

Toxoplasma gondii antibodies sheep in Lages, Santa Catarina, Brazil, and comparison using IFA and ELISA

Anticorpos toxoplásmicos em ovinos de Lages, Santa Catarina, Brasil, e comparação utilizando RIFI e ELISA

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Abstract

Toxoplasmosis in sheep is a disease of great importance in veterinary medicine, which causes economic losses in livestock and has a great impact on human health, since consumption of infected meat facilitates transmission of zoonotic infections. Blood samples from sheep (n = 360) were collected from 13 farm properties in the municipality of Lages, Santa Catarina, to estimate the prevalence of toxoplasmosis and identify risk factors associated with *Toxoplasma gondii* infection. *T. gondii* antibodies were investigated by means of the indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Animals infected with *T. gondii* were found on 100% of the farms. IFA detected 56.9% (205/360) and ELISA 42.5% of the infected sheep. Breed was the only risk factor associated with the presence of *T. gondii* antibodies. ELISA showed sensitivity of 61%, specificity of 82% and kappa of 0.41, which was considered moderate. This allows use of ELISA as an alternative technique for diagnosing *T. gondii* in sheep.

Keywords: *Toxoplasma gondii*, sheep, IFA, ELISA, risk factors.

Resumo

A toxoplasmose ovina é uma doença parasitária de elevada importância em medicina veterinária e em saúde pública, acarretando prejuízos na produção animal, gerados pelas perdas reprodutivas e econômicas, além de sua implicação na saúde humana, já que o consumo de carne infectada facilita a transmissão zoonótica. Para determinar a prevalência e identificar fatores de risco para a infecção por *T. gondii* em ovinos de Lages, Santa Catarina, amostras de sangue (n = 360) foram coletadas em 13 propriedades. Cada criador respondeu a um questionário para permitir a identificação dos fatores de risco da infecção. A pesquisa de anticorpos foi realizada por meio da Reação de Imunofluorescência Indireta (RIFI ≥ 64) e do Ensaio Imunoenzimático Indireto (ELISA). Em 100% das propriedades foram encontrados animais positivos. Pela RIFI, 205 (56,94%) ovinos apresentaram anticorpos contra *T. gondii* e pelo ELISA, 153 (42,50%). Considerando-se as técnicas sorológicas e a análise estatística, foram fatores de risco pelo ELISA: a idade, a fonte de água e a categoria animal; e pela RIFI, o tipo racial. Foi constatada sensibilidade de 61%, especificidade de 82% e concordância Kappa de 0,41 entre o ELISA e a RIFI (1:64), considerada moderada, permitindo indicar o ELISA como técnica adequada para o diagnóstico de *T. gondii* na espécie ovina.

Palavras-chave: *Toxoplasma gondii*, ovinos, RIFI, ELISA, fatores de risco.

Introduction

Toxoplasmosis is a zoonosis that is usually asymptomatic. It is caused by the coccidian *Toxoplasma gondii* and has worldwide distribution. Consumption of raw or undercooked meat containing tissue cysts is a major source of infection for carnivores and omnivores, while herbivores are primarily infected by ingestion

of oocysts. Another mode of disease transmission is transplacental transmission, which has been blamed for abortions, neonatal mortality and weakness in humans and some animal species, especially small ruminants (DUBEY; THULLIEZ, 1993). The main clinical and economic impact of toxoplasmosis in sheep is abortion, along with losses due to stillbirth or birth of weak lambs (TENTER et al., 2000).

In the state of Santa Catarina, Brazil, sheep herds on the plateau and in the western region account for more than 60% of this state's population (IBGE, 2009). The municipality of Lages is also prominent because of its sheep population. On this plateau,

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sheep are primarily raised in semi-extensive farming systems, and there is no individual control over breeding on the farm properties. Thus, the rates of pregnancy, abortion and lamb loss are not precisely known. Moreover, little is known about the causes of reproductive disorders and their relationship with *T. gondii*.

The gold standard test, which is used worldwide for final diagnosis of *T. gondii* antibodies in animals, is the immunofluorescence assay (IFA). The enzyme-linked immunosorbent assay (ELISA) is used mainly for detection of antibodies when a simpler, cheaper and faster seroepidemiological survey is necessary. It is known that IFA has greater specificity and that ELISA has greater sensitivity (BRASIL, 2002).

Considering the importance of this infection and the lack of health data relating to the sheep industry in the state of Santa Catarina, the present study was conducted with the aims of estimating the prevalence of *T. gondii* infection among sheep in the municipality of Lages using IFA, and evaluating ELISA as the test for diagnosing toxoplasmosis in sheep and identifying risk factors associated with *T. gondii* infection in these animals.

