



Epidemiological Investigation of *Toxoplasma gondii* Infections in Commercial Sheep Flock in an Endemic Area for Ocular Toxoplasmosis in Southern Brazil

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Abstract

Introduction *Toxoplasma gondii* is a widely distributed parasite and of great importance to human and animal health.

Methods The objective of this study was to assess the prevalence of *T. gondii* antibodies and risk factors associated with the infection in sheep in the Northwest region of the State of Rio Grande do Sul, Brazil; this region has a very high rate of human ocular toxoplasmosis. Ovine sera were tested by the modified agglutination test (cut-off 1:25).

Results *T. gondii* antibodies were detected in 70.2% (224 of 319). According to the logistic regression, the most significant factors associated were age and cat access to food stock facility.

Conclusion Preventive measures are discussed to reduce the risk of transmission of this zoonosis.

Keywords Toxoplasmosis · Sheep · Risk factors · MAT

Introduction

Toxoplasma gondii is a coccidian parasite that causes toxoplasmosis, one of the most prevalent parasitic infections of humans and animals [8]. The Northwest mesoregion of Rio Grande do Sul has a known epidemiological importance for human toxoplasmosis due to the high occurrence of acquired ocular toxoplasmosis [3, 11, 13, 17]. The ingestion of raw or undercooked infected meat is one the main modes of transmission, especially in Southern Brazil. Furthermore, toxoplasmosis causes abortion in sheep [8].

Rio Grande do Sul state is the biggest producer of sheep in Brazil [18] and these animals are used for the production of meat, milk and genetic material. A research on the occurrence of the disease in sheep from another mesoregion of this state suggested a production decrease equivalent to 1.87% per year, with decreased number of lambs per ewe [24]. These numbers warn on the necessity to monitor the infection and to find means to improve production efficiency [24].

Although there are many reports of serological surveys for *T. gondii* in sheep in Brazil, little is known of risk factors. Serological surveys until 2012 were summarized by Dubey et al. [11]. Since then, few additional reports were published

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[2, 6, 7, 14, 20–23]. In most of these surveys, an indirect fluorescent antibody test (IFAT) or an ELISA were used.

The objective of this study was to assess the epidemiological and risk factors associated with *T. gondii* infection in commercial sheep flocks of the northwest mesoregion of Rio Grande do Sul, using the modified agglutination test (MAT), because this is the only test that has been validated by comparing the serological data with bioassay for viable toxoplasma in ovine tissues [8].

Materials and Methods

Ethics

This study was approved by the Animal Ethics Committee of Universidade Federal Fluminense, RJ, Brazil protocol number 832 and the Animal Ethics Committee of Universidade de Passo Fundo, RS, Brazil Protocol No. 035.

Animal Sampling

Between August and October 2016, a cross-sectional study was carried out in 319 randomly selected sheep, females and males, from one to 11 years old, from 14 rural properties in Northwestern Rio Grande do Sul State, Brazil (Table 1). The software EpiInfo 7.1.5 [5] was used to determine the minimum number (n) of samples required using 20.2% as expected prevalence [24], a margin of error of 5%, 95% confidence interval and using the total population of 327,828 sheep in the studied region, according to data from the Brazilian Institute of Geography and Statistics [18].

Blood samples were collected by venipuncture of a jugular vein in 10 mL tubes without anticoagulant. After collection, the samples were stored in an isothermal container at 5 °C and taken to the laboratory for centrifugation at 1000×g for 10 min, removing of sera and conditioning in microtubes at –20 °C for subsequent serological tests.

Epidemiological Data

Individual characteristics of the animals were recorded at the time of blood collection (gender, age and breed) and farmers answered questionnaires on specific topics about the herd: production system and purpose, consortium with other animals, water source, type of sheepfold, floor of the sheepfold, location of food and water reservoirs, types of food and water reservoirs, existence or absence of veterinary care, presence of cats in the property, existence of food stock, cat access to stock facilities, cat access to water offered to sheep, if cats received commercial food, presence of rodents and dogs, and number of fetal malformations and abortions in the last 12 months (Table 1).

Serological Testing

Sera were tested for the presence of anti-*T. gondii* IgG antibodies using the MAT, as described [9]. Sera were diluted twofold in 0.01 M phosphate buffered saline (PBS) pH 7.2 from 1:25 to 1:3200 and the cut-off point was 1:25.

