Veterinary Parasitology: Regional Studies and Reports 29 (2022) 100688

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Regional report

Toxoplasma gondii in tissues of sheep slaughtered for human consumption in a highly endemic area for toxoplasmosis in Brazil

A.F. Silva [a,*], E. Frazão-Teixeira [b], F.C.R. Oliveira [c], A.B.M. Fonseca [d], A.M.R. Ferreira [e]

[a] Programa de Pós-Graduação em Medicina Veterinária, Departamento de Medicina e Cirurgia Veterinária, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[b] Laboratório de Biologia Estrutural, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Av. Brasil 4365, Rio de Janeiro, RJ 21040-361, Brazil / Laboratório de Sanidade Animal, Centro de Ciências e Tecnologias Agropecuárias, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego 2000, Campos dos Goytacazes, RJ 28013-602, Brazil
[c] Laboratório de Sanidade Animal, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil
[d] Departamento de Estatística, Universidade Federal Fluminense, Rio de Janeiro, RJ, Brazil
[e] Laboratório de Anatomia Patológica Veterinária, Programa de Pós-Graduação em Medicina Veterinária, Universidade Federal Fluminense, Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Keywords:
Toxoplasmosis
Sheep tissues
PCR
MAT
Rio de Janeiro

ABSTRACT

Toxoplasma gondii infects warm-blooded animals, including humans, and the ingestion of undercooked meat of infected animals is an important source of infection. We investigated the presence of *T. gondii* by PCR and histopathology in tissues of 25 seropositive sheep slaughtered for human consumption in Rio de Janeiro and assessed the suitability of different tissues for molecular detection of the parasite. The animals were first screened for *T. gondii* antibodies by MAT (Modified Agglutination Test), and tissues of seropositive sheep (brain, heart, lungs, kidneys, liver and diaphragm) were subjected to molecular and histological examination. A nested-PCR targeting the P43 gene of *T. gondii* was performed, and kappa Coefficient was used to assess PCR results among tissues. DNA of the parasite was detected in 60 % (9 of 15) of the animals. and tissue pairs of lungs/heart, lungs/diaphragm or heart/diaphragm had substantial agreement with the global status of the animals. The combination between these three tissues leads to an almost perfect agreement with global status results. Sheep slaughtered for human consumption in Rio de Janeiro, Brazil are infected with *T. gondii,* and pairs of fragments of lungs and heart, lungs and diaphragm or heart and diaphragm could be used for molecular identification of *T. gondii* in sheep with substantial agreement with the global status of the animals.

1. Introduction

Toxoplasma gondii is a ubiquitous parasite that infects warm-blooded animals, including humans, and it is estimated that one-third of the human population worldwide is chronically infected (Dubey, 2010). The disease typically involves a transient acute phase caused by the rapid and systemic replication of tachyzoites followed by a latent life-long stage in which bradyzoites persist within tissue cysts. It is generally well resolved in immunocompetent individuals but might lead to death by encephalitis in immunocompromised patients (Dubey, 2010). Also, congenital toxoplasmosis might occur in primarily infected women and lead to serious manifestations (Dubey, 2010). The ingestion of undercooked meat and meat products of infected animals, including sheep, is an important way of transmission to humans since they may harbor viable *T. gondii* cysts (Dubey et al., 2020).

Prevalence of antibodies anti-*T. gondii* among humans in Rio de Janeiro state, Brazil is high (up to 84 %), as well as among different species of farm animals, including pigs (20.6 %), cattle (49.4 %), chickens (54.5 %), sheep (53.3 %) and ratites (40.4 %) (Bahia-Oliveira et al., 2003; Ribeiro et al., 2008; Frazão-Teixeira et al., 2011; Frazão-Teixeira and Oliveira, 2011; Dubey et al., 2012; Cosendey-KezenLeite et al., 2014; Gallo et al., 2019). In sheep, rates ranged from 38 to 53 %, with most Modified agglutination test (MAT) titers above 1:100 (Luciano et al., 2011; Cosendey-KezenLeite et al., 2014), associated with persistent infection and increased potential to harbor viable parasites (Dubey, 2010). People who ingest sheep tissues in this region might be exposed to infection, what has led us to investigate the presence of *T. gondii* in tissues of seropositive sheep slaughtered for human consumption in Rio de Janeiro. Also, we assessed the suitability of tissues individually for molecular detection of the parasite, in order to identify

