Toxoplasma gondii: abortion outbreak in a goatherd from Southern Brazil

Toxoplasma gondii: investigação de surto em um rebanho caprino da região sul do Brasil

Mauro de Freitas Silva Filho[1]; Érika Erzinger[2]; Ivo Alexandre Leme da Cunha[1]; Felipe Monteiro Bugni[1]; Fernando Nakanishi Hamada[1]; Elizabete Regina Marangoni Marana[1]; Roberta Lemos Freire[1]; João Luis Garcia[1]; Italmar Teodorico Navarro[1*]

Abstract

The present study aimed to investigate an abortion outbreak in a goatherd from Southern Brazil. The herd had 304 goats, being 227 females and 77 males. Sixty-one animals (44.8%) out of 136 dams aborted. Indirect immunofluorescence assay (IFA) was performed for anti-*T.gondii* antibody detection and showed 59/61 (96.7%) positive females (titers ≥64). Fifty-five (93.2%) out of 59 positive dams showed titers ≥ 1024. Tissue samples from eight aborted fetuses and raw milk from their dams were collected for mouse bioassay and PCR. Two fetuses were positive for *T. gondii* infection in both bioassay and PCR. The animals were treated for five days with sulfadiazine plus trimetoprim what was enough to stop the abortion cases. Considering the results from this work the abortion outbreak was caused by *T. gondii* infection.

Key words: *Toxoplasma gondii,* abortion, goat

Resumo

O objetivo do presente estudo foi investigar um surto de aborto em um rebanho caprino ocorrido na região sul do Paraná, Brasil. O rebanho continha 304 caprinos, composto de 227 fêmeas e 77 machos. Sessenta e uma (44.8%) cabras prenhas, de um total de 136, abortaram. Para detecção de anticorpos contra *Toxoplasma gondii* foi utilizada a técnica de imunofluorescência indireta (IFI) e a positividade considerada para aqueles animais com títulos maiores que 64. Das fêmeas que abortaram 59/61 (96,7%) revelaram soropositividade na IFI, 55 (93,2%) daquelas com títulos ≥ 1024. Amostras de oito fetos e leite cru de suas mães foram avaliadas por bioensaio em camundongos e PCR. Amostras de tecido de dois fetos abortados foram positivos no bioensaio e PCR. Após um período de tratamento com sulfadiazine e trimetoprim por cinco dias os abortos cessaram no rebanho. Considerando os resultados do trabalho o agente causador do surto foi o *T. gondii*.

Palavras-chave: *Toxoplasma gondii*, aborto, caprinos

[1] Laboratório de Protozoologia, Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina-UEL, P.O. Box 6001, 86050-970 Londrina, PR, Brazil. italmar@uel.br

[2] Médico Veterinário, Guarapuava, PR, Brazil.

[*] Autor para correspondência

Recebido para publicação 05/11/07 Aprovado em 07/07/08

Introduction

Toxoplasma gondii is a worldwide parasite that usually does not produce clinic signs, but the primary infection during pregnancy in some animal species may result in abortion, fetal abnormalities or perinatal death (GILBERT; COOK; DUNN, 2000). This parasite is considered one of the main abortion causes in goat and sheep herds in the North America and Scotland (BUXTON, 1998).

The goat can act as an infection source for human beings by either consume of raw or undercooked meat containing tissue cysts or raw milk with tachyzoites (VITOR; PINTO; CHIARI, 1991; DUBEY, 1981; FIGUEIREDO et al, 2001).

T. gondii prevalence in goatherds from Brazil ranged from 11.4 to 40.7% (MACHADO; LIMA, 1987; SELLA et al., 1994; GONDIM et al., 1999; SILVA et al., 2003; MAINARDI et al., 2003; MACIEL; ARAUJO, 2004; REIS et al., 2007). To date, there are few studies relating *T. gondii* and abortion outbreak in goats. The objective of this study was to investigate a abortion outbreak in a goatherd located in Southern Brazil, and its relationship with *T. gondii*.

Material and Methods

Studied area

The abortion outbreak occurred between June and July 2005 in a property located in Guarapuava county, situated in Central-Western region of Paraná State, Southern Brazil.

