Serological survey of *Neospora caninum* and *Toxoplasma gondii* in shelter-housed cats infected with feline immunodeficiency virus, Brazil

Inquérito sorológico para Neospora caninum e Toxoplasma gondii *em um abrigo de gatos infectados com o vírus da imunodeficiência felina, Brasil*

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ABSTRACT

Felines play a leading role in the epidemiology of *Toxoplasma gondii* infection, but there is scarce information about the epidemiology of *Neospora caninum*, particularly in feline immunodeficiency virus (FIV)-infected cats. Cats seropositive to *T. gondii* do not usually show symptoms unless they are immunosuppressed, such as FIV-infected cats. The same relationship remains poorly known for *N. caninum*, although it has been associated with neurological disorders in HIV-infected people. Since FIV-infected cats are prone to develop encephalitis of unknown etiology, this study aimed to evaluate the presence of specific antibodies to *T. gondii* and *N. caninum* in a shelter for stray cats naturally infected with FIV. A total of 104 serum samples from cats living in a shelter, located in São Paulo city (Brazil), was assessed for *T. gondii* and *N. caninum* specific antibody by indirect fluorescent-antibody test (IFAT). Of the 104 cats, 25 (24%) were infected with FIV and, aside from these, 8 (32%) had antibodies against *T. gondii* (titers from 16 to 128). Only 1 (4%) of the FIV-infected cats had antibodies against *N. caninum*, which was the first record of coinfection. Among the FIV-naïve cats, 11 (14%) were positive for *T. gondii* (titers from 16 to 256) and only 1 (1.2%) had antibodies against *N. caninum*. Serologically positive reactions to *T. gondii* and *N. caninum* were not correlated with age or sex (p>0.05), and there was no correlation between FIV and the occurrence of anti-*T. gondii* or anti-*N. caninum* antibodies (p>0.05). Further studies encompassing larger cat populations from different origins and locations are essential to clarify the prevalence of *T. gondii* and *N. caninum* antibodies in FIV-positive cats.

Keywords: Neosporosis. Toxoplasmosis. Feline. FIV. Zoonosis.

RESUMO

Os felinos têm um papel importante na epidemiologia da infecção por *Toxoplasma gondii*, mas pouco se sabe sobre a epidemiologia da infecção por *Neospora caninum* em gatos, particularmente em gatos infectados com o vírus da imunodeficiência felina (FIV). Gatos soropositivos para *Toxoplasma gondii* geralmente não apresentam sintomas a não ser que estejam imunossuprimidos, como gatos infectados com FIV. A mesma relação ainda é pouco conhecida para *N. caninum*, embora tenha sido associada a distúrbios neurológicos em pessoas infectadas pelo HIV. Considerando que gatos infectados com FIV são propensos a desenvolver encefalite de etiologia desconhecida, o presente estudo teve como objetivo avaliar a presença de anticorpos específicos para *T. gondii* e *N. caninum* em gatos infectados com FIV. Um total de 104 amostras de soro de gatos residentes em um abrigo na cidade de São Paulo, Brasil, foram avaliadas para a presença de anticorpos contra *T. gondii* e *N. caninum* pelo teste de imunofluorescência indireta (RIFI). Dos 104 gatos, 25 (24%) estavam infectados com FIV e destes 8, (32%) tinham anticorpos contra *T. gondii* (titulação entre 16 e 128). Apenas 1 (4%) dos gatos infectados com FIV apresentava anticorpos contra *N. caninum*, sendo este o primeiro registro dessa coinfecção. Entre os gatos não infectados com FIV, 11 (14%) foram positivos para *T. gondii* (titulação entre 16 e 256) e apenas 1 (1,2%) tinha anticorpos contra *N. caninum*. A reação sorologicamente positiva para *T. gondii* e *N. caninum* não foi correlacionada com a idade ou sexo (p> 0,05), nem houve correlação entre FIV e ocorrência de anticorpos para *T. gondii* ou *N. caninum* (p> 0,05). Estudos subsequentes abrangendo populações maiores de gatos de diferentes origens e locais são essenciais para esclarecer a prevalência de anticorpos contra *T. gondii* e *N. caninum* em animais acometidos por FIV.

