









Research for anti - *Toxoplasma gondii* antibodies in free-living ducks (*Cairina moschata*) from Rio de Janeiro, RJ, Brazil

[Pesquisa de anticorpos anti - *Toxoplasma gondii* em patos (*Cairina moschata*) em vida livre do Rio de Janeiro, RJ, Brasil]

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ABSTRACT

Toxoplasmosis is a zoonosis determined by the protozoan *Toxoplasma gondii* with wide geographic distribution, which affects homeothermic animals, with felids being their definitive hosts and other mammals and birds being their intermediate hosts. Regarding the Latin American bird species *Cairina moschata*, there are few reports in Brazil of the exposure of these animals to this protozoan. Based on the above, this study aimed to report for the first time the exposure of *C. moschata* to infection by this parasite in Rio de Janeiro, Brazil, determining the serological frequency of anti-*T. gondii* antibodies in birds of this species. Sixty-seven specimens of *C. moschata* were captured in the vicinity of the Rio de Janeiro Zoo, and these animals at the time of capture were sorted by gender and type of feather. Blood samples from these animals were obtained by venipuncture to obtain the serum. The serum samples were subjected to IFAT for anti-*T. gondii* IgG. In general, the frequency of positive results was 58.21% (n=39), with a statistically significant association (p<0.05) between the animals with adult plumage and seropositivity. The exposure of ducks to *T. gondii* in the vicinity of the Zoo may indicate contamination of this area with protozoan oocysts.

Keyword: duck, *Toxoplasmosis*, serology, IFAT, zoo

RESUMO

Toxoplasmose é uma zoonose determinada pelo protozoário Toxoplasma gondii de ampla distribuição geográfica, que acomete animais homeotérmicos, sendo os felídeos os seus hospedeiros definitivos, e outros mamíferos e aves os seus hospedeiros intermediários. Em relação à espécie de ave latino-americana Cairina moschata, há poucos relatos no Brasil da exposição desses animais a esse protozoário. Mediante o exposto, este estudo teve como objetivo relatar pela primeira vez a exposição de C. moschata à infecção por esse parasito no Rio de Janeiro, Brasil, determinando a frequência sorológica de anticorpos anti-T. gondii em aves dessa espécie. Foram capturados, nos arredores do Zoológico do Rio de Janeiro, 67 espécimes de C. moschata, sendo esses animais, no momento da captura, triados pelo gênero e pelo tipo de pena. Amostras de sangue desses animais foram obtidas por venopunção para obtenção do soro. As amostras de soro foram submetidas à RIFI para pesquisa de anticorpos IgG anti-T. gondii. De forma geral, a frequência de positividade evidenciada foi de 58,21% (n=39), sendo observada associação estatística significativa (P<0,05) entre animais com plumagem de adultos e soropositividade. A exposição dos patos a T. gondii nos arredores do zoológico pode indicar a contaminação dessa área com oocistos do protozoário.

Palavra-chave: pato, toxoplasmose, sorologia, RIFI, zoológico

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INTRODUCTION

Toxoplasmosis is a parasitic zoonosis of worldwide distribution determined by the protozoan *Toxoplasma gondii*. This facultative heteroxene cycle protozoan has felids as its definitive hosts because they eliminate oocysts in their feces, while other mammals, including humans and birds, are their intermediate hosts (Amendoeira *et al.*, 1999; Hill *et al.*, 2005; Robert-Gangneux and Dardé, 2012). The transmission of this protozoan occurs from the ingestion of tissue cysts present in raw or undercooked meat, sporulated oocysts present in water and food contaminated by feline feces or by transplacental transmission of tachyzoites (Amendoeira, 1995; Tenter *et al.*, 2000; Montoya and Liesenfeld, 2004). In Brazil, the seroprevalence of *T. gondii* infection in the human population can vary from 21.5% to 97.4% (Souza *et al.*, 2010; Dubey *et al.*, 2012).