Characteristics of the herd

The farm had 14.54 hectares and the main activity was milk production by Angora caprine culture. The total herd was formed by 304 animals, being 227 females and 77 males. There were 157 females in reproductive age being 136 pregnant.

Questionnaire

An epidemiological questionnaire was developed to collect information on the following management variables: water origin, reproductive problems, stage of pregnancy when the reproductive problems occurred, neurological problems in lamb, presence of other animal species on the farm (rodents, cats), access of cats (food deposits, pasture).

Sample collection

Blood samples were collected from all animals by jugular vein puncture and sera stored at –20ºC until be tested. Eight aborted fetuses and raw milk from their dams were collected and processed in Laboratório de Parasitologia from Departamento de Medicina Veterinária Preventiva/ Universidade Estadual de Londrina for mouse bioassay and PCR.

Indirect immunofluorescence assay (IFA)

The presence of antibodies against *T. gondii* in serum samples of goats was determined by IFA (CAMARGO, 1974) considering as positive, sera with titer ≥ 64.

Bioassay of fetus tissues for T. gondii

Fifty grams of a pool of tissues from fetus (12.5g of each tissue: brain, liver, heart, lung) were used to evaluate the presence of *T. gondii* cysts as described previously (DUBEY, 1998). Briefly, tissues were homogenized in a blender for 30 seconds in 250 ml of saline solution (0.14M NaCl). After homogenization 250 ml of pepsin solution (50g) was added and incubated at 37 ºC for 1 h. The homogenate was filtered through 2 layers gauze and centrifuged at 1180 x *g* for 10 min. The supernatant was discarded and the sediment was resuspended in 20 ml PBS (pH 7.2) and 15 ml 1.2% sodium bicarbonate (pH 8.3) was added and centrifuged at 1180 x*g* for 10 min. The supernatant was discarded and the sediment

was resuspended in 5 ml of antibiotic saline solution (1,000 UI penicillin and 100 µl of streptomycin/ ml of saline solution) and inoculated subcutaneously into 2 mice (1ml/ mouse).

Examination of mice

Impression smears of lung from the mice that died by infection were fixed in methanol, stained with Giemsa, and examined microscopically. Blood samples were drawn from the mice that survived 60 days post-inoculation, and the brain of each mouse was examined microscopically for *T. gondii* tissue cysts by squashing a portion of brain between a coverslip and a glass slide. Serum from each mouse was diluted at 1:16 and 1:64 and examined for *T. gondii* antibodies, using IFA.

DNA extraction from samples

Fragments of tissues from fetus were stored at –20ºC prior to DNA extraction. These fragments were cut into small pieces and homogenized in 1 ml of TE buffer (200mM NaCl, 20 mM Tris, 50 mM EDTA, pH=8.0) after being transferred to a 1.5 ml tube. A volume of 500µl of the homogenized solution was transferred to a microtube to which an equal volume of extraction buffer (200 mM NaCl, 20 mM Tris, 50 mM EDTA, proteinase K 1 mg/ ml and 2% SDS) was added. This new solution was then incubated for 1 h at 56ºC. Buffered phenol (500 µl) was then added and centrifuged at 13,000 xg for 5 min. The resulting aqueous solution was transferred to another microtube, added to phenol:chloroform:isoamyl alcohol, and then centrifuged at 13,000 xg for 5 min. DNA precipitation by sodium acetate and ethanol was performed as previously described (SAMBROOK; FRITSHC; MANIATIS, 1989).

Polymerase Chain Reaction (PCR)

The amplification of *T. gondii* DNA was performed by using the method described by Homan, Vercammen and De Braekeleer (2000). Primers TOX4 (CGCTGCAGGGAGGAAGACGAAAGTTG) and TOX5 (CGCTGCAGACACAGTGCATCTGGATT) were used; these were flanking a 529 bp fragment of *T. gondii* DNA. PCR reaction was performed in a mixture containing 5 µl of extracted DNA with 20 µl (final volume of 25 µl) of mixture of 0.5 mM of each primer, 100 mM dNTP (Invitrogen), 60 mM Tris±HCl (pH=9.0), 15 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.5 U Taq DNA polymerase (Invitrogen). Amplification of parasite DNA was performed over 35 cycles in thermocycler PTC-200 (MJ-Research), using the following cycling conditions: 7 min at 94 ºC for denaturation in cycle one, followed by 33 cycles on 60 s at 94ºC for denaturation, 60 s at 55ºC for annealing and 60 s at 72ºC for extension, cycle 35 was followed by a final extension of 10 min at 72ºC. Aliquots of each PCR product were electrophoresed on 1% agarose gel. Tachyzoites of the RH strain (10^7/ ml) were diluted in tissue samples, and the DNA was extracted to be used as positive control. The negative control consisted of DNA extracted from tissue samples without *T. gondii*. A positive and negative control was included in each test.

Polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP)

Genotype analysis was determined by PCR-RFLP that amplified SAG2 gene of *T. gondii* using two nested PCRs separately to amplify the 5' and 3' ends of the gene (HOWE; SIBLEY, 1995).

Reproductive disorders

Sera from dams that aborted were also studied to other reproductive disorders, such as, *Brucella* and *Leptospira*. For the brucellosis and leptospirosis diagnosis, buffered acidified plate agglutination test using *B. abortus* as antigen and microscopic agglutination test (MAT) (RYU, 1970) was performed, respectively.

Statistical analysis

Associations among risk factors were determined by calculating odds ratios (OR) at a 95% confidence level using software EPI INFO 6.04. Statistical evaluation was carried out using Mantel–Haenszel chi-square and Fisher exact test to establish differences between the characteristics studied. We have considered as significant a p-value of ≤ 0.05.

Results

Clinical signs

During outbreak sixty-one (44.8%) out of 136 pregnant females aborted. All abortions occurred at third trimester of pregnancy. The aborted fetuses presented abnormalities, such as, mummification, maceration, deformity, and stillbirth. Some lambs birthed weak and died at first week. Clinical signs from dams were mainly anorexia, prostration, fever, and mastitis. One dam demonstrated severe symptoms and come to die. All dams were treated for five days with sulfadiazine (4 mg/kg) and trimetoprim (20 mg/kg) by IM route, and two weeks after treatment the abortions ceased.

Serological test

Comparison between IFA and variables is showed in Table 1. One hundred thirty (95.6%) out of 136 pregnant and 19/21 (90.4%) empty females had anti-*T. gondii* antibodies detected by IFA. 149 (94.9%) out of 157 females in reproductive age presented positive antibody titers for *T. gondii* (Table 1). From the herd 271/ 304 (89.1%) animals were positive for antibodies against *T. gondii*. From ewes that aborted 55 (93.2%) showed antibody titers ≥1024 (Table 2). The titers with highest frequency were 8192 (35.42%), 1024 (24.35%), 256 (17.71%), 4096 (14.39%) and 64 (8.12%).

Variables studied

Data analysis revealed an association between anti-*T. gondii* antibodies and the age of caprines (p=0.001), showing an increase of positive animals as age increases. Animals older than one year of age presented a higher prevalence (50%, 152/304) when compared to those with one year of age or less (39.1%, 119/304, p= 0.001). Seropositive distribution according to gender can also be observed in Table 1, showing that females had a greater number of seropositive cases (p = 0.03), with 208/304 (68.4%) reactive females and 63/304 (20.7%) reactive males. In the present work the serology revealed that females with antibody titers ≥1024 presented a 10-fold risk of abortion when compared to those with titers <1024 (OR=10.06; 3.04<OR<36.72, P<0.01, Table 2).

Table 1. Outcome of indirect immunofluorescence assay for anti-*Toxoplasma gondii* antibodies detection in sera from goats.

Variables	Positive (%)	Negative (%)	Total (%)	*OR* (95% CI)	P value
Females in reproductive age					
Pregnant	130(82.8)	6(3.8)	136(86.6)		
Not pregnant	19(12.1)	2(1.3)	21(13.4)		0.64[1]
Total	149(94.9)	8(5.1)	157(100)	2.28 (0.29-14.02)	
Abortion					
Yes	59 (43.4)	2 (1.5)	61 (44.9)	1.66 (0.25-13.6)	0.69[1]
No	71 (52.2)	4 (2.9)	75 (55.1)		
Total	130 (95.6)	6(4.4)	136 (100)		
Age (year)					
≤1	119 (39.2)	25 (8.2)	144 (47.4)	0,25 (0.1-0.61)	0.0010[2]
>1	152 (50.0)	8 (2.6)	160 (52.6)		
Total	271 (89.2)	33 (10.8)	304 (100)		
Gender					
Male	63 (20.7)	14 (4.6)	77 (25.3)	0,41 (0.18-0.92)	0.0292[2]
Female	208 (68.4)	19 (6.3)	227 (74.7)		
Total	271 (89.1)	33 (10.9)	304 (100)		

[1]Fisher exact [2]Mantel–Haenszel

Table 2. Results of the anti-*Toxoplasma gondii* antibody titers obtained by indirect immunofluorescence assay in serum samples from pregnant goats.