Materials and Methods

Between August 2008 and July 2009, blood samples were collected from 360 sheep on 13 farm properties in the municipality of Lages, state of Santa Catarina.

The sheep population of the municipality was estimated to be 10,581 animals (CIDASC, ICASA, 2008). The Epi Info software (version 6, CDC – Center for Disease Control and Prevention, Atlanta, USA) was used to calculate the animal sample size, assuming that the prevalence of toxoplasmosis in sheep would be around 30%, with a 95% confidence interval (CI) and error of 5% (CDC, 1996). Blood samples were properly identified, packaged and transported to the Parasitology and Parasitic Disease Laboratory, Agroveterinary Science Center (CAV), University of the State of Santa Catarina (UDESC).

Information on health and management factors that could potentially be considered to pose a risk of *T. gondii* infection was obtained through questionnaires answered by the farm owners on the day of collecting blood samples from the animals. The variables analyzed were age, sex, breed, diet, water source, contact with cats, breeding problems, rearing system and animal category.

IFA for toxoplasmosis was performed using *T. gondii* tachyzoites (RH strain) as antigen. The slides, already spotted with a tachyzoite suspension, were kindly provided by the Protozoology Laboratory, Department of Preventive Veterinary Medicine, State University of Londrina (UEL). Examinations were performed in accordance with the methods described by Camargo (1964) and Pará et al. (1995).

Samples with a reaction at dilutions $\geq 1:64$ were considered to be positive (FIGLIUOLO et al., 2004). Positive samples were subjected to four-fold dilutions, until the maximum titration reaction was reached. Positive and negative control serum samples were included in each test.

The ELISA test was performed in accordance with the methods described by Garcia et al. (2006). The reading was performed using a spectrophotometer with a 490 nm operating filter and a 600 nm differential filter. Positive and negative control serum samples were included on each plate and the corrected optical density (OD) was calculated as follows:

$$OD_{\text{corrected}} = \left[\frac{(OD_{\text{sample}} - OD_{\text{negative control}})}{(OD_{\text{positive control}} - OD_{\text{negative control}})} \right] \quad (1)$$

Serum samples were considered positive when the $OD_{\text{corrected}}$ was greater than $[OD \text{ mean (negative control)} + 2 \times \text{negative control standard deviation (SD)}]$.

Sensitivity, specificity, positive and negative predictive values, kappa index and efficacy were calculated on the basis of ELISA, using the IFA serum dilution 1:64 as reference. Association analyses were performed using the chi-square test and Fisher exact test ($p < 0.05$), in order to correlate the serological results with the variables analyzed. The magnitude of the associations was determined according to the ratio of probability of occurrence (odds ratio) for a 95% confidence interval.

This study was approved by the Ethics Committee for Animal Experimentation (CETEA) of the Agroveterinary Science Center (CAV/UDESC), on May 30, 2008, under protocol no. 1.20.08.

Results and Discussion

Animals infected with *T. gondii* were found on all of the 13 farm properties assessed. Out of the 360 serum samples analyzed using IFA, 205 (56.94%) were positive (Table 1). The titers observed were 64 (28.29%), 256 (38.05%), 1024 (22.44%), 4096 (9.76%) and 16384 (1.46%). When subjected to ELISA, 153 (42.50%) were reactive samples (Table 1).

The percentages of infected animals on the farm properties were in agreement with the results found by Figliuolo et al. (2004) and Ueno (2005), which were in similar surveys in the state of São Paulo and the Federal District, respectively. This high prevalence shows that *T. gondii* is widely distributed in sheep herds in the municipality of Lages, Santa Catarina.

The high percentage of *T. gondii* seropositive sheep found in this study may be related to environmental contamination with the parasite. Although vertical infection may occur, cats living in the same environment and the sheep's preference for grazing lower pastures, usually closer to the main buildings of the farm estate to which the cats normally have access, favor horizontal infection.

Tenter et al. (2000) reported that the incidence of antibodies in sheep populations in different countries ranged from 0 to 92%. In Brazil, the rates of *T. gondii* infection in sheep ranged from 15.2% (ESCOPELLI, 2004) in the region of Porto Alegre, in Rio Grande do Sul, to 54.6% (OGAWA et al., 2003) in the microregion of Londrina, in Paraná. The latter percentage is similar to what was obtained in the present work (56.94%). In Guarapuava, Paraná, Romanelli et al. (2007) found a prevalence of 51.47%. Lower values have been observed in Brazil and in other countries. Zonta et al. (1987) and Pappen (2008) found prevalences of 18.2% and 20.2%, respectively, in different municipalities in Rio Grande do Sul. Gondim et al. (1999) found that the incidence of infection by *T. gondii* among sheep in Bahia was 18.75%. Langoni et al. (2011) found that the prevalence was 18.6% in the eastern region of São Paulo. In the metropolitan and periurban regions of Curitiba, Paraná, Soccol et al. (2009) found that the incidence of infected animals was 25.75%.