Like other bird species, those belonging to the Anatidae family, which includes individuals popularly known as ducks, drakes' bird, geese, and swans, are also susceptible to infection by *T. gondii*. Frequencies of *T. gondii* infection in birds of the Anatidae Family from different countries, such as Mexico, Czech Republic, Egypt, China, and Brazil, ranged from 1 to 50% (Alvarado-Esquivel *et al.*, 2011; Bártová *et al.*, 2009; El -Massry *et al.*, 2000; Literák and Hejlíček, 1993; Yang *et al.*, 2012; Konell *et al.*, 2019). Except for the outbreak in domestic ducks in the Argentine chaco in 1962, there are no reports of clinical toxoplasmosis in other duck populations (Boehringer *et al.*, 1962; Dubey, 2010). Similar to chickens, these birds can also be considered bioindicators of environmental contamination by *T. gondii* oocysts due to their habit of revolving the soil with their beaks in search of food (AbouLaila *et al.*, 2011; Ibrahim *et al.*, 2018). In addition, viable forms of the protozoan were recovered from tissue samples from birds of the genus *Anas* naturally infected in Egypt, *Anas platyrhynchos* experimentally infected in the Czech Republic and in birds of the Anatidae family in China, indicating the possibility of transmission of *T. gondii* to the populations with the habit of consuming the meat of these animals (Dubey *et al.*, 2003; Bártová *et al.*, 2004; Zhao *et al.*, 2015).

Little is known about the role of domestic anatids in the epidemiology and ecology of toxoplasmosis when compared to other bird species. Within this context, the species *Cairina moschata* is included, whose subspecies *C. moschata domestica* descends from the South American wild species found from Mexico to northern Argentina, including Brazil (Sick, 1997; Mattos Junior *et al.*, 2008; WikiAves, 2015). In Brazil, there are several seroepidemiological surveys on *T. gondii* infection in different taxa of birds, domestic and wild (Gondim *et al.*, 2010; Feitosa *et al.*, 2017; Camillo *et al.*, 2018; Gonçalves *et al.*, 2018; Konell *et al.*, 2019; Sato *et al.*, 2020). There are few reports of the exposure of *Cairina moschata* to *T. gondii* infection in the national territory (Ferraroni and Marzochi, 1980; Witter *et al.*, 2020). In view of the above, this study aimed to report for the first time the exposure of *C. moschata* to infection by this protozoan in Rio de Janeiro, Brazil, determining the serological frequency of anti-*T. gondii* antibodies in birds of this species.

MATERIAL AND METHODS

This study was carried out at the Rio de Janeiro Zoo Foundation (RIOZOO) from December 2015 to June 2016. At the time of the study, this institution had an area of 138,000 m² and approximately 1300 animals under human care composed of various species, including mammals, birds, reptiles, and amphibians of national and exotic fauna. The zoo in Rio is the oldest in Brazil (Barbosa *et al.*, 2020). In addition to the animals under human care in the domains of this institution, straying domestic cats, rodents, marsupials and birds circulated, including anseriformes of the Anatidae family standing out individuals from *Cairina moschata*, popularly known in Brazil as duck-of-the-bush or Muscovy duck (Pato-do-mato (*Cairina moschata*). WikiAves, 2015; International Union for Conservation Nature. 2018).

During this period, 67 specimens of ducks that lived freely around the Zoo were captured and kept in a temporary enclosure bounded by bars and tiles, without access to the public, for approximately ten months. Blood samples were collected by venipuncture of the ulnar vein by the responsible veterinarians at the Zoo and transferred to tubes without anticoagulant. A maximum volume of 2 mL of blood was

collected, depending on the size and weight of the animal. During the collection of blood samples, the birds were inspected to categorize them by gender and type of feather. In this study, adult plumage or young feathers presence was used as a longevity parameter, since they are free-living animals of unknown age. For that, the body morphology was observed, as well as the commitment from each individual. This information was stored in the laboratory's database.

Blood samples were sent to the Toxoplasmosis and other Protozoan Diseases Laboratory at the Oswaldo Cruz Institute/Fiocruz in isothermal boxes. To obtain the serum, the blood samples were centrifuged at 3000 rpm for 10 minutes, transferred to previously identified 1.5 mL microtubes and stored at -20°C until the serological test was performed. Subsequently, the samples were subjected to the indirect fluorescent antibody test (IFAT) according to Camargo (1964). Tachyzoites of *T. gondii* strain RH kept in Swiss Webster outbred mice were used as antigens. For the detection of anti-*T. gondii* IgG, the commercial conjugate anti-duck IgG (H + L) antibody was used; Kirkegaard and Perry Laboratories - KPL® diluted in Evans Blue solution. Positive and negative control serum samples stored in the laboratory were used for each reaction. The reactions that showed total fluorescence of the surface of the tachyzoite at dilutions equal to or greater than 1:16 were considered positive (Millar et al., 2012). The statistical analysis of the data was performed using the statistical program GraphPad Prism 7.

