



## Technical Note

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# ***Toxoplasma Gondii* and *Neospora Caninum* Antibodies in Backyard Chickens in Rio Grande do Sul, Brazil**

## ABSTRACT

*Toxoplasma gondii* and *Neospora caninum* are two intracellular apicomplexan protozoa with worldwide distribution, and are responsible for reproductive disorders in sheep and cattle. These protozoa may infect a wide variety of domestic and wild animals, including birds, and backyard chickens can be used as sentinels of their infection. Parasites investigation in backyard chickens may be useful for the evaluation of environmental contamination with oocysts, of the disease cycle, and of risk factors associated with public health. The aim of this study was establish the importance of backyard chickens as *T. gondii* and *N. caninum* hosts. A number of 137 serum samples were collected from chickens in 23 farms in Rio Grande do Sul State, Brazil, and tested for toxoplasmosis and neosporosis by indirect fluorescence antibody test (IFAT). Anti-*Toxoplasma* and anti-*Neospora* antibodies were detected in 20 (87%) farms. Total prevalence of *T. gondii* was 74.4% (102/137) and 36.5% (50/137) for *N. caninum*, while 12.4% (17/137) of the chickens were positive for both protozoa. The results show that backyard chicken can used as indicators of the presenced of the protozoa *N. caninum* and *T. gondii*, emphasizing yours importance in the public health. Considering the high prevalence of toxoplasmosis in backyard chickens in the region, control measures should be taken to prevent transmission of the infection to the animals and humans.

## INTRODUCTION

*Toxoplasma gondii* and *Neospora caninum* are two apicomplexan protozoa and are biologically similar, and present worldwide, and cause reproductive failure in small ruminants and cattle (Dubey, 2002; Dubey & Schares, 2011). Toxoplasmosis is a parasitic zoonosis that affects a wide range of animals, including birds and man (Dubey, 2009). Neosporosis is important cause of abortion in cattle and has significant economic impact in the dairy and beef industries (Trees *et al.*, 1999). *T. gondii* infections are prevalent in many avian species and can cause mortality in some species of birds (Dubey *et al.*, 2010). *N. caninum* has been found in a few species of naturally-infected birds, and particularly in domestic chicken (Costa *et al.*, 2008) and in some wild birds (Darwich *et al.*, 2012). Chickens are intermediate hosts, especially those reared in backyard systems, where transmission occurs by the ingestion of oocysts from the soil that were shed by dogs and cats, which are the definitive hosts of *N. caninum* and *T. gondii*, respectively (Gondim, 2006; Dubey, 2009). For this reason, backyard chickens are considered indicators of the environmental contamination with oocysts (Furuta *et al.*, 2007; Dubey, 2009). In order to help understanding the role of chickens in the epidemiology these protozoa, the aim of the present study was estimate the seroprevalence of *T. gondii* and *N. caninum* in backyard chickens in southern Brazil.



## MATERIALS AND METHODS

Serum samples were collected from 137 clinically healthy backyard chickens from 23 farms in Rio Grande do Sul State, Brazil, between May and November 2011. Blood samples were collected by wing puncture. All samples were tested by indirect fluorescence antibody test (IFAT), for detection of *T. gondii* and *N. caninum* antibodies. The IFAT cut-off for *T. gondii* was 1:16 (Garcia *et al.*, 2000) and 1:50 for *N. caninum* (Costa *et al.*, 2008). The serum samples were diluted in phosphate buffered saline solution (PBS - 0.1M phosphate, 0.33M NaCl, pH 7.2), and positive and negative chickens for both protozoa were used as controls. IFAT was applied following the procedure described by Camargo *et al.* (2001). *T. gondii* and *N. caninum* tachyzoites, strains RH and NC-1 strains, respectively, were used as antigens, using . The commercial fluorescein-labeled anti-chicken IgY® (Sigma, St Louis, USA) was used as secondary antibody. Slides were read at x400 magnification under a fluorescence microscope (Leica CTR 4000/EBQ 100, Leica Microsystems, Germany). Samples with titers greater than 16 and 50 were considered positive for toxoplasmosis and neosporosis, respectively.

