

Serosurvey of *Toxoplasma gondii*, *Sarcocystis* sp. and *Neospora caninum* in geese (*Anser* sp.) from urban parks and captivity

Soroprevalência de *Toxoplasma gondii*, *Sarcocystis* sp. e *Neospora caninum* em gansos (*Anser* sp.) de parques urbanos e cativeiro

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Abstract

Geese, ducks, mallards, and swans are birds of the order Anseriformes, which are found in the wild, in zoos and parks, and raised for meat consumption. *Toxoplasma gondii*, *Sarcocystis* sp., and *Neospora caninum* are protozoans of several species of animals. Wild and domestic birds can serve as intermediate hosts, disseminators and potential sources of infection of these protozoa to humans through contaminated meat. The aims of this study were: (i) to perform a serological survey of *T. gondii*, *Sarcocystis* sp. and *N. caninum* in geese (*Anser* sp.) from public parks and from captivity and (ii) to compare seroprevalence between these two locations. Antibodies were detected by Immunofluorescence antibody test using the serum of 149 geese. Antibodies to *Sarcocystis* sp., *T. gondii*, and *N. caninum* were detected in 28.18%, 18% and 0.67% of geese, respectively; 57% of geese from urban parks and 26.53% of geese from captivity were seropositive for at least one protozoa. The results indicate environmental contamination, particularly for the occurrence of antibodies against *T. gondii* – a zoonosis that causes toxoplasmosis and is transmitted through oocyte ingestion. This is the first serological survey of *T. gondii*, *Sarcocystis* sp. and *N. caninum* in geese from urban parks in Curitiba, Brazil.

Keywords: Serology, IFAT, waterfowl.

Resumo

Gansos, patos, marrecos e cisnes são aves da ordem Anseriformes, encontrados em vida livre, zoológicos, parques e criados para consumo da carne. *Toxoplasma gondii*, *Sarcocystis* sp. e *Neospora caninum* são protozoários capazes de infectar diversas espécies animais. Aves domésticas e silvestres podem ser hospedeiras intermediárias e servir como disseminadoras e potenciais fontes de infecção para seres humanos por meio da carne. O objetivo do estudo foi 1) realizar a soroprevalência de *T. gondii*, *Sarcocystis* sp. e *N. caninum* em gansos (*Anser* sp.) provenientes de parques públicos e de um cativeiro e 2) comparar a soroprevalência entre os locais. Foi realizada sorologia de 149 *Anser* sp. pelo método da reação de imunofluorescência indireta. Anticorpos para *Sarcocystis* sp., *T. gondii* e *N. caninum* foram encontrados em 28,18%, 18%, e 0,67% dos animais, respectivamente; 57% dos gansos dos parques públicos e 26,53% dos animais cativos foram soropositivos para algum dos protozoários. A ocorrência de anticorpos para tais protozoários indica contaminação ambiental, ressaltando a alta prevalência de anticorpos para *T. gondii*, zoonose transmitida por ingestão dos oocistos. Sugere-se uma investigação da água e medidas ambientais para reduzir a contaminação dos animais e do ambiente. Este é o primeiro trabalho que avaliou sorologicamente gansos provenientes de parques urbanos de Curitiba, Paraná para *T. gondii*, *Sarcocystis* sp. e *N. caninum*.

Palavras-chave: Sorologia, RIFI, aves aquáticas.

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Introduction

Geese, ducks, mallards, and swans (Order: Anseriformes, Family: Anatidae) can be found in wildlife, zoos, parks, and they can also be raised in captivity for meat production. According to Corrêa (2007), waterfowl housed in the ponds of zoos and public parks are under constant risk of exposure to the pathogens of migratory birds that visit these places during a certain period every year. Monitoring both the animals and the ecosystem in areas where guest visiting occurs frequently is suggested by Chaskda et al. (2018). Due to birds' ability to fly and their wide variety of feeds, these animals have greater exposure to parasitic infections.

Toxoplasma gondii is an obligate intracellular parasite that infects mammals and birds (MOREÉ et al., 2018). Birds can be intermediate hosts for *T. gondii* and can become infected following the ingestion of raw meat of animals harboring tissue cysts, particularly given the cannibalistic or scavenger behavior of some carnivorous species; or after the ingestion of soil, water, or plant material contaminated with sporulated oocysts (TENTER et al., 2000; MURAO et al., 2008).