Table 1. *Toxoplasma gondii* antibodies among sheep in the municipality of Lages, tested by enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA).

Variable	Category	Total number (N) of sheep		Number of sheep with <i>T. gondii</i> antibodies			
		n	%	ELISA		IFA	
				n	% ¹	n	% ¹
Age	0-6 months	62	17.22	18	29.03	33	53.23
	7-12 months	64	17.78	18	28.13	29	45.31
	≥13 months	234	65.00	117	50.00	143	61.11
Sex	Males	72	20.00	29	40.28	38	52.78
	Females	288	80.00	124	43.06	167	57.99
Breed	Defined ²	169	46.94	75	44.38	85	50.30
	Mixed	191	53.06	78	40.84	120	62.83
Contact with felines	Yes	275	76.39	127	46.18	155	56.36
	No	85	23.61	26	30.59	50	58.82
Diet	Natural pasture	110	30.56	58	52.73	70	63.64
	Mixed	188	52.22	77	40.96	102	54.26
	Milk and pasture	62	17.22	18	29.03	33	53.23
Water source	Drinking troughs	46	12.78	33	71.74	27	58.70
	Ponds/springs	314	87.22	120	38.22	178	56.69
Breeding problems	Miscarriage	4	1.11	4	100.00	4	100.00
	Stillborn	1	0.28	0	0.00	1	100.00
	None	355	98.61	149	41.97	200	56.34
Animal category	Lambs	62		19	30.65	33	53.23
	Pregnant ewes	80		46	57.50	51	63.75
	Non-pregnant ewes	84		24	28.57	44	52.38
	Lactating ewes	90		44	48.89	56	62.22
	Uncastrated males	17		10	58.82	7	41.18
	Castrated males	27		10	37.04	14	51.85
Total		360		153	42.50	205	56.94

¹Relationship between the number of infected animal per category, and total number of animals within each category; ²Hampshire Down and Texel.

It was found, regarding age, that the population sampled consisted mainly of animals aged ≥ 13 months (65%). Older age is considered to be a risk factor for toxoplasmosis because adult animals have a higher likelihood of coming into contact with *T. gondii* oocysts in the environment. However, this was not observed in the results obtained from IFA, since there was no statistically significant difference ($p > 0.05$) in the seroprevalence to *T. gondii* in relation to the sheep's age (Table 2). The percentages were found to be higher among older animals by several authors: Van der Puije et al. (2000) in Ghana; Figliuolo et al. (2004) in São Paulo; Romanelli et al. (2007) in the state of Paraná; Carneiro et al. (2009) in Minas Gerais; and Tembue et al. (2009) in the arid region of the state of Pernambuco.

There was no statistically significant difference ($p > 0.05$) in the incidence of seropositivity in relation to the sex of the animals (Table 2). These results are in agreement with the majority of studies on the prevalence of *T. gondii* in sheep (ESCOPELLI, 2004; CARNEIRO et al., 2009). Discrepant results were obtained by Silva et al. (2003), Ueno (2005) and Ragozo (2007) in Pernambuco, Federal District and São Paulo, respectively, with a higher seropositivity rate among males. Van der Puije et al. (2000) conducted experiments on sheep in Ghana, and pointed out that female sex was a risk factor for toxoplasmosis because of hormonal, physical and management differences. According to Ueno (2005),

Table 2. Univariate analysis on risk factors associated with infection by *Toxoplasma gondii*, assessed through the immunofluorescence assay (≥1:64) among sheep in the municipality of Lages, Santa Catarina, in 2009.

Variables	P	OR	95% CI
Age	0.14	-	-
Sex	0.68	0.86	0.50-1.51
Breed	0.02	1.66	1.07-2.60
Contact with felines	0.78	0.90	0.53-1.52
Diet	0.23	-	-
Water source	0.58	0.80	0.41-1.57
Breeding problems	0.13	0	0-1.43
Animal category	0.14	-	-

P = Probability; OR = Odds ratio; 95% CI = 95% confidence interval.

this variable does not influence the results, given the divergence in the results, especially because of the lack of consistent biological explanations and because this variable is a random factor. In the present study, crossbred animals showed statistical differences in the seroprevalence of anti-*T. gondii* ($p < 0.05$, Table 2). These results are in agreement with those found by Silva et al. (2003), who found higher rates of infection in crossbred animals. According to those authors, this finding could be explained in terms of the

unsatisfactory hygiene conditions during herd health management among crossbred animals, thus disagreeing with the findings of Van der Puije et al. (2000) and Carneiro et al. (2009).

