Risk factors for *Toxoplasma gondii* and *Neospora caninum* seropositivity in buffaloes in Paraiba State, Brazil

Fatores de risco associados à soropositividade para *Toxoplasma gondii* e *Neospora caninum* em búfalos no Estado da Paraíba, Nordeste do Brasil

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Abstract

The aims of this survey were to determine the frequency of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies and to identify the risk factors associated with seropositivity among buffaloes in the state of Paraíba, Brazil. This survey included 136 buffaloes belonging to 14 herds. To detect anti-*T. gondii* and anti-*N. caninum* antibodies, the indirect fluorescent antibody test (IFAT) was used. Among the 136 samples analyzed, 17 (12.5%) were positive for anti-*T. gondii* antibodies with titers ranging from 64 to 1,024, and 26 (19.1%) for anti-*N. caninum* with titers from 200 to 1,600. Animals seropositive for both *T. gondii* and *N. caninum* were found in 10 of the 14 herds (71.4%). Semi-intensive management systems (odds ratio = 2.99) and presence of pigs (odds ratio = 4.33) were identified as risk factors for *T. gondii* and *N. caninum*, respectively. It can be suggested that *T. gondii* and *N. caninum* are widespread in buffaloes in Paraíba, and that additional surveys are needed in order to ascertain the importance of these agents for this species and for pigs, and the influence of the farming type on occurrences of seropositive animals.

Keywords: Toxoplasmosis, neosporosis, bubaline, seroepidemiology, control.

Resumo

Os objetivos deste trabalho foram determinar a frequência de anticorpos anti-*Toxoplasma gondii* e anti-*Neospora caninum* e identificar os fatores de risco associados com as soropositividades em búfalos do Estado da Paraíba, Brasil. Foram utilizados 136 búfalos oriundos de 14 propriedades. Para a detecção de anticorpos anti-*T. gondii* e anti-*N. caninum*, foi empregada a reação de imunofluorescência indireta (RIFI). Das 136 amostras analisadas, 17 (12,5%) foram positivas para anticorpos anti-*T. gondii*, com títulos variando de 64 a 1.024; e 26 (19,1%) para anticorpos anti-*N. caninum*, com títulos de 200 a 1.600. Das 14 propriedades, em 10 (71,4%) houve animais soropositivos tanto para *T. gondii* como para *N. caninum*. O manejo semi-intensivo (*odds ratio* = 2,99) e a presença de suínos (*odds ratio* = 4,33) foram identificados como fatores de risco para *T. gondii* e *N. caninum*, respectivamente. Sugere-se que *T. gondii* e *N. caninum* estão disseminados em búfalos do Estado da Paraíba, bem como a necessidade de mais estudos acerca da importância desses agentes nessa espécie e em suínos, e da influência do tipo de criação na ocorrência de animais soropositivos.

Palavras-chave: Toxoplasmose, neosporose, bubalinos, soroepidemiologia, controle.

Introduction

Buffaloes are known for their rusticity, tolerance and resistance to infectious and parasitic diseases, and are better adapted than cattle to adverse environmental conditions. Because they are versatile animals, they have become an excellent alternative for livestock farming in Brazil, since they have triple aptitude (milk production, meat production and traction), in addition to their easy adaptability to hostile environments, such as flood-prone lands, coastal areas, mountains and plains (NARDI, 2005).

Currently, Brazil has a prominent position within buffalo-rearing and has a headcount of more than a million animals, of which

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the Northeastern region accounts for around 122,000 animals. In the state of Paraiba, the buffalo headcount totals approximately 933 animals (IBGE, 2012).

Increasing productivity and technification of buffalo-rearing has favored introduction of infectious and parasitic diseases into the herds, as a result of changes in management, increased animal density and confinement. *Toxoplasma gondii* and *Neospora caninum* have not yet been shown to be important causative agents of abortions among buffaloes, but this possibility cannot be discarded. Investigations on the implications of these agents in relation to reproductive losses in this species are needed (GONDIM et al., 2007).

T. gondii and *N. caninum* are two morphologically related protozoa that present cosmopolitan geographical distribution. Both species may infect a broad range of animal species, including buffaloes. They have life cycles in which the definitive hosts are carnivore species and both of them cause abortions and congenital diseases in ruminants (DUBEY et al., 1988; DUBEY, 2003). Recent studies on occurrences of anti-*T. gondii* antibodies in buffaloes in Brazil have revealed rates ranging from 1.1% to 49.9% (SILVA et al., 2010; SOUZA et al., 2001). For *N. caninum*, high frequencies of antibodies in buffaloes were found in studies in the north (40.9%) (SILVA et al., 2010), southeast (64%) (FUJII et al., 2001) and northeast (35.9%) (GONDIM et al., 2007).

Because of the lack of studies on bubaline toxoplasmosis and neosporosis in the state of Paraiba, the present study aimed to determine the frequency of anti-*T. gondii* and anti-*N. caninum* antibodies in buffaloes in Paraiba, as well as to identify the risk factors associated with seropositivity.

