Seroepidemiology of *Neospora caninum* among goats (*Capra hircus*) in the state of Paraíba, northeastern Brazil

[*Soroepidemiologia de Neospora caninum em caprinos (Capra hircus) do estado da Paraíba, Nordeste do Brasil*]

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ABSTRACT

Although goat dairy farms in Brazil may have a higher risk of infection by *Neospora caninum* than beef farms, risk factor evaluation on a representative population remains to be fully established in Brazil. Accordingly, this study aimed to establish the occurrence of anti-*N. caninum* antibodies and factors associated with exposure in 406 blood samples from five dairy and three beef goat farms in the state of Paraíba, northeastern Brazil. Anti-*N. caninum* antibodies were detected by indirect immunofluorescence assay (IFA), with samples considered positive when reacting with dilution ≥ 1:50. A total of 106/406 goats (26.11%; 95% CI: 21.96-30.72%) were seroreactive comprising 2/61 (3.28%), 10/45 (22.22%), 13/50 (26.00%), 17/51 (33.33%) to 29/46 (63.04%) in dairy farms, and from 3/54 (5.56%), 12/50 (24.00%) to 20/49 (40.82%) on the beef farms. No significant associations were found in relation to age, gender, dairy versus beef farms, occurrence of abortions or mummified fetuses, and seroreactivity to *N. caninum* (P>0.05). In conclusion, goat farms in the state of Paraíba showed the highest occurrence of anti-*N. caninum* antibodies to date in Brazil.

Keywords: IFA, neosporosis, seroprevalence, small ruminants

RESUMO

Embora as criações caprinas de leite no Brasil possam ter maior probabilidade de risco de infecção por Neospora caninum *do que as de carne, a avaliação dos fatores de risco em uma população representativa ainda não está totalmente estabelecida no Brasil. Dessa forma, este estudo teve por objetivo estabelecer a soroprevalência de* N. caninum *e seus fatores associados à exposição em 406 amostras de sangue de cinco fazendas de leite e três de corte provenientes do estado da Paraíba, região Nordeste do Brasil. A detecção de anticorpos anti-*N. caninum *foi realizada utilizando-se a reação de imunofluorescência indireta (RIFI), com as amostras consideradas positivas na diluição ≥ 1:50. No total, 106/406 (26,11%; IC 95%: 21,96-30,72%) caprinos foram sororeagentes, variando de 2/61 (3,28%), 10/45 (22,22%), 13/50 (26,00%), 17/51 (33,33%) a 29/46 (63,04%) em fazendas de leite, e de 3/54 (5,56%), 12/50 (24,00%) a 20/49 (40,82%) em fazendas de corte. Não foram observadas associações significativas entre idade, sexo, criação de leite e carne, ocorrência de abortamentos ou fetos mumificados e sororreatividade para* N. caninum *(P>0,05). Em conclusão, fazendas de caprinos da Paraíba mostraram as mais altas ocorrências de anticorpos anti-*N. caninum *até o momento no Brasil.*

Palavras-chave: neosporose, pequenos ruminantes, RIFI, soroprevalência

INTRODUCTION

Neospora caninum is an obligate intracellular parasite and infection has been reportedly implicated in worldwide livestock losses, causing reproductive problems such as abortion and infertility in several species, including goats (Dubey *et al.*, 1996; Moore, 2005). Since Brazilian goat farms have been mostly (90.10%) located on Northeastern Brazil (Instituto…, 2013), which has historically reported sanitary problems due to inappropriate management practices (Pinheiro *et al.*, 2000), identification of causative agents of reproductive losses may be crucial for dairy and beef goat production.

A series of reports on the presence of infected goats with *N. caninum* has been published worldwide. Prevalence studies performed in Europe and Asia have showed data ranging from 2.3-15.5% (Cobadiova *et al.*, 2013; Czopowicz *et al.*, 2011; Diakoua *et al.*, 2013; Garcia-Bocanegra et al., 2012; Iovu *et al.*, 2012; Moreno *et al.*, 2012) and from 2.0-8.6% (Abo-Shehada e Abu-Halaweh, 2010; Nasir *et al.*, 2012; Liu *et al*, 2015), respectively. In Brazil, several States have reported anti-*N. caninum* antibodies in goats, with seroprevalence data ranging from 1.05-26.60% (Lima *et al.*, 2008; Silva *et al.*, 2009; Faria *et al.* 2007; Topazio *et al.*, 2014; Anderlini *et al.*, 2011; Figliuolo *et al.*, 2004; Varaschin *et al.*, 2011; Andrade *et al.*, 2013; Uzêda *et al.*, 2007; Modolo *et al.*, 2008; Costa *et al.*, 2012; Moraes *et al.*, 2011; Tembue *et al.*, 2011) (Figure 1).

