Comparative Immunology, Microbiology and Infectious Diseases 52 (2017) 30–35

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

# Serological survey of *Neospora caninum* and *Toxoplasma gondii* in cattle (*Bos indicus*) and water buffaloes (*Bubalus bubalis*) in ten provinces of Brazil

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## ARTICLE INFO

*Keywords:*
Buffalo
Brazil
Cattle
Neosporosis
Seroprevalence
Toxoplasmosis

## ABSTRACT

The objective of this study was to determine the prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* among 500 cattle (*Bos indicus*) and 500 buffaloes (*Bubalus bubalis*) using the indirect fluorescent antibody test (IFAT) technique. Blood samples from were collected from water buffalo and cattle in 10 municipalities in the northern region of Brazil. The frequency of cattle and water buffaloes seropositive for *Neospora caninum* in Pará state, Brazil, was 55% and 44%, respectively, and the frequency of cattle and water buffaloes seropositive for *Toxoplasma gondii* was 52% and 39%, respectively. Seropositivity for both *N. caninum* and *T. gondii* was detected in 10.6% of the cattle samples and 14.8% of the buffalo samples. The frequency of cattle positive for *N. caninum* and *T. gondii* was significantly ($p < 0.05$) higher than that of buffalo in two and three provinces, respectively. Buffaloes had a lower seroprevalence for *N. caninum* or *T. gondii* in all of the provinces studied. These results suggest that both species, when exposed to the same risks for *N. caninum* and *T. gondii* infection, have a high serological prevalence. Cattle showed a higher probability of being seropositive when exposed to the same risks for *N. caninum* and *T. gondii*. Our study, which included an extensive number of blood samples, provides important epidemiological information pertinent to buffalo production in tropical countries that can be used as a basis for disease-management practices in Latin America.

## 1. Introduction

Brazil has the largest herd of buffalo in Latin America and the largest commercial herd of cattle in the world [1]. These herds of cattle and buffalo are mainly located in the northern region of the country, where cattle constitute an important source of economic activity for the national meat industry and where buffalo are an important source of livelihood for the local communities, providing meat, milk, leather, and transportation for people and crops [1].

*Toxoplasma gondii* is the agent of a zoonosis detected worldwide and has developed several potential routes of transmission in different host species [2]. Specifically, *T. gondii* is a polyxenic parasite that is likely able to infect all warm-blooded animals, including humans [2]. Human infections are generally asymptomatic, but a severe form might occur in cases of congenital toxoplasmosis and in immunocompromised individuals. *T. gondii* infections can be acquired during pregnancy, when tachyzoites are vertically transmitted to the fetus via the placenta [2]. The horizontal transmission of *T. gondii* can involve three different life cycles: ingestion of oocysts present in the environment or shed by young or immunosuppressed cats or of tissue cysts or tachyzoites in meat or viscera from different animals [2]. Transmission can also occur via tachyzoites present in blood products, organ transplants, or unpasteurized milk. In addition, recent phylogenetic studies have shown that the *T. gondii* strains circulating among domestic animals and humans might be of the same genotype in Brazil [3].

*Neospora caninum* is the agent of a major infectious disease worldwide that causes abortions in cattle [4]. Experimental studies have shown that the domestic dog (*Canis lupus familiaris*), Australian dingo

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http://dx.doi.org/10.1016/j.cimid.2017.05.005
Received 24 November 2016; Received in revised form 4 May 2017; Accepted 8 May 2017

(*Canis lupus dingo*), and coyote (*Canis latrans*) are definitive hosts of *N. caninum* [5,6]. The vertical transmission of neosporosis was first recognized in dogs. *N. caninum* can be transmitted to neonates during the terminal stage of gestation or postnatally via milk [7]. Contrary to what occurs in cattle, the vertical transmission of *N. caninum* in dogs is highly variable and unlikely to persist in the absence of horizontal transmission [7]. In cattle, horizontal transmission is characterized by the absence of a relationship between dam and calf, by the presence of a group of animals of the same age that are positive for *N. caninum*, or by abortion outbreaks related to the parasite [8–10]. In herds in which vertical transmission is dominant, positive animals are clustered in certain families [11], in which transmission can reach 95% [12], because persistently infected cows show high rates of transplacental transmission [13,1,4]. In addition, the annual costs due to the losses caused by *N. caninum* are estimated to equal between $1.298 and $2.380 billion/year worldwide [4]. However, these estimates have not yet been studied in buffalo herds.

Although toxoplasmosis and neosporosis are widely studied in both cattle and buffalo, no study has evaluated these two herds as host species in the same area. The purpose of this study was to determine the frequency of antibodies to *T. gondii* and *N. caninum* among 500 cattle (*Bos indicus*) and 500 buffalo (*Bubalus bubalis*) in 10 municipalities in the state of Pará in the northern region of Brazil using the indirect fluorescent antibody test (IFAT) technique.