Birds are important to the biological cycle of *T. gondii* and to the epidemiology of toxoplasmosis. Poultry represent a potential risk of *T. gondii* infection to humans through the consumption of contaminated poultry products, in addition to their valuable role as sentinels since chickens are important hosts in *T. gondii* cycle, infecting animals through consume of their undercooked meat and for being resistant to clinical toxoplasmosis (GARCIA et al., 2000; DUBEY, 2010b). In the wild, birds can also be involved in the zoonotic cycle when used as a source of protein to feed felids and humans, and they may act as natural reservoirs for and sources of infection to other hosts and the ecosystem (VERMA et al., 2016).

Sarcocystis sp. is an obligate intracellular coccidian parasite that requires an intermediate and a definitive host (usually an herbivore and carnivore, respectively) and can affect several species of mammals and birds (EL-MORSEY et al., 2015). The genus is composed of more than 100 species (some of which are zoonotic) that differ in their levels of pathogenicity, host specificity, cyst structure and location (FRANCO et al., 2018).

Neospora caninum is an obligate intracellular parasite that can have domestic and wild canids as definitive hosts (GONDIM et al., 2010). In experimental infections, it has been found that Columbiformes may be susceptible to, and may serve as intermediate hosts of *N. caninum* (McGUIRE et al., 1999). Although the role of birds in the life cycle of *N. caninum* is unknown, the presence of birds has been correlated with outbreaks of abortion in cattle in Italy (OTRANTO et al., 2003), France (OULD-AMROUCHE et al., 1999) and Netherlands (BARTELS et al., 1999), suggesting that birds may be involved in the sylvatic cycle of the parasite being a mechanical vector or intermediate hosts (DONAHOE et al., 2015).

Sick birds usually mask any clinical signs of diseases (DONELEY, 2016), and they can act as reservoirs for and disseminators of pathogens given their ability to fly and migrate. Epidemiological studies in birds that share spaces with humans (captivity or free life) are extremely important when monitoring diseases with zoonotic potential as they can reduce emerging diseases and assist in biodiversity conservation through assessment of the risk that the microorganisms

may pose for the birds themselves (BRACONARO et al., 2015). Characterization of the microorganism and distribution of the disease are essential for formulating actions that aim to prevent infections, reducing the long-term complications and transmission of pathogens (IBRAHIM et al., 2018).

Serological surveys in Anseriformes are usually conducted in slaughterhouses (ABOULAILA et al., 2011; YANG et al., 2012; ZOU et al., 2017) or with migratory birds (PRESTRUD et al., 2007; SANDSTRÖM et al., 2013; VERMA et al., 2016); further studies are necessary and critical for assessing the prevalence of protozoan infections in other areas (MURAO et al., 2008). Serological surveys of *T. gondii*, *Sarcocystis* sp., and *N. caninum* in birds in Brazil are scarce, and there is no information about the seroprevalence of these protozoa in geese. Geese maintain contact with humans and migratory birds, and thus can serve as reservoir hosts and vectors of zoonotic pathogens (CORRÊA, 2007; YAN et al., 2011; CONG et al., 2012; VERMA et al., 2016).

Considering the important epidemiologic role these species may play disseminating these protozoa, the aim of this study is to determine the seroprevalence for *T. gondii*, *Sarcocystis* sp., and *N. caninum* in geese (*Anser* sp.) from urban parks. We also hypothesize that wild geese (from parks) are more exposed to protozoans than captive geese (kept in a conservationist institute). Therefore, the secondary aim of this study was to determine the seroprevalence of geese kept in captivity in southern Brazil and to compare the results obtained from animals from urban parks.

Materials and Methods

Animals

The study population comprised 149 geese (*Anser anser* and *Anser cygnoides*) caught between June 2017 until February 2018. One hundred from three public parks in the city of Curitiba, Brazil (52 from Barigui Park, 9 from Tingui Park and 39 from São Lourenço Park) and 49 from a conservational institute in São José dos Pinhais, which is the surrounding metropolitan area of Curitiba, Brazil.