Table 1. Distribution of the frequency of *Cairina moschata* sera reagents for *T. gondii* by indirect immunofluorescence (IFAT) according to gender and type of feather captured at the RIOZOO Foundation from December 2015 to June 2016

Variables		N°	IFAT %	P	OR (IC 95%)
Gender	Females	37	59.5	0.82	1.12 (0.42-2.97)
	Males	30	56.7		
Type of feather	Young Feather	25	32	0.0008*	0.17 (0.06-0.49)
	Adult Plumage	42	73.8		

*Significant association ($p < 0.05$); OR: *Odds Ratio*; IC: Confidence Interval

DISCUSSION

In general, the frequency of *Cairina moschata* positive for *Toxoplasma gondii* found in the present study was 58.2%. Lower frequencies were detected in different species of the Anatidae

To check the association between two categorical variables, Pearson's χ^2 test was performed. In assessing the impact between the variables gender and type of feather, the odds ratio (OR) values with their respective 95% confidence intervals (CI) were described. Associations where $p \leq 0.05$ were considered significant.

This study was approved by the Ethics Committee on the Use of Animals - CEUA / IOC-Fiocruz under license L-045/2016 and by the Biodiversity Authorization and Information System (SISBIO), Chico Mendes Institute for Biodiversity Conservation (ICMBio), Ministry of the Environment, under number 54797.

RESULTS

Of the total sera analyzed, 58.2% (39/67) were reagents for anti-*T. gondii* IgG on IFAT. Antibody titers ranged from 1:16 to 1:1024, with 20.5% (8/39) positive in 1:16 titration, 35.9% (14/39) in 1:64, 35.9% (14/39) in 1:256 and 7.7% (3/39) at 1:1024. Regarding the gender of birds, 59.5% of the females and 56.7% of the males had anti-*T. gondii* antibodies, with no significant difference between genders and the frequency of birds seropositive for *T. gondii* included in the study. For type of feather, the frequency of reactive individuals with adult plumage was significantly higher than that ducks with young feather. Ducks with young feather had a 0.71 times lower chance of exposure to *T. gondii* than adult plumage (Table 1).

family in the Czech Republic 24%, China 4.7% to 7.8%, and Malaysia 14.6%, as well as in *Anas platyrhynchos* (5.7%) in Germany and (10.5%) Egypt, in *Anser anser* (25.2%) in Germany and in birds of the genus *Anser* sp. (18%) in Brazil (Bártová et al., 2009; Maksimov et al., 2011;

Yang *et al.*, 2012; Puvanesuaran *et al.*, 2013; Ibrahim *et al.*, 2018; Konell *et al.*, 2019).

El-Massry *et al.* (2000) and Alvarado-Esquivel *et al.* (2011) detected seropositivity similar to this study, 50% in individuals of the family Anatidae in Egypt, as well as in *Anas platyrhynchos* and *Anas diazi* in Mexico. The difference in the frequencies detected in anatids seroreactive for *T. gondii* observed in the present study in comparison with the ones mentioned above may be due to the sample sizes included in them, as well as due to the susceptibility of different species of anatids to infection by the protozoan, including the etiological characteristics and physiological characteristics, environmental and geographical characteristics of these locations, serological test used in the study and its previously established cutoff point.

The high frequency observed in the population of *C. moschata* evaluated was already expected, considering that they were animals in free life. This correlation between free-living anatids and the high frequency of birds exposed to *T. gondii* has also been reported in China (Yang *et al.*, 2012; Zhao *et al.*, 2015). It is known that free-living birds, such as those included in the present study, end up presenting a greater chance of ingesting *T. gondii* oocysts in different environments (Millar *et al.*, 2012). It is important to note that *C. moschata* individuals cohabited the Zoo's surroundings with stray populations of domestic cats, which may have favored the parasite's prey-predator biological cycle. Even so, it cannot be ruled out that the small sample number of birds included may have contributed to the detection of high seropositivity. It is worth mentioning that the animals evaluated in the present study were free-living birds; thus, obtaining blood samples was dependent on the success of the capture, a situation not always achieved regularly, highlighting the importance and rarity of the information obtained with this biological material.

