

Prevalence and associated factors with *Neospora caninum* infection in female water buffaloes (*Bubalus bubalis*) from Pernambuco, Brazil

Prevalência e fatores associados à infecção por *Neospora caninum* em fêmeas bubalinas (*Bubalus bubalis*) em Pernambuco, Brasil

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Abstract

Neosporosis is one of the major parasitic diseases that causes reproductive problems, making it a limitation for optimal efficiency in livestock. The aim of the present study was to conduct a seroepidemiological survey for bubaline neosporosis and to analyse associated factors associated with *N. caninum* infection in female water buffaloes in the State of Pernambuco, northeast Brazil. The prevalence of *N. caninum* infection in the bubaline herds examined was 35.4% (122/345; 95% CI: 30.4%-40.7%), at least 1 *Neospora*-positive animal was found in each farm. Associated factors for *N. caninum* infection identified in this study were exclusive pasture feeding (OR: 5.91, $p < 0.001$) and purchase of animals of free fair and from reputable sellers (OR: 1.85; $p = 0.045$). In this survey, we document the occurrence of neosporosis in female water buffaloes. Associated factors should be controlled in order to reduce the risk of *N. caninum* infection in these herds.

Keywords: IFAT, neosporosis, seroepidemiology.

Resumo

A neosporose é uma das principais doenças parasitárias que causa problemas reprodutivos, torna-se possível limitar para a máxima eficiência na pecuária. Objetivou-se com este estudo realizar um inquérito soroepidemiológico e analisar os fatores associados à infecção por *N. caninum* em fêmeas bubalinas no estado de Pernambuco, Brasil. Observou-se uma prevalência de 35,4% (122/345; IC 95%: 30,4%-40,7%) para infecção por *N. caninum* e 100% das propriedades possuíam ao menos um animal positivo. Os fatores associados à infecção por *N. caninum* neste estudo foram alimentação exclusiva a pasto (OR: 5,91; $p < 0,001$) e aquisição de animais de feiras livres e criadores conhecidos (OR: 1,85; $p = 0,045$). Registra-se a ocorrência da infecção por *N. caninum* em fêmeas bubalinas e os fatores associados que precisam ser corrigidos para reduzir o risco de infecção nesses rebanhos.

Palavras-chave: RIFI, neosporose, soroepidemiológico.

Introduction

Water buffaloes (*Bubalus bubalis*) are rustic animals that are able to thrive in remote, difficult-to-access areas. Despite the tolerance of bubalines to difficult conditions and their resistance to a number of pathogens, they are still susceptible to some infectious and non-infectious diseases (ADLAKHA & SHARMA, 1992). The producer should therefore meet certain sanitary requirements in order to achieve higher reproductive rates, ensuring herd health,

and preventing the spread of pathogens, including the protozoan parasite *Neospora caninum* (CHRYSSAFIDIS et al., 2011). In buffaloes, this coccidium is one of the infectious agents that can impact animal productivity (CHRYSSAFIDIS et al., 2011), leading to reproductive losses such as abortions and recurrent estrus (CHRYSSAFIDIS et al., 2015).

In Brazil, buffaloes are bred for milk and beef, with a population of 1,970,941 million (IBGE, 2016). The northern part of the country accounts for the largest share of the Brazilian buffalo herd with 904,813 thousand head, followed by the Southeast, Northeast, South and Central-West regions. The third largest

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buffalo herd is in Northeast, with 10,437 thousand heads located in the state of Pernambuco (IBGE, 2016).

Many serological surveys on *N. caninum* infection in water buffaloes have been carried out by researchers in different parts of the world with prevalences ranging from 1.50 to 88.30% (BRASIL et al., 2015; CAMPERO et al., 2007; CHRYSALFIDIS et al., 2015; DUBEY et al., 1998; FUJII et al., 2001; FERROGLIO et al., 2003; GALVÃO et al., 2013; GONDIM et al., 2007; GUARINO et al., 2000; GENNARI et al., 2005; HUONG et al., 1998; HAMIDINEJAT et al., 2015; KONNAI et al., 2008; KONRAD et al., 2013; MEENAKSHI et al., 2007; MOORE et al., 2014; NASIR et al., 2011; NEVERAUSKAS et al., 2015; PORTELLA et al., 2016; RODRIGUES et al., 2005; ROMERO-SALAS et al., 2017; SOUZA et al., 2001; SENGUPTA et al., 2012; SILVA et al., 2010, 2014, 2017; VOGEL et al., 2006; YU et al., 2007).

