

***Toxoplasma gondii* and *Neospora caninum* in domestic cats from the Brazilian semi-arid: seroprevalence and risk factors**

[*Toxoplasma gondii* e *Neospora caninum* em gatos no semiárido nordestino: soroprevalência e fatores de risco]

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ABSTRACT

In the present study we aimed to establish the seroprevalence of *T. gondii* and *N. caninum* in stray and domiciled cats from the municipality of Patos, Paraíba state, Brazil. Blood samples were collected from 201 animals: 132 domiciled cats and 69 stray cats. An epidemiological questionnaire was conducted with all cat owners. Indirect immunofluorescent antibody test (IFAT) was performed at cut-offs of 1:16 and 1:50 for *T. gondii* and *N. caninum*, respectively. Overall prevalence of seroreagent cats for *T. gondii* was 43.8%. We found a prevalence of 47.7% in domiciled cats and 36.2% in stray cats. Antibody titers ranged from 1:16 (cut-off) to 1:8192; 1:128 was the most frequent titer. No statistical difference was observed between domiciled cats and stray cats. Correlation was verified between seroreagent for *T. gondii* and age and hunting habit ($P<0.05$). No animals tested seroreagent for *N. caninum*. It was possible to conclude that there is high prevalence of cat seroreagent for *T. gondii* and that *N. caninum* is not present in cats from the area studied.

Keywords: domiciled cat, stray cat, toxoplasmosis, zoonosis

RESUMO

Este trabalho teve como objetivo determinar frequência de Toxoplasma gondii e Neospora caninum em gatos domiciliados e errantes do município de Patos, mesorregião do sertão paraibano. Foram coletadas amostras de sangue provenientes de 201 gatos, 132 domiciliados e 69 errantes. Nos domiciliados, foi aplicado um questionário epidemiológico aos proprietários. A reação de imunofluorescência indireta (RIFI) foi realizada levando-se em consideração os pontos de corte 1:16 e 1:50 para T. gondii e N. caninum, respectivamente. Foi obtida uma prevalência total de 43.8% de gatos sororreagentes para T. gondii. Nos domiciliados, a prevalência foi de 47,7% (63/132), e nos errantes de 36,2% (25/69), com títulos variando de 1:16 a 1:8192, sendo a titulação 1:128 a mais frequente. Não se observou diferença estatística entre animais domiciliados e errantes. Verificou-se correlação entre sororreagentes a T. gondii e idade e hábito de caçar (P<0.05). Nenhum animal foi sororreagente para N. caninum. Concluiu-se que é alta a prevalência de gatos sororreagentes para T. gondii e que o N. caninum não está presente em gatos na área estudada.

Palavras-chave: gatos domiciliados, gatos errantes, toxoplasmose, zoonose

INTRODUCTION

Toxoplasma gondii is a protozoan able to infect humans and several warm-blooded animals

(Dubey, 2004), and cats play an important role in the transmission of the agent, because they are the only hosts capable of eliminating oocysts in the environment, being responsible for the natural cycle of the disease (Dubey and Beatle,

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1988). Cats are able to eliminate about 20 million oocysts between three and 18 days post infection (Dabritz *et al.*, 2007). Serological studies in such animals are used to estimate the environmental contamination, considering that oocysts are rarely found in feces (Dubey *et al.*, 1995; Miró *et al.*, 2004).

Neospora caninum is an obligate intracellular protozoan that has dogs, coyotes and dingoes as definitive hosts (Dubey *et al.*, 2007; King *et al.*, 2010). Although there is no documented case report of natural infection with *N. caninum* in cats, experimental infection has already been performed in these animals (Dubey and Lindsay, 1989). There is little information on the seroprevalence of *N. caninum* in cats in Brazil, being observed only in the state of São Paulo, with frequencies ranging from 11.9 to 24.5% (Dubey *et al.*, 2002; Bresciani *et al.*, 2007), and in the state of Maranhão, with prevalence of 27% (Braga *et al.*, 2012).

Several works have been carried out on *T. gondii* and *N. caninum* infection in pigs, goats, and sheep (Araújo Neto *et al.*, 2008; Azevedo *et al.*, 2010; Faria *et al.*, 2010) from the semiarid mesoregion of northeastern Brazil, but no research was carried out to determine the status of these agents in cats. Thus, in the present study, we aimed to determine the frequency of seroreagent for *T. gondii* and *N. caninum* and possible risk factors in domiciled and stray cats in the municipality of Patos, Paraíba state.

MATERIAL AND METHODS

This survey was conducted in the municipality of Patos (7°13'S, 37°16'W), Paraíba state, northeastern Brazil, between May and August 2011. No probabilistic criteria were used for the selection of domiciled animals: the choice was dependent on owner consent. Blood samples were collected from 201 animals: 132 domiciled cats and 69 stray cats. Collection was made by jugular vein puncture and the blood was centrifuged to obtain serum and subsequently stored at -20 °C until processing of analyzes.

The experiments conducted in this work comply with current regulations in Brazil and were approved by the Research Ethics Committee of UFCG, protocol 27-2012.

