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Neospora caninum serostatus is affected by age and species variables in cohabiting water buffaloes and beef cattle

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ABSTRACT

The aim of this study was to investigate how *Neospora caninum* serostatus may be affected by variables such as host species (water buffaloes or cattle) and age in animals cohabiting in the same ranch. A convenience cross-sectional study was performed on four ranches in the Northeast of Argentina, where water buffalo are cohabitating with beef cattle. Blood samples were collected from 1350 female water buffaloes (*Bubalus bubalis*) and 880 female beef cattle (*Bos taurus* and *Bos indicus* crossbreeds) from four ranches. Calving and weaning percentages at herd level for each ranch were also recorded. *N. caninum* antibody levels were measured by an indirect fluorescent antibody test (IFAT) (reciprocal antibody titers $\geq$100). Serological results were classified into 2 categories (0: negative; 1: positive). A logistic regression model was used to describe the relationship between *N. caninum* serostatus and specie (water buffalo or cattle), age or ranch and their interactions. Likelihood ratio tests were used to assess the significance of the model and their terms. Odds ratios were estimated and 95% profile likelihood (LR) and Wald confidence intervals (CI) obtained. Overall, specific antibody titers were found in 43.3% (584/1350) of water buffaloes and 28.6% (252/880) of cattle. Seropositive water buffaloes and cattle were observed on all ranches. Age was statistically significant ($p = 0.01$) with an overall estimate of logit (log odds) of age of 0.03 for both species. This indicates that for every one year increase in age, the expected change in log odds of being seropositive increased by 0.03. On three of four ranches a water buffalo was 4.48, 1.54 and 2.25 times more likely to be seropositive than cattle for animals of the same age. The *N. caninum* serostatus was affected by age in the first place, but also by species on at least three of the four ranches. Calving and weaning percentages were higher in water buffaloes than in beef cattle ($p < 0.05$). Even though the low pathogenicity that *N. caninum* seems to have in water buffaloes, this study reinforces the importance of this specie as maintenance of the disease.

1. Introduction

Neospora caninum is a coccidian parasite that causes costly disease in cattle (Reichel et al., 2013) and dogs worldwide (Dubey et al., 2007). Transplacental transmission appears to be the main mechanism by which the

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parasite persists in cattle (Anderson et al., 2000). Nevertheless, after recognizing the dog as the definitive host of the parasite (McAllister et al., 1998), epidemiological work has also established the association between the presence of dogs and the disease in cattle (Dubey et al., 2007). In addition, it has been established that intensive herd management may be associated with increased seroprevalence to *N. caninum* (Sanderson et al., 2000; Barling et al., 2000; Dubey et al., 2007). Many advances in the understanding of the epidemiology of *N. caninum* have been achieved in the last decades (Dubey et al., 2007), nevertheless, the frequency of postnatal transmission is not fully understood.

Evidence of *Neospora*-infections has been reported for many domestic and wild species including water buffaloes (*Bubalus bubalis*) (Dubey et al., 1998; Huong et al., 1998; Meenakshi et al., 2007; Nasir et al., 2011) where vertical transmission also occurs naturally (Chryssafidis et al., 2011). An increasing prevalence of antibodies to *N. caninum* with age was found in water buffalo populations from Argentina (Campero et al., 2007), southeastern Brazil (Fujii et al., 2001), southern Italy (Guarino et al., 2000) and Southern Peninsular India (Sengupta et al., 2012). It has also been demonstrated that this species is susceptible to experimental *Neospora*-infection causing fetal death (Konrad et al., 2012) and the occurrence of spontaneous abortions caused by *N. caninum* buffaloes has been recently reported (Auriemma et al., 2014).

Water buffaloes are better adapted to the wet tropical and subtropical environment than cattle and the water buffalo population is growing in many areas of the world (Food and Agriculture Organization, 2012) including the North East of Argentina (NEA) (Campero et al., 2007). Although a high seroprevalence to *N. caninum* in water buffaloes was previously described in the NEA (Campero et al., 2007), it is still uncertain whether neosporosis is a serious cause of reproductive failure in this species. The aim of this study was to investigate how *N. caninum* serostatus may be affected by variables such as species (water buffaloes or cattle) and age in animals cohabiting the same area. Also reproductive and productive parameters for water buffaloes and beef cattle at herd level are described.

