DOI: 10.5433/1679-0359.2016v37n3p1397

Neospora spp. and *Toxoplasma gondii* infection in sheep flocks from Rio Grande do Sul, Brazil

Infecção por *Neospora* spp. e *Toxoplasma gondii* em rebanhos ovinos no Rio Grande do Sul, Brasil

Maiara Sanitá Tafner Ferreira[1*]; Fernanda Silveira Flores Vogel[2]; Luis Antonio Sangioni[2]; Alfredo Skrebsky Cezar[3]; Fernanda Rezer de Menezes[4]

Abstract

Neospora caninum and *Toxoplasma gondii* are coccidian parasites (protozoa: Apicomplexa) that cause reproductive losses in ruminants. Although, in sheep, abortion is generally related to *T. gondii*, potential damage caused by *N. caninum* must be considered. The aims of this study were to evaluate the frequencies of antibodies against *Neospora* spp. and *T. gondii,* and to identify the risk factors related to neosporosis and toxoplasmosis in sheep from ten flocks located in Central-Western, Northwestern, Southwestern, and Southeastern mesoregions of the Rio Grande do Sul state, southern Brazil. Blood samples were collected from 300 sheep (30 per flock) and an epidemiological questionnaire was completed by the farmers. The presence of canids, felids, and rodents, the sources of water for the animals, and their reproductive history were investigated as potential epidemiological factors related to the studied infections. Serum samples were tested by the indirect fluorescent antibody test for the presence of antibodies against *Neospora* spp. (cutoff 1:50) and *T. gondii* (cutoff 1:64), with respective frequencies detected being 16.3% (49/300) and 41.3% (124/300). Seropositivity to both parasites was observed in 8% (24/300) of the animals. *Neospora* spp. and *T. gondii* were present in 90% (9/10) and 100% (10/10) of the flocks, respectively. The presence of cats in the pastures, pens, and barns was significantly associated (p=0.047) with high frequencies (53.3% to 70%) of antibodies against *T. gondii* in the flocks. However, no associations were detected (p>0.05) between each of the other risk factors and the frequency of antibodies against *Neospora* spp. or *T. gondii*. Results of this study showed a broad distribution of both protozoa in evaluated sheep flocks.
Key words: Antibodies. Epidemiological survey. Neosporosis. Toxoplasmosis. Serology. Ifat.

Resumo

Neospora caninum e *Toxoplasma gondii* são parasitos coccídeos (protozoa: Apicomplexa) que causam perdas reprodutivas em ruminantes. Embora o aborto em ovinos geralmente esteja associado a *T. gondii*, potenciais perdas causadas por *N. caninum* devem ser consideradas. Os objetivos deste estudo foram

[1] Discente do Curso de Doutorado no Programa de Pós-Graduação em Medicina Veterinária, Deptº de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, UFSM, Santa Maria, RS, Brasil. Bolsista CAPES. E-mail: matafner@hotmail.com

[2] Profs., Programa de Pós-Graduação em Medicina Veterinária, Deptº de Medicina Veterinária Preventiva, UFSM, Santa Maria, RS, Brasil. E-mail: fefevogel@gmail.com; lasangioni@gmail.com

[3] Pós-Doutorando no Programa de Pós-Graduação em Economia & Desenvolvimento, PPGE&D, Deptº de Ciências Econômicas, UFSM, Santa Maria, RS, Brasil. Programa Nacional de Pós-Doutorado. Bolsista PNPD/CAPES. E-mail: alfredosps@hotmail.com

[4] Discente do Curso de Mestrado no Programa de Pós-Graduação em Economia e Desenvolvimento, UFSM, Santa Maria, RS, Brasil. Bolsista CAPES. E-mail: fernandarezer@gmail.com

[*] Author for correspondence

Recebido para publicação 23/05/15 Aprovado em 21/12/15

avaliar as frequências de anticorpos contra *Neospora* spp. e *T. gondii* e identificar fatores de risco para neosporose e toxoplasmose em ovinos de dez rebanhos localizados nas mesorregiões Centro-Ocidental, Noroeste, Sudoeste e Sudeste, no estado do Rio Grande do Sul, Brasil. Foram coletadas amostras de soro sanguíneo de 300 ovinos (30 por rebanho) e foi aplicado inquérito epidemiológico aos proprietários dos rebanhos. A presença de canídeos, felídeos e roedores, as fontes de água dos animais, e seu histórico reprodutivo, foram investigados como potenciais fatores epidemiológicos relacionados às infecções estudadas. As amostras de soro foram testadas pela técnica de imunofluorescência indireta para presença de anticorpos contra *Neospora* spp. (ponto de corte 1:50) e *T. gondii* (ponto de corte 1:64), sendo encontradas frequências de 16,3% (49/300) e de 41,3% (124/300), respectivamente. Soropositividade para ambos os agentes foi observada em 8% (24/300) dos animais. *Neospora* spp. e *T. gondii* estavam presentes em 90% (9/10) e 100% (10/10) dos rebanhos, respectivamente. A presença de gatos nas pastagens, currais e depósitos esteve significativamente associada (p=0,047) com altas frequências de anticorpos (entre 53,3% a 70%) contra *T. gondii* nos rebanhos. Não houve associação (p>0,05) entre os demais fatores de risco e a frequência de anticorpos contra *Neospora* spp. ou *T. gondii*. Os resultados deste estudo demonstraram ampla distribuição de ambos os protozoários nos rebanhos ovinos avaliados.
Palavras-chave: Anticorpos. Inquérito epidemiológico. Neosporose. Toxoplasmose. Sorologia. Rifi.