Figure 1. Geographical illustration of serologic occurrence of *Neospora caninum* in goats from Brazil based referenced data. Geographic Information System, Quantum GIS, Version 2.8 WIEN.

Interestingly, dairy farms in Brazil may have a higher risk likelihood of infection than beef farms, probably due to the longer lifetime of dairy herds on the farm. Despite the seroprevalence data, risk factor evaluation on a representative population remains to be fully established in Brazil. Accordingly, this study aimed to evaluate the occurrence and factors associated with anti-*N. caninum* antibodies in dairy and beef goats in the state of Paraíba, northeastern Brazil.

MATERIALS AND METHODS

This study was approved by the Ethics Committee for Animal Experimentation and Animal Welfare at the Federal University of Paraíba, northeastern Brazil (protocol 3305/14).

The sample size was calculated as previously described (Thrusfield, 1995). The total number of goats in the state of Paraíba was estimated at 478.000 (Instituto…, 2013) and the expected prevalence was assumed to be 50%. The minimum sample size required for detecting a difference with 95% confidence level at 5% was estimated as n=384. A total of 406 goat blood samples (35 males and 371 females) were randomly collected from five dairy and three beef farms (~50 animals per farm) in the state of Paraíba.

The dairy farms were located in the following municipalities Algodão de Jandaíra (6° 53.531' S 35° 59.096' W), Caturité I (7° 24.562' S 36° 0.096' W), Caturité II (07° 23.261'S 036° 03.878'W), Gurjão (7° 14.747' S 36° 33.084' W), and Serra Branca (7° 29.259' S 36° 42.335' W), while the beef farms were located at Cuité (6° 38.356' S 36° 09.733' W), Juarez Távora (7° 09.437' S 35° 36.051 W), and Olivedos (06° 53.039' S 36° 13.923' W). The farms are located in two different climate regions of the state semi-umid coastal and semi-arid tropical.

Blood samples were collected using commercial tubes containing separating gel clots (BD Vacutainer®, Franklin Lakes, NJ USA). These were kept at room temperature (25°C) until clot retraction became visible. The samples were centrifuged at 1500g for 5min and the serum was then separated out, appropriately aliquoted, identified and stored at -20ºC.

The farm owners answered an epidemiological questionnaire, which included questions on goat gender and age, abortions or mummified fetuses, and presence of dogs. Goats were age-stratified into groups of ≤ 1 year and > 1 year. The beef farm at Juarez Távora presented high-quality management practices and was considered to be a reference farm for statistical comparison.

The indirect immunofluorescence antibody assay (IFA) was applied to detect antibodies anti-*N. caninum* using a commercial kit (Imunoteste Neospora (RIFI) - caprino, Imunodot®, Jaboticabal, São Paulo, Brasil), in accordance with the manufacturer´s instructions, except for using conjugated rabbit anti-Goat IgG-FITC as the secondary antibody. Samples were considered positive when the tachyzoites showed total peripheral fluorescence at 1:50 dilution (Lindsay et al., 1995).

The Chi-square or Fisher's exact test was used to determine whether the factors evaluated were associated with seropositivity for *N. caninum*. Odds ratio (OR), 95% confidence interval and P values were calculated separately for each variable. Results were considered significantly different when $P<0.05$. Data were generated and analyzed using software available free-of-charge (Epi Info™ 7.5.1.0, CDC, Atlanta, USA).

RESULTS

A total of 106/406 (26.11%; 95% CI: 21.96-30.72%) goats were seroreactive for *N. caninum*. All farms had at least one *N. caninum* seroreactive animal. With the exception of the dairy farm at Serra Branca, goats from all the remaining farms were more likely to be seroreactive for *N. caninum* than those from beef farm at Juarez Távora. No associations between seroreactivity to *N. caninum* and gender (P=0.1199), age (P=0.5881), occurrences of abortions (P=0.2090) or mummified fetuses (P=0.9851), and production system (P=0.32725) were observed. All the farms reported that dogs were present. The occurrence of anti-*N. caninum* antibodies within each variable evaluated are summarized in Table 1.

