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Caetano de Oliveira, Fernando; Aguiar de Oliveira, Plínio; Azevedo da Cunha Filho, Nilton; Guidotti Aguiar, Cintia Lidiane; Pappen, Felipe Geraldo; Lopes Ruas, Jeronimo; da Rosa Farias, Nara Amélia

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## The incidence and productive significance of ovine toxoplasmosis in Southern Brazil

### Incidência e significância econômica da toxoplasmose ovina no sul do Brasil

Fernando Caetano de Oliveira<sup>1\*</sup> Plínio Aguiar de Oliveira<sup>1</sup> Nilton Azevedo da Cunha Filho<sup>1</sup>  
Cintia Lidianne Guidotti Aguiar<sup>1</sup> Felipe Geraldo Pappen<sup>II</sup>  
Jeronimo Lopes Ruas<sup>1</sup> Nara Amélia da Rosa Farias<sup>1</sup>

#### ABSTRACT

This research aimed to determine incidence of *Toxoplasma gondii* infection in Corriedale sheep in southern Brazil during pregnancy and the extent of associated losses. Blood samples were collected from 411 Corriedale sheep in two flocks at different locations in the state of Rio Grande do Sul (Brazil). Presence of *T. gondii* was diagnosed by the Indirect Fluorescent Antibody Technique (IFAT). Seroprevalence of *T. gondii* during pre-mating was 20.2%, with no significant difference between the two flocks studied. Infection by *T. gondii* was influenced by ewes' age ( $P \leq 0.05$ ). The pre-mating seroprevalence did not influence either returning to estrus or pregnancy rates. Weaning rate was significantly higher in pre-positive dams compared to negative ones (87.9 and 74.1%, respectively -  $P < 0.05$ ). The incidence of seroconversion was 16.5% (54/328). In seroconverted ewes, a high rate of return to estrus was observed ( $P \leq 0.05$ ). The incidence of toxoplasmosis suggests production losses equivalent to 1.87% per year; in this system production. In addition, toxoplasmosis decreased the number of lambs per ewe. Therefore, to improve livestock performance, *T. gondii* infection should be monitored and controlled in the studied area.

**Key words:** *Toxoplasma gondii*, ewe, pregnancy, losses.

#### RESUMO

Este trabalho tem como objetivo determinar a incidência da infecção pelo *T. gondii* em ovinos, durante o período gestacional e a extensão das perdas associadas. Amostras de sangue foram coletadas de 411 ovelhas Corriedale em dois rebanhos, em diferentes locais do Rio Grande do Sul, Brasil. Presença de *T. gondii* foi diagnosticada pela técnica de imunofluorescência indireta (RIFI). A soroprevalência no pré-acasalamento foi de 20,2%, sem diferença significativa entre os dois rebanhos estudados. A infecção por *T. gondii* foi influenciada pela idade ( $P \leq 0,05$ ). A soroprevalência no pré-acasalamento não influenciou a taxa de retorno ao estro e a taxa de prenhez. Taxa de desmame foi significativamente maior nas ovelhas positivas na pré-cobertura em comparação com as negativas (87,9 e 74,1%, respectivamente -  $P < 0,05$ ). A incidência de soroconversão foi de 16,5% (54/328). Em ovelhas que soroconverteram, uma alta taxa de retorno ao estro foi observada ( $P \leq 0,05$ ). A incidência da toxoplasmose sugere perdas de produção equivalentes a 1,87% ao ano nestes sistemas. Além disso, a toxoplasmose diminuiu o número de cordeiros por ovelha. Portanto, para

melhorar o desempenho produtivo nos rebanhos ovinos, a infecção por *T. gondii* deve ser monitorada e controlada na área estudada.

**Palavras-chave:** *Toxoplasma gondii*, ovelhas, gestação, perdas.

#### INTRODUCTION

*Toxoplasma gondii* (Protozoa: *Apicomplexa*) is a cyst-forming protozoa with worldwide distribution and low parasitic specificity. Sheep is one of the intermediate hosts more susceptible to acute infection (HILL & DUBEY, 2013), which may lead to abortion and prenatal death (FTHENAKIS et al., 2012).

Hence, toxoplasmosis directly affects sheep flocks, being the main cause of losses in many countries, such as Australia (WALDELAND, 1977), New Zealand (CHARLESTON, 1994), Uruguay (FREYRE et al., 1997) and Czech Republic (BÁRTOVÁ et al., 2009). In Brazil, toxoplasmosis prevalence in sheep fluctuates depending on flock management strategies (SOARES et al., 2009). In Greece, ANASTASIA et al. (2013) observed difference in seroprevalence between cattle and sheep under mixed grazing, being higher in sheep, probably due to differences in grazing habits.