* Corresponding author.
E-mail address: andressafsilva@ufrrj.br (A.F. Silva).

https://doi.org/10.1016/j.vprsr.2022.100688
Received 2 May 2021; Received in revised form 2 December 2021; Accepted 12 January 2022
Available online 20 January 2022

tissues that may be representative of the animal's positivity.

2. Material and methods

2.1. Ethics

This study was submitted to the Animals Ethics Committee of Universidade Federal Fluminense (UFF), Niterói, RJ, Brazil, and approved under license # 0011109.

2.2. Sample collection and processing

Sheep from Rio de Janeiro, Brazil and slaughtered for human consumption were screened for IgG antibodies anti-*T. gondii* by the modified agglutination test (MAT) and tissues of 15 seropositive females presenting reproductive disorders were submitted to an investigation of *T. gondii* DNA by PCR. Sampling was not randomized and animals were chosen by their reproductive disorders status and seropositivity for anti-*T. gondii* antibodies. The 15 seropositive animals were selected from the 292 ewes screened in the group's previous work (Cosendey-KezenLeite et al., 2014).

Blood and tissues were collected by convenience during slaughter by venipuncture of a jugular vein in 10 mL tubes without anticoagulant and sent to the laboratory in isothermal containers at 5 °C. Blood samples were centrifuged at 1,000 x *g* for 10 min, sera removed and stored at 20 °C until submission to serological tests. Fragments of brain, heart, lungs, kidneys, liver and diaphragm were collected and part of these samples was kept at -20 °C for DNA extraction and another part in 10% buffered formalin for histopathology.

2.3. Modified agglutination test (MAT)

Serologic testing of sheep was performed by the MAT according to Dubey and Desmonts (1987). In short, sera were diluted in phosphate buffered saline (PBS) pH 7.2 and those with agglutinating activity at a dilution of 1:25 were considered positive (Sousa et al., 2009). Additional dilutions were tested in the ewes screened in the group's previous work (Cosendey-KezenLeite et al., 2014).

The antigen was constituted by formalin-fixed *T. gondii* tachyzoites of the RH strain and the test was performed in U-shaped 96-well plates, where 25 μL of the antigen solution was mixed with 25 μL of the previously diluted sera. The positive control was serum from a mouse experimentally infected with *T. gondii*. The negative control was serum from a mouse known to be negative for *T. gondii* infection. Both controls were added to the test and incubated at 37 °C for 12 hours. Results were based on the sedimentation profile of the tachyzoite suspension, where the formation of a blue button means negative and a clear bottom means positive.

2.4. Polymerase chain reaction (PCR)

Tissue fragments of approximately 25 mg were submitted to DNA extraction using Qiagen's DNeasy® Blood and Tissue kit following the manufacturer's recommendations. A nested-polymerase chain reaction (nPCR) targeting the P43 gene of *T. gondii* was performed using the following primer set described by Grigg et al. (2001): CAACTCTCACCATTCCACCC and TCTTGTCGGGTGTTCACTCA (external primers) and GCGCGTTGTTAGACAAGACA and CACAAGGAGACCGAGAAGGA (internal primers). In the first amplification round, 5 μL of extracted DNA samples was added to PCR mix and, in the nested round, 1 μL of round 1 products. Both rounds used 5 μL of PCR buffer in a final volume of 50 μL containing 15 mM $MgCl_2$, 0.1 mM dNTP mix, 10 pmol of each primer, and 1.5 U of Taq DNA polymerase. Each of the 35 cycles consisted of 94°C for 30 secs, 60°C for 30 s, and 72°C for 30 s.

