

Serology for Toxoplasmosis and Neosporosis in Ewes in the State of Mato Grosso Do Sul, Brazil

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Abstract

Toxoplasma gondii and *Neospora caninum* are protozoans that can cause reproductive problems in sheep. The aim of the present study was to determine the frequency of anti-*N. caninum* and anti-*T. gondii* antibodies among sheep herds in some counties in the state of Mato Grosso do Sul. Four hundred sixteen serum samples were collected from ewes in eight counties. The sera samples were evaluated by using an indirect fluorescent antibody test with a 1:50 dilution. The seroprevalences found for *T. gondii* and *N. caninum* were 33.7% and 52.2%, respectively. The rate of animals testing positive simultaneously for *Neospora* and *Toxoplasma* was of 17.5%. The results of the present study demonstrate that sheep raised in these counties are exposed to *T. gondii* and *N. caninum* and that sheep can be co-infected with both parasites.

Keywords: Coccidia; Parasitic disease; Serology; Indirect immunofluorescence; Reproductive problems

Introduction

Toxoplasmosis is caused by the *Toxoplasma gondii* and Neosporosis by the *Neospora caninum*. Both parasites are protozoans of the Sarcocystidae family and have a wide geographic distribution. There are reports of anti-*T. gondii* and anti-*N. caninum* antibodies in several species, including humans [1]. The definitive hosts of *T. gondii* are felids, and the definitive hosts of *N. caninum* are certain canids, such as the domestic dog, the wolf and the coyote [2-6]. The relevant intermediate hosts of *T. gondii* and *N. caninum* are livestock, such as sheep, cattle and goats [2]. These parasites may cause abortions and stillbirths [7], and, consequently, economic losses. Studying the intermediate hosts may help to identify *T. gondii* and *N. caninum* circulation patterns in different environments [8].

Brazil has favorable geographical and climatic conditions for sheep production. Its current sheep population is estimated to be 16.789.492 animals [9], with the state of Mato Grosso do Sul (MS) having 498.064 animals. Even though sheep breeding never became an economic activity of equal importance as cattle breeding, sheep meat has always been part of the Mato Grosso do Sul population's diet. The sheep's economic importance increased with the arrival of immigrants who had the tradition of lamb meat consumption, such as the gaúchos, northeastern Brazilians and Syrian-Lebanese. In Campo Grande, 92% of consumers have tried lamb meat, and most have a favorable opinion about it [10].

The Mato Grosso do Sul state was studied by Andreotti et al. [11], Marques et al. [12] and Pinto et al. [13], who reported the presence of anti-*N. caninum* and - *T. gondii* antibodies in sheep, using different techniques and obtaining different results. Other diseases related to abortion were mentioned by Juliano et al. [14] in the municipality of Corumbá. They found a 12.8% seropositivity for ovine brucellosis in a herd of 1.198 animals, through the use of the IDGA method. In the Nhecolândia region, also in the municipality of Corumbá, wild and domestic animals sera (including ovines) were analyzed for antibody titles against serological variants of leptospira, and it was found that 9% of animals were contaminated, in a group of 110 [15].

In Mato Grosso do Sul, sheep breeding is usually done extensively, being a secondary activity to cattle breeding; the production is mainly used for the farmer's own consumption [16]. Nonetheless, the production system has been changing over the years, but disease reports are still low in the state.

Considering the data previously reported, showing that the toxoplasmosis may be a source of infection for humans and that neosporosis may cause abortion, and that currently there are few reports about the infection of these parasites in sheep in the state of Mato Grosso do Sul, the objective of this study was to verify the occurrence of infection by *T. gondii* and *N. caninum* in sheep from farms located at the intersection of the four mesoregions of the state of Mato Grosso do Sul.

Material and Methods

Animals and samples

Blood samples were collected in 2010 from 410 ewes of reproductive age, each having several offspring. The blood samples (5.0 mL) were stored in tubes without anticoagulant. The serum was separated by centrifugation and stored at -20°C, until the analysis was done. The samples were collected in the most representative region (comprising 79 counties; Table 1) regarding the presence of flocks in the state of Mato Grosso do Sul, Brazil (Figure 1).