Of the total sample, 130 geese were adults and 19 were young. The young were determined when the animal weight less than 2,5kg, had initial plumage and notched tips of tail feathers. The geese were captured by placing a net on the animal and submitted to individual physical examination (assessment of body condition, examination of eye, beak, oral cavity, feathers, wings and legs, palpation of the abdomen, cardiac and respiratory auscultation and search for ectoparasites). All captured animals were sampled and added to the study population. Blood samples were collected via medial metatarsal venipuncture using 5 mL sterile syringes with 25×7 mm gauge needles. After sampled, a plastic numbered leg band was placed on the left leg of each animal for further evaluation of migratory behavior. This study was approved by the animal ethics committee of the Federal University of Paraná (Protocol number 125/2016) and by the Parks and Squares Committee of Curitiba City Hall.

Parks and conservationist institute

Barigui Park (25°25'34.52 "S 49°18'27.03" W) has an area of 1,400,000 m² located on the banks of the Barigui River. Flocks of geese (*A. anser* and *A. cygnoides*) are commonly sighted cohabiting with ducks, caracaras, capybaras, preas, agoutis, opossums, and different species of passerines. These geese feed mostly on grass and food provided by park visitors (e.g., popcorn, corn, and bread).

Tingui Park (25°23'36.05 "S 49°18'21.71" W) is also located on the banks of the Barigui River and has an area of 380,000 m². Approximately 10 geese cohabit with a large diversity of birds, reptiles, and mammals and feed on grass and various foods provided by visitors.

São Lourenço Park (25°23'2.400"S 49°15'55.440"W) has an area of 204,000 m², it is located on the banks of the Belem River and is home to flocks of up to 70 individuals of geese (*A. anser* and *A. cygnoides*). Geese receive daily feeds of corn by park employees, but they also receive bread and popcorn from park visitors. A large amount of birds (raptors, ducks, galliformes, and passerines) and mammals are seen in the vicinity of the park.

The conservationist institute (25°35'17.624"S 49°10'49.184"W) is located in São José dos Pinhais, surrounding the metropolitan area of Curitiba, Brazil. It is home to several species of mammals and birds, including 49 domestic geese (*A. anser*). The geese are kept in two large open enclosures (approximately 3000m² each) containing artificial lakes, and they cohabit with ducks and swans. They have had their wings clipped and are fed on grass, commercial food, fruits, and vegetables. Due their monitored feeding and environment, they were considered as a "captivity control" in comparison to wild animals (from urban parks).

Laboratory testing

Blood samples were immediately conditioned in tubes without anticoagulant and transported at room temperature to the laboratory. Samples were centrifuged for 5 minutes at 5,000 rpm to obtain the serum and then stored at -20°C until serological analysis. Detection of specific *T. gondii*, *S. neurona* and *N. caninum* antibodies were carried out by an immunofluorescent antibody test (IFAT). Slides were prepared with tachyzoites of the *N. caninum* NC-1 strain, tachyzoites of the *T. gondii* RH strain, and merozoites of the *S. neurona* SN37R strain with carbonate-buffered glycerin (pH 9.5) and a coverslip. All the strains were from *in vitro* culture in Vero cells at the Laboratory of Veterinary Clinical Pathology of

Federal University of Paraná. Geese serum samples were diluted in phosphate buffered saline (PBS; pH 7.2) at a concentration of 1:25 (MARTINS et al., 2011), and conjugated anti-Duck immunoglobulin (Ig)G (KPL®, Milford, MA, USA) was used as a secondary antibody at 1:50 dilution. Dog sera positive for *N. caninum* and *T. gondii* were used to validate a positive control of positive geese sera, which was used for further analysis. A positive serum sample obtained from a bird of prey was used as a positive control for *Sarcocystis* sp. Only samples that exhibited fluorescence of the entire parasitic surface were considered to be positive (LOCATELLI-DITTRICH et al., 2006; RIBEIRO et al., 2016). Endpoint titers were determined by serial dilutions from 1:25 to 1:150.