All procedures of animal handling and experimentation were performed under veterinary supervision and according to guidelines of Institutional Ethics and Animal Welfare Committee (UFSM, approved under protocol #009/2011).

## RESULTS AND DISCUSSION

Anti-*Toxoplasma* and anti-*Neospora* antibodies were detected in 20 (87%) of the 23 farms. In 19 (82.6%) out of the 23 farms, antibodies against *T. gondii* were detected, while anti-*N. caninum* were detected in 14 (60.9%) out of the 23 farms. In the backyard chickens tested, antibodies against *T. gondii* were detected in 74.4% (102/137) chickens and against *N. caninum* in 36.5% (50/137) chickens. Mixed infections were found in 17 out of 137 backyard chickens tested (12.4%), and in 13 (56.5%) of the 23 farms. Therefore, 85 (62%) and 33 (24.1%) of the serum samples were positive only for *T. gondii* and *N. caninum*, respectively.

Higher antibody titers against *T. gondii* compared with *N. caninum* were determined in the sera of the tested chickens. Studies in different regions of Brazil showed that the seroprevalence of *T. gondii* ranged between 38% and 66% in chickens (Dubey, 2009). *T. gondii* infection has been extensively reported in birds, but only a few reports in backyard chickens

showed their importance as hosts, which deserves further study (Tilahun *et al.*, 2013). As for *N. caninum*, Costa *et al.* (2008) and Martins *et al.* (2011) found 23.5% and 39.5% positive serum samples in backyard chickens respectively, indicating significant exposure to this protozoan. These results are epidemiologically important because chickens are cosmopolitan animals consumed by many animal species, including dogs and cats which are the definitive hosts of those parasites (McAllister *et al.*, 1998; Dubey, 2002).

Sources of infection for humans depend on local culture, geographical location, and eating habits differences, and *T. gondii* has been detected in different in various tissues of chickens (Tenter *et al.*, 2000; Dubey *et al.*, 2003; Dubey *et al.*, 2004). *T. gondii* can be isolated from infected tissues of backyard chickens, and therefore, these birds may contribute for the epidemiological characterization of strains on the protozoan present in the environment (Dubey, 2009). Moreover, meat of infected backyard chickens may be a source of *T. gondii* infection of humans and animals. The presence of free-range poultry on dairy farms presenting abortions caused by neosporosis has been considered a risk factor for *N. caninum* infection in some studies (Bartels *et al.*, 1999; Ould-Amrouche *et al.*, 1999).

The presence of antibodies against *T. gondii* and *N. caninum* in backyard chickens may be used as an indication of the contamination of the environment with oocysts. Those birds feed on the ground and tissues of infected chickens are considered a source of infection of others animals, such as dogs and cats, allowing the life cycle of this protozoan to be completed (Costa *et al.*, 2008; Dubey, 2009).

The presence of antibodies for both parasites indicates the environmental contamination of the evaluated farms. Although chickens can be naturally infected by these protozoa, mainly *T. gondii* (Dubey, 2009), further studies are necessary to define the role of chickens in epidemiology of *N. caninum*. Backyard chickens are raised in the region where farms were evaluated for meat and egg production, and these products are consumed the households. The birds are slaughtered at home or in uninspected slaughter facilities. Meat and viscera of infected backyard chickens may be an important source of infection of humans and animals when improperly handled or consumed (Dubey, 2009). Considering the results, biosecurity measures in backyard production are required to prevent human infection. In conclusion, the results of the present study suggest that backyard



chickens can be intermediate hosts of *T. gondii* and *N. caninum*, and their possible role in the maintenance of the life cycle of these protozoan. The results indicate a widespread exposure of backyard chickens to *T. gondii* and *N. caninum* in the state of Rio Grande do Sul, Brazil.

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