The study was conducted in counties belonging to 03 of 04

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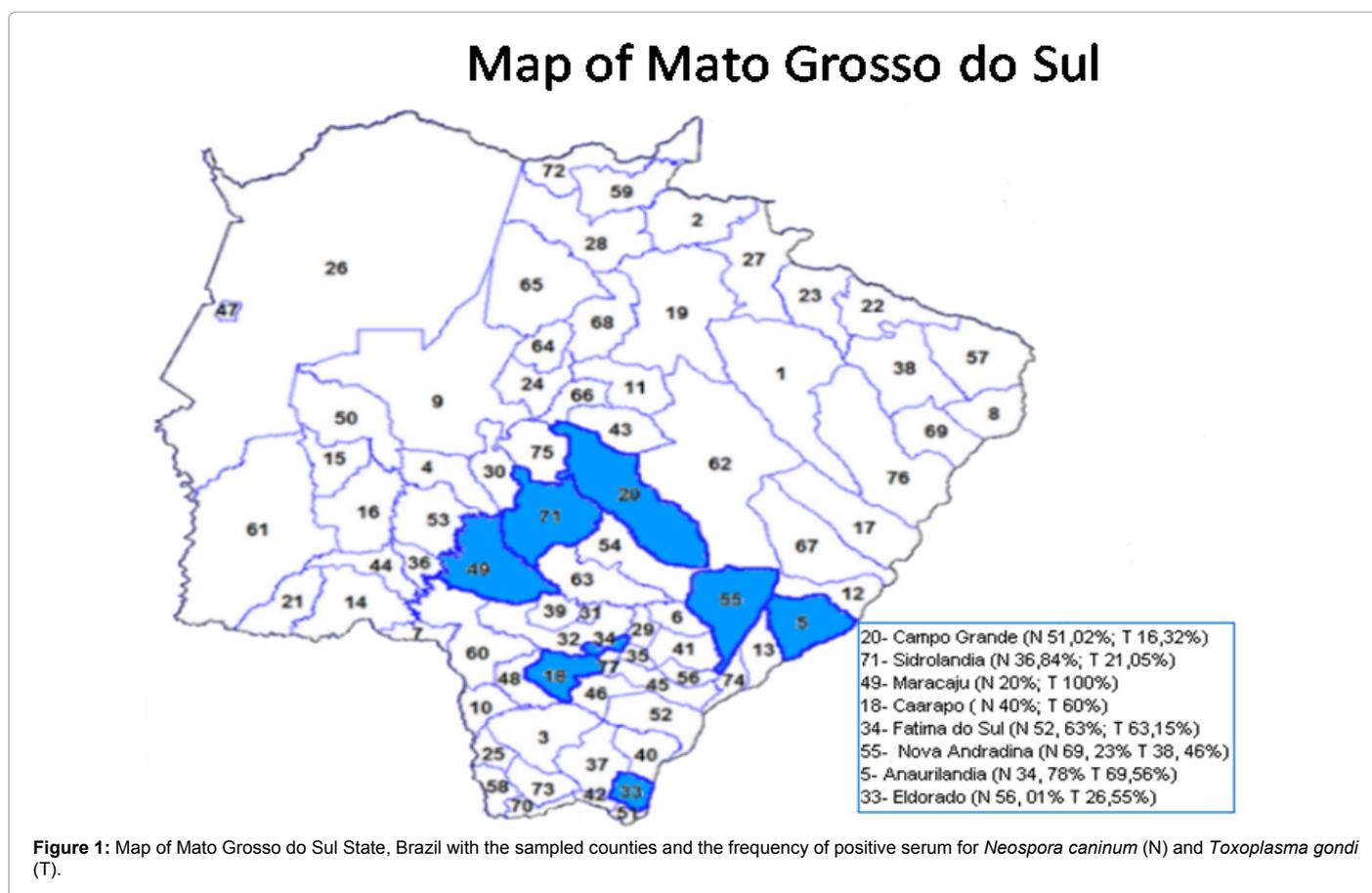
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Counties (n)	<i>Neospora caninum</i>		<i>Toxoplasma gondii</i>		Chi-square P-value
	Positive	Frequency	Positive	Frequency	
Anaurilandia (46)	16	34.8%	32	69.6%	p=0.0914
Caarapo (05)	2	40.0%	3	60.0%	p=0.1921
Campo Grande (49)	25	51.0%	8	16.3%	p=0.6997
Eldorado (241)	135	56.01%	64	26.6%	p<0.0001
Fátima do Sul (19)	10	52.6%	12	63.2%	p=4371
Maracaju (05)	1	20.0%	5	100%	p=0.3711
Nova Andradina (26)	18	69.2%	10	38.5%	p=0.6143
Sidrolandia (19)	7	36.8%	4	21.1%	p=0.2312
Total (416)	214	52.2%	138	33.7%	p<0.0001

n: number of samples collected in each county.