Statistical Analysis

Data obtained from the epidemiological questionnaires were used for the analysis (independent variables or explanatory variables) of possible risk factors associated with seropositivity for antibodies anti-*T. gondii*. After the selection of the explanatory variables based on frequency distribution, those were analyzed using the Chi-square test to evaluate the association between serology results and explanatory variables. The explanatory variables that presented a significance level of $p < 0.05$ in Chi squared were all tested together in the forward conditional regression model.

Results

Antibodies to *T. gondii* were detected in 70.2% (224/319) of sheep and titers ranged from 1:25 to 1:3200 (Table 2). All herds had seropositive animals and most positive animals (79.5%; 178 of 224) had antibodies with titers equal to or higher than 1:800.

Explanatory variables selected by Chi square are shown in Table 1 and were included in the logistic regression model. According to this multivariate analysis, sheep had 3.8 more chances to be exposed to *T. gondii* (odds ratio 4.8 and confidence interval 2.7–8.5) when cats had access to food stock facilities ($p < 0.001$). Also, sheep older than 2 years had 6.5 more chances to be infected (odds ratio 7.5 confidence interval 3.8–14.6) ($p < 0.001$) according to Nagelkerke coefficient ($R^2 = 0.276$) and Hosmer and Lemeshow test for model fit ($p = 0.658$). All other variables tested significant in the Chi-square test were not considered significant in the regression model.

Discussion

All herds had seropositive animals and frequency within each farm was also high, as 12 of the 14 farms had 50% or more seropositive sheep. It is noteworthy that 79.5% of seropositive sheep had MAT titers of 1:800 or higher.

There are no studies specifically designed to test the validities of different serological tests for the diagnosis of ovine toxoplasmosis [8]. In one study, viable *T. gondii* was

Table 1 Chi square results for selection of the explanatory variables to be included in the Logistic Regression analysis for detection of the risk factors for the presence of *Toxoplasma gondii* antibodies in sheep from northwestern Rio Grande do Sul, Brazil

Variable	Category	Negative		Positive		<i>p</i>
		<i>n</i>	%	<i>n</i>	%	
Gender	Male	22	37.3	37	62.7	0.162
	Female	73	28.1	187	71.9	
Age	1 year	39	52.7	35	47.3	<0.001
	2 years	26	40	39	60	
	≥ 3 years	30	16.7	150	83.3	
Breed	Various breeds ^a	28	23.9	89	76.1	0.082
	Texel	67	33.2	135	66.8	
Production objective	Textile	67	33.2	135	66.8	0.199
	Meat	40	32.3	84	67.7	
	Mixed	18	37.5	30	62.5	
	Genetics	37	25.2	110	74.8	
Production system	Intensive	12	50	12	50	0.024
	Semi-intensive	83	28.1	212	71.9	
Consortium with other animal	Yes	40	27	108	73	0.317
	No	55	32.2	116	67.8	
Water source	Mixed	45	39.5	69	67.6	0.003
	Weir and cistern	24	32.4	50	80.2	
	Artesian well	26	19.8	105	50	
Type of sheepfold	Absent	12	50	12	50	0.004
	Suspended	28	21.1	105	78.9	
	Ground floor	55	34	107	66	
Floor of the sheepfold	Beaten or absent	44	43.6	57	56.4	<0.001
	Ripped	17	15.5	93	84.5	
	Cement	34	31.5	74	68.5	
Water supply	Reservoir inside	30	20.8	114	79.2	0.001
	Reservoir outside	22	46.8	25	53.2	
	Direct from the source	12	50	12	50	
	Mixed	31	29.8	73	70.2	
Type of drinking reservoir	Cement	6	30	14	70	<0.001
	Wood	2	9.5	19	90.5	
	Absent	12	50	12	50	
	Mixed	15	71.4	6	28.6	
	Plastic	60	25.8	173	74.2	
	Reservoir inside	55	23.1	183	76.9	
Reservoir outside	16	59.3	11	40.7		
Mixed	12	40	18	60		
Type of feeder	Absent	12	50	12	50	<0.001
	Wood	68	24.8	206	75.2	
	Mixed	15	71.4	6	28.6	
Veterinary care	Yes	89	29.8	210	70.2	0.982
	Not	6	30	14	70	
Presence of cats	Yes	85	31.6	184	68.4	0.100
	Not	10	20	40	80	
Stock of food	Yes	83	28.1	212	71.9	0.024
	Not	12	50	12	50	
Cat has access to food stock facility	Yes	31	17.8	143	82.2	<0.001
	Not	64	44.1	81	55.9	