However, few studies have reported the associated factors for *N. caninum* infection in buffaloes. According to studies carried out by Moore et al. (2014) and Nasir et al. (2011), the age of the water buffaloes and the presence of dogs in the premises are considered to be associated factors for the occurrence of neosporosis, respectively. *N. caninum* infection in the water buffalo species may be clinically silent and epidemiological studies should be carried out on a regular basis in order to characterize the dynamics of infection and identify associated factors for the occurrence of the disease. Such studies would help in the future to minimize production losses associated with neosporosis in bubaline herds (CHRYSALFIDIS et al., 2015).

Considering the scarcity of data and economic losses related to neosporosis in water buffaloes, the aim of the present study was to conduct a seroepidemiological survey for *N. caninum* infection in buffaloes and identify associated factors with positive serology in female water buffaloes in the State of Pernambuco, northeast Brazil.

Materials and Methods

Study area

Farms included in the survey were located in the counties of the Agreste and Zona da Mata mesoregion in the State of Pernambuco, northeast Brazil, where the largest water buffalo herds are found according to data provided by the Agriculture Defense of State of Pernambuco (ADAGRO).

Sampling

A cross-sectional study was carried out using a convenience, non-probability sampling technique. The sample size required for this seroepidemiological survey was estimated considering a bubaline population of 10,437 heads (IBGE, 2016) and an expected prevalence for *N. caninum* infection of 19.1% (BRASIL et al., 2015). Based on this data, a minimum sample size of 238 buffaloes was deemed necessary, considering a 95% confidence interval and statistical error of 5% (THRUSFIELD, 2004).

A total of 345 blood samples from of female water buffaloes in reproductive age, from different herds in the following counties of the State of Pernambuco were collected: Água Preta city (n = 90);

Canhotinho city: (n = 21); Maraial city (n = 23); Rio Formoso city (n = 48), and Ribeirão city (n = 163) (Figure 1). Total of 10 properties were sampled based on availability, by non-probability sampling, considering the number of females in reproductive age in each premise, using the aforementioned parameters.

Sample collection

Blood samples were collected without anticoagulant from the lateral coccygeal veins by venipuncture. Disposable needles (40 X 12 mm) with Vacutainer tubes were used. Each sample was identified and transported to the Laboratory in isothermal boxes. Serum was obtained from each blood sample after centrifugation at 300 rpm for 10 minutes and stored in labeled polypropylene microtubes at -20°C until the serological test was performed.

Serology

Search for anti-*N. caninum* antibodies on serum samples of water buffaloes was performed using the Indirect Fluorescence Antibody Test (IFAT) (DUBEY et al., 1998), using slides pre-sensitized with the NcSp7 strain, with fluorescein isothiocyanate-labeled anti-IgG bovine (Sigma, St. Louis, USA) (KENGRADOMKIJ et al., 2015). A cut-off value with the IFAT at 1:100 dilution was used (PORTELLA et al., 2016). IFAT slides were examined by fluorescence microscopy using an epifluorescence microscope (Olympus Bx 51, Olympus Inc., Tokyo, Japan) at 400x magnification. Samples were regarded positive when *N. caninum* tachyzoites exhibited complete peripheral fluorescence. Previously tested positive and negative buffalo serum samples were included in every slide as controls.

Associated factors with *Neospora caninum* infection

Associated factors were assessed using structured questionnaires, which included the following objective questions: production system (dairy, beef, dairy and beef); breeding system (intensive; semi-intensive; extensive); type of breeding system (open herd, closed herd); feeding system (grass, forage/creep feeding); consortium creation (yes; no); animal purchasing (independent seller, fairs/ independent seller); quarantine animals (yes; no); presence of wildlife in the farm (yes; no) and presence of dogs in the farm (yes; no).

