


Serological evidence of exposure to *Toxoplasma gondii* and *Neospora caninum* in free-ranging Orinoco goose (*Neochen jubata*) in Brazil

Evidência sorológica de exposição à *Toxoplasma gondii* e *Neospora caninum* em Ganso-do-Orinoco (*Neochen jubata*) de vida livre no Brasil

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Abstract

Toxoplasma gondii and *Neospora caninum* are Apicomplexan intracellular protozoan parasites that affect numerous animal species, thus leading to severe diseases and economic losses, depending on the vertebrate species involved. The role of the avian species in maintaining and transmission of these coccidia has been studied for several years as they tend to serve as a potential source of infection for mammals and humans. The present study aimed to assess the serological exposure of Orinoco goose (*Neochen jubata*) to *T. gondii* and *N. caninum*. Between 2010 and 2013, 41 free-ranging Orinoco geese were captured in the Araguaia River, Brazil. The presence and titration of IgY antibodies to both coccidia were assayed via indirect immunofluorescent antibody test (IFAT). While IgY antibodies for *N. caninum* were present in 5 animals, with titers of 20, the antibodies for *T. gondii* were found in 35 animals, with titers ranging from 20 to 640. Considering that the Orinoco goose's meat is consumed by the local population in the studied area, it may represent an important source of *T. gondii* infection for humans. Due to its migratory behavior, this goose may play a pivotal role in the natural dispersion of both parasites. Furthermore, molecular studies are required for genotyping the isolates of *T. gondii* that occurs in this avian species.

Keywords: Orinoco goose, serology, toxoplasmosis, neosporosis.

Resumo

Toxoplasma gondii e *Neospora caninum* são parasitas protozoários intracelulares do filo Apicomplexa que afetam uma vasta gama de espécies animais, causando sérias doenças e levando a perdas econômicas, dependendo da espécie envolvida. O papel das aves na manutenção e transmissão destes coccídios tem sido estudado por anos, já que eles são potenciais fontes de infecção para outros animais e humanos. O objetivo deste estudo foi avaliar a exposição do Ganso-do-Orinoco (*Neochen jubata*) a *T. gondii* e *N. caninum* por meio de técnicas sorológicas. Entre os anos de 2010 e 2013, 41 Gansos-do-Orinoco de vida livre foram capturados no Vale do Rio Araguaia, Brasil. A presença e titulação de anticorpos IgY para ambos os coccídios foi obtida utilizando-se a Reação de Imunofluorescência Indireta (RIFI). Enquanto a presença de anticorpos IgY para *N. caninum* foi detectada em 5 aves, com titulação 20, anticorpos para *T. gondii* foram encontrados em 35 aves, com títulos variando de 20 a 640. Considerando que a carne do Ganso-do-Orinoco é uma fonte de alimento para a população da área estudada, a ave pode representar uma importante fonte de infecção de *T. gondii* para humanos. Devido ao seu comportamento migratório, esta espécie assume grande importância na dispersão de ambos os parasitas. Estudos moleculares são necessários a fim de caracterizar genotipicamente os isolados de *T. gondii* que ocorrem nesta espécie de ave.

Palavras-chave: Ganso-do-Orinoco, sorologia, toxoplasmose, neosporose.

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Toxoplasma gondii and *Neospora caninum* are Apicomplexan intracellular protozoan parasites that affect various animal species (DUBEY, 1986; DUBEY et al., 1988). Both parasites are responsible for causing severe diseases, being an important cause of abortion, leading to economic losses in the production system (MASALA et al., 2003). Despite the morphological similarities, *T. gondii* and *N. caninum* present distinct biological properties (INNES & MATTSSON, 2007). The canids are the definitive hosts for *N. caninum* (MCALLISTER et al., 1998; DUBEY & SCHARES, 2011), which affect mainly cattle and dogs and are not considered as an important zoonotic agent; whereas, felines are the definitive hosts for *T. gondii* (DUBEY, 2009), which affect several warm-blooded animals, causing reproductive disorders in small ruminants and humans (DUBEY et al., 2002).

