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ORIGINAL PAPER

Seroepidemiology of *Toxoplasma gondii*, *Neospora caninum*, and *Leishmania* spp. infections and risk factors for cats from Brazil

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Abstract The seroprevalence of infection by *Toxoplasma gondii*, *Neospora caninum*, and *Leishmania* spp. was detected through an indirect immunofluorescence in 70 cats from the Andradina Municipality, São Paulo State, Brazil. Anti-*T. gondii* antibodies (titer >64) were detected in 15.7% (11/70) of animals, whereas positivity for *N. caninum* (titer 16) was not observed in any animal. Of the cats from urban and rural areas, 10.4% (5/48) and 27.2% (6/22) were positive for *T. gondii*, respectively. Breed, age, food, and contact with animals of other species were significant for considering the positivity for *T. gondii* ($P \leq 0.0001$). Cats having access to streets (17.1%, 11/64), cats cohabiting with rats (19.6%, 10/51), and cats feeding on homemade food and raw milk (27.2%, 6/22) were positive for *T. gondii*. In addition, 4.2% (3/70) of the cats were positive for *Leishmania* spp. by ELISA technique and negative by IFAT without coinfection with *T. gondii* and *Leishmania* spp. There was no serological positivity against feline immunodeficiency virus or feline leukemia virus. In conclusion, *T. gondii* infection in part of the feline population from Andradina is not linked to immunosuppressions or coinfections but probably to postnatal infection in association with the type of diet and presence of rats.

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Introduction

The *Toxoplasma gondii* and *Neospora caninum* are obligate parasites that can infect several animal species including humans (Garcia et al. 1999; Dubey and Lindsay 2006; Lobato et al. 2006; Benetti et al. 2009; Dubey 2010). The occurrence of these protozooses has been correlated to management, environment (Modolo et al. 2008a; Modolo et al. 2008b), livestock by-products (Hiramoto et al. 2001), and even dissemination through water (Dubey et al. 2004).

In cats, toxoplasmosis can be associated with the feline immunodeficiency virus (FIV) (Lucas et al. 1998). The occurrence of *Leishmania* infection in animals without immunossupression suggests that other factors must determine the susceptibility, although the literature has reported cases of leishmaniasis in cats positive for both viruses, whose action is essentially immunosuppressive (POLI et al.

2002; GREVOT et al. 2005). In theory, this favors parasitic infection in these animals, which have high degree of natural immunological resistance (VITA et al. 2005).

Thus, the aim of this study was to investigate the occurrence of antibodies against *T. gondii*, *N. caninum*, and *Leishmania* spp. in cats from Andradina Municipality, São Paulo State, Brazil, and the existence of an association between these parasites and infections by FIV and feline leukemia virus (FeLV). The influence of several factors on the occurrence of these parasites was also evaluated including cat age, sex, breed, origin, access to extra-household environment, and contact with other animal species, especially mice.

Material and methods

Animals and sample collection

The experimental group consisted of 70 male and female cats of different breeds and ages from urban and rural areas of the microregion of the Andradina Municipality (−20.866007° S, −51.307152° W, and hypsometry of 405 m), São Paulo State, Brazil. This study was carried out between 2007 and 2009 with the approval of the Animal Experimentation Ethics Committee of Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, protocol no. 2007–003276.

Blood (5 ml) was collected by venipuncture in siliconized Vacutainer tubes without an anticoagulant and centrifuged at 3,000 rpm for 5 min. The obtained serum was divided into two aliquots, then transferred to sterile plastic tubes and immediately frozen at −20°C.

Laboratory techniques

Serum samples were analyzed through an indirect immunofluorescence antibody test (IFAT) to investigate the presence of immunoglobulin G against *T. gondii* and *N. caninum.* Serological diagnosis was done according to the technique described by Camargo (1964), with cutoffs fixed in the dilutions of 1:64 for *T. gondii* and 1:16 for *N. caninum*.

Sera were base two diluted until the last dilution in which fluorescence was observed. The presence of total fluorescence around the tachyzoite surface was considered positive and the reactions of apical or partial fluorescence negative. Positive and negative control sera were included in each slide. Antibodies against FIV and FeLV were detected using the ELISA commercial kit (Snap-Combo®, IDEXX Systems, Portland, USA), following the manufacturer's recommendations.