Congenital toxoplasmosis is not only a serious public health problem to humans, but it also has a huge economic importance for sheep industry (WANG et al., 2011). Abortion losses caused by *T. gondii* infection in the UK are in the range of 1.2-2.2% (BUXTON et al., 2007). In Uruguay, FREYRE et al. (1997) estimated losses in sheep industry around 1.4 and 3.9%, reaching US\$ 4.7 million per year. Furthermore, toxoplasmosis represents a risk to public health, since ingestion of raw or rare ovine meat is an important source of human infection, provoking occasional outbreaks (BONAMETTI et al., 1997).

<sup>1</sup>Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas (UFPEL), s/n, CP 354, 96010-900, Pelotas, RS, Brasil. E-mail: fcoliveiravet@gmail.com. \*Corresponding author.

<sup>II</sup>Instituto Federal Catarinense, Concórdia, SC, Brasil.

Due to its flourishing sheep industry, it is important to monitor the impact of toxoplasmosis in ovine stock in this region. The present study aimed to determine *Toxoplasma* infection rate in ewes during pregnancy and to estimate productive losses in ewes of contracting the protozoa infection in extensive farming in Southern Brazil.

## MATERIALS AND METHODS

### Experimental animals

Two sheep flocks were studied, containing 131 and 280 Corriedale breed sheep, from farm A (31° 42' 38.14"S 52° 54' 42.23"W) and farm B (31° 58' 44.78"S 53° 27' 31.55"W) respectively. Ewes were monitored in the pre-mating period and subsequently when lambs weaned. The period for natural sheep mating was 45 days, with rams of verified fertility. Pregnancy diagnosis was performed by ultrasonography 30 days after mating period, with an estimated time of pregnancy. The adopted farming system is considered extensive and mixed (sheep with cattle) and uses food supplement during critical periods, such as pre-mating and pre-partum.

### Blood samples

Blood was collected from 411 sheep in reproductive age, during pre-mating and post-partum periods, with intervals of seven months between each collection. Both farms showed history of abortion and neonatal mortality. Samples were collected using vacutainer-type tubes without anticoagulant by jugular venipuncture. Collected blood was allowed to stand at room temperature for clot formation, and then cooled down (2°C). Serum was centrifuged at 2.500G for 10 minutes and stored at -20°C.

### Serological examination

Serum samples were examined for *T. gondii* IgG antibodies using an indirect fluorescence antibody test (IFAT), as described by CAMARGO (1974). An anti-sheep IgG conjugate antibody (Sigma Chemical®) and a commercial antigen obtained from WAMA Diagnostic® (ME 49 strain of *T. gondii*) were used in immunological tests. The cut-off point was 1:64. Positive and negative control sheep sera were always included in antibody detection assays.

### Statistical analyses

Seroprevalence rates were defined according to the percentage of samples tested with antibodies against *T. gondii*. Incidence was calculated considering seronegative animals under risk of acquiring infection (ORTEGA-PACHECO et al., 2011), along with those becoming seropositive throughout the experimental period. Significance production loss was

estimated essentially as described by FREYRE et al. (1997), by the formula:  $A - B \times 4/7 \times 0.25$ , where A: post-partum seroprevalence, B: pre-mating seroprevalence, 4 is the period (in months) where susceptible ewes may suffer abortion due to toxoplasmosis, 7 is the time elapsed (in months) between the first and second seroprevalence analysis and 0.25 is the abortion rate (considering a natural infection scenario, in agreement with WALDELAND, 1977).

P values were obtained with the chi-square test (for low frequencies) or the Fisher's exact test, employing the Statistix® 9.0 software package (Analytical Software, Tallahassee, FL, USA).

## RESULTS AND DISCUSSION

The overall seroprevalence during the pre-mating period was 20.2% (83/411). It was significantly influenced by increase of age ( $P < 0.01$ ). The increase in prevalence depending on ewes' age observed in Rio Grande do Sul suggests the occurrence of horizontal infection by oocysts, as likewise pointed out by BUXTON et al. (2007) in Scotland. In Paraná State (Brazil), OGAWA et al. (2003) reported that the percentage of ewes seropositive to *T. gondii* is greater when they are older than two years.

Therefore, 79.8% (328/411) of reproductive dams did not show antibodies against *T. gondii*, and were susceptible to protozoal infection. No difference was observed between seroprevalence of flocks A and B, including age groups of ewes, both for pre-mating, and post-partum ewes. Therefore, subsequent analysis of data was performed considering both flocks as a single unit.

Flock seroprevalence in post-partum period was significantly higher than in the pre-mating period ( $P < 0.05$ ) (Table 1). Post-partum seroprevalence was 33.3% (137/411). Incidence rate was 16.5% (54/328). Statistical difference between the pre-mating and post-partum seroprevalence is due to the high number of animals that seroconverted during the experimental period. In Uruguay, a similar study conducted by SAVIO & NIETO (1995), reported that only 10.8% of dams were seroconverted during the reproductive period, with only 34.2% weaned lambs.