Materials and Methods

Sampling

This study was carried out on 14 farmed buffalo herds in the counties of Alagoa Nova, Areia, Campina Grande, Guarabira, Juripiranga, Santa Helena, Sapé, Rio Tinto, Santana dos Garrotes, Itatuba, Solânea and Cacimbas (Figure 1). The study population was formed of bubaline females with an aptitude for meat and milk, of mixed breed and Murrah breed, with ages ≥ 24 months. To calculate the number of animals to be sampled, the formula (Equation 1) for simple random sampling (THRUSFIELD, 2007) was used:

$$n = \frac{Z^2 P(1-P)}{d^2} \qquad (1)$$

where:

n = number of animals to be sampled
Z = normal distribution value for the 95% confidence level
P = expected prevalence of 50% (for maximization of the sample)
d = error of 10%

In total, 136 bubaline females with ages ≥ 24 months were selected from these 14 herds. Blood samples were collected between November 2012 and July 2013, by puncturing the jugular vein, using a disposable needle and a vacuum tube (without anticoagulant) with capacity for 15 ml. At the time of the blood collection, an epidemiological questionnaire was applied in order to obtain data for use in the risk factor analysis. The variables and respective categories used were as follows: management system (intensive, semi-intensive or extensive); type of exploitation (meat, milk or mixed); type of milking (manual or mechanical); number of
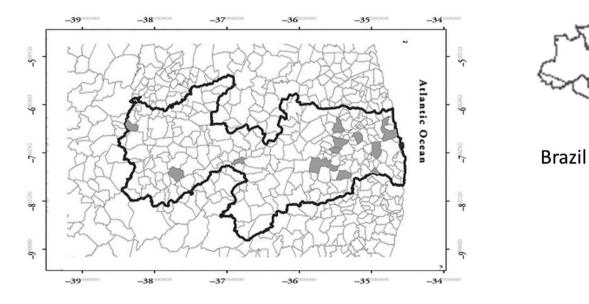

Figure 1. Geographical distribution of counties used. Detail shows the State of Paraíba – Brazil.

milkings per day (none, once a day or twice a day); presence of other animal species (cattle, horses, goats/sheep, pigs, poultry, dogs or cats); presence of wildlife (yes or no); occurrences of miscarriages during the last 12 months (yes or no); presence of rodents (yes or no); use of rodent control (yes or no); feeding on native pasture (yes or no); water source (drinking troughs or watering points); animal purchases (yes or no); pasture rental (yes or no); presence of flooded areas (yes or no), presence of maternity pens (yes or no); separation of young from adult animals (yes or no); and presence of veterinary assistance (yes or no).

Serological diagnosis

To detect anti-*T. gondii* and anti-*N. caninum* antibodies, the indirect fluorescent antibody test (IFAT) was performed at the Laboratory of Parasitic Diseases, Federal University of Campina Grande, Patos, PB, Brazil. For *T. gondii*, the technique was carried out in accordance with the methodology described by Camargo (1974), using tachyzoites of the RH strain as antigens and a cutoff point of 1:64 (SILVA et al., 2010). For *N. caninum*, the cutoff point was 1:200 (GONDIM et al., 2007; SILVA et al., 2010) and the technique was carried out in accordance with Paré et al. (1995), using an NC-1 sample of *N. caninum* cultured in bovine monocytes as the antigen.

Statistical analysis

Risk factor analysis was performed in two steps: univariable and multivariable analysis. Univariable analysis was performed using the chi-square test or Fisher's exact test, and variables that presented $P \leq 0.20$ were used for multivariable logistic regression. The multivariable analysis was then performed, using the stepwise forward method (HOSMER & LEMESHOW, 2000). Collinearity between independent variables was verified by a correlation analysis; for those variables with a strong collinearity (correlation coefficient > 0.9), one of the two variables was excluded from the multiple analysis according to the biological

plausibility (DOHOO et al., 1997). The significance level in the multivariable analysis was 5%, and the SPSS 20.0 software for Windows was used.

Results and Discussion

Among the 136 animals, 17 (12.5%) were seropositive for *T. gondii*, and the titers and respective frequencies were 64 (29.4%), 128 (29.4%), 256 (17.6%), 512 (11.8%) and 1,024 (11.8%). For *N. caninum*, 26 (19.1%) of the 136 animals were seropositive, with titers of 200 (19.2%), 400 (34.6%), 800 (30.7%) and 1,600 (15.3%). Animals seropositive for both *T. gondii* and *N. caninum* were found in 10 of the 14 herds (71.4%).

Tables 1 and 2 show the variables associated with seropositivity for *T. gondii* and *N. caninum* ($P \leq 0.2$), from the univariable analysis. In the final logistic regression model (Table 3), the semi-intensive management system was identified as a risk factor for toxoplasmosis (odds ratio = 2.99) and the presence of pigs was a risk factor for neosporosis (odds ratio = 4.33).