Data analysis

To identify factors associated with *N. caninum* infection, a univariate analysis of the variables of interest was performed using the Pearson's chi-squared test or Fisher's exact test, when deemed appropriate. Subsequently, a logistic regression was performed considering the serological status of the animal (positive or negative) as the dependent variable. The independent or explanatory variables considered in the final model were those that presented statistical significance <0.05. This probability was stipulated to prevent that possible factors associated with *N. caninum*, were excluded from

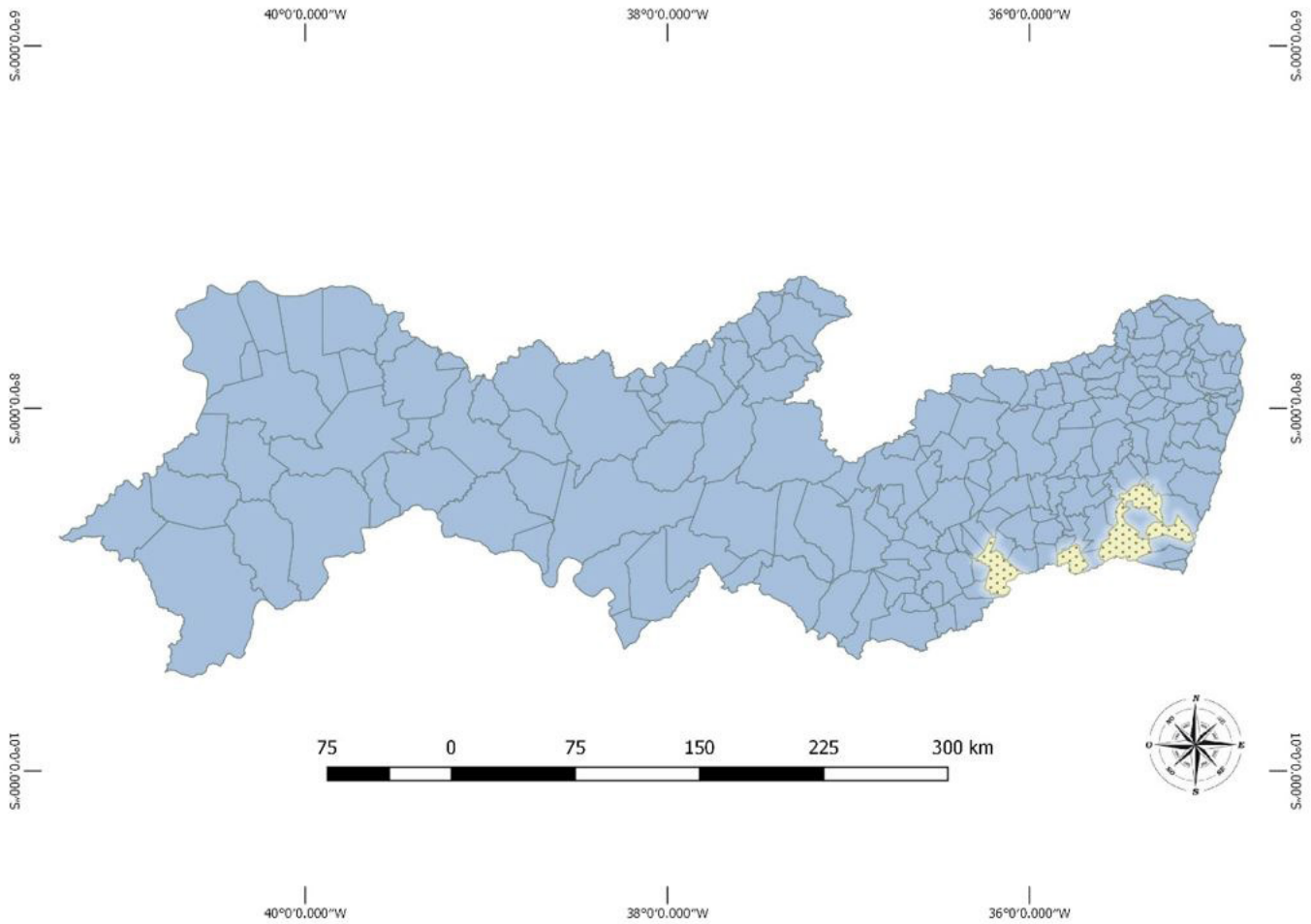


Figure 1. Distribution map of the municipalities sampled, located in the state of Pernambuco, Brazil.

the analysis. The Epi-Info™ 7.0 software was used to perform the statistical calculations (HOSMER & LEMESHOW, 1989).

Research ethics

The research proposal was approved by the Committee on Animal Research and Ethics of the University (license nº 104/2016).

Results

In the present study, the prevalence of *N. caninum* in female water buffaloes was 35.4% (122/345, C.I. 30.4% - 40.7%). There was at least one *Neospora*-positive animal in each farm. Prevalences of *N. caninum* infection in the bubaline herds examined ranged from 7.0% to 47.61%. The prevalence of anti-*N. caninum* antibodies by municipality is shown in table 1. When evaluating the occurrence of abortion, it was present in only one of the evaluated herds (1/10).

Associated factors with the positive serology for *N. caninum* infection in the female water buffaloes studied are shown in Table 2. Associated factors with the positive serology for *N. caninum*

Table 1. Anti-*Neospora caninum* antibody prevalence in female water buffaloes from the Agreste and Zona da Mata mesoregion of the State of Pernambuco, northeast Brazil.

City	IFAT				Total
	Positive		Negative		
	A.F.	R.F.%	A.F.	R.F.%	
Água Preta	39	43.3	51	56.7	90
Canhotinho	9	42.9	12	57.1	21