Table 1: Frequency of positive serum for *T. gondii* and *N. caninum* in sheep in the state of Mato Grosso do Sul, Brazil.



mesoregions, according to IBGE/CIDER [9]. These are the mesoregions with the highest sheep population in the state of Mato Grosso do Sul. The city of Campo Grande, where the only slaughterhouse in the state is located, is within one of these mesoregions.

For the purpose of epidemiological representation and adequate statistical analysis, the non-probability sampling method was used to select the producers. This decision was taken due to the small number of sheep producers in Mato Grosso do Sul, which impairs the use of random sampling.

The available literature regarding the mentioned parasites in the Mato Grosso do Sul state is small. One of the studies reported the inexistence of the *T. gondii* in sheep from the municipality of Eldorado

[12]. Regarding the *N. caninum*, antibodies were found in 30.8% of the sheep tested [11].

The minimum number of samples to be tested (n) was statistically calculated [17] considering an expected prevalence of 5% (based on the lowest prevalence found in the literature for the studied region and considering both diseases), a sampling error of 20% and a confidence level of 95% (z=1.96), the number of samples to be tested was calculated at 410.

Serological diagnosis

The indirect fluorescent antibody test (IFAT) was used to detect anti-*T. gondii* and anti-*N. caninum* antibodies, according to the methods described by Camargo [18] and Dubey et al. [19] respectively.

According to Dubey and Lindsay [7], for each serological test in each region, there are several serological techniques and various cutoff, so there are several positive values.

The *T. gondii* RH and *N. caninum* NC-1 strains were used as antigens in the IFAT. *N. caninum* tachyzoites were maintained in Vero cell cultures, and *T. gondii* tachyzoites were obtained by intraperitoneal passage in mice. At the Embrapa Gado de Corte laboratory, the antigens were processed and maintained in Vero cell cultures [20,21] at 37°C and without CO₂.

The sera dilution used for the IFAT was 1:50 [22,23]. Positive and negative control sera were used on each slide. The secondary antibody used was the monoclonal FITC-conjugated anti-sheep IgG (Sigma-Aldrich F 5137) at a 1:160 dilution. Samples with complete peripheral fluorescence, when viewed under immunofluorescence microscopy, were considered positive [22].

Statistics

The Chi-square test was applied to statistically differentiate the seroprevalence between counties and in the total sample.

Results and Discussion

Of the 416 samples examined in the present study, 52.2% and 33.7% were positive for *N. caninum* and *T. gondii*, respectively ($p < 0.0001$; Table 1). These results show that *N. caninum* is highly distributed among the sheep population. Across the counties studied, there were animals contaminated simultaneously with *N. caninum* and *T. gondii*; however, the simultaneous seroprevalence rate was low (17.5%; 73/416).

In all the counties studied, there was at least one animal contaminated with *N. caninum* or *T. gondii*. The municipality of Nova Andradina had the highest prevalence of anti-*N. caninum* antibodies (69.2%). Maracaju city had the highest prevalence of anti-*T. gondii* (100%) and the smallest of anti-*N. caninum* (20%), but there was no statistically significant difference ($p = 100\%$; Table 1), probably because of the small sample (5 samples). The county of Campo Grande had the smallest seroprevalence for *T. gondii* (16.3%).

Serology studies of the presence of anti-*N. caninum* and anti-*T. gondii* antibodies have been made in various parts of the world and in Brazil. However, it is hard to compare the results due to different cutoff points and methods used for diagnosis [1,24].

There are several serological methods to evaluate the presence of antibodies against *T. gondii* and *N. caninum* in sheep. One of the most used is the IFAT, although other methods are also suitable for analyzing different animal species [25,26].

A study conducted in Jaboticabal, state of São Paulo, found, through the use of IFI, an anti-*T. gondii* antibody prevalence of 52.05% (254/488). This high prevalence was associated with the presence of felids, directly related to the spread of oocysts in the properties studied; the importance of sheep in *T. gondii* transmission to humans was emphasized [27]. Therefore, the *T. gondii* rate of contamination in Jaboticabal was higher than the one found in this study (33.7%; 138/416); the difference is that this one included several municipalities [28] evaluated the presence of *T. gondii* in sheep sera in 23 municipalities of the state of Alagoas, and showed a prevalence of 32.9% (142/432). The results found can be considered as a base reference for this study, since the herds that were analyzed belonged to different municipalities. Although the results are similar, the studies are not comparable because

of the different number of surveyed municipalities, different number of animals and the different cutoff points in each study.