In the present research, seroconversion rate was higher, but it did not affect the weaning rate. Such a difference may be ascribed to virulence variations between *Toxoplasma* strains (DUBREMETZ & LEBRUN, 2012). Group of dams that seroconverted during pregnancy had higher rates of estrus return ( $P < 0.05$ ), but had no significant differences were reported between pregnancy and weaning rates (Table 2).

Toxoplasmosis is known to cause resorption, abortions and neonatal deaths in lambs (BUXTON, 2007). Such cases were observed among the studied animals

Table 1 - Seroprevalence (RIFI) for *T. gondii* in sheep (dams) in extensive farming, related to reproductive cycle period.

Period	Reactive serum %	Non reactive %	-----Univariate analysis-----	
			X <sup>2</sup>	P*
Pre-mating	20.2% (83/411)	79.8% (328/411)	200	0.0001
Post-partum	33.3% (137/411)	66.7% (274/411)		

\* $P < 0.05$  by the chi-square test.

Table 2 - Association between seroconversion occurrence to *T. gondii* in sheep (dams) during pregnancy, and their productive parameters.

Productive parameter	Seroconversion	%	-----Univariate analysis-----	
			OR (95%)	P*
Estrus return	Non-converted	29.4% (105/357)	1	0.02
	Converted	44.4% (24/54)	1.92 (1.07-3.43)	
Pregnancy rate	Non-converted	95.0% (339/357)	1	0.07
	Converted	88.9% (48/54)	2.35 (0.89-6.22)	
Weaning rate	Non-converted	76.2% (272/357)	1	0.39
	Converted	81.5% (44/54)	0.72 (0.35-1.50)	

OR value (CI=95%) by two-by-two analysis.

\*P<0.05 by the chi-square test.

and were assumed to be due to toxoplasmosis. However, confirmation of this diagnosis was not possible due to extensive farming practices. In UK, a questionnaire-based epidemiological study reported that 72.0% of sampled ovine livestock farms suffered abortion problems caused by *T. gondii* (LONGBOTTOM et al., 2013).

Seropositive animals in the pre-mating period, (which were theoretically protected from *T. gondii* infection), had lower rate of estrus return and increased pregnancy rates, but such differences were not statistically significant. Weaning rate was significantly higher in this group ( $P<0.05$ ); moreover, seropositive ewes were 2.55 times more likely to wean lambs (OR=2.55, Table 3).

Data obtained in the present research indicated that toxoplasmosis reduced productive significance by 1.87%. Expanding these results to the state of Rio Grande do Sul, taking into account the sheep production, it is possible to estimate a loss around 35 thousand lambs or US\$ 1.491 million annually, caused by *T. gondii*.

According to BLEWETT & TREES (1987), incidence of toxoplasmosis is difficult to define, however they concluded that in UK, there is around 1 to 2% neonatal losses in sheep per year. If such figure is accurate, it can be assumed that about 1.25 million lambs perished due to *Toxoplasma* infection in the European Union in 2003

(BUXTON et al., 2007). In the UK, DUNCANSON et al. (2001) reported losses of 11.6% of pregnancies and 9% of lambs, suggesting that losses occurred in several ways: animals that did not get pregnant, ewes that were unable to complete pregnancy and animals born with severe health problems which did not survive.

Sheep flock in the state of Rio Grande do Sul is 4 million (IBGE, 2012), of which 2.4 million corresponds to dams breeding in the region (SEAPA, 2012). Twenty percent of those were probably immunized against toxoplasmosis by natural infection (PAPPEN, 2008), so that there were yet 1.9 million sheep susceptible to *T. gondii*. According to the present study, this would cause a deficit of more than thirty-five thousand lambs per year. With an average selling price of weaned lamb at state fairs of US\$ 42.00 each (RODRIGUES, 2013), this implies an annual loss of about \$1.491 million.

## CONCLUSION

Toxoplasmosis infected ewes during pregnancy in two flocks of Southern Brazil, adding a substantial risk to lamb production in this region, with important financial consequences for ovine production in this country.

Table 3 - Association between serological status for *T. gondii* of dams pre-mating period and productive variables of flock in extensive farming.

Variables	Serostatus	%	-----Univariate analysis-----	
			OR (95%)	P*
Estrus return	Reactive	24% (20/83)	1	0.10
	Non-reactive	33.2% (109/328)	1.56 (0.90-2.72)	
Pregnancy rate	Reactive	96.4% (80/83)	1	0.33
	Non-reactive	93.6% (307/328)	1.82 (0.53-6.28)	
Weaning rate	Reactive	88.0% (73/83)	1	0.007
	Non-reactive	74.1% (243/328)	2.55 (1.26-5.17)	

OR value (CI=95%) by two-by-two analysis.

\*P<0.05 by the chi-square test.

## BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This research was conducted with the approval of the Ethics Committee on Animal Experimentation at the Universidade Federal de Pelotas, as granted by Opinion No. 2301/2013.

## CONFLICT OF INTEREST STATEMENT

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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