Indirect immunofluorescence assay (IFAT) was used to detect antibodies anti-*T. gondii* and antibodies anti-*N. caninum* using cut-offs of 1:16 and 1:50, respectively (Cruz *et al.*, 2011, Dubey 1988). In order to detect antibodies anti-*T. gondii*, we used RH strain tachyzoites of *T. gondii* fixed on slides (Camargo 1974); while for the detection of *N. caninum*, Nc-1 tachyzoite samples fixed on slides were utilized (Dubey *et al.*, 1988). Conjugated anti-cat IgG (SIGMA, St. Louis, MO, USA) was used for both agents.

The frequency of antibodies anti-*T. gondii* and anti-*N. caninum* was estimated from the ratio of positive results for the total number of cats examined, with exact binomial confidence interval of 95% (Thrusfield, 2004), using EpiInfo, version 6.04. The association between wandering domiciled animals regarding the frequency of positivity was verified by the Chi-square test (Zar, 1999), with a significance level of 5%.

An epidemiological questionnaire was conducted with the owners of domiciled cats concerning the following issues: age, sex, type of food, access to street environment, place where animals live and defecate, and hunting habit. We used the information obtained from the questionnaires in the analysis of risk factors, which was conducted in two phases: univariate analysis and multivariate analysis. For the univariate analysis, each independent variable was matched with the dependent variable, and those that presented $p \leq 0.20$ value by the Chi-square test or Fisher's exact test were selected for multivariate analysis, using multiple logistic regression. The final model fit was verified by the Hosmer - Lemeshow test, whose $P > 0.05$ value indicates a good fit (Hosmer and Lemeshow, 2000). A significance level of 5% was adopted for the multivariate analysis. All analyses were performed with SPSS 20.0 for Windows.

RESULTS

Eighty-eight (43.8%, 95% CI = 36.8% - 50.9%) of the 201 examined cats were seroreagent for *T. gondii*. For stray cats, 25 (36.2%, 95% CI = 25.0% - 48.7%) of the 69 animals were seroreagent. For domiciled cats, 63 (47.7%, 95% CI = 38.9% - 56.6%) of the 132 animals were seroreagent. There was no statistical difference between stray and domiciled cats regarding the

frequency of seropositivity ($p = 0.159$). No animals were seropositive for *N. caninum* in this study.

The titers of antibodies to *T. gondii* ranged from 16 to 8192, with 128 and 2048 being the most frequent in stray animals. This variation was also observed in domiciled cats, with greater frequency of titers: 128, 1024, and 2048 (Table 1).

Table 1. Prevalence of antibodies anti-*Toxoplasma gondii* by IFAT in 201 sera from domiciled and stray cats in Paraíba State, Northeast region of Brazil, at different dilutions

Titer	Seroreagent animals		Total (%)
	Number of stray cats (%)	Number of domiciled cats (%)	
16	3 (12)	3 (4.8)	6 (6.8)
32	2 (8)	-	2 (2;3)
64	1 (4)	6 (9.5)	7 (7.9)
128	5 (20)	10 (15.9)	15 (17)
256	2 (8)	9 (14.3)	11 (12.5)
512	3 (12)	4 (6.4)	7 (7.9)
1024	3 (12)	10 (15.9)	13 (14.8)
2048	4 (16)	10 (15.9)	14 (15.9)
4096	-	8 (12.7)	8 (9)
8192	2 (8)	3 (4.8)	5 (5;7)
Total	25 (100)	63 (100)	88 (100)

Results from univariate analysis of risk factors for *T. gondii* are shown in Table 2. The variables age, type of food, and hunting habit were selected ($P \leq 0.20$) for multivariate analysis. The following variables were identified as risk factors by logistic regression (Table 3): age 1-4 years (odds ratio = 4.85), age > 4 years (odds ratio = 16.54), and hunting habit (odds ratio = 2.43). The final model showed satisfactory fit by the Hosmer - Lemeshow test (Chi-square = 2248, $P = 0.690$).

DISCUSSION

The high frequency of seroreagent cats found (43.8%) indicates that there is considerable environmental contamination by oocysts, suggesting that cats play a prominent role in the

epidemiology of toxoplasmosis in the area studied. The high contamination is a concern because it favors the appearance of disease outbreaks in humans and animals due to contamination of water and food supplies, or even by inhalation (Dubey et al., 2004; Carmo et al., 2010).

Different from the results found in this study, Rosa et al. (2010) and Cruz et al. (2011) found low prevalence of *T. gondii* in southern Brazil, attributing this result to the way animals were raised, since most of them presented strictly domiciled habits and ingested only industrialized products. In this research, most of the animals had access to the street and did not consume only industrialized food (71.2%).