The detection of antibodies against *Leishmania* spp. was also done by ELISA using protein A as the antigen (Lima et al. 2005) in triplicate. The cutoff was established based on the mean added to the standard deviation of the optical density obtained during the assay with serum from animals from a nonendemic area. Also, IFAT was performed by using promastigote forms of *Leishmania* (L.) *chagasi* as antigen, being considered seropositive titer ≥40. Animal records included individualized sheets containing species, breed, sex, age, place of origin, diet type, contact with animals, access to external or extra-household environments, and the presence of mice (*Mus musculus)* and rats (*Ratus ratus)* on the property.

Statistical analysis

Association between the analyzed variables was determined by the chi-square (χ^2) test using the software Minitab Version 11.

Results

Anti-*Leishmania* spp. antibodies were observed in 4.2% (3/70) of cats from the urban area by ELISA and all negative by IFAT. All positive animals were of undefined breed including an asymptomatic young female and two adult males. The occurrence of *Leishmania* spp. in the cats of this study was not correlated to the variables sex, breed, age, FIV, and FelV ($P>0.05$).

Of the serum samples, 15.7% (11/70) of cats from the rural area were considered positive or reactive to *T. gondii* with titers equal to or higher than 1:64. There was no seropositivity for *N. caninum* with IFAT ≥1:16. As to racial pattern, only animals without a definite breed and those with access to the external environment were reactive to *T. gondii* without correlation with sex.

A higher tendency of occurrence of *T. gondii* was noted for young cats, which had access to the external environment, cohabited with mice, and fed on homemade food and raw milk. All animals that received this type of food lived in rural properties and had contact with other pets and livestock ($P\leq0.0001$). The variables and positivity for *T. gondii* are shown in Table 1.

None of the 70 analyzed cats presented antibodies against FIV and FeLV. Coinfection by *T. gondii*, *N. caninum*, and *Leishmania* spp. was not detected in the analyzed animals.

Discussion

Of the cat population in this study, 5.76% (3/52) were positive for *L.* (L.) *chagasi*, and these animals were not serologically reactive to the viruses FIV and FeLV.

Table 1 Occurrence of seropositivity for *T. gondii* in 70 cats from Andradina Municipality, São Paulo State, Brazil according to the analyzed variables

T. gondii				
Variable	Category	Positive	Negative	Frequency (%)
Breed	DB	0	14	14 (20)
	WDB	11	45	56 (80)
Sex	Male	5	21	26 (37.1)
	Female	6	38	44 (62.8)
Age	Young	10	44	54 (77.1)
	Adult	1	15	16 (22.8)
Origin	Urban	5	43	48 (68.5)
	Rural	6	16	22 (31.4)
Diet	Both	5	33	38 (54.2)
	Commercial cat food	0	10	10 (14.2)
	Homemade food + raw milk	6	16	22 (31.4)
Contact with animals	Cats	1	14	15 (21.4)
	Dogs	1	6	7 (10)
	Cats + dogs	0	4	4 (5.7)
	Dogs + cattle + equines + chickens + pigs	6	16	22 (31.4)
	Dogs + cats + chickens	0	2	2 (2.8)
	No contact with animals	1	9	10 (14.2)
Presence of rats	Yes	10	41	51 (72.8)
	No	1	18	19 (27.1)
Access to extra-household environment	Yes	11	53	64 (91.4)
	No	0	6	6 (8.5)

+ contact with animals, *DB* determined breed, *WDB* without determined breed

Similarly, Martín-Sánchez et al. (2007) carried out an epidemiological study on leishmaniasis in cats from Spain and found no association between *Leishmania* infection and feline immunodeficiency. Although IFAT has been used in epidemiological studies (Ayllon et al. 2008; Poli et al. 2002; Vita et al. 2005), the positive animals of the present study were not seroreactive by IFAT but by ELISA, results similar to that obtained by Figueiredo et al. (2009), who evaluated antibodies anti-*Leishmania* in 43 cats from Barra Mansa Municipality, Rio de Janeiro State, Brazil by the methods IFAT and ELISA and detected only one reactive animal, 2.4% (1/43), by the latter technique only.

Bresciani et al. (2010) found 0.7% (2/283) of cats positive for *Leishmania* spp. by the IFAT technique in the municipality of Araçatuba, São Paulo State, Brazil. In this study, two females were positive, a young mongrel and an adult feline. The occurrence of *Leishmania* spp. in the cats of the present study was not correlated to the variables sex, breed, and age. The polymerase chain reaction was considered a better diagnostic method than the serology to define *Leishmania* infection in cats (Martín-Sánchez et al. 2007).