Table 1 (continued)

Variable	Category	Negative		Positive		<i>p</i>
		<i>n</i>	%	<i>n</i>	%	
Cat access to water offered	Yes	58	25.9	166	74.1	0.020
	Not	37	38.9	58	61.1	
Cats received specific food	Yes	27	60	18	40	<0.001
	Not	68	24.8	206	75.2	
Presence of dogs	Yes	95	31.8	204	68.2	0.001
	Not	0	0	20	100	
Presence of rodents	Yes	80	26.8	218	73.2	<0.001
	Not	15	71.4	6	28.6	
Fetal malformation in the last 12 months	Yes	9	22.5	31	77.5	0.215
	Not	74	32.3	155	67.7	
Abortion in the last 12 months	Yes	45	26.8	123	73.2	0.217
	Not	50	33.1	101	66.9	

^aOther breeds had very few number of animals each and were analyzed as a whole: Dorper, Lacaune, Poll Dorset, Suffolk and crossbred

*Significance level of *p* < 0.05

Table 2 Seroprevalence and titers of *Toxoplasma gondii* antibodies in sheep from northwestern Rio Grande do Sul State, Brazil

Municipalities	Farm No	<i>n</i>	% Pos.	No. of animals with titers of:							
				25	50	100	200	400	800	1600	> 3200
Passo Fundo	1	30	60	2	2			5	3	6	
Vila Maria	2	30	80	4	2	1		6	6	5	
Marau	3	21	90					6	5	8	
Nova Araça	4	20	100		2			5	5	8	
Nova Bassano	5	20	85		1	1		8	2	5	
Soledade	6	24	50	2	2			1	2	5	
Guaporé	7	23	52	1				5	2	4	
Erechim	8	23	87	1	2			9	5	3	
Erechim	9	20	80		2			6	2	6	
Gaurama	10	20	75			1		11		3	
Erechim	11	20	100		1	1		13	1	4	
Cruz Alta	12	27	41	3	1		2	3		1	
Passo Fundo	13	20	70	3				2	1	1	
Soledade	14	21	29	3	1					2	
	Total	319	70.2	19	10	9	3	5	76	37	65

isolated from 53 (77.9%) of 68 naturally exposed lambs with MAT titers of 1:50 or higher but not from 44 seronegative lambs with titers of < 1:25 [10]. It is likely that most of the seropositive sheep in the present study had persistent infection with *T. gondii*.

Toxoplasmosis is a foodborne disease and the consumption of raw or undercooked mutton might be a source of infection to humans [4, 15].

Toxoplasma gondii causes fetal death, mummification, stillbirth, abortion or births of debilitated animals [12]. Congenital toxoplasmosis is relevant for sheep producers in the sampled region, which is a reference in the breeding and commercialization of sheep genetic material. In the

present study, there was no correlation between abortion and seropositivity but seroprevalence was high among groups of ewes with history of reproductive disorders (Table 2).

The high occurrence for anti-*T. gondii* antibodies in most of the farms studied also indicates an inadequate management in the herds, possibly due to the environmental contamination following the excretion of oocysts by infected cats reared near or inside the flock. Felines are the definitive hosts of *T. gondii* and their presence inside the farms should be taken in consideration as a risk factor for animals [1, 19, 25]. Cats were present in all farms and, therefore, we could not assess their direct influence on sheep seropositivity. On the other hand, their access to sheep food stock had

significant impact on animal's exposure to *T. gondii*, possibly through the shedding of oocysts inside these facilities.

Review of literature indicates that *T. gondii* seropositivity increases with age. Thus, adult sheep are more prone to become infected by *T. gondii* [2, 16, 26] due to the prolonged time of exposition to the oocyst-contaminated environment. Results of the present study support this conclusion.

In conclusion, sheep (70%) and the environment were highly contaminated with *T. gondii* because the ingestion of oocysts is the main mode of transmission of *T. gondii* in sheep. Efforts are needed to control felid population on sheep farms. All mutton should be cooked properly before human consumption to minimize risk of transmission to humans.

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