Statistical analyses

Statistical analyses were performed using EZR 1.37 (KANDA, 2013), which is a graphical interface for R commander (The R Foundation for Statistical Computing, Vienna, Austria, version 3.4.4). Association between age (adult or young), collection point, and the presence of antibodies against each protozoa were analyzed using a chi-squared test. Statistical significance was set at $P < 0.05$ for all analyses.

Results

Antibodies to either *T. gondii*, *Sarcocystis* sp. and/or *N. caninum* were detected in 70 of 149 geese (Table 1). Antibodies to *Sarcocystis* sp. were detected in 42 samples, *T. gondii* in 27 samples, and *N. caninum* in one sample. Co-infection with *T. gondii* and *Sarcocystis* sp. was reported in 8% (12 of 149) of geese – one young and 11 adults.

Seropositive animals were found in all places where this study was performed. Antibodies to *N. caninum* were detected in only one animal, which was kept in captivity (conservationist institute) (Figure 1). The detection of *T. gondii* antibodies was significantly higher in animals from urban parks ($X^2=5.9303$; $P=0.015$) than in animals from captivity, although significant differences were not observed for neither *N. caninum* ($X^2=0.1336$; $P=0.715$) nor *Sarcocystis* sp. ($X^2=2.7932$; $P=0.095$).

Serum antibodies titers ranged from 1:25 to 1:150. Antibodies to *T. gondii* were detected at 1:25 titer in 25 animals (all locations) and at 1:50 titer in two animals from Barigui Park. Antibodies to *Sarcocystis* sp. were detected at 1:25 titer in 22 animals

Table 1. Occurrence of antibodies to *Toxoplasma gondii*, *Sarcocystis* sp., and *Neospora caninum* and their co-infection in geese (*Anser* sp.) from urban parks and captivity in Curitiba and metropolitan area, Brazil.

	Public Parks			Captivity
	Barigui (n=52)	Tingui (n=9)	São Lourenço (n=39)	Captivity (n=49)
<i>T. gondii</i>	13 (25%)	2 (22.2%)	9 (23%)	3 (6.1%)
<i>Sarcocystis</i> sp.	13 (25%)	7 (77.7%)	13 (33.3%)	9 (18.3%)
<i>N. caninum</i>	0	0	0	1 (2.04%)
Total	26 (50%)	9 (100%)	22 (56%)	13 (26%)
Co-infection*	5	2	4	1

n = total number of animals from each park and captivity). *Co-infection: Seropositivity for both *Toxoplasma gondii* and *Sarcocystis* sp.

(all locations), at 1:50 titer in 17 animals (all locations), at 1:100 titer in two animals (from captivity) and at 1:150 titer in one animal (from captivity). Antibodies to *N. caninum* were detected at 1:25 titer in one animal from captivity.

Age was not significant for the detection of *T. gondii* ($X^2=0.36151$; $P=0.548$), *N. caninum* ($X^2=5.9761e-30$; $P=1$), and *Sarcocystis* sp. ($X^2=0.0062$; $P=0.937$) antibodies. 63 adults and seven young animals were seropositive to at least one of the protozoa (Table 2).

Discussion

The occurrence of antibodies to *Sarcocystis* sp. and to *T. gondii* was 28% and 18% of all geese, respectively. The percentage of seropositive animals for *N. caninum* was low, accounting for 0.74% of the total population.

Antibodies to *Sarcocystis* sp. were detected in geese from all urban parks and from captivity. Most of the geese (90%) in