Introduction

Neosporosis and toxoplasmosis are diseases caused by the coccidian protozoa *Neospora* spp. (*N. caninum* and *N. hughesi*) and *Toxoplasma gondii*, respectively, which are obligate intracellular parasites that belong to the phylum Apicomplexa (DUBEY, 2003, 2009). Cats are definitive hosts of *T. gondii*, which causes major losses in sheep production due to reproductive disorders, such as abortion, fetal abnormalities, stillbirth, weakness, and neurological signs in newborn lambs (DUBEY et al., 1990; BÁRTOVÁ et al., 2009). Furthermore, toxoplasmosis is an important zoonotic disease (DUBEY, 2009).

Canids are definitive hosts of *N. caninum* that often causes neosporosis in dogs and cattle (DUBEY et al., 2007). However, *N. caninum* can cause reproductive disturbances in sheep, in a similar way to toxoplasmosis (DUBEY; LINDSAY, 1990). Nevertheless, infection by *N. caninum* is less frequent in sheep than in cattle (KOBAYASHI et al., 2001; SOARES et al., 2009; UENO et al., 2009).

In Brazil, infection rates found for both parasites in naturally exposed sheep are variable, depending on the flock's management (SOARES et al., 2009). Epidemiological studies regarding these parasites in sheep are commonly based on serological diagnosis, but different techniques and cutoffs can be used for positive sample identification (FIGLIUOLO et al., 2004). The recent resumption in Brazilian sheep production reinforces the importance of studies on diseases, such as neosporosis and toxoplasmosis, which can cause major economic losses to sheep farms (MUNHÓZ et al., 2010).

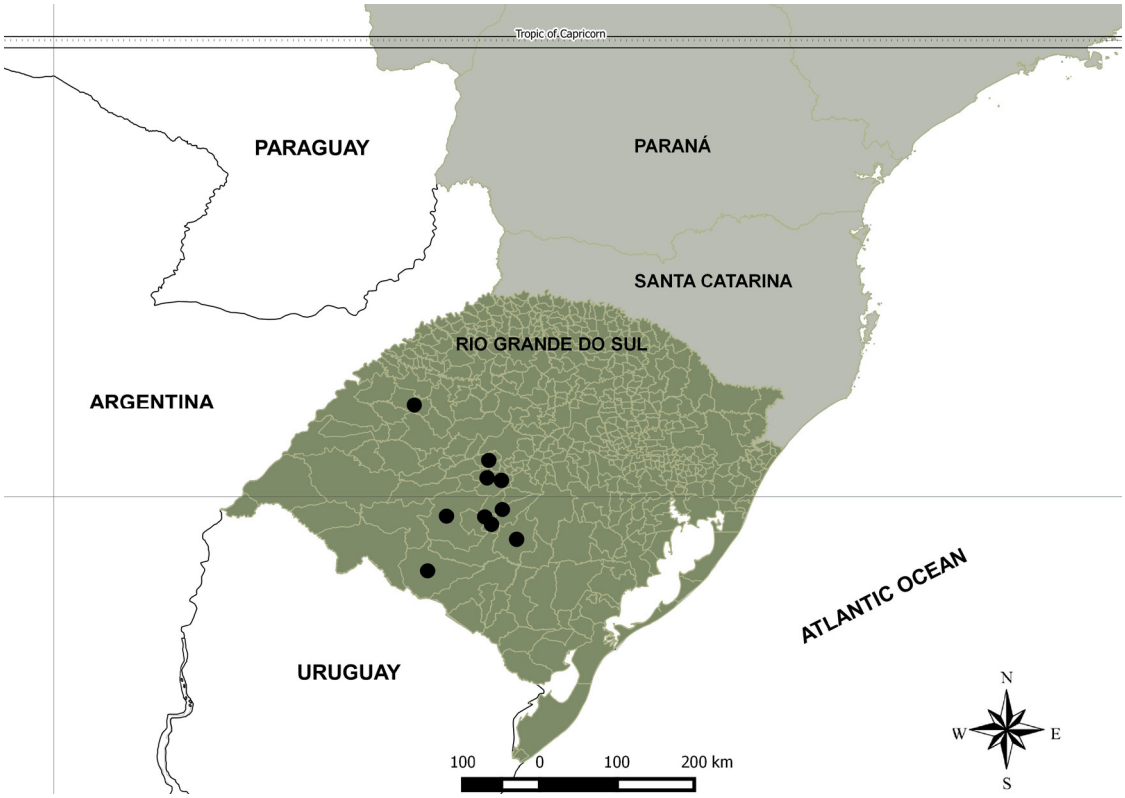
The aims of this study were to assess the frequency of antibodies against *Neospora* spp. and *T. gondii*, and to identify risk factors associated with infection by these parasites, in sheep flocks located in Rio Grande do Sul, Brazil.