It is known that buffaloes are considerably resistant to *T. gondii* infection (DUBEY, 1988). However, surveys carried out in Brazil such as those of Souza et al. (2001) and Silva et al. (2013), in which the frequencies of antibodies were 49.9% and 36%, respectively, have suggested that there is a need for further studies on this parasitosis in buffaloes. In the present survey, the presence of antibodies was indicative of *T. gondii* circulation in buffalo herds in the state of Paraíba. This is a public health concern, given that in Brazil there has been an increase in the consumption of raw or undercooked beef, especially in areas with no federal inspection service (GUERRA et al., 2014).

The frequency of seropositivity for *N. caninum* among these buffaloes in the state of Paraíba was 19.1%, and serological surveys for *N. caninum* in buffaloes in Brazil have shown frequencies ranging from 40.9% (SILVA et al., 2010) to 49.9% (SOUZA et al., 2001). In cattle, neosporosis is considered to be the greatest cause of abortions, but its impact on buffaloes' reproductive sphere remains unclear. Given that there have been

Table 1. Results from the univariable analysis, showing the variables most associated ($P \leq 0.2$) with seropositivity for *T. gondii* in buffaloes, between November 2012 and July 2013, in the state of Paraíba, Brazil.

Variable	Category	Total number of animals	Number of positive animals (%)	*P*
Management system	Semi-intensive	32	7 (21.9)	
	Extensive	104	10 (9.6)	0.122
Production system	Beef	97	11 (11.3)	
	Dairy	20	5 (25)	0.143
		19	1 (5,3%)	
Presence of pigs	No	121	13 (10.7)	
	Yes	15	4 (26.7)	0.179
Use of rodent control	No	48	9 (18.8)	
	Yes	88	8 (9.1)	0.175
Access to watering points	No	58	4 (6.9)	
	Yes	78	13 (16.7)	0.149
Abortions during the last 12 months	No	109	11 (10.1)	
	Yes	27	6 (22.2)	0.106

Table 2. Results from the univariable analysis, showing the variables most associated (P ≤ 0.2) with seropositivity for *N. caninum* in buffaloes, between November 2012 and July 2013, in the state of Paraiba, Brazil.

Variable	Category	Total number of animals	Number of positive animals (%)	P
Management system System	Semi-intensive	32	11 (34.4)	
	Extensive	104	15 (14.4)	0.024
Production system	Beef	97	14 (14.4)	
	Dairy	20	8 (40)	0.029
		19	4 (21,1)	
Presence of pigs	No	121	18 (14.4)	
	Yes	15	8 (53,3)	0.001
Presence of goats and sheep	No	85	21 (24.7)	
	Yes	51	5 (9.8)	0.056
Animal purchases	No	84	11 (13.1)	
	Yes	52	15 (28.8)	0.041
Milk cooling	No	126	20 (15.9)	
	Yes	10	6 (60)	0.003

Table 3. Risk factors for *T. gondii* and *N. caninum* seropositivity among buffaloes, between November 2012 and July 2013, in the state of Paraiba, Brazil.

Risk factor	Odds ratio	95% CI	P
Toxoplasma gondii			
Semi-intensive management system	2.99	[0.99 – 8.98]	0.050
Neospora caninum			
Presence of pigs	4.33	[1.34 – 13.98]	0.014

reports of isolation of *N. caninum* in buffalo brains and fetuses in Brazil (RODRIGUES et al., 2004; CHRYSSAFIDIS et al., 2011), neosporosis needs to be investigated as a possible cause of abortions in this species.

It is believed that the low frequency of seropositive animals for both *T. gondii* and *N. caninum* compared with other surveys conducted in Brazil could be explained by the low occurrence of definitive hosts in the herds since in 60% of the positive herds for *T. gondii* there wasn't report of cats, and in 40% of the positive herds for *N. caninum* the presence of dogs was not reported (data not shown).

Surveys on risk factors for toxoplasmosis among buffaloes are scarce in Brazil. In the present work, the semi-intensive management system was identified as a risk factor, as also found by Albuquerque et al. (2011) for cattle. This can be explained by the greater chances of contact with oocysts in the environment when this system is used, probably due to the fact that such contacts are favored both in the confinement and in the extensive management system. In the semi-intensive system, the animals remain part of the day free in pastures and part of the day confined, and are thus exposed to both environments.

The presence of pigs was identified as a risk factor for occurrences of anti-*N caninum* antibodies in buffaloes in the state of Paraíba. Even though the presence of pigs does not has direct importance

with regard to transmission of this agent to buffaloes, Azevedo et al. (2010) reported findings of pigs positive for *N caninum* in Paraiba, using IFAT and immunoblotting with total and purified antigen (p38 – NcSRS2) of *N. caninum* tachyzoites. This suggests that complementarity of life cycles in the herds may exist when definite hosts are present. Therefore, studies aiming to isolate the agent in pigs and buffaloes are necessary in order to elucidate the real importance of neosporosis in these species.

The frequencies of buffaloes seropositive for *T. gondii* and *N. caninum* in the state of Paraíba, northeastern Brazil suggest that both of these agents are spreading in this region. From our risk factor analysis, it is suggested that further surveys on the importance of these agents in this species and in pigs are needed, as well as evaluation of the influence of the type of management system on occurrences of seropositive animals.

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