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Original article

Seroprevalence of anti-*Neospora caninum* antibodies in sheep from the rapidly expanding flock of Rio de Janeiro, Brazil

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ABSTRACT

Neosporosis is primarily a disease of cattle and dogs, but *Neospora caninum* has been linked to abortion and neonatal mortality in sheep. Since the economic, clinical and epidemiological importance of the infection in sheep remains uncertain, this work investigated the seroprevalence of anti-*N. caninum* antibodies and associated factors in the rapidly expanding flock of Rio de Janeiro state. Blood samples from 388 sheep of 12 farms were collected and sera tested by a commercial Enzyme-Linked Immunosorbent Assay. Seroprevalence at the animal-level was of 6.2% (24/388) and, at the herd-level, 50% (6/12) of the studied farms had at least one seropositive animal. Multivariate analysis detected that occasional veterinary assistance ($P < 0.05$) was significantly associated to higher seroprevalence, which is also associated to age ($P < 0.001$) and gender ($P < 0.0001$). Farmers' investments should focus on making technical assistance more frequent and future studies should assess the association of veterinary assistance with anti-*N. caninum* antibodies in sheep flocks.

1. Introduction

Neosporosis is primarily a disease of cattle and dogs, but its etiologic agent, *Neospora caninum,* can also infect other mammals and birds (Dubey and Schares, 2011). Canids are definitive hosts (McAllister et al., 1998; Gondim et al., 2004; Dubey et al., 2011; King et al., 2010). Herbivores and other intermediate hosts may become infected by ingested contaminated pasture, water or through vertical transmission (Basso et al., 2009; Dubey et al., 2011).

Clinical manifestations of bovine infection are well established as the protozoan is a major cause of abortion either in beef or dairy cattle (Dubey, 2003; Dubey et al., 2007). Although etiology and frequency need definitive evidence, *N. caninum* infection was linked to abortion and neonatal mortality in sheep (Dubey and Schares, 2011). Antibody and DNA analysis indicate transplacental transmission, but its economic, clinical and epidemiologic importance remains uncertain.

Ovine production is expanding in Brazil, especially in regions without tradition in this type of economic activity. This is the case of Rio de Janeiro state, where flocks expanded almost 60% in 10 years (IBGE, 2012). Consequently, it is crucial to be aware of potential health problems, mainly reproduction disorders, which have direct effects on production (Machado et al., 2011; da Silva Andrade et al., 2012).

Seroprevalence studies on sheep anti-*N. caninum* antibodies in Brazil varied from 1.8 to 64.2% (Soares et al., 2009; Tembue et al., 2011) and parasite was isolated from naturally infected sheep (Pena et al., 2007). Recently, an extremely high incidence of 62.2% (23/37) was detected in the Northeastern state of Pernambuco (Filho et al., 2017).

In the state of Rio de Janeiro, *N. caninum* is prevalent among cattle (Munhoz et al., 2006; Boa-Morte and Oliveira, 2009). However, to date, there is no information on the seroprevalence of *N. caninum* in this rapidly expanding sheep flock and the factors associated with the maintenance of the parasite inside farms. Therefore, the present work investigated the seroprevalence of anti-*N. caninum* antibodies among sheep bred in this state and factors associated.

2. Material and methods

2.1. Ethics

All procedures involving animals were approved by the Animal

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2.2. Study area and sampling

A representative number of sheep from Rio de Janeiro State, Brazil was calculated by Epi Info™ 3.5.3 based on data of the national animal census, confidence interval of 95%, absolute error of 0.05 and expected frequency of anti-*N. caninum* antibodies in sheep of 19.4% based on average of previous studies conducted in Brazil and reviewed by Cerqueira-Cézar et al. (2017).

Twelve farms of eight counties were selected by convenience of contact with owners and blood samples of 388 ewes and rams were collected from the jugular vein of all animals within each given property. Samples were centrifuged, sera removed and stored at −20 °C.

Risk factors for the seroprevalence of anti-*N. caninum* antibodies in sheep were assessed based on epidemiological questionnaires applied to the owners, which raised information on region, gender, age, presence of rodents, dogs, production system, sheep flock presentation, water source, frequency of veterinary assistance and hygiene measures inside the properties.

Production systems assessed were extensive and semi-intensive. Extensive systems were characterized by small inputs of labor, fertilizers and capital in comparison to the land area being farmed. In the semi-intensive system, animals received some kind of food supplement.

Regular veterinary assistances were those in which technical visits were made on a weekly basis with administration of medicines, including anti-parasitic drugs, vaccination, diagnosis of reproductive disorders and suggestion of hygiene-sanitary measures. Occasional veterinary assistances were those in which technical visits occurred only if animals presented clinical signs of illness.