Material and Methods

Blood samples were collected from 300 clinically healthy ewes, raised in extensive systems in ten farms, named Flock *A* to *J* (30 animals/farm), located in Rio Grande do Sul (RS), southern Brazil, in the following counties: Santa Maria (two farms), Vila Nova do Sul (two farms), São Martinho da Serra, and São Sepé, within the Central-Western mesoregion (RS); Caçapava do Sul, within the Southeastern mesoregion (RS); São Gabriel, and Dom Pedrito, within the South-Western mesoregion (RS); and Bossoroca, within the North-Western

mesoregion (RS) (see Figure 1). Blood samples were obtained by jugular venipuncture into vacutainer tubes, and transported in cooled boxes to the laboratory. Serum samples were obtained after blood centrifugation (1000 rpm, 10 min), and stored (-20°C) until Immunofluorescent Antibody Tests (IFAT) for detection of antibodies to *Neospora* spp. and *T. gondii* were completed.

Figure 1. Ten studied sheep flocks located in eight counties from Rio Grande do Sul state, southern Brazil. Black spheres depict the location of the farms.



The IFAT reactions were performed in slides covered by *N. caninum* (NC-1 strain) or *T. gondii* (RH strain) tachyzoites, and fluorescein-conjugated anti-sheep IgG© (Sigma, St Louis, MO, USA) was used as secondary antibody. However, the diagnosis was expressed for the genus *Neospora* spp. due to serological cross-reactivity between *N. caninum* and *N. hughesi* (GONDIM et al., 2009). Serum samples were diluted in phosphate-buffered saline solution (PBS; phosphate 0.1M, NaCl 0.33M, pH 7.2). Positive and negative sheep serum samples were used as controls for IFAT to each parasite. Slides were examined using a fluorescence microscope under a 400x magnification (Leica CTR 4000/ EBQ 100, Leica Microsystems GmbH, Germany). Dilution cutoffs used were 1:50 for *Neospora* spp. (DUBEY; LINDSAY, 1996) and 1:64 for *T. gondii* (OPEL et al., 1991; GARCIA et al., 1999).

An epidemiological survey assessing the presence of canids, felids and rodents, the kinds of water sources for the animals, and the history of reproductive problems in the flocks, was performed to identify risk factors related to infection by *N. caninum* and *T. gondii*. These data were used to assess the possible influence of searched risk factors on the frequency of antibodies against *Neospora*

spp. and *T. gondii* in sheep. The frequency of seropositive sheep per farm was compared by the Chi-square test (95% confidence interval) regarding each parasite. Thus, all ten flocks were classified into upper, intermediate, or lower strata, which were categorized according to the frequency of antibodies against *Neospora* spp. (Figure 2), or *T. gondii* (Figure 3). Association between risk factors and the frequencies of antibodies against *Neospora* spp. or *T. gondii* were evaluated by comparing the frequencies of each risk factor in the flocks within upper, intermediate, and lower strata by the Fisher's exact test (95% confidence interval).

Figure 2. Distribution and strata (upper, intermediate, lower) of the frequency of *Neospora* spp. seropositive sheep in each farm (A, B, C, D, E, F, G, H, I, J). Different lowercase letters on each column (a, b, c, d, e) indicate significant differences, assessed by the Chi-square test at 95% confidence interval.

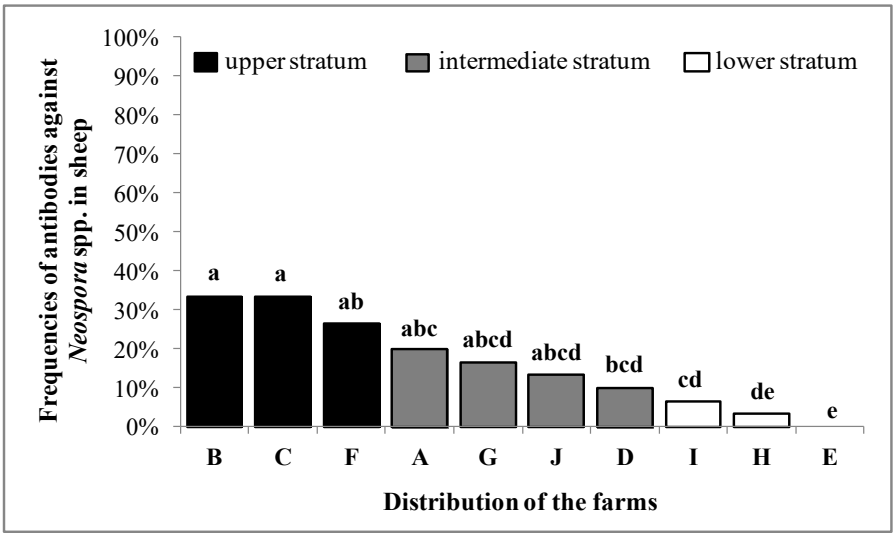
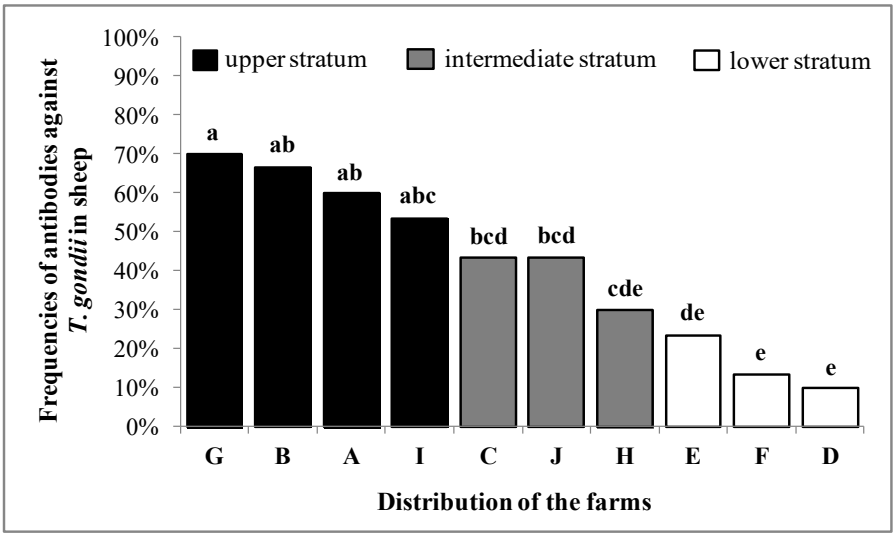