	Titers		
	≥ 1024	64 to1024	
Abortion			Total(%)
Yes	55 (42.3%)	4 (3.1%)	59(45.4)
No	41 (31.5%)	30 (23.1%)	71(54.6)
Total (%)	96 (73.8)	34(26.2)	130(100)

Fisher exact OR=10.06 (95% CI, 3.04<OR<36.72), P< 0.0001)

Antibodies against Leptospira and Brucella abortus

All females were negative for *B. abortus* and only one was reactive for *Leptospira* serovar *Autumnalis autumnalis* (1:100).

Mouse bioassa and PCR

It was possible to isolate *T. gondii* in two (25%) of the eight fetuses tested using mouse bioassay, and in those the detection of the parasite DNA was possible using PCR. The other fetuses and the milk samples were negative in both bioassay and PCR. The genetic marker of the isolated strain was type III (data not shown).

Discussion

Goats are one of the most susceptible species to *T. gondii* (DUBEY; ADAMS, 1990). Moreover, they act as a source of infection for humans, since the transmission can occur via the consumption of "in natura" milk or meat which was not properly cooked (VITOR; PINTO; CHIARI, 1991; DUBEY, 1981; FIGUEIREDO et al., 2001).

Chiari and Neves (1984) and Dubey (1980) have shown the risk of transmission of the parasite through the milk of experimentally infected goats. Also, Vitor, Pinto and Chiari (1991) have verified the elimination of tachyzoites in milk at 434 days after inoculation. However, the milk samples collected in this work were negative in the bioassay tests and also in PCR, although only three samples were tested.

With results observed here we could speculate that the seroprevalence observed in goatherds with epidemic abortion due to *T. gondii* is higher than in normal herds. We verified a high prevalence of anti-*T. gondii* antibodies in the animals studied (89.1%). In addition, Slosarkova et al. (2000), in the Czech Republic, reported on a toxoplasmosis outbreak in a goatherd. Those authors verified a seroprevalence of 66% in IFA, and from 44 pregnant females, five aborted. These seroprevalence were higher than those verified by Reis et al. (2007) and Sella et al. (1994), which studies goatherds without abortion problems in Paraná state, they found a seroprevalence of 44.7% and 30.7%, respectively. All these studies used RIFI and the positivity was considered for titers ≥ 64.

Similar observation may be done among antibody titers, we observed the most of positive animals with titers ≥ 1024, and this was higher than those found by Reis et al. (2007) and by Figliuolo et al (2004). It should be noticed that the described articles evaluated the prevalence of *T. gondii* in goat herds, while the present paper evaluated an outbreak in a single herd.

Additionally, in the present study we observed a high association between *T. gondii* antibody titers and abortion. Similar result was also seen by Vitor, Pinto and Chiari (1991) who observed a correlation between high antibody titers and abortion. Dubey, Sundberg and Matiuck (1981) in Connecticut-USA reported cases of abortion and stillborns in goats and sheep, having *T. gondii* as the most likely cause. They observed high antibody titers (1024 to 4096) in the affected goats. Dubey et al. (1986) through MAT verified high titers for *T. gondii* in sera for three goats that aborted or had dystocia and in the fetal peritoneal liquid. High antibody titers can be found in acute infections and in the reactivation of *T. gondii* (ROBERT; CHABASSE; HOCQUET, 1981). Thus, epidemic abortion in a goatherd associated with titers of antibodies against *T. gondii* ≥ 1024 could be used as an indicative of *T. gondii* as causative agent. However, Dubey (1981) reports that high titers of anti-*T. gondii* antibodies in goats can be kept for long periods, independently of the existence of symptoms, and therefore could not be considered as a diagnosis of a recent infection.