Farms with good hygiene measures were those in which animals' environment was daily cleaned, while farms with bad hygiene measures were those where animals were kept under poor hygiene conditions. The main poor hygiene conditions considered were contact of dogs with ovine placentas, uterine discharges and carcasses, which present biological importance in the epidemiology of this parasite.

Farms with bad flock presentation were those with animals presenting with visible signs of cachexia, including weight loss and muscle atrophy, while in farms with good flock presentation had animals showing good physical condition.

2.3. Serologic tests

Sera were tested for anti-*N. caninum* antibodies using a commercial ELISA kit for ruminants (Chekit* Neospora, Idexx, Liebefeld, Switzerland). Refinement of this same kit was made by Reichel et al. (2008) and established a 11.8% threshold cut-off specifically for sheep, which we adopted in this work. This test has sensitivity of 97.56% and specificity of 98.53% (Wu et al., 2002) and the true prevalence (TP) was calculated as follows: TP = (apparent prevalence + specificity −1)/(sensitivity + specificity −1).

2.4. Statistical analysis

For analysis of associated factors, logistic regression models were used as described by Lemeshow and Hosmer Jr. (1984). Univariate analysis (Chi-square test) was performed to test associations between the dependent variables and each independent variable in the logistic regression model (associated factors). The decision criteria for potential independent variables for logistic regression were those in which the association presented a Chi-square *P*-value ≤ 0.2. Series of logistic regression models were applied having as dependent variable (response variable) the serologic results and, as independent variables, the characteristics filled out in the questionnaires. After selecting the final model of logistic regression, the beta (β) coefficients of each independent variable were observed to estimate *odds ratio* (OR).

Table 1
Chi-square test for the association between frequency of anti-*Neospora caninum* antibodies in sheep and associated factors in Rio de Janeiro, Brazil.

Factors	Categories	Positive		Negative		P-value[a]
		n	%	N	%	
Region	North	16	6.9	215	93.1	0.335
	Midsouth	0	0	45	100	
	Metropolitan	8	7.1	104	92.9	
Gender	Male	14	16.1	73	83.9	< 0.001
	Female	10	3.3	291	96.7	
Age						< 0.001
	1–6 mo	3	15.0	17	85.0	
	7–12 mo	11	21.2	41	78.8	
	> 12 mo	10	3.2	306	96.8	
Presence of rodents	Yes	8	6.0	125	94.0	0.920
	No	16	6.3	239	93.7	
Presence of dogs	Yes	24	6.2	364	93.8	–
	No	0	0.0	0	0.0	
Production system	Extensive	3	3.1	93	96.9	0.221
	Semi-intensive and intensive	21	7.2	271	92.8	
Flock presentation	Good	8	2.6	304	97.4	< 0.001
	Bad	16	21.1	60	78.9	
Source of water	Well	16	8.5	173	91.5	0.140
	Pond	7	4.9	137	95.1	
	Spring	1	1.8	54	98.2	
Veterinary assistance	Regular	9	2.7	324	97.3	< 0.001
	Occasional	15	27.3	40	72.7	
Hygiene measures	Good	13	4.3	324	96.1	< 0.001
	Bad	11	21.6	40	78.4	

[a] Confidence interval of 95%.

3. Results

Overall animal-level seroprevalence was 6.2% (24/388) with seroprevalence varying from 0 to 36.7% among the 12 farms. At a herd-level, 50% of farms had at least one animal that was seropositive. True prevalence was lower (4.9%), based on sensitivity of 97.56% and specificity of 98.53% of the test (Wu et al., 2002).

Age, gender, hygiene measures, flock presentation and veterinary assistance were significant associate factors at univariate analysis (Table 1). Multivariate analysis pointed at significant association of seroprevalence with gender ($P < 0.0001$, OR = 16.585, 95% CI 4.279–64.283), age ($P < 0.001$, OR = 6.851, 95% CI 2.208–21.253), and veterinary assistance ($P < 0.05$, OR = 13.436, 95% CI 1.112–162.336) (Table 2).

Dogs were observed in all farms, thus univariate analysis could not rule this factor out. Five seropositive ewes from two different farms - two from one farm and three from another - had a history of reproductive disorders, including four abortions, but the number of animals was insufficient for statistical analysis.

4. Discussion

In the present work, we performed a cross-sectional study with a multivariate analysis of possible risk factors to identify those associated with sheep exposure to the parasite *N. caninum* in Rio de Janeiro state, Brazil. This type of study allows observation of exposure and outcome at the same time point and is therefore suitable for investigation of the burden of the disease or condition and useful for prevention planning. Although most of the information on the factors assessed and acquired through the survey could be confirmed on site, some extent of bias may be expected as the questionnaires were answered by farm owners. We should also consider the limitations of convenience sampling methods.

