



Revista Brasileira de Parasitologia
Veterinária

ISSN: 0103-846X

zacariascbpv@fcav.unesp.br

Colégio Brasileiro de Parasitologia
Veterinária
Brasil

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Revista Brasileira de Parasitologia Veterinária, vol. 19, núm. 3, julio-septiembre, 2010, pp.
148-151
Colégio Brasileiro de Parasitologia Veterinária
Jaboticabal, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=397841477003>

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Prevalence of *Neospora caninum* antibodies in sheep flocks of Uberlândia county, MG

Prevalência de anticorpos anti-*Neospora caninum* em rebanhos de ovinos do município de Uberlândia, MG

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Received February 3, 2010

Accepted April 19, 2010

Abstract

Neosporosis is a parasitic disease that occurs in sheep and is associated with reproductive failure. The aim of this study was to verify seroprevalence of *Neospora caninum* antibodies in 12 sheep flocks in Uberlândia county, Minas Gerais, Brazil, using Indirect Immunofluorescence Assay (IFA). A total of 334 sheep blood samples were analyzed and an epidemiologic questionnaire was applied for each farm in order to correlate with risk factors of neosporosis: gender, age, breed, abortion problems, contact with cattle and dogs, and presence of wild canids. The prevalence of *Neospora caninum* antibodies in sheep was 8.1%, with titers ranging from 50 to 3200 and 83.3% of farms detected at least one ovine reactive. There was statistically significant difference in flocks with abortion problems associated with the presence of *Neospora caninum* antibodies ($p < 0.05$).

Keywords: Neosporosis, sheep, immunofluorescence assay, seroprevalence.

Resumo

A neosporose é uma doença parasitária que acomete os ovinos e está associada a perdas reprodutivas. O objetivo deste estudo foi verificar a prevalência de anticorpos anti-*Neospora caninum* em 12 rebanhos de ovinos do município de Uberlândia, MG, utilizando a Técnica de Imunofluorescência Indireta (RIFI). Foram analisadas 334 amostras de sangue e, em cada propriedade, aplicou-se um questionário epidemiológico para avaliar os fatores de risco envolvidos na transmissão desse protozoário: sexo, idade, raça, problemas de aborto, contato com bovinos e caninos e presença de canídeos silvestres. A prevalência de anticorpos anti-*Neospora caninum* em ovinos foi de 8,1%, com títulos variando de 50 a 3200 e 83,3% das propriedades apresentaram pelo menos um ovino reagente. Houve diferença estatística significativa para rebanhos com problemas de aborto associados à presença de anticorpos anti-*Neospora caninum* ($p < 0,05$).

Palavras-chave: Neosporose, ovina, reação de imunofluorescência, soroprevalência.

Introduction

Neosporosis is an infection caused by the coccidian parasite *Neospora caninum* (*N. caninum*). Until now dogs and coyotes have been identified as the definitive hosts, although infection with *N. caninum* has been occasionally reported in other animal species such as ovines, caprines, cervids, and equines (DUBEY, 2003; GONDIM et al., 2004).

Infected dogs release oocysts of *N. caninum* in the soil with the feces, contaminating the environment and infecting susceptible species (HELMICK et al., 2002). Herbivorous animals are infected

by ingestion of food and water contaminated by oocysts while dogs become infected by ingestion of tissues containing bradyzoites. Another form of infection is by vertical transmission across the placenta, when the tachyzoites of *N. caninum* are transferred to the fetus (DUBEY et al., 2007).

Infection with *N. caninum* in sheep does not have the same relevance as in cattle. However, in farms where there is joint raising of cattle and sheep, they may eventually participate in the epidemiology of infection (VOGEL et al., 2006). Occasionally the infection can cause miscarriages and sick newborns (BÁRTOVÁ et al., 2009).

The presence of the parasite in sheep flocks can be verified by the detection of serum antibodies or by isolation and molecular identification of the parasite. Many countries, including

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Slovakia, have reported antibodies against *N. caninum* in their sheep flocks (SPILOVSKÁ et al., 2009). In Brazil, serological surveys have demonstrated prevalence of infection ranging from 6.4 to 29% (AGUIAR et al., 2004; FIGLIUOLO et al., 2004; ROMANELLI et al., 2007; UENO et al., 2009). Pena et al. (2007) reported the first isolation and molecular characterization of *N. caninum* in the brain of naturally infected sheep.

Hassig et al. (2003) were the first to report abortion in sheep by neosporosis. Howe et al. (2008) isolated *N. caninum* from aborted ewes, suggesting an association of this agent with the reproductive failure. Although not a zoonoses, infection with *N. caninum* is associated with reproductive failure, causing economic losses in affected herds (DUBEY et al., 2007).