In the goats studied, a positive association between the high antibody titers and the increase in age can be observed, with the prevalence on animals older than 1 year (50.0%) was significantly higher

than on those ≤ 1year (39.1%). A similar condition was highlighted by Machado and Lima (1987), Dubey and Adams (1990), Sella et al. (1994) and Figliuolo (2003). Females (64.4%) presented greater seropositivity than the males (20.7%). This result was similar to that found by Reis et al. (2007).

In the present study, *T. gondii* was isolated in two fetuses (25%), both by bioassay and PCR. Vitor, Pinto and Chiari (1991) isolated *T. gondii* from 5/10 (50%) caprine fetuses aborted and tested by bioassay. The higher isolation reached by the second one could be related with the time from collection until examination, once that the study was an experimental inoculation.

T. gondii infection in goatheards is important both for Public Health and the development of caprine culture. In this way, the present study showed important approaches for pathogenicity of this parasite in the goats. In conclusion, we demonstrated abortion cases in a goatherd caused by *T. gondii*.

References

BUXTON, D. Protozoan infections (*Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp.) in sheep and goats: recent advances. *Veterinary Research*, Les Ulis, v. 29, n. 3-4, p. 289-310, 1998.

CAMARGO, M. E. Introdução às técnicas de imunofluorescência. *Revista Brasileira de Patologia Clinica*, Rio de Janeiro, v. 10, n. 4, p. 143-171, 1974.

CHIARI, C. A.; NEVES, D. P. Toxoplasmose humana adquirida através da ingestão de leite de cabra. *Memórias do Instituto Osvaldo Cruz*, Rio de Janeiro, v. 79, n. 3, p. 337-340, 1984.

DUBEY, J. P.; MILLER, S.; DESMONTS, G.; THULLIEZ, P.; ANDERSON, W. R. *Toxoplasma gondii*-induced abortion in dairy goats. *Journal of the American Veterinary Medical Association*, Schaumburg, v. 188, n. 2, p. 159-162, Jan. 1986.

DUBEY, J. P. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. *Veterinary Parasitology*, Amsterdam, v. 74, n. 1, p. 75-77, 1998.

_____. Persistence of encysted *Toxoplasma gondii* in caprine livers and public health significance of toxoplasmosis in goats. *Journal of the American Veterinary Medical Association*, Schaumburg, v. 177, n. 12, p. 1203-1207, 1980.

_____. *Toxoplasma:* induced abortion in dairy goats. *Journal of the American Veterinary Medical Association*, Schaumburg, v. 178, n. 7, p. 671-674, 1981.

DUBEY, J. P.; ADAMS, D. S. Prevalence of *Toxoplasma gondii* antibodies in dairy goats from 1982 to 1984. *Journal of the American Veterinary Medical Association*, Schaumburg, v. 196, n. 2, p. 295-296, 1990.

DUBEY, J. P.; SUNDBERG, J. P.; MATIUCK, S. W. Toxoplasmosis associated with abortion in goats and sheep in Connecticut. *American Journal of Veterinary Research*, Chicago, v. 42, n. 9, p. 1624-1626, 1981.

FIGLIUOLO, L. P. C.; RODRIGUES, A. A. R.; VIANA, R. B.; AGUIAR, D. M.; KASAI, N.; GENNARI, S. M. Prevalence of anti-*Toxoplasma gondii* and anti-Neospora caninum antibodies in goat from São Paulo State, Brazil. *Small Ruminant Research*, Amsterdam, v. 55, n. 1-3, p. 29-32, 2004.

FIGUEIREDO, J. F.; SILVA, D. A. O.; CABRAL, D. D.; MINEO, J. R. Seroprevalence of *Toxoplasma gondii* infection in goats by the indirect haemagglutination, immunofluorescence and immunoenzymatic test in the region of uberlandia, Brazil. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v. 96, n. 5, p. 687-692, 2001.

GILBERT, R.; COOK, A.; DUNN, D. Sources of *Toxoplasma* infection in pregnant women: a European multicenter case-control study. *British Medical Journal,* London, v. 321, n. 7254, p. 142-147, 2000.