Figure 3. Distribution and strata (upper, intermediate, lower) of the frequency of *Toxoplasma gondii* seropositive sheep in each farm (A, B, C, D, E, F, G, H, I, J). Different lowercase letters on each column (a, b, c, d, e) indicate significant differences, assessed by the Chi-square test at 95% confidence interval.



All procedures involving experimentation with animals were performed under veterinarian supervision, and in accordance with the project approved by the Ethics Committee on Animal Use of the Federal University of Santa Maria (process #069/2014).

Results

Considering all the 300 samples evaluated, 16.3% (49/300) were positive to *Neospora* spp., with seropositive sheep in 90% (9/10) of the farms, and 41.3% (124/300) were positive to *T. gondii*, which was detected in 100% of the farms (10/10). Only 8% (24/300) of sheep were seropositive for both parasites.

Figure 2 shows the frequency of antibodies against *Neospora* spp. in each flock. Flocks B, C, F constituted the upper stratum, due to their significantly higher infection frequencies in comparison to the lower stratum flocks I, H, E ($p<0.05$). Flocks A, G, J, D showed intermediate infection frequencies, with no significant difference to the upper and lower strata.

Frequencies of antibodies against *T. gondii* (Figure 3) were higher ($p <0.05$) in flocks G, B, A, I (upper stratum) than in flocks E, F, D (lower stratum). Intermediate infection rates were found in the flocks J, C, H, with no significant difference to the upper and lower strata.

Epidemiological factors and the infection frequencies by *Neospora* spp. and *T. gondii* in each farm are described in the Table 1. Dogs and cats, with free access to water sources, pastures, corrals and feed deposits, were present in 100% (10/10) and 50% (5/10) of the farms, respectively. Wild rodents in deposits of feed, pens or barns, besides wild canids and felids accessing pastures and feed or hay deposits, were reported in 90% (9/10) of the farms. Sheep's water supplies were from weirs (80%; 8/10) or streams (30%; 3/10). Reproductive problems, such as abortion, stillbirth, mummified fetuses, or birth of weak lambs, were reported in 30% (3/10) of the flocks. The higher frequencies of antibodies against T. gondii in the upper stratum flocks G, B, A, I was significantly associated ($p=0.047$) with the higher frequency of cats present in these flocks, in comparison to the lower stratum flocks E, F, D (see the Table 1). On the other hand, none significant association between each of the other epidemiological factors and the frequency of antibodies against *Neospora* spp. or *T. gondii* in sheep was detected ($p>0.05$).

Table 1. Frequencies of *Neospora* spp. and *Toxoplasma gondii* seropositive sheep and risk factors observed in each studied farm.

Farms	Seropositive sheep (%)[a]		Presence/absence of potential risk factors as informed by the farms' owners						
	Neosp.	*Toxopl.*	Dogs	Cats[b]	Wild canids/ felids	Wild rodents	Weir water	Stream water	Reproductive problems
A	20.0	60.0	Yes	Yes	Yes	Yes	No	Yes	No
B	33.3	66.6	Yes	No	Yes	Yes	Yes	No	No
C	33.3	43.3	Yes	Yes	Yes	Yes	Yes	No	Yes
D	10.0	10.0	Yes	No	Yes	Yes	Yes	No	No
E	0	23.3	Yes	No	No	Yes	Yes	No	No
F	26.6	13.3	Yes	No	Yes	Yes	Yes	Yes	Yes
G	16.6	70.0	Yes	Yes	Yes	Yes	Yes	No	No
H	3.3	30.0	Yes	No	Yes	No	Yes	No	No
I	6.6	53.3	Yes	Yes	Yes	Yes	No	Yes	No
J	13.3	43.3	Yes	Yes	Yes	Yes	Yes	No	Yes
Total (%)	16.3	41.3	100	50.0	90.0	90.0	80.0	30.0	30.0

[a] Percentages of *Neospora* spp. (Neosp.) and *T. gondii* (Toxopl.) seropositive sheep in each of the ten farms (30 sheep samples per farm).
[b] Presence of cats in the farm was significantly associated ($p = 0.047$) with high frequencies of *T. gondii* seropositive sheep in the flock, as assessed by the Fisher's exact test at 95% confidence interval.