The objective of this study was to assess the prevalence of *N. caninum* antibodies in sheep flocks raised in Uberlândia county, Minas Gerais, Brazil and associate it with the following risk factors: gender, age, race, abortion, and contact with cattle and canids.

Material and Methods

We used 334 blood samples from sheep derived from 12 farms located in the outskirts of Uberlândia, Minas Gerais. To determine the prevalence of *N. caninum* antibodies the simple random selection method and calculations were performed as described by Thrusfield (2004). We considered an expected prevalence of 29%, which was the prevalence of *N. caninum* antibodies found by Aguiar et al. (2004) in sheep flocks from Monte Negro, Rondonia, Brazil. We used 95% confidence interval and estimation error of 0.05, resulting in a sample of 317 sheep.

The number of blood samples per farm was determined using proportional stratified sampling, according to Ayres et al. (2007). Blood samples were collected randomly from apparently healthy sheep of both genders and of different ages and breeds, by puncturing the jugular vein, using disposable needles and vacuum tubes.

After blood collection, a questionnaire was applied to obtain data regarding gender, age, race, herds with abortion problems, contact between cattle and sheep, contact between dogs and sheep and presence of wild canids, according to Thrusfield (2004). We followed the rules regarding the use of animals in research and obtained permission (054/09) to perform this work from the Ethics Committee of the Federal University of Uberlândia.

Following blood collection, samples were packed in Styrofoam boxes with ice, and sent to the Laboratory of Infectious-Contagious Disease of the Faculty of Veterinary Medicine of the Federal University of Uberlândia where clot retraction to obtain serum was performed. The resulting serum was stored at - 22 °C until serological tests were done.

Detection of *N. caninum* antibodies was performed at the Laboratory of Viral Diseases of Bovine Animal Center of the Biological Institute (São Paulo) by Indirect Immunofluorescence Assay (IFA), using a cut-off at a dilution of 1/50, according to Lima et al. (2008). In each slide we used previously established positive and negative control sera and sheep-specific anti-human IgG labeled with fluorescein isothiocyanate at a dilution of 1/10

(KPL, Gaithersburg, USA). Tachyzoites of *N. caninum* (strain NC-1) that were maintained by continuous passages in cultures of VERO cells CCL81 were used as antigens. Reactions were considered positive when tachyzoites showed total peripheral fluorescence, and serum samples were titrated to obtain the highest positive dilution by IFA.

Statistical analysis was performed using the program Bioestat 5.0 (AYRES et al., 2007), using a nonparametric test *Odds Ratio* for two independent samples to pool the results obtained, and risk factors at a significance level of 5%.

Results and Discussion

Among all blood samples collected, 8.1% (27/334) had antibodies to *Neospora caninum*, with titers ranging from 50 to 3200 (Table 1). Similar results were found in other states of Brazil, showing prevalence of 9.2% of anti-*N. caninum* in São Paulo, 9.5% in Guarapuava (Paraná), and 8.81% in Brasília (FIGLIUOLO et al., 2004; ROMANELLI et al., 2007; UENO et al., 2009). By contrast, in Mossoró (Rio Grande do Norte), Soares et al. (2009) found a prevalence of 1.81% while in the state of Rondonia (AGUIAR et al. 2004) detected 29% positive, demonstrating the variability of results according to the region studied.

Studies performed in other countries showed similar results to those found in Brazil. In the Czech Republic and in Slovakia, studies reported a prevalence of 12 and 3.8% of anti-*N. caninum*, respectively (BÁRTOVÁ et al., 2009; SPILOVSKÁ et al., 2009).

Of all the studied farms, 83.3% (10/12) had at least one sheep that was seropositive for *N. caninum*, and the prevalence of this infection varied from 2.9 to 16.3% per farm. These results suggest that infection with *N. caninum* may be disseminated among the sheep flocks in Uberlândia county, Brazil. Similar results were observed in other studies: in the State of São Paulo prevalence was found to range from zero to 86.7% of herds; and in Guarapuava (Paraná) seroprevalence ranged from 4.7 to 17% (FIGLIUOLO et al., 2004; ROMANELLI et al., 2007). Aguiar et al. (2004) reported the highest occurrence of anti-*N. caninum* compared with other regions of Brazil, detecting 66.6% of the farms as containing animals seropositive for the infection.

There was no significant association ($p > 0.05$) between sheep seropositive for *N. caninum* and gender, age, or race (Table 2), a result already shown by Romanelli et al. (2007) in Paraná. In a study conducted in Brasília, there was no significant difference in gender (UENO et al., 2009), both sexes being susceptible to infection.

Sheep were grouped according to age, distributed in intervals of 6 to 12 months, one to four years, and over four years. Among the pure breeds commonly found in the region, we highlight the Santa Ines and Dorper. Among crossbreds, the crosses between these two races are predominant. Thus, although blood samples were obtained from sheep of different breeds, this study found no statistically significant difference between gender, age, and race.