Maraial	4	17.4	19	82.6	23
Ribeirão	49	30.1	114	69.9	163
Rio Formoso	21	43.7	27	56.3	48

Acronyms: IFAT – Indirect Fluorescence Antibody Test; A. F. – absolute frequency; R. F. – relative frequency.

infection identified in the present study were: (1) water buffaloes' grazing (OR: 5.91, 95% CI: 2.60-13.39), and (2) animals of free fair and/or from reputable sellers (OR: 1.85, 95% CI: 1.01-3.39). In the present study, domestic dogs were present in 60% (6/10) of the bubaline farms studied. In the present study, domestic dogs were present in 60% (6/10) of the bubaline farms studied with seropositive buffaloes. The presence of domestic dogs

Table 2. Assessed factors associated with the positive serology for *N. caninum* infection in bubaline farms from the Agreste and Zona da Mata mesoregion of the State of Pernambuco, northeast Brazil.

Variable	N	IFAT Positive (%)	p value	Logistic regression		
				OR	CI 95%	p value
Production system						
Dairy	124	46 (37.1%)	<0.001	0.79	[0.48; 1.30]	0.372
Beef	181	61 (33.7%)		2.12	[0.92; 4.86]	0.074
Mixed (dairy/beef)	40	15 (37.5%)				
Breeding system						
Intensive	-	-	<0.000			
Semi-intensive	155	38 (24.5%)		1.48		
Extensive	190	84 (44.2%)			[0.88; 2.49]	0.138
Type of breeding system						
Open herd	252	95 (37.7%)	0.084	0.67	[0.40;1.13]	0.136
Closed herd	93	27 (29.0%)				
Feeding system						
Grass	279	115 (41.2%)	<0.001	4.50	[1.83; 11.03]	0.001
Forage/Creep feeding	66	7 (10.6%)				
Breeding partnership						
Yes	44	20 (45.5%)	0.092	0.61	[0.32; 1.16]	0.136
No	301	102 (33.9%)				
Animal purchasing						
From reputable sellers	295	98 (33.2%)		1.85	[1.01; 3.39]	0.045
Animals of free fair and from reputable sellers	50	24 (48.0%)				
Quarantine animals						
Yes	23	11 (47.8%)	0.143	0.57	[0.24; 1.34]	0.200
No	322	111 (34.5%)				
Presence of wildlife in the farm						
Yes	231	86 (37.2%)	0.180	0.77	[0.48; 1.25]	0.302
No	114	36 (31.6%)				
Farm dogs						
Yes	141	50 (35.5%)	0.532			
No	204	72 (35.3%)				