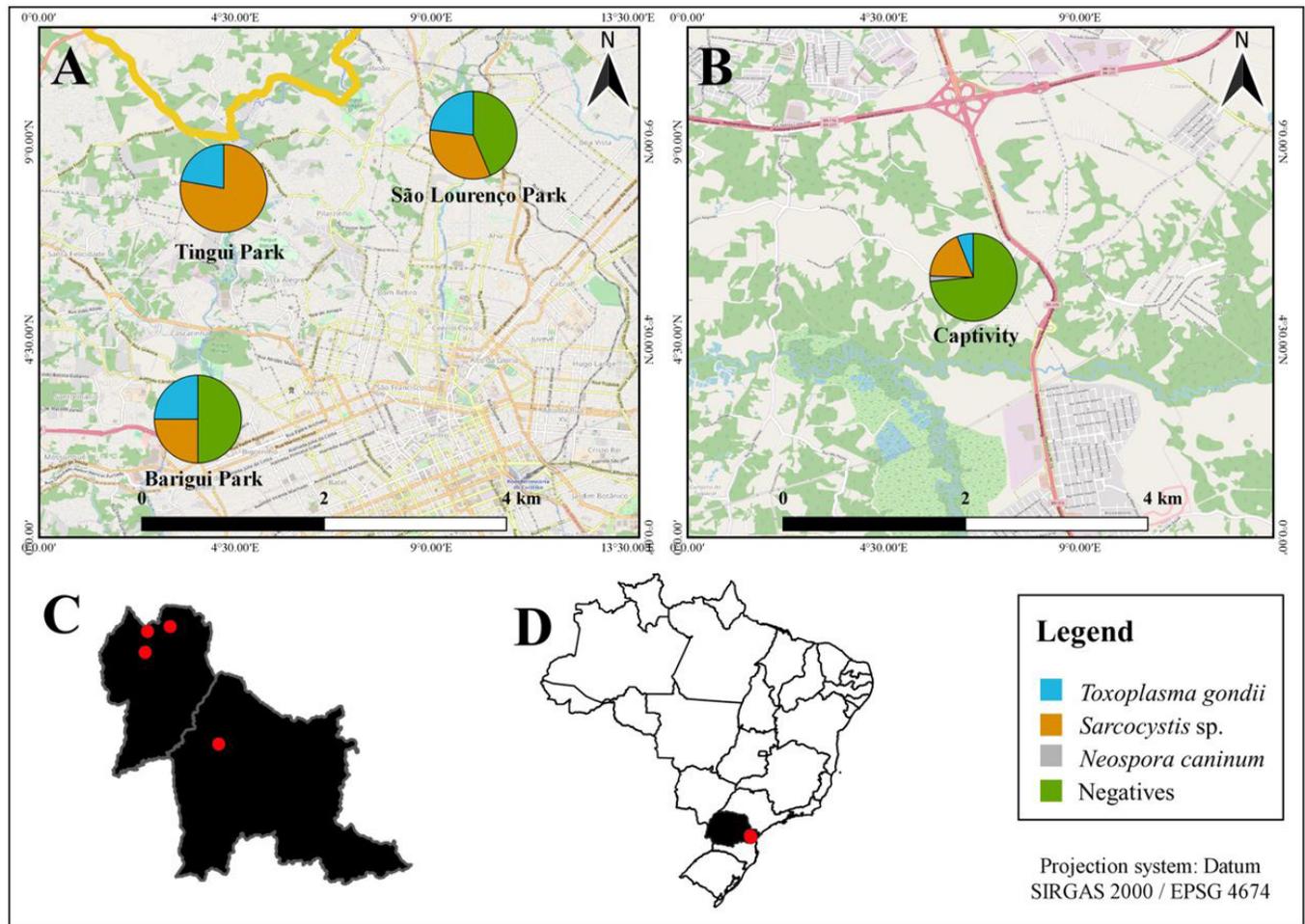


Figure 1. Map showing the location of the study area, including public parks and a breeding center in Paraná, Brazil, and the respective seroprevalence of *Toxoplasma gondii*, *Sarcocystis* sp., and *Neospora caninum* at each locality. (A) Location of public parks and the respective seroprevalence of *Toxoplasma gondii*, *Sarcocystis* sp., and *Neospora caninum*; (B) Location of the conservationist institute with seroprevalence of *T. gondii*, *Sarcocystis* sp., and *N. caninum*; (C) Map of Curitiba and the surrounding area, pinpointing the location of urban parks and the breeding center; (D) Map of Brazil showing the location of Paraná state and Curitiba city.

Table 2. Percentage and absolute numbers of young and adult geese seropositive to *Toxoplasma gondii*, *Sarcocystis* sp. and/or *Neospora caninum*, divided in parks or captivity from Curitiba and metropolitan area, Brazil.

