviance chi-squared test found to be not significant ($P > 0.05$).

It was found significant relation between the presence of neural issue and being tested se-

ropositive for *T. gondii* infection, tested positive represented a PR of 1.02 (1.00-1.03) $P < 0.001$ for the presence of neural problems been reported. On the other hand it was found no significant relation between reproduction issue and *T. gondii* infection ($P = 0.92$).

Table 1. Univariate analysis of risk factors for toxoplasmosis infection in goats from Santa Catarina (West side), southern Brazil. Period of August 2012 and March 2013.

Variable	No. of animals	Frequency (%)	*P* - value	PR* (IC 95%)
Region	654			
Mountain		25	< 0.001	-
West		75		0.91 (0.87-0.96)
Sex	654			
Male		20	0.04	-
Female		80		0.94 (0.90-0.99)
Age				
6 months -1year	654	7	0.01	-
1year – 2 years		15		0.89 (0.81-0.97)
>2 years		78		0.92 (0.85-0.98)
Diet	654		0.44	
With concentrate		47		0.98 (0.94-1.02)
Without concentrate		52		-
Water source	654		0.17	
Natural source		53		-
River		9		0.94 (0.86-1.02)
Wellwater		30		0.97 (0.92-1.02)
Piped water		8		0.97 (0.90-1.05)
Breed	654		< 0.001	
Mixed breed		70		-
Boer		27		1.10 (1.06-1.15)
Anglo		3		1.08 (0.95-1.22)
Production type	654		0.45	
Milk		3		-
Meat		87		0.99 (0.85-1.15)
Mixed		10		0.93 (0.79-1.10)
Contact with cats	654		0.19	
Yes		53		1.03 (0.94-1.02)
No		47		-

*PR: prevalence ratio.

Table 2. Multivariate analysis of risk factors for toxoplasmosis infection in goats from Santa Catarina state (West side), southern Brazil. Period of August 2012 and March 2013.

Variable	Estimate (β)	*P* - value	PR* (CI: 95%)
Region		< 0.001	
Mountain	-		-
West	0.14		1.16 (1.10-1.22)
Breed			
Mixed breed	-	-	-
Boer	0.15	< 0.011	1.64 (1.11-1.22)
Anglo	0.10	0.11	1.11 (0.97-1.27)
Contact with cats			
Yes	0.04	0.04	1.04 (1.00-1.09)
No	-		-

*PR: prevalence ratio.

DISCUSSION

The prevalence of approximately 33% for *T. gondii* in goats in Santa Catarina, was lower than in Paraná state (44.68%) [31], and higher than reported in Rio Grande do Sul state (30%) [22], the two Brazilian states that make territorial boundary with Santa Catarina state. Statistically and numerically higher prevalence was found in the western region of the state. According to literature, the prevalence of toxoplasmosis varies from region to region, been influenced by the climate, geographical factors, human and animals habits [16]. Another study has also already reported difference between regions in the State of Bahia [17], where the number of positive animals was higher in the region of with higher temperatures and humid climate, similar to what occurred in this study, that because this climate favors sporulation, and thus enables the oocysts to become contaminants to the animals [21,23].

In this study, similar to other epidemiological studies with goats and *T. gondii* [31,32], in a univariate analysis, gender of the animal was identified as risk factor for *T. gondii*, occurring in greater numbers in females. According to the literature, some factors may contribute to this situation, as the females stay longer on the farm (with greater chance of exposure to the parasite) and/or immunosuppression during pregnancy and lactation [34]. A higher prevalence of *T. gondii* infection in adult animals was found, similar to that reported by other researchers [26], who observed a positive association between older animals and the presence of antibodies, and thus greater chance of exposure to the etiological agent [5,13,19]. In this study, unlike the univariate analysis, the multifactorial model, did not kept the variables sex and age as risk factors for *T. gondii* infection in goats.

The goat's breed was considered statistically a risk factor for disease in the current study; the animals belonging to Boer breed were mainly affected, although 70% of the goats were without race defined animals. Otherwise, others demonstrated a higher (32.7%) susceptibility wend animals were Mongrel [1], compared to goats purebred (18.6%). Despite some justification for breed been a risk factor, the authors consider difficult to find an explanation for breed to be a risk factor for toxoplasmosis since the infection is related to exposure to the etiological agent by the oral route (oocyst ingestion) or vertical, it has never been published any description about breed resistant or susceptibility of infection by the parasite.

The type of food and water source given to the animals was not considered a risk factor for *T. gondii* infection, although some authors mention that cat feces contaminated with oocysts of the parasite can be more easily ingested in the diet with concentrated (cats have access) and natural water sources [14,28]. According to literature [15], the most common form of infection among goats is by ingestion of oocysts agent that are present in soil, food and water contaminated by cat feces (definitive host). This study showed that 53% of the animals studied had contact with cats, and this variable was considered a risk factor for disease, ie, most of the goats that had seropositive results had contact with cats. A similar result was reported by other researcher [2], where the labeling index was higher in farms where goats contact with cats.

Infection by *T. gondii* in sheep is usually associated with reproductive problems [35]. However in our study, statistically, unverified relationship between reproductive problems and goats seropositive for *T. gondii*, although of approximately 24% of the animals studied showed some reproductive problems (abortion, mummified or heat repetition). Similarly, researcher also report that reproductive failure, alone, were not associated with seropositivity to *T. gondii* [6], unlike cases of coinfection (*T. gondii* and virus infectious arthritis goat). Other study also observed no relationship between seropositive goats and reproductive failure [32]. Therefore, other infectious diseases may be related to reproductive problems reported in goats.

In the effect-cause analysis, neurological issues were related to infection by *T. gondii* in goats. This can easily be explained because the parasitic cysts of *T. gondii* may persist in the central nervous system (CNS) for a long period [11]. Consequently, the parasite in the brain can cause severe inflammatory infiltrates and histological lesions, and hence to enable the occurrence of encephalitis, described in goats with toxoplasmosis [8]. The relationship with toxoplasmosis and neurological disorders in humans [20], dogs [30] and experimentally infected rodents [18] are well described.

Our serological results were quite similar to those obtained from other studies throughout the Brazil, showing relatively low positivity for *T. gondii*.

However, our results show some risk factors to *T. gondii* infection in goats such as region, breed, and contact with cats, as well as neurologic problems is a cause of disease. Thus, it is possible to conclude that toxoplasmosis is present in approximately one third of goats of the western and mountain regions of the state of Santa Catarina, Brazil, and is associated with some important risk factors.

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***Ethical approval*.** The procedure was approved by the Animal Welfare Committee of Universidade do Estado de Santa Catarina, number 1.02.12.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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