Discussion

Neospora spp. and *T. gondii* seropositive sheep were found, respectively, in 90% (9/10, exception being farm E) and 100% (10/10) of the flocks. However, a higher frequency of seropositivity (p = 0.0001) to *T. gondii* (41.3%, 124/300) was found in comparison to *Neospora* spp. (16.3%, 49/300), considering all the sheep tested. Simultaneous presence of antibodies against both parasites was detected in 8% (24/300) of the sheep. This low frequency could be considered noticeable if compared to the 3.5% found in a similar study in the state of Sao Paulo, Brazil (FIGLIUOLO et al., 2004). Prevalence studies, demonstrating the susceptibility of sheep to these protozoa, show infection rates from 0 to 64.2% for *Neospora* spp., and from 6.3% to 100% for *T. gondii* (DUBEY; LINDSAY, 1996; DUBEY, 2003; TEMBUE et al., 2011; GUIMARÃES et al., 2013; MOURA et al., 2014). Infection rates range depending on the diversity of epidemiological factors that occur in each flock (SOARES et al., 2009). Serological data comparable to those found in this study have been described in sheep flocks in other regions of Brazil.

Neospora spp. was detected in 91.7% (11/12) of the farms evaluated in the Sao Paulo state (LANGONI et al., 2011). In the Paraná state, *Neospora* spp. was found in 88.9% (8/9) of the farms (ROMANELLI et al., 2007), and in 81.8% (9/11) of the farms, with infection rates ranging from 0 to 32.3% (MUNHÓZ et al., 2010). In contrast, only 42.4% (39/92) of the farms had *Neospora* spp. seropositive sheep, with seroprevalence rates from 0 to 40%, in a study performed in the Plateau region of Santa Catarina state (MOURA et al., 2014).

Compared to *Neospora* spp. serological surveys in sheep from other Brazilian regions, the overall frequency of seropositivity (16.3%, 49/300, 1:50 cutoff) observed in the present study was similar to: 13.1% (64/488) found from 63 municipalities of Minas Gerais state (ANDRADE et al., 2012); 13.2% (105/795) which included 31 farms from nine counties located in the southeastern region of the Bahia state (ROCHA et al., 2014); and 13.9% (53/381) in 11 flocks from the Paraná state (MUNHÓZ et al., 2010). However, lower seroprevalences have been found using the same diagnostic technique, such as: 7% (92/1308) in the Plateau region of the Santa Catarina state (MOURA et al., 2014); 8.75% (90/1028) at Federal District (UENO et al., 2009); 9.2% (55/597) at São Paulo state (FIGLIUOLO et al., 2004); 9.5% (29/305) at Paraná state (ROMANELLI et al., 2007), and only 1.8% from the Mossoró municipality, Rio Grande do Norte state (SOARES et al., 2009). On the other hand, higher seroprevalences were reported from Mato Grosso do Sul state, with rates of 29% (41/141) (ANDREOTTI et al., 2009) and 33.7% (140/416) (OSHIRO et al., 2015); Minas Gerais state, 47.1% (73/155) (ROSSI et al., 2011); and Pernambuco state, 64.2% (TEMBUE et al., 2011).

Neospora spp. infection frequencies from 0 to 33.3% were found in the studied flocks (Figure 2). Similar rates, from 2.5 to 25.8%, were reported from Santa Catarina state flocks (DALLA ROSA et al., 2011). However, seroprevalence ranging from 20 to 69.2% was detected in the Mato Grosso do Sul state (OSHIRO et al., 2015). Studies indicate that such variations can result from differences related to factors as: flock management, water sources, presence (or quantity) of dogs on the farm, farm size, climatic conditions, presence of other animal species on grazing areas, animals' origin, age and breed, and veterinary supervision (ROSSI et al., 2011; DALLA ROSA et al., 2011; MOURA et al., 2014).

The presence of antibodies against *T. gondii* in 100% of the farms indicates the relevance of this parasite in the flocks studied, as described in other regions of Brazil (SAKATA et al., 2012; GUIMARÃES et al., 2013). Widely variable frequencies of *T. gondii* seropositive sheep were found (10 - 70%) within each of the ten flocks studied (Figure 3), as reported in Paraná state (28.6 - 84%) (ROMANELLI et al., 2007). Moreover, the

overall frequency of *T. gondii* seropositive sheep in the present study (41.3%, 124/300, 1:64 cutoff) was similar to 46.5% (72/155) that was reported in Uberlândia county, Minas Gerais state (ROSSI et al., 2011). However, other studies have shown higher frequencies in Brazil: 56.9% (205/360) at Lages county, Santa Catarina state (SAKATA et al., 2012); 51.5% (157/305) at Paraná state (ROMANELLI et al., 2007); 52% (254/488) at Jaboticabal microregion, São Paulo state (LOPES et al., 2010); and 60.8% (97/305) at Fernando de Noronha archipelago, Pernambuco state (COSTA et al., 2012). On the other hand, lower (but remarkable) frequencies were detected at Central region of São Paulo state (34.7%; 207/597) (FIGLIUOLO et al., 2004), also at the states of Alagoas (32.9%; 142/432) (PINHEIRO JÚNIOR et al., 2009), Bahia (30.2%; 240/795) (GUIMARÃES et al., 2013), and Sergipe (28.2%; 263/932) (MENDONÇA et al., 2013), and at the Mossoró county, Rio Grande do Norte state (20.8%; 85/409) (SOARES et al., 2009).

