

Neospora caninum and Toxoplasma gondii in Urban Dogs from Espírito Santo, Brazil: Seroprevalence, Coinfection, and Associated Factors

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ABSTRACT

Background: *Neospora caninum* and *Toxoplasma gondii* are globally distributed intracellular protozoa of veterinary and public health importance. Dogs are the definitive hosts of *N. caninum* and act as epidemiological sentinels of environmental contamination by *T. gondii*. However, information regarding canine exposure to these protozoa in Espírito Santo State, Brazil, remains limited. Therefore, this study investigated the seroprevalence of anti-*N. caninum* and anti-*T. gondii* antibodies in dogs from an urban area of Espírito Santo State and evaluated factors associated with infection.

Materials, Method & Results: A total of 103 canine serum samples were analyzed by the Indirect Fluorescent Antibody Test (IFAT). Epidemiological information regarding sanitary management, feeding practices, housing conditions, and contact with other animal species was obtained through questionnaires applied to dog owners. Descriptive statistical analysis was performed to determine seroprevalence, followed by univariate and multivariate analyses to identify factors associated with seropositivity. Anti-*Neospora caninum* antibodies were detected in 24 (23.3%) dogs, whereas 33 (32.0%) were seropositive for *Toxoplasma gondii*. Coinfection was identified in 9 (8.7%) animals. For *N. caninum*, antibody titers ranged from 1:50 to 1:3200, with predominance of the 1:50 titer. For *T. gondii*, titers ranged from 1:16 to 1:2048, with the highest frequencies at 1:16 and 1:128. None of the evaluated variables was associated with *N. caninum* seropositivity ($P > 0.05$). For *T. gondii*, dogs living in houses showed a significantly higher seroprevalence than those living in apartments in the univariate analysis ($P \leq 0.05$); however, no significant association remained after multivariate logistic regression.

Discussion: The seroprevalence observed in this study is consistent with previous reports demonstrating the circulation of *Neospora caninum* and *Toxoplasma gondii* in canine populations from different regions of Brazil. The wide variation reported among studies probably reflects differences in environmental conditions, management practices, diagnostic cut-off values, and characteristics of the evaluated canine populations. Previous studies conducted in Espírito Santo have also demonstrated exposure of dogs to both protozoa, confirming their circulation in different canine populations throughout the state. Dogs play an important epidemiological role because they are the definitive hosts of *N. caninum* and serve as sentinels of environmental contamination by *T. gondii*. The predominance of low anti-*N. caninum* antibody titers suggests previous exposure in most seropositive animals, whereas the heterogeneous distribution of anti-*T. gondii* titers may indicate differences in antigenic stimulation or host immune response. The occurrence of coinfection supports the simultaneous circulation of both protozoa in the same urban environment. Although household type was associated with *T. gondii* seropositivity in the univariate analysis, this association was not maintained in the multivariate model, suggesting that exposure is influenced by multiple environmental or behavioral factors. Overall, the high seroprevalence of anti-*T. gondii* and anti-*N. caninum* antibodies, together with the detection of coinfection, demonstrates the circulation of both protozoa in the studied urban environment. These findings reinforce the role of dogs as epidemiological sentinels and highlight the importance of continued surveillance and preventive measures within a One Health perspective.

Keywords: neosporosis, toxoplasmosis, sentinel animals, urban environment, One Health.

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INTRODUCTION

Neospora caninum and *Toxoplasma gondii* are obligate intracellular protozoa of the phylum Apicomplexa, with dogs and cats acting as their definitive hosts, respectively [24]. These parasites have a worldwide distribution and infect a wide range of vertebrate hosts, causing neosporosis and toxoplasmosis, diseases of major importance in veterinary medicine and, in the case of *T. gondii*, also in public health [13,23].

Dogs play an important epidemiological role as definitive hosts of *N. caninum* and as sentinels of environmental contamination by *T. gondii*, reflecting exposure to contaminated soil, water, and food shared with humans and other domestic animals [13]. Although *N. caninum* is not considered zoonotic, antibodies have been detected in humans, highlighting the need for continued surveillance [7,17]. In contrast, toxoplasmosis is one of the most prevalent parasitic infections worldwide and may cause severe disease in immunocompromised individuals [12,13].

In Espírito Santo State, Batista *et al.* [11] reported seroprevalences of 13.2% for *N. caninum* and 12.5% for *T. gondii* in slaughtered cattle, demonstrating the circulation of both protozoa in the state. However, studies evaluating canine exposure remain limited, despite the importance of dogs in the epidemiology of these infections and as indicators of environmental contamination [1,4].

Therefore, this study investigated the seroprevalence of anti-*Neospora caninum* and anti-*Toxoplasma gondii* antibodies in dogs from Espírito Santo State, Brazil, and evaluated factors associated with infection.