Domestic and wild felids play a pivotal role in the epidemiology of *T. gondii* infection as they produce and shed resistant oocysts responsible for the perpetuation of the environmental contamination (DUBEY, 2009). Similar to humans, animals can be infected horizontally, ingesting *T. gondii* tissue cyst from the preys or oocysts from the contaminated environment, as well as vertically, through transplacental transmission. Besides being a good indicator of the environmental contamination with parasite oocysts, birds can serve as a potential source of *Toxoplasma* infection for humans (BÁRTOVÁ et al., 2009). Additionally, toxoplasmosis has been associated with mortality in certain avian species, such as doves (*Columba livia*) and canaries (*Serinus canarius*) (DUBEY, 2002). Since the early 1900, *T. gondii* infected several avian species, showing the high prevalence of this parasite worldwide (DUBEY, 2002).

Neospora caninum has a limited host range compared to *T. gondii* and causes diseases in different host species (DUBEY & SCHARES, 2011). Distinct from *T. gondii*, *N. caninum* only recently has been investigated to cause infection in avian species, and few reports are available on natural infections, with immunohistochemistry and/or molecular assays demonstrating the presence of the parasite in brain and/or muscle of chickens (*Gallus gallus*), crows (*Corvus cornix* and *C. monedula*) sparrows (*Passer domesticus*), Red-and-green macaws (*Ara chloropterus*), Amazon parrots (*Amazona aestiva*), magpies (*Pica pica*), and common buzzards (*Buteo buteo*) (COSTA et al., 2008; GONDIM et al., 2010; MINEO et al., 2011; DARWICH et al. 2012; SALANT et al., 2015).

As the *Toxoplasma* and *Neospora* infections in avian have gained immense interest by researchers, the molecular and serologic tests have revealed that avian species play a pivotal role as intermediate hosts for both parasites. The employment of both techniques expanded the knowledge about the parasites' host range (DUBEY, 2002; BARROS et al., 2018). The present study aimed to detect antibodies for *T. gondii* and *N. caninum* in the serum samples of free-ranging Orinoco goose (*Neocheen jubata*).

This project was approved by the Ethics Committee on Animal Use of the School of Agricultural and Veterinarian Sciences (FCAV/Unesp) under the protocol number 012273/11 and by Institute Chico Mendes for Conservation of Biodiversity (ICMBio) under the permission number 21650-4.

In the years 2010 and 2013, during the molting period, when geese became flightless, 41 free-ranging Orinoco geese were captured in the Araguaia River, state of Goiás, Brazil (13°13'02.1"S 50°34'37.8"W). After the birds were manually restrained, whole

blood samples were collected in tubes without anticoagulant by ulnar vein puncturing. After that, the geese were released in the same places where they were captured. The samples were centrifugated for 5 minutes at 5,000 rpm to obtain serum, and then stored at -20 °C until further analysis.

In order to detect the IgY antibodies of *T. gondii* and *N. caninum*, the serum of each goose was subjected to indirect fluorescent antibody test (IFAT), as previously described (ANDRÉ et al., 2010). *Neospora caninum* NC-1 strain (DUBEY et al., 1988; MINEO et al., 2009) and *T. gondii* RH strain tachyzoites (DOMINGUES et al., 1998) were used as antigens in the serological reactions. Antigen slides were removed from storage and were allowed to thaw at room temperature for 30 min. Thereafter, 10 µL of sera at a dilution of 1:20 (cut-off for both *N. caninum* and *T. gondii*) were placed in wells on the antigen slides. Bird serum samples, positive and negative for *T. gondii* and *N. caninum*, obtained from the serum bank of the Laboratory of Immunoparasitology (VITALIANO et al., 2010; MINEO et al., 2009), Department of Veterinary Pathology of UNESP at Jaboticabal, SP, Brazil, were also used in the serological reactions. Slides were incubated at 37 °C in a moist chamber for 45 min, washed thrice in phosphate-buffered saline (pH 7.2) for 5 min, and were then air-dried at room temperature. Immunoglobulin G (IgG) anti-chicken conjugate (whole molecule with fluorescein isothiocyanate, dilution of 1:32; Sigma[®], St. Louis, Missouri) was diluted according to the manufacturer's instructions and added to each well. This conjugate has showed cross-reactivity with different IgY from several avian species (CRAY & VILLAR, 2008; JUSTIZ VAILLANT et al., 2013). The slides were incubated, washed and dried, as above described. After that, they were overlaid with buffered glycerin (pH 8.7), covered with glass coverslips, and examined using an epifluorescence microscope (Olympus, Japan).