Controls for extraction and nPCR steps were included, as follows. For the extraction step, an infected mouse lung fragment was included as a positive control and non-infected mouse lung fragment as a negative control. These extracted DNA samples were inserted in the first PCR round along with another positive control (DNA sample known positive for *T. gondii*) and another negative control (PBS as template). In the nPCR round, we included an extra negative control using PBS as a template. The controls were included in each reaction and amplification products were separated by electrophoresis in a 2% agarose gel visualized by UV transillumination. For staining of the amplicons we used GelRed (Biotium, Hayward, CA, USA), following the manufacturer specifications.

2.5. Histopathology

Tissue fragments fixed in 10% buffered formalin were also routinely processed in paraffin for light microscopy, according to Silva et al. (2013).

2.6. Statistical analysis

All PCR results for individual tissues were combined into a single global status where animals were classified as negatives only if all individual PCR results were also negative. For this analysis we used only the 15 sheep in which we had all tissues available. Kappa Coefficient was used to measure agreement between each pair of PCR results for individual tissues and also between a PCR result and animal global status. A significance level of 0.05 was adopted for testing hypothesis of agreement randomness in each comparison using McNemar test. The analysis was done using the SPSS software (Statistical Package for Social Science, version 18).

3. Results

Toxoplasma gondii DNA was detected in tissues of 60% (9/15) of the seropositive sheep, and lung, and heart were the most prevalent (4/15; 26.7%), followed by brain and diaphragm (3/15; 20.0%), kidney (2/15; 13.3%) and liver (1/15; 6.7%) (Table 1). Kappa coefficients indicated only a fair agreement between PCR results of brain, lung, heart and diaphragm separately and those of animal global status, while only a slight agreement was observed between liver and the same status (Table 2). In these comparisons, most hypotheses of random distribution of disagreements were rejected, indicating that there were systematic differences in classification for PCR results for these tissues and for global status results. Lung and heart were the only two tissues where this hypothesis was accepted ($p = 0.063$, each).

Although a moderate agreement was found between lung and kidney PCR results (Kappa = 0.595), they had only two positive samples in common and most of this agreement came from 11 negative samples. Also, we found another moderate agreement (Kappa = 0.444) between brain and each one of these tissues: lungs, heart and liver, but again only two samples were found positive at the same time in both PCR results. A moderate agreement (Kappa = 0.444) was also found between diaphragm and liver PCR results, which again was mainly based on 13 negative results. The hypothesis of random distribution of disagreements was accepted in all cases (Table 3).

Combined results from two tissues, lungs and heart, lungs, and diaphragm or heart and diaphragm increased the Kappa coefficient increases and indicate a substantial agreement between these results, and global status for the animals (Table 4). It was also noticed that the combination between these three tissues leads to an almost perfect agreement with global status results (Kappa = 1,000; McNemar test p-value = 1,000). In these cases, McNemar test results show that any disagreements between these classifications can be treated as random, and there was no indication of any systematic bias of classification between them.

Table 1
Toxoplasma gondii DNA in tissues of seropositive sheep slaughtered for human consumption in Rio de Janeiro state, Brazil.

Animal# [a]	Brain	Lungs	Kidney	Heart	Diaphragm	Liver	Global status
1		+[b]	+				+
2	+	+	+	+	+	+	+
3				+			+
4	+	+					+
5					+		+
6		+					+
8					+		+
12				+			+
13	+			+			+
Total	3 (20%)	4 (26,7%)	2 (13,3)%	4 (26,7%)	3 (20%)	1 (6,7%)	9 (60%)

[a] Animals #7, 9, 10, 11, 14, 15 had no positive tissue by PCR.
[b] Tissue positive by PCR.

Table 2
Kappa Coefficient for equivalence between individual tissues and animal global status on *Toxoplasma gondii* DNA detection in sheep slaughtered for human consumption in Rio de Janeiro state, Brazil.

Tissues	Kappa	*p-value*[*]
Brain	0,286	0,031
Lungs	0,390	0,063
Kidney	0,186	0,016
Heart	0,390	0,063
Diaphragm	0,286	0,031
Liver	0,091	0,008

[*] McNemar test: $p \leq 0.05$ are significant.