MATERIALS AND METHODS

Study site and sampling

This survey was conducted among dogs attended at the Small Animal Internal Medicine Service of the Veterinary Hospital Professor Ricardo Alexandre Hippler, at Universidade Vila Velha, located in Vila Velha, state of Espírito Santo, Brazil (20°21'03"S, 40°18'05"W). Blood samples were collected during routine clinical care and submitted for standard laboratory analyses at the Clinical Pathology Laboratory of the hospital. After routine testing, 100 µL of serum from each sample was aliquoted into microtubes and stored for use in this study.

The sample size was calculated based on an expected prevalence of 50% for *N. caninum* and *T. gondii* infections, due to the absence of previous studies in dogs from the study region. A minimum confidence level of 95% was considered. The minimum required sample size was 98; however, a total of 103 samples were ultimately collected.

Serological analysis

Serological diagnosis was performed using the Indirect Fluorescent Antibody Test (IFAT) at the Laboratory of Immunology and Infectious Diseases, Instituto Federal da Paraíba (IFPB), Campus Sousa.

For the detection of anti-*N. caninum* IgG antibodies, tachyzoites of the NC-1 strain fixed on slides were used as antigen, adopting a cutoff point of 1:50 to determine seropositivity [3]. For the detection of anti-*T. gondii* IgG antibodies, tachyzoites of the ME-49 strain were used as slide-fixed antigens, with a cutoff value of ≥ 16 [11]. A fluorescein isothiocyanate (FITC)-conjugated anti-dog IgG¹ (whole molecule) was used. Positive and negative controls were included in all reactions. Samples were considered positive when tachyzoites exhibited complete peripheral fluorescence. Positive samples were subsequently subjected to twofold serial dilutions until a negative result was obtained, as described by Sousa *et al.* [21].

Statistical analysis

During clinical care, all animal owners completed an investigative questionnaire previously validated by a specialist, containing objective questions regarding sanitary management, feeding practices, and contact of dogs with other animal species.

Descriptive statistical analysis was performed to calculate the frequencies of IFAT results. To identify factors associated with the prevalence of anti-*N. caninum* and anti-*T. gondii* antibodies, data obtained from the epidemiological questionnaires were analyzed in 2 stages: univariate and multivariate analyses. In the univariate analysis, each independent variable was tested against the dependent variable (seropositivity), and variables with $P \leq 0.05$, according to the chi-square test, were selected for multivariate analysis using multiple logistic regression, adopting a significance level of 5%.

To assess potential collinearity among variables, a correlation test was applied. When the correlation coefficient exceeded 0.9, 1 of the variables was excluded based on biological plausibility. The chi-square and Omnibus tests were used to evaluate the goodness-of-fit of the model. Statistical analysis were performed using GraphPad Prism version 8.0.12.

RESULTS

Among the analyzed samples, 24 (23.3%) were positive for anti-*N. caninum* antibodies, while 33 (32.0%) were seropositive for *T. gondii*. Simultaneous presence of antibodies against both protozoa was observed in 9 (8.7%) dogs, indicating coinfection (Figure 1).

The distribution of anti-*N. caninum* antibody titers showed a predominance of the 1:50 titer (41.7%), followed by 1:200 (25.0%) and 1:400 (16.7%). Higher titers were observed at lower frequencies, including 1:800 (4.2%) and 1:3200 (4.2%), while no dogs presented a titer of 1:1600 (Table 1).

Regarding anti-*T. gondii* antibodies, a heterogeneous distribution of titers was observed, with higher frequencies at 1:16 (33.3%) and 1:128 (18.2%), followed by 1:32 (15.2%) and 1:512 (12.2%). Higher titers, such as 1:1024 (6.1%) and 1:2048 (3.0%), were also identified among seropositive dogs (Table 1).

Univariate analysis of potential factors associated with *N. caninum* seropositivity included demographic, environmental, and management variables (Table 2). None of the evaluated variables showed a significant association with seropositivity ($P > 0.05$).

Similarly, univariate analysis of factors associated with *T. gondii* seropositivity included demographic, environmental, and management variables (Table 3). Among the evaluated variables, only the type of household environment showed a significant association ($P \leq 0.05$), with a higher frequency of positive animals among dogs living in houses (44.0%; 22/50) compared to those living in apartments (20.8%; 11/53). Multivariate analysis was performed; however, no significant associations were identified between the evaluated variables and seropositivity.