Protozoa	Young	Parks	Captivity	Adult	Parks	Captivity
<i>T. gondii</i>	1 (5.6%)	1 (5.6%)	0	26 (20%)	23 (17.6%)	3 (2.3%)
<i>Sarcocystis</i> sp.	6 (31.5%)	6 (31.5%)	0	36 (27.6%)	27 (20.7%)	9 (6.9%)
<i>N. caninum</i>	0	0	0	1 (0.76%)	0	1 (0.76%)
Total	7 (36.8%)	7 (36.8%)	0	63 (48.4%)	50 (38.4%)	13 (10%)

which antibodies to *Sarcocystis* sp. were detected were considered healthy at clinical examination (without any changes in evaluated parameters). Four animals seropositive to *Sarcocystis* sp. exhibited nonspecific clinical signs at physical examination, such as cloacal prolapse, diarrhea, and the presence of crackles on chest auscultation. Clinical signs in *Sarcocystis* sp. infection are more evident in old-world psittacines, and they are usually related to neurological and respiratory disorders (CRAY et al., 2005; ARCA-RUIBAL, 2016).

Antibodies to *Sarcocystis* sp. were detected in geese from all urban parks and from captivity. Anseriformes are intermediate hosts of at least four species of *Sarcocystis*: *S. rileyi*, *S. anasi*, *S. albifrons*, and *S. wobeseri* (PRAKAS et al., 2014). However, there are no studies reporting the detection of antibodies to *Sarcocystis* sp. in geese. Antibodies to *Sarcocystis* sp. have been detected in psittacines either with no clinical signs or with nonspecific signs, suggesting that seroconversion did not represent an active infection in these birds but might be related to exposure to the parasite or subclinical infections (CRAY et al., 2005). Diagnosis of sarcosporidiosis is usually performed by post-mortem examinations (KUTKIENĖ et al., 2010, 2012; PRAKAS et al., 2014), and ante mortem examinations are performed by muscle biopsies (CRAY et al., 2005), being therefore underdiagnosed.

Due to the wide variety of *Sarcocystis* species, and the wide variety of definitive and intermediate hosts (FRANCO et al., 2018; PRAKAS & BUTKAUSKAS, 2012), it is difficult to identify the source of goose contamination. Waterfowl that inhabit and feed in shallow waters are more commonly infected than those that inhabit deeper waters (CHEN et al., 2015), suggesting that the infection occurs most commonly in shallow and marshy waters. This result indicates that the population of geese are exposed to *Sarcocystis* sp. in both public parks and captivity probably infected with sporocysts naturally excreted from original host.

The seroprevalence of *T. gondii* in geese in the present study was 18%. There are no previous reports of serological surveys of *T. gondii* antibodies in *Anser* sp. in Brazil. In Europe, seroprevalence ranges from 4.8%–43% (BARTOVÁ et al., 2009; MAKSIMOV et al., 2011; SANDSTRÖM et al., 2013) and from 4.7%–17% in Asia (MURAO et al., 2008; YAN et al., 2011; YANG et al., 2012; RONG et al., 2014). Geese populations from these studies comprised wild, domestic, and hunted animals.

Several serological tests have been employed to detect antibodies to *T. gondii* in anseriformes, such as enzyme-linked immunosorbent assay (ELISA) (MURAO et al., 2008; MAKSIMOV et al., 2011; IBRAHIM et al., 2018), direct agglutination test (DAT) (PRESTRUD et al., 2007), indirect hemagglutination (IHA) (HARFOUSH & TAHOON, 2010; RONG et al., 2014; NUNES, 2016), and modified agglutination test (MAT) (YANG et al., 2012; MANCIANTI et al., 2013; SANDSTRÖM et al., 2013; VERMA et al., 2016). However, there is still disagreement regarding the ideal method for detecting antibodies due to the variability of antigenic proteins, as well as differences in the sensitivity, and quality of commercial kits (SANTOS, 2012).

In two serological surveys of geese in Europe, IFAT was also employed: Maksimov et al. (2011) detected antibodies to *T. gondii* in 25.2% of samples and Bartová et al. (2009) detected them in 43% of samples. It is important to emphasize that these studies

were performed on animals from production farms, which are intended for meat consumption. This information is important since *T. gondii* is a zoonosis, the consumption of raw or undercooked meat is one of the main routes of infection (YAN et al., 2011).