Table 3
Kappa Coefficient for equivalence between tissues on *Toxoplasma gondii* DNA detection in sheep slaughtered for human consumption in Rio de Janeiro state, Brazil.

Tissues compared	Kappa	*p-value*[*]
Brain / Lungs	0,444	1,000
Brain /Kidney	0,286	1,000
Brain / Heart	0,444	1,000
Brain / Diaphragm	0,167	1,000
Brain / Liver	0,444	0,500
Lungs / Kidney	0,595	0,500
Lungs / Heart	–0,023	1,000
Lungs / Diaphragm	0,074	1,000
Lungs / Liver	0,328	0,250
Kidney / Heart	0,189	0,625
Kidney / Diaphragm	0,286	1,000
Kidney / Liver	0,634	1,000
Heart / Diaphragm	0,074	1,000
Heart /Liver	0,328	0,250
Diaphragm / Liver	0,444	0,500

[*] McNemar test: $p \leq 0.05$ are significant.

4. Discussion

The occurrence of *T. gondii* DNA in tissues of the seropositive sheep observed in the present study was higher than observed in slaughterhouses from São Paulo, Brazil (33.3%) and Iran (37.5%) (Asgari et al., 2011; Silva et al., 2011). Rate differences among regions or countries depend on many factors, like natural variations of *T. gondii* infection, the size of tissue samples analyzed and processing methods for DNA isolation (Adamska, 2018). It is important to consider the possible influence of the small tissue fragment (25 mg) used for DNA extraction in the sensibility of the test, as the parasite could be present in another portion of the tissue and not in the one sampled.

Identifying *T. gondii* in the tissues of sheep slaughtered for human consumption is a matter of public health (Dubey et al., 2020). The ingestion of mutton has been identified as a risk factor for seroprevalence among pregnant women (Berger et al., 2007) and was also confirmed to be the cause of an outbreak of acute toxoplasmosis in Brazil (Bonametti et al., 1997). The consumption of mutton is significantly associated to toxoplasmosis in humans and special care should be taken, especially because it is often eaten undercooked and *T. gondii* is present in virtually all edible ovine tissues (Dubey et al., 2020).

Table 4
Kappa Coefficient for equivalence between each pair of tissues and animal global status on *Toxoplasma gondii* DNA detection in sheep slaughtered for human consumption in Rio de Janeiro state, Brazil.

Pair of Tissues	Kappa	*p-value*[*]
Brain and Lungs	0,500	0,125
Brain and Kidney	0,390	0,063
Brain and Heart	0,500	0,125
Brain and Diaphragm	0,500	0,125
Brain and Liver	0,286	0,031
Lungs and Kidney	0,390	0,063
Lungs and Heart	**0,737[a]**	**0,500[b]**
Lungs and Diaphragm	**0,615[a]**	**0,250[b]**
Lungs and Liver	0,390	0,063
Kidney and Heart	0,500	0,125
Kidney and Diaphragm	0,390	0,063
Kidney and Liver	0,186	0,016
Heart and Diaphragm	**0,615[a]**	**0,250[b]**
Heart and Liver	0,390	0,063
Diaphragm and Liver	0,286	0,031

[*] *McNemar test: $p \leq 0.05$ are significant. [a]combinations of tissues that most agree with global status [b]eventual disagreement between the tissues and the global status that occur randomly

Prevalence rates of antibodies anti-*T. gondii* are high among humans in the Rio de Janeiro state (Bahia-Oliveira et al., 2003; Ribeiro et al., 2008) as well as in several species of farm animals (Frazão-Teixeira et al., 2011; Frazão-Teixeira and Oliveira, 2011; Dubey et al., 2012; Cosendey-KezenLeite et al., 2014; Gallo et al., 2019). These animals are, therefore, potential sources for human infection, but real risk is mainly inferred when viable parasites are isolated from the animals' tissues. Although detecting *T. gondii* DNA does not prove the tissues harbor viable parasites, it proves the tissue was once infected. Viable *T. gondii* has been recovered from tissues of persistently infected sheep and most isolations were made in Brazil, Ethiopia and France (Dubey et al., 2020). Rani et al. (2020) proved that even 5-10 g of meat might harbor viable tissue cysts. In this way, by detecting DNA of *T. gondii* in tissues of the sheep slaughtered for human consumption in Rio de Janeiro, including muscles (diaphragm and heart), we can infer that sheep tissues from this area may be an important source for human infection.