Palavras-chave: Neosporose. Toxoplasmose. Felino. FIV. Zoonose.

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Introduction

Neosporosis is caused by *Neospora caninum*, which is an obligate intracellular coccidian protozoan parasite with worldwide distribution. To date, dogs and some canid species are the definitive hosts to *N. caninum* (Dubey et al. 2011; Gondim et al., 2004; Lindsay et al., 1999). In the first description of natural exposure of domestic cats to *N. caninum*, performed in Brazil, antibodies to *N. caninum* were found in 60 (11.9%) out of 502 cats by the *Neospora* agglutination test (NAT), with titers ranging from 40 to 800 (Dubey et al., 2002).

Toxoplasma gondii is a parasite closely related to *N. caninum* (Dubey et al., 1988; Goodswen et al., 2013). Toxoplasmosis is a cosmopolitan anthropozoonosis affecting virtually all homeothermic animal species, including humans (Dubey & Lappin, 2006), and felines are the definitive hosts (Goodswen et al., 2013). Prevalence of *T. gondii* infection may vary with animal age and lifestyle, and it is greater in older cats or cats allowed outdoors (Dubey, 1994; Lappin, 2010). The latter occurs because free-roaming cats are exposed longer and tend to hunt small rodents, which serve as parasite reservoirs (Dorny et al., 2002; Lappin, 2010). Prevalence of anti-*T. gondii* antibodies in feline immunodeficiency virus (FIV)-infected cats have been well described (Akhtardanesh et al., 2010; Dorny et al. 2002; Lucas et al., 1998; Witt et al., 1989). *T. gondii* infected cats are usually asymptomatic unless immunosuppressed with FIV (Platt & Olby, 2013).

Natural or iatrogenic immunosuppression induced by exogenous corticoids may exacerbate lesions caused by neosporosis and toxoplasmosis (Anfray et al., 2005; Donahoe et al., 2015; Dubey, 2010; Dubey et al., 1990; Dubey & Lindsay, 1990; Kul et al., 2011). Although Lobato et al. (2006) found high seropositivity rates to *N. caninum* in human immunodeficiency virus (HIV)-infected patients, to date, there are few reports of coinfection between FIV-infected cats and *N. caninum* (Coelho et al. 2011; Munhoz et al. 2017). While shelters aim to be safe for animals (Galdioli et al., 2020), there is a lack of information regarding the occurrence of pathogens in cats living in these environments. In this sense, for a better understanding of the health status, in this study, we performed a serological survey of *N. caninum* and *T. gondii* in stray cats naturally infected with FIV in a shelter in Southeast Brazil.

Material and Methods

A total of 104 mongrel cats of both sexes and various ages were evaluated. The animals belonged to an animal shelter located in the city of São Paulo, Brazil. The cats were presumed healthy based on caretaker observations and physical examinations performed by veterinarians. The age of each cat was estimated during the shelter admission, on the physical examination, or by information provided by people who had previous and provisional ownership of the animals. Whole blood samples (2-4 mL) from all cats were collected into serum separator tubes and centrifuged at 12,000 g for 10 min. Serum was transferred into 1.5 mL microcentrifuge tubes (Eppendorf AG, Germany) and stored at -20°C before testing for FIV infection and indirect fluorescent-antibody testing (IFAT) for *T. gondii and N. caninum*. The samples were collected from October 2006 to December 2007.