Of the 41 serum samples of Orinoco goose analyzed via IFAT, 35 (85.3%) presented IgY antibodies for *T. gondii*, with titers of 20 ($n = 21$), 40 ($n = 10$), 80 ($n = 1$), 160 ($n = 1$), 320 ($n = 1$), and 640 ($n = 1$); whereas, 5 (12.1%) revealed IgY antibodies to *N. caninum*, with titers of 20. The five animals positive for *N. caninum* were also positive for *T. gondii*, with titers of 20 ($n = 3$), 40 ($n = 1$), and 160 ($n = 1$). None of the geese presented antibodies for only *N. caninum*.

Orinoco goose belongs to Anatidae family and it is widely distributed in South America. In Brazil, this species is present in central and Amazon regions (ENDO et al., 2014). These geese are terrestrial grazers that live in pairs or in families, joining big groups during the molt. They present migratory behavior, moving in an expressive longitudinal direction inside the Amazon Basin, and eventually reaching the Llanos de Moxos, in north Bolivia (DAVENPORT et al., 2012). The species is classified as *near threatened*, as the geese count is reducing due to the hunting pressure and habitat loss for livestock and husbandry (BRESSAN et al., 2009).

Herein, it was demonstrated that the sampled Orinoco geese presented antibodies against *N. caninum* and *T. gondii*, suggesting previous contact with the parasites. Antibodies against *N. caninum* have also been described in common ravens (*Corvus corax*) in Spain and Israel, with prevalence rates of 35,8% and 16,4%, respectively (MOLINA-LÓPEZ et al., 2012; SALANT et al., 2015), and in

ducks and mallards (*Anas* sp.) in Italy, with prevalence of 34% (ROCCHIGIANI et al., 2017). Antibodies against *T. gondii* have been previously described in geese and ducks in the Czech Republic with prevalence of 43% (BÁRTOVÁ et al., 2009), as well as in China, with prevalence ranging from 4.7% to 17% (YAN et al., 2011; YANG et al., 2012; RONG et al. 2014). In Brazil, several studies have been performed considering the serological exposure of avian species to *T. gondii* (GONDIM et al., 2010; GENNARI et al., 2014; FEITOSA et al., 2017) and *N. caninum* (summarized by BARROS et al., 2018), demonstrating that these agents are widely distributed among the native wild avian species, in both free-ranging and captive specimens. A survey performed in Brazil detected antibodies to *T. gondii* and *N. caninum* in captive and free-living geese (*Anser* sp.), with prevalence rates lower than that presented in the present study (18% and 0.67%, respectively, n=149). While the titers for *T. gondii* in that study (150) were also lower than those found in the present study, the titers for *N. caninum* were slightly higher (25) (KONELL et al., 2019). To the best of our knowledge, this is the first serological evidence of exposure to *T. gondii* and *N. caninum* in the Orinoco goose.

Avian species that feed directly from the soil, such as sparrows, chicken, doves, and geese, are exposed to infection by coccidial parasites, and may indicate the presence of *N. caninum* and *T. gondii* in the environment. Previous studies related to the presence of avian species on dairy farms with increasing seroprevalence and reproductive issues were associated with *N. caninum* infection in cattle, suggesting that these birds may play a pivotal role in parasite dispersion in the environment (OTRANTO et al., 2003). Moreover, certain avian species may be preyed by canids and felids, thus contributing to the transmission of both parasites (GONDIM et al., 2010). Due to its migratory behavior, the Orinoco goose may gain immense importance in studies exploring the natural dispersion of parasites.

The occurrence of neosporosis in avian species is less explored. While the clinical symptoms during natural infections have not been reported, the results of the experimental infections contribute to the present knowledge related to pathogenesis (BARROS et al., 2018). Although infection with *N. caninum* has been identified in several avian species via immunological and molecular techniques, to date, no viable parasites have been isolated (BARROS et al., 2018). This may be related to the higher body temperature of the birds since previous studies have reported that *N. caninum* tachyzoites were unable to grow *in vitro* at temperatures between 39 °C and 41.5 °C (REZENDE-GONDIM et al., 2017). In the present study, the prevalence of antibodies for *N. caninum* was lower than that found for *T. gondii*. This could be explained by the transient detectable antibodies, as previously described in crested caracaras (*Caracara plancus*) and chickens (*Gallus gallus*), experimentally infected with *T. gondii* and *N. caninum*, respectively, suggesting a different kinetics for the immune-humoral response in birds (FURUTA et al., 2007; VITALIANO et al., 2010).