In a study conducted in 04 nearby municipalities in the state of São Paulo, a survey was made to identify the presence of anti-*N. caninum* and anti-*T. gondii* antibodies. A sample of 597 animals was used, predominantly composed of female animals intended for human consumption, for the manufacture of wool and reproduction. The result found a presence rate of 34.7% for *T. gondii* and 9.2% for *N. caninum* [29]. This study is close to the current study because there is at least one animal that tested positive for *T. gondii* in each municipality studied.

Regarding *T. gondii* infection, Pinheiro et al. [28] reported a prevalence of 32.9% (142/432) in a study performed in 23 municipalities in the state of Alagoas. The results found by these authors were similar to those found in our study. Similar results were also found in a study performed in the state of São Paulo, in which 34.7% (207/597) of the animals were seropositive and at least one animal had anti-*T. gondii* antibodies in all of the properties studied [29]. A study conducted in Jaboticabal, state of São Paulo, found an anti-*T. gondii* antibody prevalence of 52.05% (254/488), which is higher than the prevalence of the two other cited studies. This high prevalence was associated with the presence of felids in the properties studied, and the importance of sheep in *T. gondii* transmission to humans was emphasized [27].

The present study found a higher serology of *N. caninum* (52.2%) than *T. gondii* (33.7%). These data differ from those reported by Rossi et al. [30], who found a seropositivity of 47.1% for *N. caninum* and 46.5% for *T. gondii*. In relation to *N. caninum*, the result above is close to ours, but with a different cutoff. In the state of Pernambuco, Tembue et al. [31] reported a *N. caninum* prevalence rate of 64.2% (52/81) after surveying 23 properties in the municipality of Ibirimir. In the study by Tembue et al. [31], 10 males and 127 females were evaluated, and there was no significant difference between genders. In the present study, only females were assayed.

In Brazil, there is a large variation between positive and negative results for both *T. gondii* and *N. caninum*. This variation can be explained by the large sheep population, the different breeds raised in the country, the food resources available and the animal age, among other factors [32]. Ueno et al. [33] reported that different serological tests, cutoff values and sampling methods might increase the difficulty of comparing the prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies between different studies performed in different regions.

Until now, there had been only three reports on the presence of anti-*N. caninum* and/or anti-*T. gondii* antibodies in sheep in the state of Mato Grosso do Sul. Among these reports, Andreotti et al. [11], in a study performed in 2008 in Campo Grande-MS, reported a prevalence of 30.8% for *N. caninum* (136/441) in ewes using the IFAT and indirect ELISA tests with the rNcSRS2 recombinant antigen. Andreotti et al. [11] also reported a seropositivity increase among ewes from 2008 to 2010 in the same region. The current study is the first to demonstrate the simultaneous occurrence of anti-*T. gondii* and anti-*N. caninum* antibodies in sheep from Mato Grosso do Sul.

Marques et al. [12] studied the presence of anti-*T. gondii* antibodies in sheep in Eldorado - MS, and did not find serum antibodies using the direct agglutination test (1:25). This result differs from the current study, which found a 33.7% positivity using the IFAT method (1:50).

Pinto et al. [13] reported an abortion outbreak in a herd of 268 ewes in the municipality of Aquidauana, MS. Among all the animals, 186 were ewes in the third trimester of gestation, and 10 of them

aborted. Of the four fetuses evaluated three were from the same ewe. Immunohistochemical examination of the fetuses showed strong positive staining for *N. caninum* and weak positive staining for *T. gondii*. *N. caninum* infection was confirmed, demonstrating the importance of the *N. caninum* as a potential abortion agent for sheep.

Conclusion

In conclusion, our work shows that *T. gondii* and *N. caninum* infections occur in sheep of this region. In all the municipalities studied, there was at least one animal with anti-*T. gondii* or anti-*N. caninum* serum antibodies. Therefore, regular monitoring of toxoplasmosis is important due to its zoonotic potential and the reproductive disorders it can cause in ovine flocks, leading to economic losses. This is also a problem regarding neosporosis, which also has the potential to cause reproductive disorders. The high prevalence percentages of *N. caninum* and *T. gondii* should serve as a warning that the control of these diseases in sheep farming should be taken more seriously.

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