Serological testing for FIV-infection was performed using a commercial test kit (Snap combo FeLV antigen/FIV antibody test kit, IDEXX Laboratories, Westbrook, Maine, USA). The tests were conducted and interpreted according to the manufacturer´s instructions. For the *T. gondii* survey, the serum samples were screened at 1:16 dilution. All sera positive at this dilution were subsequently submitted to serial dilutions up to 1:4096. For indirect fluorescent-antibody testing (IFAT) for *N. caninum*, the serum samples were screened at 1:50 dilution. The IFAT was an in-house assay similar to that used in prior studies to evaluate circulating *T. gondii* and *N. caninum* IgG antibodies (Camargo, 1974; Dubey et al., 1988).

Fisher's exact test was used to determine the statistical significance of the studied parameters. An unpaired t-test was used to compare the means of the ages. Differences in occurrence were considered as significant when $p \leq 0.05$.

The GraphPad InStat software (GraphPad Software, San Diego, California, USA) was used to assist calculations.

Results

Twenty-five (24%) of all evaluated cats were FIV infected, while 79 (76%) were negative. The mean age and standard error of FIV-infected cats were 6.3±2.52 years, and 5.2±2.65 years of the non-infected cats. There was no statistical significance between FIV infection and animal age or sex (p>0.05). Additionally, there was no correlation of FIV infection with IFAT positive samples for *T. gondii* and *N. caninum* (Table 1).

Antibodies to *T. gondii* were found in 19 (19/104, 18.3%) of the studied cats. Among the FIV-positive cats, 8 (8/25, 32%) had antibodies to *T. gondii*, and 11 (11/79, 14%) of the FIV-naïve cats had *T. gondii* antibodies with titers ≥16. However, antibodies to *N. caninum* were found in only 2 (2/104, 1.9%) cats. One of these cats was FIV-positive (1/25, 4%) and the other was FIV-negative (1/79, 1.2%) (Table 1).

Discussion

Serum samples were obtained from 104 stray cats living in a shelter in São Paulo city, Brazil. The prevalence of FIV infection in the present study was similar to that found in Australian cats (Norris et al., 2007), but higher than those found in the UK (3-11%) (Stavisky et al., 2017) and New Zealand shelters (13.7%) (Gates et al., 2017).

Since animals had been indiscriminately accepted to the shelter, without any serological testing for retroviruses (FIV and Feline Leukemia Virus), it was unclear whether the infection was spread among animals after confinement or whether animals had been already infected before being admitted to the shelter. Thus, studies on FIV transmission in shelters with this same profile are limited. Therefore, comparison with other studies is compromised (Akhtardanesh et al., 2010).

Although forms of *N. caninum* transmission among felids are unknown (Dubey et al., 2009), it is known that cats may get infected and develop neosporosis (Dubey et al, 1990, 2007). Such affirmations corroborate the studies that have

shown a serological prevalence of IgG class antibodies to *N. caninum* among felids (Bresciani et al., 2007; Dubey et al., 2002). The overall prevalence of *N. caninum* and *T. gondii* observed in this shelter was lower than previous studies conducted in Brazil for other shelters (Bresciani et al., 2007; Lucas et al., 1998; Pereira et al., 2018), free-roaming (Sousa et al., 2014), or even domestic cats (Meneses et al., 2014). Indeed, antibodies to *N. caninum* are rare among cats (Coelho et al., 2011; Dubey et al., 2009; Munhoz et al., 2017), although it has been found in a variety of species of wild felids (André et al., 2010).

Interestingly, Lobato et al. (2006) reported high *N. caninum* seropositivity rates in HIV-infected human patients. Yet so far no published studies are reporting the coinfection of FIV and *N. caninum* in cats. Additionally, our findings demonstrate an extremely low occurrence of *N. caninum* and *T. gondii* antibodies in FIV-infected cats. Such a small occurrence of *N. caninum* in our cat population may reflect its minor role in the epidemiology of neosporosis (Hornok et al., 2008). It is noteworthy to remark that although data were collected from serum samples of cats confined in a shelter, some were free-roaming cats removed from the streets and then introduced to the colony. Given that, our results may not represent the occurrence of *N. caninum* and *T. gondii* in a general population of naturally FIV-infected cats. Shelter animals normally do not follow hygienic-sanitary standards and could be more exposed to risk factors for toxoplasmosis and neosporosis than household animals.