Variations in infection rates among flocks indicate that differences related to animals' management and the presence or absence of specific risk factors may be influencing the occurrence of toxoplasmosis. Thus, some factors can be considered, such as vertical transmission of *T. gondii* and the presence of felids and rodents with access to food deposits, barns, corrals, and pastures. Likewise, improper disposal of carcasses and abortion debris facilitates the infection of cats by *T. gondii*, and its consequent horizontal transmission to sheep and humans (WEISS, 2007; DUBEY, 2009; BRAGA FILHO et al., 2010).

Another factor that may be influencing the transmission of *T. gondii* and *N. caninum* is the variable risk of contamination of water sources as fountains, weirs, dams, and streams, with oocysts excreted in the feces of definitive hosts. Furthermore, the contact with wild canids and felids from woods next to the farms can favor infection of the flock (BLOOD; RADOSTITS, 1991). In the present study, the presence of wild canids and felids was reported by 90% of the owners. These factors, combined with carelessness or lack of knowledge about hygiene of facilities and about disposal of carcasses and fetal-placental debris, can increase *T. gondii* transmission. As indicated by our results, other studies showed that contact with cats increases the risk of toxoplasmosis in sheep (ANDRADE et al., 2012; GUIMARÃES et al., 2013; MENDONÇA et al., 2013). Therefore, adoption of preventive measures to avoid contamination of the sheep's food and water by cat feces, and to restrict the access of carnivores to sheep carcasses and placental/fetal debris after lambing/abortion, can reduce the risks of *T. gondii* transmission to cats, humans and sheep, and can reduce reproductive disorders in the flock.

As observed by Figliuolo et al. (2004) and Dalla Rosa et al. (2011), the relationship between the presence of dogs and *N. caninum* infection rates could not be assessed, due to the presence of dogs in 100% (10/10) of the farms. Furthermore, it was suggested that the maintenance of neosporosis in sheep flocks may be mainly due to vertical (transplacental) transmission, similarly to what occurs in cattle (ANDRADE et al., 2012). Additionally, health education measures could be taken to control the spread of *Neospora* spp. in sheep flocks, through advising farmers to avoid the access of canids to fetal and placental tissue of potentially infected sheep and other host species.

Despite the low infection rates by Neospora spp. found in each flock, this protozoan was detected in 90% of the farms studied, showing its presence within the Central-Western, Northwestern, Southwestern and Southeastern regions of the Rio Grande do Sul state, possibly causing reproductive losses for sheep farming operations. In the present study, reproductive problems were noticed in 30% (3/10) of the farms, similarly to 38.7% (12/31) described at Federal District, Brazil (UENO et al., 2009). However, as reported by Moura et al. (2014), this factor was not significantly associated with the frequency of antibodies against *Neospora* spp. in the flocks.

Sheep play a significant role regarding human toxoplasmosis, as a direct source of *T. gondii* transmission through undercooked meat consumption. In addition, toxoplasmosis causes great economic losses in sheep production. Neosporosis causes neonatal mortality and abortion in ruminants. The wide occurrence of *Neospora* spp. in the flocks showed that its economic impact on the sheep industry could be underestimated. Therefore, toxoplasmosis and neosporosis are diseases that should be considered in the health management of sheep flocks in Rio Grande do Sul state, Brazil.

Conclusion

This study showed that *Neospora* spp. and *T. gondii* are spread through sheep flocks from Central-Western, Northwestern, Southwestern and Southeastern of the Rio Grande do Sul state, Brazil. The occurrence of these parasites may be related to multifactorial causes identified in the farms. However, no association was found between the frequencies of *Neospora* spp. and *T. gondii* seropositive sheep and each potential risk factor evaluated, except in regards to the presence of cats, which was associated with higher frequencies of antibodies against *T. gondii* within the flocks.

Ethics Committee on Animal Experimentation

All procedures with animals and experimentation were performed under supervision of veterinarians and in accordance with the recommendations imposed by the *Comitê de Ética em Experimentação Animal* (CEUA) of the Universidade Federal de Santa Maria (UFSM) (process number 069/2014).

References

ANDRADE, G. S.; BRHUNA, F. R. P.; ROCHA, M. B. M.; GUIMARÃES, A. S.; GOUVEIA, A. M. G.; GUIMARÃES, A. M. Seroprevalence and risk factors for *Neospora caninum* in sheep in the state Minas Gerais, southeastern Brazil. *Veterinary Parasitology*, Amsterdam, v. 188, p. 168-171, 2012.

ANDREOTTI, R.; MATOS, M. F. C.; GONÇALVES, K. N.; OSHIRO, L. M.; LIMA-JUNIOR, M. S. C.; PAIVA, F.; LEITE, F. L. Comparison of indirect ELISA based on recombinant protein NcSRS2 and IFAT for detection of *Neospora caninum* antibodies in sheep. *Revista Brasileira de Parasitologia Veterinária*, São Paulo, v. 18, n. 2, p. 19-22, 2009.

BÁRTOVÁ, E.; SEDLÁK, K.; LITERÁK, I. *Toxoplasma gondii* and *Neospora caninum* antibodies in sheep in the Czech Republic. *Veterinary Parasitology*, Amsterdam, v. 161, n. 1, p. 131-132, 2009.

BLOOD, D. C.; RADOSTITS, O. M. *Clínica veterinária.* Rio de Janeiro: Guanabara Koogan, 1991. 1121 p.