The variable of contact with felines related to the presence of domestic cats on the farm property. Stray cats and wild felines were not categorized within this variable, because they have habits and habitat characteristics that differ from those of domesticated animals, and do not have a close relationship with sheep. In this study, no statistically significant difference in seropositivity ($p > 0.05$) was found in relation to the presence and/or contact of sheep with felines (Table 2), which is in agreement with the data obtained by Soares et al. (2009). These results disagree with those found by Romanelli et al. (2007) in the state of Paraná and by Pappen (2008) in the southern region of Rio Grande do Sul. These authors stated that access by cats to the feed storage barn, presence of young cats and raising sheeps near to the farmhouse were factors associated with a greater chance of ingesting oocysts. According to Hill and Dubey (2002), there is a relationship between the presence of definitive hosts and the epidemiology of *T. gondii* infections, since the transmission of *T. gondii* to sheep is mainly attributed to oocysts shed by infected cats over pasture and water.

There was no statistically significant difference in seropositivity ($p > 0.05$) in relation to the type of diet among the sheep (Table 2), although almost half of the animals received a mixed diet. In Minas Gerais, Carneiro et al. (2009) also observed that the diet of animals was not a factor that contributed to the risk of toxoplasmosis in sheep.

The IFA results showed that there was no statistically significant correlation between the water source and *T. gondii* infections ($p > 0.05$, Table 2). However, Romanelli et al. (2007) and Vesco et al. (2007) found a correlation between *T. gondii* seropositivity and surface water sources, as these were more likely to be contaminated with *T. gondii* oocysts.

There was no statistically significant difference in seropositivity ($p > 0.05$) in relation to reproductive problems (abortion and stillbirth) among the sheep according to IFA, although all the sheep that aborted were seropositive for *T. gondii*. This result was in agreement with what was found by Ogawa et al. (2003) in Paraná and by Carneiro et al. (2009) in Minas Gerais.

The ELISA and IFA serological tests are considered to be suitable for epidemiological studies on toxoplasmosis in sheep because they are practical techniques with high sensitivity and specificity (SAWADOGO et al., 2005). In the present study, the sensitivity (61%) and specificity (82%) of ELISA, at dilutions $\geq 1:64$ (Table 3), were lower than those reported by Van der Puije et al. (2000), who used IFA and ELISA in seroprevalence studies conducted in Ghana, Africa. In Cairo, Egypt, Shaapan et al. (2008) found *T. gondii* antibody prevalences of 37% using IFA, 41.7% using ELISA, 43.7% using the modified agglutination test (MAT) and 34% using the dye test (DT). When data from the first three serological tests were compared with the DT, which was used as the reference for toxoplasmosis, MAT was the test that showed the highest sensitivity (96%), followed by ELISA (90.1%) and IFA, which showed low sensitivity (80.4%). On the other hand, IFA showed the highest specificity (91.4%), followed by MAT (88.9%) and ELISA (85.9%).

Table 3. Sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), kappa and efficacy of the immunosorbent assay (ELISA), using the immunofluorescence assay (IFA) as reference (≥ 64), among 360 sheep serum samples from the municipality of Lages, Santa Catarina, Brazil.

ELISA Result	IFA Result		Total
	Positive	Negative	
Positive	125	28	153
Negative	80	127	207
Total	205	155	360

SE = 61.0%; SP = 81.9%; PPV = 81.7%; NPV = 61.3%; Kappa index = 0.41; Efficacy = 70%.

In the present study, ELISA conducted on sheep serum samples was found to be more specific than sensitive. The moderate agreement (kappa = 0.41) between ELISA and IFA (1:64) allows to consider that ELISA is an alternative technique for diagnosing *T. gondii* in sheep. Pereira-Bueno et al. (2004) found perfect agreement between IFA and ELISA (kappa = 1.00) in a study conducted on sheep in Spain. However, in a study on sheep in Rio Grande do Sul, using indirect hemagglutination (HAI) and IFA techniques, Escopelli (2004) found a kappa value of 0.51, thus showing moderate agreement between the two techniques. Carneiro et al. (2009) compared ELISA and IFA (1:64) in Minas Gerais, and observed that the kappa value showed substantial agreement (0.68).

Conclusions

- Infection by *T. gondii* is widespread in sheep herds in the municipality of Lages, Santa Catarina, and seropositive animals were detected on 100% of the farm properties analyzed;
- Sheep in the municipality of Lages, Santa Catarina, showed high prevalence of antibodies to *T. gondii*: 56.94% according to IFA and 42.50% according to ELISA;
- Breed was associated with the risk of acquiring *T. gondii* infection; and
- The kappa agreement level between IFA and ELISA was considered to be moderate, and therefore ELISA can be used as an alternative technique for diagnosing toxoplasmosis in epidemiological studies among sheep.

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