Acronyms: N – total number of samples analyzed; IFAT – Indirect Fluorescence Antibody Test; OR – odds ratio; CI – confidence interval.

was not deemed an associated factor with positive serology for *N. caninum* infection.

Discussion

To the authors' knowledge, this is the first study reporting the occurrence of *N. caninum* infection in female water buffaloes in the State of Pernambuco, Northeastern Brazil. In Brazil, the prevalence of *N. caninum* infection in water buffaloes varies from 14.6% to 88.0% (FUJII et al., 2001; SOUZA et al., 2001; GENNARI et al., 2005; RODRIGUES et al., 2005; VOGEL et al., 2006; GONDIM et al., 2007; SILVA et al., 2010, 2014, 2017; GALVÃO et al., 2013; BRASIL et al., 2015; CHRYSSAFIDIS et al., 2015; PORTELLA et al., 2016). Comparing results across seroepidemiological surveys carried out by different researchers is particularly challenging, as the methodology applied, including sampling and diagnostic tests can vary between studies, especially concerning IFAT cut-off values.

Environmental and climatic factors may also influence the prevalence of infectious and parasitic diseases in farm animals in different regions of the country (NASIR et al., 2011; KENGRADOMKIJ et al., 2015). Gondim et al. (2007) mentioned that seroepidemiological surveys for antibodies to *N. caninum* carried out in different parts of the world may vary according to the diagnostic assay used and geographic area where animals are raised.

In the present study, *N. caninum* infection was detected in all bubaline herds examined, with at least one animal from each farm was positive for *N. caninum*. From an epidemiological standpoint, these results raise concern over management practices and show wide dissemination of this protozoal disease throughout the Bubaline herds of the State of Pernambuco, Brazil. Some factors may have favored the high number of positive animals in each herd including intra-herd and inter-herd infection, geographic location and environmental contamination.

Intra-herd infection by *N. caninum* has been confirmed in water buffaloes by PCR. *N. caninum* DNA was detected in samples from aborted fetuses of naturally infected females (AURIEMMA et al., 2014; CHRYSsafIDIS et al., 2011). Other studies have demonstrated that vertical (transplacental) transmission can occur in bubalines when pregnant females are infected with a high infection dose of a highly pathogenic strain (isolate) and with a low pathogenic strains of *N. caninum* (KONRAD et al., 2012; CHRYSsafIDIS et al., 2014). Rodrigues et al. (2005) showed that the serum of female water buffaloes had detectable levels of anti-*N. caninum* antibodies for 12 months. The authors considered the possibility of maternal transfer of antibodies via milk in these animals that remained seropositive for prolonged periods.

There is limited data available in the veterinary literature regarding endogenous and exogenous transplacental infection in water buffaloes and the occurrence of persistently infected animals (REICHEL et al., 2015) and this warrants further research. In cattle, vertical (transplacental) transmission is the main route by which *N. caninum* is disseminated between animals. Infected animals are fundamental for the transmission and maintenance of *N. caninum* within seropositive herds (GOODSWEN et al., 2013).

Considering the inter-herd transmission factor, it was noted that the studied herds were composed of animals purchased from reputable breeders, and that the purchases and sales of animals were performed without knowing the *N. caninum* serological status of each of these individuals. Buying cattle from reputable breeders without knowing their sanitary status allows the movement of infected animals and the perpetuation of *N. caninum* epidemiological cycle (WILLIAMS & WINDEN, 2014). Asmare et al. (2013) and Beck et al. (2010) demonstrated that animals purchased without any sanitary records had a higher association with *N. caninum* infection.