There was only a fair agreement between PCR results of brain, lungs, heart and diaphragm separately and those of animal global status (Table 2). But, combining results of tissues in pairs, Kappa coefficient increased above 0.6 for some of them, showing substantial agreement between pairs of lungs/heart, lungs/diaphragm or heart/diaphragm and the global status of the animals. This means that each of these pairs could be chosen when assessing the presence of *T. gondii* DNA in sheep.

This outcome might improve detection methods and reduce costs since one could choose a simple pair of tissues to represent the positivity of the animal as a whole. Additionally, the combination of the triad lungs/heart/diaphragm led to perfect agreement with global status results, turning into the best option for molecular testing the global status of sheep with only a few tissue specimens.

There are few studies on *T. gondii* predilection sites in sheep and these performed experimental infections using type II clonal strains. *T. gondii* strains infecting sheep from Brazil are genetically much more diverse and there is no data so far on any difference in predilection sites. From the 35 sheep isolates genotyped, only three were type II and the rest were classified in 15 different genotypes. Esteban-Redondo et al. (1999) compared the presence of *T. gondii* DNA in brains, heart and skeletal muscle of experimentally infected sheep and the parasite was more frequently detected in brains and hearts. Juránková et al. (2015) tested predilection sites for *T. gondii* by PCR in the brain, lung, heart, kidney, liver, spleen and skeletal muscle of sheep. Brain (6/6) followed by lung and heart (3/6) were the most suitable tissues for *T. gondii* molecular detection. Verhelst et al. (2015) experimentally tested *T. gondii* concentration in different sheep tissues and brain, heart and skeletal muscle had the highest parasite burdens.

Those studies are in accordance with the present results as the heart/lungs pair had the highest Kappa coefficient. It is interesting to notice that the brain was not found to be important as a tissue for molecular detection of *T. gondii* in sheep in the present study, although this parasite is known to have strong tropism to it. Instead, we could identify the diaphragm as another option when in pair with lungs or heart. Also, the liver was not in substantial agreement either individually or in pairs, but it was identified as an organ of choice for the detection of *T. gondii* in sheep by IHC (Silva et al., 2013). It is worth mentioning that the liver is a viscera used for feeding and some physicians recommend its ingestion for children and pregnant women with iron deficiency or anemia, which could increase the risk of *T. gondii* infection (Asgari et al., 2011).

We conclude that sheep slaughtered for human consumption in Rio de Janeiro, Brazil are infected with *T. gondii*. Investigators might test pairs of fragments of lungs and heart, lungs and diaphragm or heart and diaphragm for molecular identification of *T. gondii* in sheep with substantial agreement with the global status of the animals.

Funding acknowledgement

The authors acknowledge the financial support given by CAPES, FAPERJ and CNPq.

Declaration of Competing Interest

This work was supported Conselho Nacional de Pesquisa - CNPq / Brazil. The authors declare they have no competing financial interests.

References

Adamska, M., 2018. Molecular detection of *Toxoplasma gondii* in natural surface water bodies in Poland. J Water Health 16. https://doi.org/10.2166/wh.2018.236, 657-660. 5.

Asgari, Q., Sarnevesht, J., Kalantari, M., Sadat, S.J.A., Motazedian, M.H., Sarkari, B., 2011. Molecular survey of *Toxoplasma* infection in sheep and goat from Fars province, Southern Iran. Trop Anim Health Prod 43, 389–392. https://doi.org/10.1007/s11250-010-9704-1.

Bahia-Oliveira, L.M., Jones, J.L., Azevedo-Silva, J., Alves, C.C., Oréfice, F., Addiss, D.G., 2003. Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. Emerg Infect Dis 9, 55–62. https://doi.org/10.3201/eid0901.020160.