## 2. Materials and methods

### 2.1. Representative samples

A total of 500 cattle (*Bos indicus*) and 500 buffalo (*Bubalus bubalis*) blood samples were randomly collected in December 2011 from different farms in 10 municipalities in Pará state (Figs. 1 and 2) and simultaneously analyzed using the IFAT technique to determine the serological prevalence of *N. caninum* (Fig. 1) and *T. gondii* (Fig. 2), as demonstrated in Table 1. The number of samples required to assess the prevalence of *N. caninum* and *T. gondii* in cattle and water buffaloes was determined using the formula recommended by the Pan American Zoonosis Center (CEPANZO, 1979) for the study of chronic diseases: $N = p\ (100 - p)\ Z^2/(dp/100)^2$, where N is the number of samples, p is the expected prevalence, Z is the confidence level, and d is the error margin. The estimated prevalence of *N. caninum*- or *T. gondii*-infected buffaloes was 40%, and a confidence level of 95.0% and an error margin of 5.0% were established. We thus estimated that 500 samples should be analyzed.

The serological samples were collected from 18 farms located in six provinces of Marajó Island (Source, Salvaterra, Muaná, Chaves, Ponta de Pedra and Cachoeira do Arari; three farms per province) and eight farms in four mainland provinces (Curionópolis, Xinguara, Marabá and Rio Maria; two farms per province). Thus, sera from 320 and 180 cattle and 340 and 160 buffaloes on the mainland and island, respectively, were sampled.

### 2.2. Criteria for the selection of provinces and farms

The following criteria were used to select the provinces and farms included in this study: (1) provinces with at least 10 farms that kept cattle and buffaloes under the same management and in the same area, ensuring that these herds were at the same risk of being infected by the pathogens; (2) farms having both cattle and buffalo herds with more than 100 and less than 500 animals; and (3) farms with a recorded presence of domestic cats and dogs as well as rodents, wild canids, and felids. The farms were pre-selected during the 2010 vaccination campaign against foot-and-mouth disease. One thousand females with a mean age of 3 years from 28 farms, including 15–25 animals per farm, were selected for the study. The water buffaloes and beef cattle were pastured together, and all of the studied animals had been vaccinated against brucellosis (*Brucella abortus*) and foot-and-mouth disease (*Aphthovirus*) and annually tested negative for tuberculosis (*Mycobacterium bovis*). In addition, all of the animals were medicated with ivermectin (1 mg/kg, Merial, Brazil) every 3 months. The animals were raised extensively in large swamp areas and native grasslands along the river floodplains of Marajó Island, where the vegetation predominantly consists of floodplains and Amazon forest (rainforest).

### 2.3. Serological analyses

Sera from cattle and buffaloes were evaluated by an IFAT against the RH strain of *T. gondii* previously described by Camargo [15] and against the NC-Bahia strain of *N. caninum* previously described by Gondim et al. [16]. The cattle and buffalo serum samples were diluted 1:64 and 1:128 in phosphate-buffered saline (PBS pH 7.2, 130 mM NaCl, 2.7 mM KCl, 5.6 mM $Na_2HPO_4$, 1.0 mM $KH_2PO_4$, and 0.8 mM $NaH_2PO_4$). A 10-μL aliquot of the diluted serum was deposited in each well of slides containing *N. caninum* and *T. gondii* antigens. Sera from cattle that were known to be positive or negative for both pathogens were used as controls. The slides were incubated at 37 °C for 30 min in a moist chamber and subsequently washed in PBS (pH 7.2) and dried. Ten microliters of a conjugate (fluorescein isothiocyanate-labeled anti-bovine IgG) diluted as recommended by the manufacturer (catalogue N F7887, Sigma, St. Louis, MO, USA) was then added to each slide. The material was then incubated for an additional 30 min at 37 °C in a moist chamber. After further washing and drying, the slides were examined using a fluorescence microscope (E400 Eclipse, Nikon, Kawasaki, Japan). A positive reaction resulted in the observation of fluorescence, and the fluorescence of the samples was then compared to that of the positive and negative controls [15,17]. Two sera samples obtained from calves before they suckled colostrum were used as negative controls. Two cattle and two buffaloes that tested positive for *T. gondii* or *N. caninum* were used as positive controls in the serological assays.

### 2.4. Statistical analysis

An individual animal served as the unit of analysis. The serological results (positive or negative) in this study were the response variables for *T. gondii* and *N. caninum*. Chi-squared test with Yates' continuity correction was used to compare the seroprevalence values among and between animal species and provinces. The operational procedures were performed using StatCalc of Epi Info 7 (CDC, Atlanta, GA, USA, 2011).

### 2.5. Spatial analysis

The maps were produced using ArcGIS version 10.4 software (ESRI).

## 3. Results

The overall seroprevalence of *N. caninum* (Fig. 1) and *T. gondii* (Fig. 2) according to the IFAT results was significantly higher in cattle (52.0% and 54.4%, respectively) than in buffaloes (39.0% and 38.5%, respectively) (Table 1). However, the overall prevalence of *N. caninum* and *T. gondii* did not present significant differences within each species. Among the animals studied, 10.6% (53/500) of the cattle and 14.8% (74/500) of the buffaloes were seropositive for both pathogens.

The prevalence of *N. caninum* was higher in cattle (Fig. 1-B) than in buffaloes (Fig. 1-A) in the provinces of Chaves and Rio Maria. The prevalence of *N. caninum* in buffaloes was higher than that in cattle only in the province of Xinguara. The prevalence of *N. caninum* in buffaloes raised in the mainland was higher than that observed on Marajó Island (p = 0.001). No difference in the prevalence of *N. caninum* was found between the cattle from the mainland and that from Marajó Island. The prevalence of *T. gondii* in cattle (Fig. 2-B) was significantly higher than that in buffaloes (Fig. 1-A) in the provinces of Soure and Ponta de