Contamination of water by protozoa has also been acknowledged as an important source of infection. The presence of an aquatic environment is one of the few similarities featured in different studies that document seropositive animals from different environments and continents (BARTOVÁ et al., 2009). *T. gondii* oocysts were shown to be viable in the aquatic environment for several months (DUBEY et al., 2003; 2007); hence, surveys of waterfowl infection can serve as good indicators of water and environmental contamination (DUBEY, 2010a; WAAP et al., 2008). Infection of *T. gondii* possibly occurred due to the ingestion of sporulated oocysts in contaminated pasture and water. Oocysts are shed by definitive hosts (felines), as free-roaming cats have unconditional access to urban parks and they have also been sighted in the conservationist institute.

Waterfowls can be a source of toxoplasmosis infection to humans and domestic animals (MURAO et al., 2008), particularly in places where these animals are hunted and consumed, such as in Canada, Europe, and the United States. The presence of visible muscular cysts (macrocysts) is not common in geese, enabling the meat to be consumed without suspicion of contamination (WOBESER et al., 1981). According to park employees from the urban parks documented in this study, it is not unusual for geese to disappear from parks, which may be due to predation by animals and humans. A serological survey of *T. gondii* antibodies in capybaras from Tingui Park revealed the presence of antibodies to *T. gondii* in 62.5% of animals (TRUPPEL et al., 2010). In the present study, 22% of geese were seropositive for *T. gondii* and 77.7% were seropositive for *Sarcocystis* sp., endorsing the need to monitor environmental contamination in Curitiba's urban parks.

Nunes (2016) performed a serological survey of *T. gondii* antibodies in ducks (*Cairina moschata*) sent to a slaughterhouse in Pará, Brazil, and verified a seroprevalence rate of 16.28%, similar to the results of the present study (18%). According to Nunes (2016), the risk of infection is higher in animals raised in extensive systems, a fact that was also supported by the findings of our study, where we found seropositivity to *T. gondii* to be higher in animals from public parks.

The role of birds in the *N. caninum* cycle is still unknown. It has been suggested that they might participate as intermediate hosts, particularly since the report of an immune response to and the presence of parasite DNA in raptors and passerines (GONDIM et al., 2010; DARWICH et al., 2012; MOLINA-LOPEZ et al., 2012). Rocchigiani et al. (2017) reported seropositivity in 34.5% of ducks and mallards (*Anas* sp.) via IFAT in Italy, and suggested that birds that feed on pasture or water plants (like waterfowl) can ingest oocysts and contribute to the transmission of the parasite in the sylvatic cycle as a potential source of infection. In the present study, antibodies to *N. caninum* were detected in only one goose (from captivity). It is possible that the low occurrence of antibodies to *N. caninum* in this study might take place due to the low rate of seroconversion for *N. caninum* in *Anser* sp. or due to different types of feed.

There was no significant difference between the age of the animals and the detection of antibodies to any of the protozoa ($P>0.05$), however, Sandström et al. (2013) evaluated the occurrence of *T. gondii* in geese in the Arctic and in temperate areas and reported that seroprevalence increases with age, suggesting that these birds undergo rapid seroconversion. This author also suggests that juveniles must be sampled in greater numbers to directly link the site of infection with the environment; since the study population in the present study had few samples from young animals (13% of total), further studies are encouraged to evaluate seroconversion in juvenile geese exposed to urban parks in Curitiba.

In conclusion, we observed that wild animals have a higher occurrence of antibodies to *T. gondii* than animals kept in captivity. Waterfowl are considered good indicators of water and environmental contamination (DUBEY et al., 2012; WAAP et al., 2008), suggesting that urban parks are more contaminated with oocysts in water and soil. *Anser anser* and *A. cygnoides* presented with high seropositivity to *T. gondii* and *Sarcocystis* sp., indicating they may have been exposed to these protozoa in urban parks, and that they may serve as a potential source of infection to other animals. This is the first serological survey of *T. gondii*, *N. caninum*, and *Sarcocystis* sp. in geese from urban parks.

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