COSTA, D.G.C.; M. F. V., MARVULLO, J. S. A., SILVA; S. C., SANTANA; F. J. R., MAGALHÃES; C. D. F., LIMA FILHO; V. O., RIBEIRO; L. C., ALVES; R. A., MOTA; J. P., DUBEY; J.C.R., SILVA. Seroprevalence of *Toxoplasma gondii* in Domestic and Wild Animals From the Fernando de Noronha, Brazil. *Journal of Parasitology*, Lawrence, v. 98, n. 3, p. 679-680, 2012.

DALLA ROSA, L.; MOURA, A. B. de; GÜTHS, M. F.; BELLATO, V.; SARTOR, A. A.; SOUZA, A. P. de. Prevalência e fatores de risco para infecção por *Neospora caninum* em ovinos no município de Lages, Santa Catarina, Brasil. *Revista de Ciências Agroveterinárias*, Lages, v. 10, n. 2, p. 127-137, 2011.

DUBEY, J. P. *Neospora caninum* and neosporosis in animals. *Korean Journal of Parasitology,* Seoul*,* v. 41, n. 1, p. 1-16, 2003.

_____. Toxoplasmosis in sheep-the last 20 years. *Veterinary Parasitology,* Amsterdam, v. 163, p. 1-14, 2009.

DUBEY, J. P.; LINDSAY, D. S. *Neospora caninum* induced abortion in sheep. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians,* v. 2, n. 3, p. 230-233, 1990.

DUBEY, J. P.; SCHARES, G.; ORTEGA-MORA, L. M. Epidemiology and control of neosporosis and *Neospora caninum*. *Clinical Microbiology Reviews*, v. 20, n. 2, p. 323-367, 2007.

DUBEY, J. P.; SONN, R. J.; HEDSTROM, O.; SNYDER, S. P.; LASSEN, E. D. Serologic and histologic diagnosis of toxoplasmic abortions in sheep in Oregon. *Journal of the American Veterinary Medical Association,* New York, v. 196, n. 2, p. 291-294, 1990.

DUBEY, J. P.; LINDSAY, D. S. A review of *Neospora caninum* and neosporosis. *Veterinary Parasitology,* Amsterdam, v. 67, p. 1-59, 1996.

FIGLIUOLO, L. P. C.; KASAI, N.; RAGOZO, A. M. A.; PAULA, V. S. O. de; DIAS, R. A.; SOUZA, S. L. P.; GENNARI, S. M. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in ovine from São Paulo State, Brazil. *Veterinary Parasitology*, Amsterdam, v. 123, p. 161-166, 2004.

BRAGA FILHO, E.; RAMOS, O. S.; FREITAS, J. A. Inquérito sorológico de *Toxoplasma gondii* em ovinos na microrregião Castanhal, Pará, Brasil. *Arquivos do Instituto Biológico*, São Paulo, v. 77, n. 4, p. 707-710, 2010.

GARCIA, J. L.; NAVARRO, I. T.; OGAWA, L.; OLIVEIRA, L. de; OLIVEIRA, R. C. de. Soroepidemiologia da toxoplasmose em gatos e case de propriedades rurais do município de Jaguapita, Estado do Paraná, Brasil. *Ciência Rural*, Santa Maria, v. 29, n. 1, p. 99-104, 1999.

GONDIM, L. F. P.; LINDSAY, D. S.; MCALLISTER, M. M. Canine and bovine *Neospora caninum* control sera examined for crossreactivity using *Neospora caninum* and *Neospora hughesi* indirect fluorescent antibody tests. *Journal of Parasitology*, Lawrence, v. 95, n. 1, p. 86-88, 2009.

GUIMARÃES, L. A.; BEZERRA, R. A.; ROCHA, D. S.; ALBUQUERQUE, G. R. Prevalence and risk factors associated with anti-*Toxoplasma gondii* antibodies in sheep from Bahia state, Brazil. *Revista Brasileira de Parasitologia Veterinária*, Jaboticabal, v. 22, n. 2, p. 220-224, 2013.

KOBAYASHI, Y.; YAMADA, M.; OMATA, Y.; KOYAMA, T.; SAITO, A.; MATSUDA, T.; OKUYAMA, K.; FUJIMOTO, S.; FURUOKA, H.; MATSUI, T. Naturally-occurring *Neospora caninum* infection in an adult sheep and her twin fetuses. *The Journal of Parasitology*, West Lafayette, v. 87, n. 2, p. 434-436, 2001.

LANGONI, H.; GRECA JÚNIOR, H.; GUIMARÃES, F. F.; ULLMAN, L. S.; GAIO, F. C.; UEHARA, R. S.; ROSA, E. P.; AMORIM, R. M.; SILVA, R. da. Serological profile of *Toxoplasma gondii* and *Neospora caninum* infection in commercial sheep from São Paulo State, Brazil. *Veterinary Parasitology*, Amsterdam, v. 177, n. 1, p. 50-54, 2011.

LOPES, W. D. Z.; SANTOS, T. R.; SILVA, R. S.; ROSSANESE, W. M.; SOUZA, F. A.; RODRIGUES, J. D. F.; MENDONÇA, R. P.; SOARES, V. E.; COSTA, A. J. Seroprevalence of and risk factors for *Toxoplasma gondii* in sheep raised in the Jaboticabal microregion, São Paulo State, Brazil. *Research in Veterinary Science*, v. 88, n. 1, p. 104-106, 2010.