In the present study, domestic dogs were present in 60% (6/10) of the bubaline farms studied. In the epidemiological chain of *N. caninum*, these animals are considered the definitive hosts of the protozoan parasite and oocysts are shed in canine feces, which contaminate the environment (DUBEY & SCHARES, 2011). A study carried out on water buffaloes by researchers in Pakistan showed that there is a significant association between the presence of dogs in the farms and the prevalence of *N. caninum* infection in the premises ($p < 0.05$) (NASIR et al., 2011). According to these authors, farm dogs contaminate the feed of water buffaloes resulting in increased prevalence of this protozoal infection. Our findings are corroborated by the results published by Nasir et al. (2011).

In our survey, water buffaloes' grazing was identified an associated factor for the occurrence of neosporosis and in 100% of the properties the source of water was rivers and/or stream. This finding reinforces the hypothesis of contamination of the environment by *N. caninum* oocysts which are shed in the feces of dogs and of transmission of the disease by the fecal-oral route. Horizontal (postnatal) transmission of *N. caninum* in water buffaloes occurs through ingestion of food or water contaminated with oocysts of the parasite (fecal-oral transmission) (REICHEL et al., 2015). The higher prevalence of *N. caninum* infection in older animals is due to the fact that these individuals are exposed over long periods of time to an environment contaminated with

oocysts of the protozoal organism (CAMPERO et al., 2007; MOORE et al., 2014).

Wetlands inhabited by water buffaloes are an adequate environment for the survival of *N. caninum* oocysts (KENGGRADOMKIJ et al., 2015). In order to assess the viability of *N. caninum* oocysts, Alves et al. (2011) confirmed the resistance of the infective form of *N. caninum* in the environment, from the evaluation of the viability of the oocyst under chemical and physical treatments.

The present study was carried out in water buffalo herds located in sugarcane producing areas and in the Agreste zone near the Atlantic Forest in Brazil. The environmental conditions in these sites favor survival and widespread dissemination of *N. caninum* oocysts. Bubalines stay submerged in large water collections many hours a day, which may favor *N. caninum* infection through ingestion of water contaminated with oocysts of the parasite (ABLAS et al., 2007).

In the present survey, only one water buffalo herd had history of abortion. In a study on bubaline neosporosis that was carried out by Gondim et al. (2007), the role of *N. caninum* as a possible cause of abortion was investigated. The presence of the protozoal infection in these animals was not associated with the occurrence of abortions as no abortions were reported in the water buffalo herds studied.

N. caninum can cause abortions in the water buffalo. However, the occurrence of abortions in cases of bubaline neosporosis may depend on the infecting dose (CHRYSsafIDIS et al., 2015). Sporadic abortions may go unnoticed in farms that are not under strict sanitary programmes. This partly explain the lack of reports of abortions (AURIEMMA et al., 2014; CHRYSsafIDIS et al., 2015; KONRAD et al., 2012). Another factor that may contribute to the lack of information on reproductive parameters in cases of bubaline neosporosis is the seasonal estrous activity of female water buffaloes (CHRYSsafIDIS et al., 2015).

We highlight the importance of assessing the serological status for *N. caninum* and the epidemiological indicators in bubaline herds, in order to determine the economic impact of this disease in water buffaloes from Brazil. Based on the results of the present study, we suggest that more research is needed on the association between *N. caninum* and pregnancy loss and other fertility problems in female water buffaloes. The *N. caninum* strains circulating in Brazilian bubaline herds should be isolated and identified for evaluation of their pathogenicity and virulence. Environmental samples should be tested for the presence of oocysts and the role of persistently infected animals in the transmission of the disease should be further investigated.

Conclusion

This study documents the occurrence of *N. caninum* infection in female water buffaloes in Pernambuco, Brazil. Associated factors with the positive serology for *N. caninum* infection should be controlled or eliminated in order to reduce the prevalence of *N. caninum* infection in these herds. Associated factors with the positive serology for *N. caninum* should be identified before a control plan is put into action.

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