2. Material and methods

2.1. Animals and sampling

A convenience cross-sectional study was performed on four ranches in the NEA, where water buffalo are cohabitating with beef cattle. Descriptive data about area, total number of animals and reproductive parameters including calving and weaning percentages at herd level for each ranch were recorded.

Blood samples were collected from a jugular vein from 1355 female water buffaloes (*B. bubalis*) and 880 female beef cattle (*Bos taurus* and *Bos indicus* crossbreeds). Samples were obtained at the end of the calving season and before the breeding season starts; March–April for water buffaloes and October–November for beef cattle. The age of the animals was also recorded at the moment of sampling and ranged from 2 to 19 and 3 to 8 years old for water buffaloes and beef cattle, respectively. The serum samples were kept at −20 °C until analysis.

2.2. Serological analysis

2.2.1. Parasites and antigen slide preparation

Antigen slides were prepared using tachyzoites of the NC-1 *N. caninum* strain kindly provided by Dr MC Venturini, La Plata Veterinary College, Argentina. They were grown continuously in stationary monolayer cultures of VERO cells as described previously (Konrad et al., 2012). Culture medium consisted of Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% (v/v) heat-inactivated adult equine serum, 2 mM-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin (DMEM-HS). Parasite-infected cultures were maintained in 75 cm^2 flasks incubated at 37 °C in an atmosphere of 5% CO_2. Parasites were harvested for antigen preparation when 80% of the VERO cells in the culture flask were infected with clusters of tachyzoites. The infected monolayer was removed from the flask by scraping into the medium and then passed 3 times through a 25-ga needle to disrupt the cells. The suspension was passed through a 5-μm filter to remove cellular debris, and tachyzoites were pelleted by centrifugation at 1300 g for 10 min. After removing the supernatant, the pellet was washed twice in sterile phosphate-buffered saline (PBS) (pH 7.2) and then resuspended in a modified PBS saline (137 mM NaCl, 3 mM KCl, 3 mM $Na_3C_6H_5O_7–2H_2$,O, 0.4 mM $NaH_2PO_4·H_2O$, 12 mM $NaHCO_3$, 6 mM glucose) to a final concentration of approximately 10^7 tachyzoites/ml. Aliquots of 10 μl of tachyzoite suspension were dispensed into each 4-mm well on 12-well heavy-teflon-coated antigen slides. Slides were air dried at room temperature (RT) and stored at −20 °C.

2.2.2. Indirect fluorescent antibody test (IFAT)

N. caninum antibody levels either from water buffaloes or beef cattle were assayed by IFAT as previously mentioned (Fujii et al., 2001). Briefly, antigen slides and sera were thawed at RT prior to use. The serum samples were tested at a dilution of 1:100 (Rodrigues et al., 2004). Ten microliters of diluted test or control sera were placed in separate wells on the antigen slides. Positive and negative control sera from buffalo were provided by Dr. S.M. Gennari, Veterinary College, São Paulo University, Brazil. Slides were incubated at 37 °C for 1 h in a moist chamber, washed 3 times for 5 min each in PBS, and then tapped gently to remove excess PBS. A polyclonal rabbit anti-bovine IgG labeled with fluorescein isothiocyanate (Sigma, St. Louis, MO) diluted 1:200 in PBS was added in 10 μl aliquots to each well. Slides were incubated at 37 °C for 30 min, washed 3 times with PBS for 5 min each, tapped to remove excess PBS, cover-slipped with buffered glycerol (25% [w/v] glycerine in Tris-HC1 [pH 9.0]), and examined at 200 x using a fluorescence microscope. The IFAT was considered to be positive when the typical peripheral staining pattern of the tachyzoites was observed.