In this study, sheep flocks with abortions had a higher frequency ($p < 0.05$) of anti-*N. caninum*, suggesting that infection with *N. caninum* may be one of the possible causes underlying reproductive problems found in sheep herds in the region. By

contrast Helmick et al. (2002), evaluating sheep with a history of miscarriages in England and Wales, detected a low frequency of *N. caninum* antibodies (0.45%). The study demonstrated that the reproductive failure associated with infection by *N. caninum* in these countries is low and that serological tests to study this infection in outbreaks of abortion in sheep are not justified.

Among the infectious agents that can cause abortion in sheep, *Brucella ovis*, *Campylobacter* spp., *Chlamydia abortus*, *Toxoplasma gondii*, *Listeria monocytogenes*, and *Leptospira interrogans* stand

out. There are few published data on the occurrence of natural *N. caninum* infection in sheep (MENZIES, 2007), demonstrating the need for further studies on abortion in sheep caused by this parasite (MOORE, 2005).

There was no statistical difference ($p > 0.05$) when contact with cattle and dogs was analyzed (Table 2). Vogel et al. (2006) reported that sheep can participate in the epidemiology of the infection when they are raised together with bovines because cattle are considered the main intermediate host in the infection

Table 1. Prevalence of antibodies to *Neospora caninum* in sheep according to ownership and serological titles in Uberlândia county, Minas Gerais, Brazil, 2009.

Farms	N	n	Titer (%)						Total of reagents (%)	Prevalence %
			50	100	200	400	1600	3200		
A	345	24	1	2	-	-	-	-	3	12.5
B	591	43	3	2	-	-	1	1	7	16.3
C	153	14	-	-	-	1	-	-	1	7.1
D	382	30	1	-	1	-	-	-	2	6.7
E	580	41	3	-	-	-	-	-	3	7.3
F	165	12	1	-	-	-	-	-	1	8.3
G	500	35	4	1	-	-	-	-	5	14.3
H	1000	70	1	-	1	-	-	-	2	2.9
I	512	38	1	1	-	-	-	-	2	5.3
J	200	15	-	-	1	-	-	-	1	6.7
L	35	3	-	-	-	-	-	-	-	-
M	90	9	-	-	-	-	-	-	-	-

^NTotal number of sheep in each farm; ⁿTotal number of sheep used in the experiment.

Table 2. Distribution of sheep seropositive for *Neospora caninum* according to gender, age, race, and risk factors in Uberlândia county, Minas Gerais, Brazil, 2009.

Variables	Ovines			OR ^a	CI ^b 95%	p
	Total	Reagent	Frequency (%)			
Gender						
Male	18	1	5.6	1.52	0.19-11.91	0.97
Female	316	26	82	-	-	-
Age						
6 to <12 months	62	2	3.2	0.28 ^c	0.06-1.26	0.14
1 to <4 years	101	7	6.9	-	-	-
≥4 years	171	18	10.5	-	-	-
Race						
Mixed	173	12	6.9	1.38	0.62-3.04	0.55
Pure	161	15	9.3	-	-	-
Herds with abortion problems						
Yes	105	14	13.3	2.55	1.16-5.65	0.03
No	229	13	5.7	-	-	-
Contact between cattle and sheep						
Yes	39	4	10.3	1.35	0.44-4.14	0.83
No	295	23	7.8	-	-	-
Contact between dogs and sheep						
Yes	211	22	10.4	2.75	1.01-7.45	0.06
No	123	5	4.1	-	-	-
Presence of wild canids						
Yes	225	16	7.1	0.68	0.31-1.52	0.46
No	109	11	10.1	-	-	-

^aOdds Ratio; ^bConfidence Interval; ^cOR carried out for the biggest difference between proportions; $p < 0.05$ shows significant difference.

of *N. caninum*. According to the epidemiological survey, only 16.7% (2/12) of the farms kept cattle and sheep in close contact, although all farms owned herds of cattle.

The lack of association with the presence of canids is consistent with Romanelli et al. (2007), who also found no significant differences in the occurrence of seropositive sheep. However all farms studied owned dogs and 66.7% (8/12) had dogs in direct contact with sheep. Figliuolo et al. (2004) found no statistically significant difference between the presence of seropositive sheep and wild canids, a result that was also confirmed in this study.

This study demonstrated the presence of antibodies to *Neospora caninum* in sheep flocks in Uberlândia county, Brazil and the importance of this infection as a diagnostic aspect possibly underlying the causes of abortion occurring in sheep flocks in the region. Dubey et al. (2007) stressed the importance of implementing control measures for this infection, such as limiting the access of dogs to places where sheep herds are kept and the proper disposal of placental membranes and aborted fetuses.

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