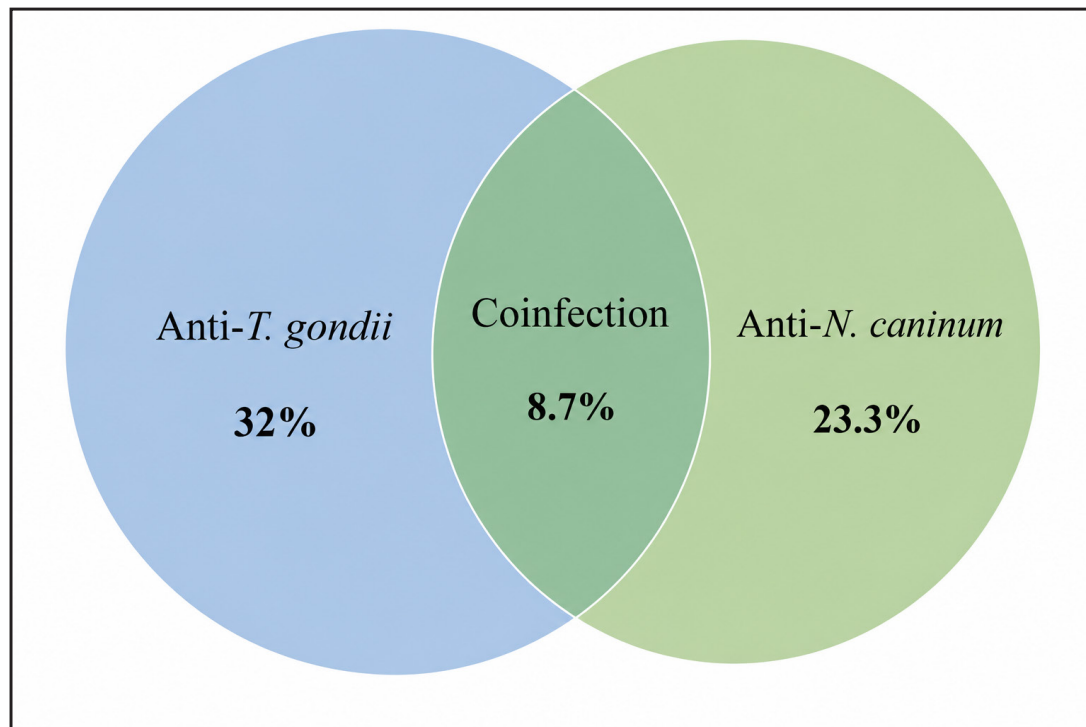


Figure 1. Venn diagram showing the distribution of dogs seropositive for *Neospora caninum* and *Toxoplasma gondii*.

Table 1. Seroprevalence and distribution of anti-*Neospora caninum* and anti-*Toxoplasma gondii* antibody titers in dogs from the state of Espírito Santo, Brazil.

Overall seroprevalence						
Parasite			Positive		Prevalence (%)	
<i>N. caninum</i>			24 / 103		23.3	
<i>T. gondii</i>			33 / 103		32	
Coinfection			9 / 103		8.7	
Positivity for anti- <i>N. caninum</i> antibodies						
Titration	1:50	1:200	1:400	1:800	1:1600	1:3200
Total (%)	10 (41.7)	6 (25)	4 (16.7)	1 (4.2)	0 (0)	1 (4.2)
Positivity for anti- <i>T. gondii</i> antibodies						
titration	1:16	1:32	1:128	1:512	1:1024	1:2048
Total (%)	11 (33.3)	5 (1.2)	6 (18.2)	4 (12.2)	2 (6.1)	1 (3)

Table 2. Univariate analysis of factors associated with the seroprevalence of anti-*N. caninum* antibodies in dogs attended at a veterinary hospital in the state of Espírito Santo, Brazil.

Variable	Category	Univariate analysis			
		Total	Positives	%	<i>P</i>
Sex	Female	61	15	24.6	0.709
	Male	42	9	21.4	
Breed	SRD	50	13	26	0.529
	Purebred	53	11	20.8	
Domiciliary environment	House	50	14	28	0.273
	Apartment	53	10	18.9	
Age group	1 year	12	7	58.3	0.799
	2-7 years	45	15	33.3	
	> 8 years	22	2	9.1	
Contact with other animals	No	31	5	16.1	0.258
	Yes	72	19	26.4	
Contact with dogs	No	39	7	17.9	0.315
	Yes	64	17	26.6	
Type of feeding	Commercial feed	61	14	23	0.919
	Mixed feeding	42	10	23.8	

Table 3. Univariate and multivariate analyses of factors associated with the seroprevalence of anti-*Toxoplasma gondii* antibodies in dogs attended at a veterinary hospital in the state of Espírito Santo, Brazil.