Table 1. Occurrence of anti-*Neospora caninum* antibodies in eight different goat farms from Paraíba State, Northeastern Brasil and correspondent testing of risk factors

Farm	Purpose	N. caninum (IFA)		OR	95% CI	P-value
		+/n	(%)			
Algodão	Dairy	29/46	63.04	29.0000	7.83-107.40	0.00000
Caturité I	Dairy	17/51	33.33	8.5000	2.31-31.24	0.00030
Caturité II	Dairy	13/50	26.00	5.9730	1.59-22.47	0.00390
Gurjão	Dairy	10/45	22.22	4.8571	1.25-18.92	0.01450
Serra Branca	Dairy	2/61	3.28	1.7353	0.28-10.79	0.55010
Cuité	Beef	20/49	40.82	11.7241	3.21-42.86	0.00002
Olivedos	Beef	12/50	24.00	5.3684	1.41-20.36	0.00740
Juarez*	Beef*	3/54	5.56			
Age	>1	87/340	25.59	1.17	0.65-2.11	0.58810
	≤1	19/66	28.79			
Gender	Male	13/35	37.14	1.7664	0.85-3.65	0.11999
	Female	93/371	25.07			
Abortion	Yes	89/355	25.07	0.6692	0.35-1.25	0.20900
	No	17/51	33.33			
Mummified	Yes	13/50	26.00	0.9936	0.51-1.95	0.98510
fetuses	No	93/356	26.12			
Production	Dairy	70/252	27.78	1.2607	0.79-2.00	0.32725
system	Beef	36/154	23.38			

+, Number of positive animals; n, number of samples; 95% CI, 95% confidence interval. * Reference goat farm.

DISCUSSION

In the present study, an overall occurrence of anti-*N. caninum* antibodies of 26.11% (ranging from 3.28% to 63.04%) was found among goats in the state of Paraíba, which is within the range found in previous Brazilian studies on goats, from 1.05% to 26.6% (Faria *et al.* 2007; Uzêda *et al.*, 2007; Lima *et al.*, 2008; Anderlini *et al.*, 2011; Tembue *et al.*, 2011; Moraes *et al.*, 2011). The differences between studies may be due mainly to the type of population and sample size, management system, climatic variances, diagnostic method and the respective cut-offs used. However, in the present study, we found the highest occurrence regarding all the studies developed in Brazil. An occurrence of 63.04% was found in the dairy farm from Algodão, where the herd has less than 50 animals, there was no rotational grazing and more than one animal species was bred in the same property (data not shown). Previous studies have reported that goat breeding without any rotational grazing associated to the direct contact with other animal species, such as in the present study, may facilitate the infection by oocysts (Atwill *et al.*, 1999).

Although previous studies reported that age (Tembue *et al.*, 2011) and gender (Uzêda *et al.*, 2007) were associated with seropositivity for *N. caninum*, neither age nor gender were associated with seroreactivity in the present study. The disproportionate number of female (371 females and 35 males) and animals >1 year (340>1 year and 66≤1 year) may have interfered in the statistical analyses. However, previous studies have stated that the lack of association between gender or age and seropositivity to *N. caninum* in goats indicates that the presence of this protozoan is homogenous among the herd (Figliuolo *et al.*, 2004; Modolo *et al.*, 2008).

In the present study, even though approximately 25% of the seroreactive goats were from properties with a historical of abortions or mummified fetuses, no association between these factors and seroreactivity to *N. caninum* was observed (P>0.05). In fact, the most common clinical manifestations of neosporosis in goats include abortions or mummified fetuses. Previous studies have stated that goats experimentally infected with *N. caninum* do not develop clinical disease (Dubey and Lindsay, 1996). Thus, other infectious (and non-infectious) agents, which unfortunately were not

evaluated in the present study, particularly *Toxoplasma gondii*, may lead to similar reproductive problems and should be further investigated.

In the present study, no statistical differences were observed between dairy and beef farms (P=0.32725). However, the intra-group variation among dairy farms (3.28%, 22.22%, 26.00%, 33.33% and 63.04%) and beef farms (5.56%, 24.00% and 40.82%), associated to the fact that dogs were present in all farms may have impaired statistical comparisons. Therefore, other factors associated with *N. caninum* infection, such as the type of the water source and its quality (Figlioulo *et al.*, 2004), not covered in the present study may have accounted for this discrepancy in seropositivity. To the author's knowledge, no study to date has focused on comparisons between dairy and beef farms, and therefore this topic needs to be further addressed.

CONCLUSION

In conclusion, anti-*N. caninum* antibodies were highly prevalent in dairy and beef goats from the state of Paraíba. Even though no statistical differences between the production system and seroreactivity to this protozoon have been observed, other factors associated with infection should be further investigated. Finally, goat farms in the state of Paraíba showed the highest occurrence of anti-*N. caninum* antibodies to date in Brazil.

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