MENDONÇA, C. E.; BARROS, S. L. B.; GUIMARÃES, V. A. A.; FERRAUDO, A. S.; MUNHOZ, A. D. Prevalence and risk factors associated to ovine toxoplasmosis in northeastern Brazil. *Revista Brasileira de Parasitologia Veterinária*, Jaboticabal, v. 22, n. 2, p. 230-234, 2013.

MOURA, A. B.; GÜTHS, M. F.; FARIAS, J. A.; SOUZA, A. P.; SARTOR, A. A.; QUADROS, R. M. *Neospora caninum* seroprevalence and risk factors for ewes from Santa Catarina Plateau, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 35, n. 5, p. 2591-2600, 2014.

MUNHÓZ, K. F.; LUCA NETO, M. de; SANTOS, S. M. A.; GARCIA, J. L.; GUIMARÃES JUNIOR, J. S.; VIDOTTO, O.; HEADLEY, S. A.; YAMAMURA, M. H. Occurrence of anti-*Neospora caninum* antibodies in sheep from farms located in northern Parana, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 31, n. 4, p. 1031-1040. 2010.

OPEL, U.; CHARLESTON, W. A. G.; POMROY, W. E.; ROMMEL, M. A survey of the prevalence of *Toxoplasma* infection in goats in New Zealand and a comparison of the latex agglutination and indirect fluorescence tests. *Veterinary Parasitology*, Amsterdam, v. 40, n. 3, p. 181-186, 1991.

OSHIRO, L. M.; REIS, F. A.; DITTRICH, R. L.; CUNHA, R. C.; ANDREOTTI, R. Serology for Toxoplasmosis and Neosporosis in Ewes in the State of Mato Grosso do Sul, Brazil. *Journal of Veterinary Science & Technology*, Los Angeles, v. 6, n. 4, p. 233, 2015.

PINHEIRO JÚNIOR, J. W.; MOTA, R. A.; OLIVEIRA, A. A. F.; FARIA, E. B.; GONDIM, L. F. P.; SILVA, A. V. da; ANDERLINI, G. A. Prevalence and risk factors associated to infection by *Toxoplasma gondii* in ovine in the State of Alagoas, Brazil. *Parasitology Research*, Berlin, v. 105, p. 709-715, 2009.

ROCHA, D. S.; GUIMARÃES, L. A.; BEZERRA, R. A.; MENDONÇA, C. E. D.; DÓREA, T. G.; MUNHOZ, A. D.; ALBUQUERQUE, G. R. Seroprevalence and factors associated with Neospora caninum infection in sheep from southeastern Bahia, Brazil. *Revista Brasileira de Medicina Veterinária*, Rio de Janeiro, v. 36, n. 4, p. 443-447, 2014.

ROMANELLI, P. R.; FREIRE, R. L.; VIDOTTO, O.; MARANA, E. R. M.; OGAWA, L.; PAULA, V. S. O. de; GARCIA, J. L.; NAVARRO, I. T. Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Paraná State, Brazil. *Research in Veterinary Science*, Oxford, v. 82, n. 2, p. 202-207, 2007.

ROSSI, G. F.; CABRAL, D. D.; RIBEIRO, D. P.; PAJUABA, A. C. A. M.; CORRÊA, R. R.; MOREIRA, R. Q.; MINEO, T. W. P.; MINEO, J. R.; SILVA, D. A. O. Evaluation of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Uberlândia, Minas Gerais State, Brazil, by different serological methods. *Veterinary Parasitology*, Amsterdam, v. 175, n. 3, p. 252-259, 2011.

SAKATA, F. B. L. S.; BELLATO, V.; SARTOR, A. A.; MOURA, A. B.; SOUZA, A. P.; FARIAS, J. A. *Toxoplasma gondii* antibodies sheep in Lages, Santa Catarina, Brazil, and comparison using IFA and ELISA. *Revista Brasileira de Medicina Veterinária*, Jaboticabal, v. 21, n. 3, p. 196-200, 2012.

SOARES, H. S.; AHID, S. M. M.; BEZERRA, A. C. D. S.; PENA, H. F. J.; DIAS, R. A.; GENNARI, S. M. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in sheep from Mossoró, Rio Grande do Norte, Brazil. *Veterinary Parasitology*, Amsterdam, v. 160, n. 3, p. 211-214, 2009.

TEMBUE, A. A. S.; RAMOS, R. A. N.; SOUSA, T. R.; ALBUQUERQUE, A. R.; COSTA, A. J.; MEUNIER, M. J.; FAUSTINO, M. A. G.; ALVES, L. C. Serological survey of *Neospora caninum* in small ruminants from Pernambuco State, Brazil. *Revista Brasileira de Parasitologia Veterinária*, Jaboticabal, v. 20, n. 3, p. 246-248, 2011.

UENO, T. E. H.; GONÇALVES, V. S. P.; HEINEMANN, M. B.; DILLI, T. L. B.; AKIMOTO, B. M.; SOUZA, S. L. P. de; GENNARI, S. M.; SOARES, R. M. Prevalence of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Federal District, central region of Brazil. *Tropical Animal Health and Production*, Edinburgh, v. 41, n. 4, p. 547-552, 2009.

WEISS, L. M. *Toxoplasma gondii.* The model apicomplexan: perspectives and methods. New York: Elsevier Ltd., 2007. 800 p.