Variable	Category	Univariate analysis			
		Total	Positives	%	P
Sex	Female	61	20	32.8	0.844
	Male	42	13	31	
Breed	SRD	50	19	38	0.207
	Purebred	53	14	26.4	
Domiciliary environment	House	50	22	44	0.011*
	Apartment	53	11	20.8	
Age group	1 year	19	4	21.1	0.26
	2-7 years	60	23	38.3	
	> 8 years	24	6	25	
Contact with other animals	No	31	6	19.4	0.07
	Yes	72	27	37.5	
Contact with cats	No	53	14	26.4	0.207
	Yes	50	19	38	
Type of feeding	Commercial feed	61	17	27.9	0.274
	Mixed feeding	42	16	38.1	

*Significance of $P \leq 0.2$.

DISCUSSION

The seroprevalence of *Neospora caninum* observed in this study falls within the range reported in previous studies conducted in Brazil, which varies from 0.75% to 73.9% [1,2,13,18,25]. This wide variation probably reflects differences in environmental conditions, management practices, diagnostic cut-off values, and characteristics of the evaluated canine populations. Nevertheless, the present findings reinforce that *N. caninum* infection is widely distributed throughout different regions of Brazil.

Dogs have particular epidemiological importance because they are the definitive hosts of *N. caninum* and can shed viable oocysts into the environment [9]. Consequently, canine infection contributes directly to maintaining the parasite life cycle and represents an important source of environmental contamination. This is particularly relevant for livestock production, especially cattle, in which neosporosis is recognized

as one of the leading causes of reproductive losses and abortion [14].

The seroprevalence of *Toxoplasma gondii* observed in the present study was relatively high, indicating widespread environmental contamination and reinforcing the role of dogs as epidemiological sentinels [12]. Although dogs are not definitive hosts of *T. gondii*, infection generally occurs through ingestion of contaminated water, soil, or food, reflecting the level of environmental contamination shared with humans and other domestic animals [8,20]. Therefore, canine seropositivity can be considered an indirect indicator of environmental exposure, particularly in urban settings [6].

Previous studies conducted in the state of Espírito Santo have also demonstrated exposure of dogs to both protozoa. Acosta *et al.* [1] reported seroprevalences of 47.1% for *T. gondii* and 11.8% for *N. caninum* in rural dogs from northern Espírito Santo, whereas

Batista *et al.* [5] detected anti-*T. gondii* antibodies in 10.7% of dogs from the metropolitan region. These differences among studies may be associated with variations in environmental characteristics, host density, management practices, and the populations evaluated. Overall, these studies, together with the present findings, confirm the circulation of both protozoa in different canine populations throughout Espírito Santo.

The predominance of anti-*N. caninum* antibody titers up to 1:400 suggests previous exposure to the parasite. Although IFAT cannot determine the time of infection or whether oocyst shedding is occurring, the detection of higher titers (1:800 and 1:3200) may indicate stronger antigenic stimulation resulting from recent or repeated exposure or from individual differences in immune response [10].

Likewise, the predominance of anti-*T. gondii* titers of 1:16 and 1:32 is consistent with previous serological surveys in dogs [16]. On the other hand, the relatively high proportion of animals presenting titers $\geq 1:128$ suggests more intense antigenic stimulation in part of the studied population, possibly reflecting repeated exposure or individual variability in immune response [22].

Coinfection with *T. gondii* and *N. caninum* was detected in 8.7% of the evaluated dogs. Although the clinical implications of simultaneous exposure remain poorly understood, this finding demonstrates the concurrent circulation of both coccidian parasites in the same environment and further supports the usefulness of dogs as epidemiological sentinels [2,19]. The occurrence of coinfection also suggests that dogs may be simultaneously exposed to different sources of environmental contamination, reinforcing their epidemiological importance within the One Health framework.

In addition to the serological findings, the analysis of epidemiological factors showed that no variables were associated with *N. caninum* seropositivity, suggesting that exposure may occur broadly across

the evaluated canine population [12,15]. For *T. gondii*, dogs living in houses showed higher seropositivity than those living in apartments in the univariate analysis, possibly reflecting greater environmental exposure [6,16]. However, this association was not maintained in the multivariate model, indicating that exposure is probably influenced by multiple environmental and behavioral factors rather than by household type alone. Therefore, these findings should be interpreted cautiously, since other unmeasured variables may also contribute to the risk of infection.

CONCLUSIONS

Overall, the high seroprevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies, together with the detection of coinfection, demonstrates the circulation of both protozoa in the studied urban environment. These findings reinforce the role of dogs as epidemiological sentinels and highlight the importance of continued surveillance and preventive measures within a One Health perspective.

MANUFACTURERS

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

Artificial intelligence statement. The authors declare that artificial intelligence ChatGPT was used exclusively for linguistic correction, grammatical refinement, and reference standardization.

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