Shelter medicine was formally recognized by the American Veterinary Medical Association as a specialty in 2014 (Galdioli et al., 2020). The basic preventative measures (in addition to the structural part relating to quarantine and isolation) consist of hygiene management, which is an important part of any health care plan, and infection control. When plans are implemented correctly, the introduction and spread of pathogenic organisms can, in most cases, be minimized or avoided (Galdioli et al., 2020).

Although the co-infection of FIV and *T. gondii* in cats has been already demonstrated (Chi et al., 2021), epidemiological and clinical data remain scarce. It is

Table 1 – Antibody titers to *N. caninum* and *T. gondii* in cats positive and negative to feline immunodeficiency virus (FIV) from a shelter in Sao Paulo, Brazil. The samples were collected from October 2006 to December 2007

IgG Titer	Positive samples/Total (%)					
	N. caninum	*T. gondii*				
	50	16	32	64	128	256
FIV Positive	1/25 (4.0)	1/25 (4.0)	4/25 (16.0)	2/25 (8.0)	1/25 (4.0)	0/25 (0.0)
FIV Negative	1/79 (1.2)	2/79 (2.5)	3/79 (3.8)	3/79 (3.8)	2/79 (2.5)	1/79 (1.2)
Total	2/104 (1.9)	3/104 (2.8)	7/104 (6.7)	5/104 (4.8)	3/104 (2.8)	1/104 (0.9)

believed that toxoplasmosis could have serious clinical implications in FIV-positive cats. FIV has been associated with immunosuppression syndromes since this virus presents tropism to lymphoid cells (Rivetti et al., 2008). In this regard, some studies have demonstrated a reduction of T lymphocytes CD4+ in domestic cats with prolonged FIV infections (about 25 months) (Rivetti et al., 2008). Thus, cats can present decreased ability to respond to T-dependent antigens, decreasing their humoral immune response (Chi et al., 2021).

Prophylactic measures for toxoplasmosis have been proposed to reduce the incidence of infections in felines and the subsequent elimination of oocysts in the environment. Considering that cats' predatory habits include intermediate hosts or mechanical vectors of *T. gondii* (*e.g.,* cockroaches, earthworms, and rodents), the confinement reduces the risk of infection by *T. gondii.* Moreover, since the prevalence of feline toxoplasmosis has been higher in countries where animals are fed raw meat products (Dubey & Lappin, 2006), cats should preferably be fed with commercially processed dry or canned food. Regarding neosporosis, there are only a few reports on naturally acquired infections, and literature about *N. caninum* in cats remains scarce (Bresciani et al., 2007; Hornok et al., 2008). Therefore, there is an urgent need for further research to elucidate the role of cats in the epidemiology of this disease, and to find out whether this parasite can affect the health of these animals (Feitosa et al., 2014).

Further investigations addressing the association between FIV and/or feline viral leukemia with opportunistic infections such as toxoplasmosis and neosporosis are desirable for a better understanding of the clinical impacts of these co-infections. Unfortunately, in this study, the shelter did not provide any information regarding the cats' arrival date and time of stay, which is considered crucial data in this kind of investigation. Lastly, considering that shelter medicine has recently emerged as a specialty that demands specific veterinary knowledge, more studies in this field are warranted.

Conclusion

The present study reports the presence of antibodies to *T. gondii* and *N. caninum* in FIV-infected cats sharing the same food and sanitary management and therefore equally exposed to risks of *N. caninum* and *T. gondii* infection. Further studies encompassing larger cat populations from different origins and locations are essential to clarify the prevalence of *T. gondii* and *N. caninum* antibodies in FIV-positive cats, to provide new data regarding the correlation of such infectious diseases for the area of medicine from domestic cat shelters.

Conflict of Interest

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Ethics Statement

The owner of the feline patient provided written informed consent for both diagnostic assessment and publication of this case report, with accompanying images.

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