Considering that the genomes of *T. gondii* and *N. caninum* present similarities in size and in the protein groups involved in biological functions (LORENZI et al., 2016), cross-reactivity may be observed in the serological tests. The IFAT revealed higher specificity when compared to the enzyme-linked immunosorbent assay (ELISA) during antibody detection for these parasites in

the dog sera (HIGA et al., 2000). In particular, the IFAT has been previously demonstrated to be specific for the diagnosis of neosporosis in mammals, with no cross-reactivity with *T. gondii*, using polyclonal antisera obtained from rabbits (DUBEY et al., 1996). Dilutions of 1:50 or higher have been found to be appropriate to avoid cross-reactivity between *N. caninum* and *T. gondii* in the dog sera (HIGA et al., 2000; SILVA et al., 2007) and humans (LOBATO et al., 2006) using IFAT. Noteworthy, the avian species reveals a different kinetics for the immune-humoral response, which may influence the antibody titers. Chickens and crested caracaras experimentally infected with *N. caninum* and *T. gondii*, respectively, presented peak IgG production between 15 and 30 days post infection, with negative antibody detection after 2 months of infection in chickens and 45 days of infection in the crested caracaras (FURUTA et al., 2007; VITALIANO et al., 2010). Pigeons (*Columba livia*) experimentally infected with *T. gondii* presented IgG profiles similar to those infected with *N. caninum*, although the antibody titers were higher in *T. gondii*-infected pigeons during acute phase (MINEO et al., 2009). In that work, the authors also reported the lack of serological cross-reactions between the antibodies for *T. gondii* and *N. caninum*, even at dilutions of 1:20 with IFAT (MINEO et al., 2009). In this study, we found five *N. caninum*-seropositive geese, using a cutoff of 1:20. Considering the short duration of antibody titers in the avian species, as demonstrated in previous experimental studies, whether a higher cutoff had been used, antibodies for *N. caninum* might not have been detected. However, it is noteworthy that a second serological test would confirm the evidence of serological exposure to *N. caninum* in the sampled Orinoco geese.

Although antibodies against *N. caninum* have been described in several hosts, including humans, the zoonotic potential remains unclear, since no reports are available on the clinical infections in humans. Presumably, the cases of human neosporosis have been misdiagnosed as toxoplasmosis, as the infection by *N. caninum* in healthy individuals may follow a course similar to that of *T. gondii*, with most infections being asymptomatic (TRANAS et al., 1999). Previously, the antibodies for *N. caninum* were detected predominantly in the HIV-infected patients and those with neurological disorders, whereas newborns and healthy subjects presented lower seropositivity rates (LOBATO et al., 2006).

EDTA-blood samples from the same geese of the present study have been previously analyzed by molecular assays targeting arthropod-borne agents, which showed the presence of genetic material of *Ehrlichia*, *Anaplasma*, *Babesia*, *Plasmodium*, and *Haemoproteus* (WERTHER et al., 2017). The coinfection with several parasite species might lead to impairment in the avian health, making them more susceptible to be preyed by a carnivorous animal, or caught by human.

It is known that the Orinoco geese are consumed as a food source by the local population. Although there could be several sources of *T. gondii* infection in humans residing in that area, a survey on the seroprevalence for *T. gondii* in the local population may be helpful in understanding the role of this anatid species in the transmission of toxoplasmosis in the local population. Toxoplasmosis is a zoonotic health issue, and therefore, the population should be aware of the risk of encountering this parasite when exposed to the infected avian species.

Presently, the molecular and serological studies have revealed that *N. caninum* and *T. gondii* have numerous intermediate hosts. Birds, in particular, may contribute to the dissemination of both parasites. Furthermore, studies on different avian species, with the isolation and molecular genotyping of these agents are needed, in order to understand the real importance of these birds in the epidemiology and ecology of the aforementioned diseases, thus aiming to control them and decrease their health and economic impact.

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