GONDIM, L. F. P.; BARBOSAJR., H. V.; RIBEIRO FILHO, C. H. A.; SAEKI H. Serological survey of antibodies to *Toxoplasma gondii* in goat, shepp, cattle and water buffaloes in Bahia State, Brazil. *Veterinary Parasitology*, Amsterdam, v. 82, n. 4, p. 273-276, 1999.

HOMAN,W.L.;VERCAMMEN,M.;DEBRAEKELEER, J. Identification of a 200 to 300 fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. *International Journal for Parasitology*, Oxford, v. 30, n. 1, p. 69-75, 2000.

HOWE, D. K.; SIBLEY, L. D. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *Journal Infection Diseases*, Chicago, v. 172, n. 6, p. 1561-1566, 1995.

MACHADO, T. M. M.; LIMA, J. D. Freqüência de anticorpos anti-*Toxoplasma gondii* em caprinos criados sob diferentes formas de exploração no Estado de Minas Gerais. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 39, n. 2, p. 255-264, 1987.

MACIEL, K. P.; ARAUJO, F. A. P. Inquérito sorológico para detecção de anticorpos de *Toxoplasma gondii* em caprinos (capra hircus) criados nos municípios de Gravataí e Viamão, Região da Grande Porto Alegre, Rio Grande do Sul, Brasil. *Revista de Ciências Agroveterinárias*, Lages, v. 3, n. 2, p. 121-125, 2004.

MAINARDI, R. S.; MODOLO, J. R.; STACHISSINI, A. V. M.; PADOVANI, C. R.; LANGONI, H. Soroprevalência de *Toxoplasma gondii* em rebanhos caprinos do Estado de São Paulo. *Revista da Sociedade brasileira de Medicina Tropical***,** Rio de Janeiro, v**.** 36, n. 6, p. 759-761, 2003.

REIS, C. R.; LOPES, F. M. R.; FREIRE, R. L.; GONÇALVES, D. D.; GARCIA, J. L.; NAVARRO, I. T. Occurrence of anti-Toxoplasma gondii antibodies in caprines from Pitanga city, Paraná State, Brazil. *Brazilian Journal of Veterinary Research and Animal Science*, São Paulo, v. 44, n. 5, p. 358-363, 2007.

ROBERT, R.; CHABASSE, D.; HOCQUET, P. Anti-*Toxoplasma* IgM studied by indirect immunofluorescence and hemagglutination elimination of false positives and negatives by adsorption of IgG on immobilized protein A. *Biomedicine*, Paris, v. 35, n. 1, p. 61-65, 1981.

RYU, E. Rapid microscopic agglutination test for leptospira without non-specific reaction. *Bulletin Official of International Epizooties*, Paris, v. 73, n. 1-2, p. 49-58, 1970.

SAMBROOK, J.; FRITSHC, E. F.; MANIATIS, T. *Molecular cloning*: a laboratory manual. 2.ed. New York: Cold Spring Harbor Laboratory Press, 1989.

SELLA, M. Z.; NAVARRO, I. T.; VIDOTTO, O.; FREIRE, R. L.; SHIDA, P. N. Epidemiologia da toxoplasmose caprina, levantamento sorológico do *Toxoplasma gondii* em caprinos leiteiros na micro região de Londrina, Paraná, Brasil. *Revista Brasileiro de Parasitologia Veterinária*, Rio de Janeiro, v. 3, n. 1, p. 13-16, 1994.

SILVA, A. V.; CUNHA, E. L. P.; LANGONI, H.; MOTA, R. A.; MEIRELES, L. R.; GOTTSCHALK, S. Toxoplasmose em ovinos e caprinos: estudo soroepidemiológico em duas regiões do Estado de Pernambuco, Brasil. *Ciência Rural*, Santa Maria, v. 33, n. 1, p. 115-119, 2003.

SLOSARKOVA, S.; LITERAK, I.; SKRIVANEK, M.; SVOBODOVA, V.; SUCHY, P.; HERZIG, I. Toxoplasmosis and iodine deficiency in Angora goats. *Veterinary Parasitology*, Amsterdam, v. 81, n. 2, p. 89-97, 2000.

VITOR, R. W. A.; PINTO, J. B.; CHIARI, C. A. Eliminação de *Toxoplasma gondii* através de urina, saliva e leite de caprinos experimentalmente infectados. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 43, n. 2, p. 147-154, 1991.