This is the first study to assess the seroprevalence of *N. caninum* antibodies and associated factors in sheep from this region. Previous

Table 2
Logistic regression models for which response variables were the likelihood of positive results for anti-*Neospora caninum* antibodies in sheep from Rio de Janeiro, Brazil.

Explanatory variable	Category	OR	95% CI risk	
			Below	Above
Gender	female[a]			
	male	16.585**	4.279	64.283
Age	> 12 months[a]			
	1–6 months	4.855	0.931	25.320
	7–12 months	6.851*	2.208	21.253
Veterinary assistance	regular[a]			
	occasional	13.436*	1.112	162.336
Hygiene measures	Good			
	Bad	0.296	0.061	1.430
Flock presentation	Good			
	Bad	4.615	0.282	75.556

* $P < 0.05$.
** $P < 0.0001$.
[a] Reference category.

studies in this area had shown high prevalence among cattle and maintenance of the parasite inside herds through vertical transmission (Munhoz et al., 2006; Boa-Morte and Oliveira, 2009). Although 50% (6/12) of the present flocks have been exposed to *N. caninum*, at animal level, ovine seroprevalence is much lower.

In the present work, multivariate analysis results pointed at the association of veterinary assistance, age and gender to seroprevalence of antibodies anti-*N. caninum* in sheep bred in Rio de Janeiro (RJ) (Table 2). Rates seems to increase as lambs get older, markedly during the first year of life (Table 2) and the biological rationale for this is usually the continued exposure to oocyst-contaminated environments. But it is interesting that after the first year of life, seropositivity oddly decreased, when tendency should be the opposite. To better understand this scenario, one must consider the other important factor associated to prevalence in these RJ sheep: veterinary assistance. Occasional veterinary assistance was also significantly associated to increased chances of exposure to *N. caninum* in sheep from the studied region. It is important to stress that, although regular veterinary assistance may not necessarily be due to sheep with neosporosis, the farm might have being culling animals with reproductive disorders and, indirectly, reducing possible carriers of *N caninum* inside the flock. The identification and discarding of aborting or empty ewes might have influenced the total number of seropositive ewes and, consequently, the total number of seropositive adult sheep. Considered significant by a multivariate analysis, these three associated factors (veterinary assistance, age and gender) acted as a whole to influence chances of *N. caninum* exposure in sheep. Also, veterinary prophylactic measure appear to help maintain a healthier flock and guide flock management to limit exposures.

One must also consider the possibility of in uterus transmission. Filho et al. (2017) identified vertical transmission in 11% of naturally infected sheep from Brazil and Syed-Hussain et al. (2014) demonstrated that antibodies might be detectable for over two years. This infection may lead to future failure to conceive and culling of the ewe, with reduction in the number of seropositive adult females.

No previous studies have implicated gender with *N. caninum* seroprevalence in sheep. Occasional veterinary assistance was also associated to higher seroprevalence in Jordan (Abo-Shehada and Abu-Halaweh, 2010) but, interestingly, the assessment of this factor has been neglected by others. Infrequent veterinary assistance was also associated to the prevalence of *T. gondii* and *Leptospira* in sheep and cattle from RJ (Lilenbaum and Souza, 2003; Cosendey-Kezenleite et al., 2014).

Five of 10 seropositive ewes of this research had reproductive problems – four abortions and one estrus repetition. But these numbers were insufficient to allow assessment of a cause/effect association. An interesting point regarding the importance of *N. caninum* as a cause of abortion in sheep from Brazil remains. Although the association between ovine reproductive disorders and seroprevalence in sheep have been assessed in 13 studies, only three detected statistical significance for this association (Filho et al., 2017). Etiology and frequency of abortions still lack definitive evidence (Dubey and Schares, 2011).

Of note, the findings of this study suggest that the breeding system was not associated with seroprevalence of *N. caninum* in sheep, which is in agreement with the majority of previous studies (Romanelli et al., 2007; Ueno et al., 2009; Faria et al., 2010; Munhóz et al., 2010; da Silva Andrade et al., 2012; Paiz et al., 2015). This is economically important, since indicates that neosporosis prevention in sheep is not influenced by any attempt to increase inputs of labor, fertilizers and capital in the lands.

In conclusion, sheep from the rapidly expanding flock of Rio de Janeiro are exposed to *N. caninum*, although the seroprevalence of specific antibodies is low. Farmers' investments should focus on making veterinary assistance more frequent and future worldwide studies should include assessment of the association of veterinary care with anti-*N. caninum* antibodies in sheep flocks.

Conflict of interest statement

The authors declare that this work provides no conflict of interest.

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