Infection by *T. gondii* was detected in 15.7% of the cats evaluated in the present study. Higher prevalence of anti-*Toxoplasma* antibodies in cats, detected by IFAT, was reported by Garcia et al. 1999 in the Paraná State, Langoni et al. (2001) in the states of São Paulo and Paraná, by Pinto et al. (2009) in the state of Rio Grande do Sul, and by Ortolani et al. (2005) in native Indian villages in the state of São Paulo, where occurrences were 19.4% (37/191), 37.9% (93/245), and 46.4% (13/28), respectively, with titers ≥1:16. Different percentages of positivity can be found in several studies on the soroepidemiology, especially due to the cutoffs adopted for IFAT.

Using the IFAT, other researchers adopted the cutoff at 1:64 to investigate seroprevalence of *T. gondii* in cats and observed a higher (Bresciani et al. 2007) or similar (Dalla Rosa et al. 2010) percentage than that found in our study. Higher percentage of infection by *T. gondii* was detected through IFAT by Garcia et al. (1999) in cats from rural areas in the state of Paraná. This fact corroborates the findings of the present study.

Garcia et al. 1999 suggest a statistical association for age groups, especially in young animals, as observed in the present study. These findings corroborate the results of Lucas et al. (1998), in which the highest percentage of cats reactive to toxoplasmosis was detected among adult

animals, confirming the predominant occurrence of toxoplasmic infection in the postnatal stage. The association between other variables and sex was not detected as to seropositivity for *T. gondii* in felines (Bresciani et al. 2007) similar to the present study.

As to the racial pattern, only animals without a definite breed and those with access to the external environment were reactive to *Toxoplasma*. The breed of the evaluated animals had no influence on the occurrence of *T. gondii* (Langoni et al. 2001; Bresciani et al. 2007; Pinto et al. 2009; Dalla Rosa et al. 2010).

Reports on *N. caninum* infection in cats still remain scarce. In Araçatuba Municipality, São Paulo State, Brazil, Bresciani et al. (2007) studied anti-*N. caninum* antibodies in cats and observed that 24.5% (98/400) were reactive, with IFAT ≥1:16, and found a higher occurrence to *N. caninum* in older cats, whereas in the present study, no animal was infected. This finding is interesting especially due to the proximity between Araçatuba and Andradina Municipalities and the differences in positivity for such a protozoan in this animal species.

Using agglutination test, Dubey et al. (2002) analyzed the serum of Brazilian cats and observed that 11.9% (60/502) were reactive. A higher percentage was detected in Italian cats using the same technique; 24.8% (70/282) reacted positively. The association between the ages of animals infected by *N. caninum* was not statistically significant (Ferroglio et al. 2005).

Although the cats of the present study did not present antibodies against *N. caninum,* even cohabiting with mice, several researchers have observed that some mouse species can be positive for such a parasite through serological (Huang et al. 2004) and molecular (Ferroglio et al. 2007; Jenkins et al. 2007) techniques, suggesting thus that mice can serve as a reservoir of this protozoosis for predator animals.

As to immunosuppression, Lucas et al. (1998) studied 115 blood samples from cats negative for FeLV, of which 22 were positive for FIV; there was a strong association between occurrence of the immunodeficiency virus and anti-*T. gondii* antibodies, probably due to the reactivation of a latent infection. This finding was not observed in our study, as well the coinfection by *Leishmania*, *Toxoplasma*, and *Neospora*, which suggests a good specificity of this technique to diagnose these parasites minimizing the possibility of cross-reactions.

Among the 11 seropositive cats, 13.1% (5/38) received homemade food with commercial cat food and 27.2% (6/22) received homemade food and raw milk. The 22 animals from the countryside ingested viscera of livestock eventually slaughtered in the property and 27.2% of them were positive for *T. gondii*. On the other hand, animals fed exclusively with commercial cat food presented no immunoglobulins against *T. gondii* and *N. caninum*. This highlights the importance of adopting sanitary–hygienic measures for the preparation of meat by-products from the several livestock categories that can serve as a source of infection when improperly cooked (Dubey and Lindsay 2006) and handled (Millar et al. 2007), so that *Toxoplasma* may remain infectious even in bovine milk and cheese (Hiramoto et al. 2001).

Conclusion

Based on the current study, we can conclude that the infection by *T. gondii* and *Leishmania* spp. present in the cats was not associated with immunosuppression caused by the viruses; in addition, there was a trend of correlation between seropositivity by *T. gondii* and cat access to the external environment, the presence of rats, as well as diet type.

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