Despite the high frequency detected of *T. gondii* seropositive *C. moschata*, the birds evaluated in the present study did not show clinical signs suggestive of toxoplasmosis at the time of containment. A similar panorama was found with *A. platyrhynchos* in the Czech Republic, infected with *T. gondii* oocysts orally, who did not develop any clinical signs after 28 days of

infection, although they were seroconverted (Bártová *et al.*, 2004). The findings of the present study, added to what was exposed in the literature, indicate that these anatids, although susceptible to infection by *T. gondii*, can develop infections with an asymptomatic profile.

Most seroepidemiological surveys that assessed the exposure of anatids to *T. gondii* are restricted to countries where these animals are consumed by the human population, such as Southeast Asia and Egypt (El-Massry *et al.*, 2000; Dubey *et al.*, 2003; Yan *et al.*, 2009; Yang *et al.*, 2012; Zhao *et al.*, 2015; Ibrahim *et al.*, 2018). Similar to other homoeothermic animals intended for human consumption, anatids can harbor tissue forms of *T. gondii* in their meat. Thus, the consumption of raw or undercooked meat from these animals can represent an important risk factor for the human population that consumes them. In their free lives, anatids such as *C. moschata* in the present study can be prey for countless species of mammals and carnivorous birds. In addition, these anatids are generally territorial, but they fly in need of food and shelter, which may favor the exchange of genetic lines of *T. gondii* when chronically infected birds are preyed upon by felids.

Most of the studies that evaluated the frequency of anti-*T. gondii* antibodies was performed with anatids of the genus *Anas*, mainly in the species *A. platyrhynchos* (Dubey *et al.*, 2003; Bártová *et al.*, 2004; Alvarado-Esquivel *et al.*, 2011; Maksimov *et al.*, 2011; Ibrahim *et al.*, 2018). Only AbouLaila *et al.* (2011) in Egypt, among the recovered articles, previously reported the detection of anti-*T. gondii* antibodies in *C. moschata*, popularly known as duck-of-the-bush. In addition to the present study, the other two reports of *T. gondii* infection in *C. moschata* in Brazil are presented by Ferraroni and Marzochi (1980) and Witter *et al.* (2020). The first found anti-*T. gondii* antibodies in 40% of ducks evaluated in Manaus, northern Brazil, by indirect hemagglutination test (Ferraroni and Marzochi, 1980). More recently, Witter *et al.* (2020) detected the parasite DNA in tissues of free-living *C. moschata* from Midwestern Brazil. In this context, *T. gondii* infection in *C. moschata* should be further investigated in future studies seeking to assess the role of these birds as bioindicators of environmental contamination by oocysts and as an intermediate host in the

transmission cycle and in the epidemiology of toxoplasmosis in Brazil.

In the present study, high titers of anti-*T. gondii* IgG were detected by IFAT, up to 1:1024. In the study by Bártoová *et al.* (2009), anti-*T. gondii* antibody titers ranged from 1:40 to 1:320 in *Anas platyrhynchos* in the Czech Republic using the same technique. However, most other reports used the modified agglutination test (MAT) as a method for the detection of anti-*T. gondii* IgG (Dubey *et al.*, 2003; Yan *et al.*, 2009; Alvarado-Esquivel *et al.*, 2011; AbouLaila *et al.*, 2011; Yang *et al.*, 2012; Puvanesuaran *et al.*, 2013; Zhao *et al.*, 2015). The absence of a gold standard technique for the diagnosis of avian toxoplasmosis makes comparisons between the few studies carried out with these birds difficult.

From the results obtained, both genders of *C. moschata* that circulate around the RIOZOO Foundation are equally exposed to infection by *T. gondii*. A similar result was observed in *Anas platyrhynchos* from different provinces in Egypt (Ibrahim *et al.*, 2018). Regarding of adult plumage or young feather presence, the greatest exposure was found in birds with adult plumage. This result was already expected, considering that birds with adult plumage have a higher presumed age and, consequently, a greater chance of exposure to the possibly contaminated environment throughout their lives compared to individuals with young feather.

CONCLUSION

From this serological survey, it was possible to demonstrate that the *Cairina moschata* birds that circulated around the RIOZOO Foundation were exposed to infection by *T. gondii*, indicating the possible environmental contamination of this area with oocysts of the parasite, since they are birds with territorial behavior. This is the first report of *T. gondii* infection in *C. moschata* in the southeastern region of Brazil.

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