Table 2. Univariate analysis for association between the presence of anti-*Toxoplasma gondii* antibodies and risk factors in cats from Paraíba State, Brazil

Variable	Number of cats	Seroreagent cats (%)	P
Sex			
Males	72	33 (45.8)	0.762
Females	60	30 (50)	
Age (years)			
0-1	42	8 (19)	< 0.001
1-4	66	36 (54.5)	
> 4	24	19 (79.2)	
Type of food			
Industrialized	48	15 (31.2)	0.007
Industrialized + meat	84	48 (57.1)	
Access to the street			
No	36	16 (44.4)	0.790
Yes	96	47 (49.0)	
Environment where animals live			
Land	80	38 (47.5)	1.000
Ceramic	52	25 (48.1)	
Where animals defecate			
Sand box	31	13 (41.9)	0.594
In the street	101	50 (49.5)	
Hunting habit			
No	52	18 (34.6)	0.024
Yes	80	45 (56.2)	

Table 3. Final logistic regression model for risk factors associated with *Toxoplasma gondii* in cats from Paraíba State, Brazil.

Variable	Odds ratio (IC 95%)	P
Age 1-4 years old	4,85 (1.92-12.25)	0.001
Age > 4 years old	16,54 (4.61-59.28)	0.001
Hunting habit	2,43 (1.08-5.42)	0.031

Hosmer and Lemeshow test: chi-square = 2.248; P = 0.690

One can infer that the prevalence rates observed in this and other surveys depend on several factors, including the techniques and cut-off points adopted. There are no experiments in cats standardizing the cut-off, and there is no data for *T. gondii* in cats in the studied region, so we preferred to use a cut-off according to Garcia *et al.* (1999), Lucas *et al.* (1999), Pinto *et al.* (2009) and Cruz *et al.* (2011). The lack of standardization of diagnostic techniques complicates the comparison of results. Many studies work with different cut-offs. In the state of Maranhão, Braga *et al.* (2012) used IFAT with a cut-off of 1:40, observing prevalence of 50.5%.

In the state of Santa Catarina, Rosa *et al.* (2010) used a cut-off of 1:64 and obtained 14.33% prevalence. Other researchers used different techniques, such as Indirect Hemagglutination Test and Modified Agglutination Test, noting prevalence between 26.9% and 35.4% (Pena *et al.*, 2006; Pinto *et al.*, 2009). In this study there was no statistical difference in positivity between domiciled and stray cats, probably because most of the domiciled cats in this study have free access to the street, also being exposed to the agent. DeFeo *et al.* (2002) also found no difference between these categories. However, several authors indicate that stray cats tend to

present higher prevalence when compared to domiciled cats (Miró *et al.*, 2004; Raeghi *et al.*, 2011).

Regarding the sex variable, no difference was observed in either domiciled or stray animals, indicating that the genders have an equal chance of being seroreagent for *T. gondii*, corroborating other authors that obtained the same results in their researches (Rosa *et al.*, 2010; Cruz *et al.*, 2011).

The majority of animals showed serorescent titer ≥ 128 . With the titration of IgG, it is only possible to suggest that infection is recent or active when it presents titers above 256, and that infection is latent when titers are below 256 (Lappin, 2004). Bresciani *et al.* (2007) reported that titers above 1024 strongly indicate that the animal is asymptotically infected with *Toxoplasma* or presenting clinical signs; 27 animals presented titers above 1024 in this work.

The age variable was considered a risk factor for the presence of antibodies for *T. gondii*., corroborating the works by Miró *et al.* (2004), Pena *et al.* (2006) and Pinto *et al.* (2009). This association can be explained by the fact that older animals are more likely to become infected because they have been exposed more often. However, some authors found no difference regarding this variable in their studies (Cruz *et al.*, 2011).

Studies show that access to the street is a risk factor for the seroreagent of cats (Pena *et al.*, 2006; Rosa *et al.*, 2010), but, in this research, none of the possible risk factors, such as access to the street, place where animals defecate, and environment where animals live were significant for *T. gondii* infection.

The prevalence of antibodies in animals that fed only on industrialized products was 31.1%, while the animals that consumed meat with processed feed had a prevalence of 57.1%, with positive association in the univariate analysis. The hunting habit variable was also significant for *T. gondii*. These results can be explained by the biology of the parasite, since one of its routes involves the formation of bradyzoites in the tissues of animals; cats can then become infected by the ingestion of tissues containing the parasite. Raeghi *et al.* (2010) also observed

positive association between meat intake and positivity of cats.

Corroborating this work, Arunvipas *et al.* (2012) found no animals seroreagent for *N. caninum*. However, Dubey *et al.* (2002) made the first detection of positive cats in the world, by natural infection with *N. caninum*, in a study carried out in Brazil, São Paulo State. Positive animals and positive correlation between *T. gondii* and *N. caninum* were found in other studies conducted in Brazil. In Maranhão State, 27% of seropositive animals were found, and in São Paulo state there was 24.5% (Bresciani *et al.* 2007; Braga *et al.*, 2012). There are only a few reports on naturally acquired seropositivity and studies on *N. caninum* in cats is limited (Hornok *et al.*, 2008; Bresciani *et al.*, 2007). There is need for further research to elucidate the role of cats in the epidemiology of this disease, and to find out whether this parasite can harm the health of these animals.

CONCLUSION

We conclude that the prevalence of seroreagent cats for *T. gondii* is high and that there are no differences in seropositivity of domiciled and stray cats. The age and hunting habit are significant risk factors for infection by this parasite. The presence of *N. caninum* was not observed in the studied animals.

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