Berger, F., Goulet, V., Le Strat, Y., De Valk, H., Désenclos, J.C., 2007. La toxoplasmose en France chez la femme enceinte en 2003: séroprévalence et facteurs associés Maladies infectieuses. Institut de veille sanitaire, Saint Maurice, France.

Bonametti, A.M., Passos, J.N., Silva, E.M.K., Bortoliero, A.L., 1997. Surto de toxoplasmose aguda transmitida através da ingestão de carne crua de gado ovino. Rev. Soc. Bras. Med. Trop. 30, 21–25. https://doi.org/10.1590/S0037-86821997000100005.

Cosendey-KezenLeite, R.I., de Oliveira, F.C., Frazão-Teixeira, E., Dubey, J.P., de Souza, G.N., Ferreira, A.M., Lilenbaum, W., 2014. Occurrence and risk factors associated to *Toxoplasma gondii* infection in sheep from Rio de Janeiro, Brazil. Trop Anim Health Prod 46, 1463–1466. https://doi.org/10.1111/j.1863-2378.2008.01187.x.

Dubey, J.P., 2010. Toxoplasmosis of Animals and Humans, Second edition. U.S.A. Taylor & Francis Group, USDA/ARS, Beltsville, Maryland.

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339. https://doi.org/10.1111/j.2042-3306.1987.tb01426.x.

Dubey, J.P., Lago, E.G., Gennari, S.M., Su, C., Jones, J.L., 2012. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitology 139, 1375–1424. https://doi.org/10.1017/S0031182012000765.

Dubey, J.P., Murata, F.H.A., Cerqueira-Cezar, C.K., Kwok, O.C.H., Su, C., 2020. Economic and public health importance of Toxoplasma gondii infections in sheep: the last decade. Veterinary Parasitology. https://doi.org/10.1016/j.vetpar.2020.109195.

Esteban-Redondo, I., Maley, S.W., Thomson, K., Nicoll, S., Wright, S., Buxton, D., Innes, E.A., 1999. Detection of *Toxoplasma gondii* in tissues of sheep and cattle following oral infection. Vet. Parasitol. 86, 155–171. https://doi.org/10.1016/S0304-4017(99)00138-7.

Frazão-Teixeira, E., Oliveira, F.C.R., 2011. Anti–*Toxoplasma gondii* antibodies in cattle and pigs in a highly endemic area for human toxoplasmosis in Brazil. J. Parasitol. 97, 44–47. https://doi.org/10.1645/GE-2457.1.

Frazão-Teixeira, E., Sundar, N., Dubey, J.P., Grigg, M.E., Oliveira, F.C.R., 2011. Multilocus DNA sequencing of *Toxoplasma gondii* isolated from Brazilian pigs identifies genetically divergent strains. Vet. Parasitol. 175, 33–39. https://doi.org/10.1016/10.1016/j.vetpar.2010.09.030.

Gallo, S.S.M., Frazão-Teixeira, E., Ederli, N.B., Oliveira, F.C.R., 2019. Prevalence of anti-*Toxoplasma gondii* antibodies in ratites from Brazil. J Parasitol. 105, 733–737. https://doi.org/10.1645/19-101.

Grigg, M.E., Ganatra, J., Boothroyd, J.C., Margolis, T.P., 2001. Unusual Abundance of Atypical Strains Associated with Human Ocular Toxoplasmosis. J Infect Dis. 184, 633–639. https://doi.org/10.1086/322800.

Juránková, J., Basso, W., Neumayerová, H., Frencová, A., Baláž, V., Deplazes, P., Koudela, B., 2015. Predilection sites for *Toxoplasma gondii* in sheep tissues revealed by magnetic capture and real-time PCR detection. Food Microbiology 52, 150–153. https://doi.org/10.1016/j.fm.2015.07.005.

Luciano, D.M., Menezes, R.C., Ferreira, L.C., Nicolau, J.L., Neves, L.B., Luciano, R.B., Dahroug, M.A.A., Amendoeira, M.R.R., 2011. Occurrence of anti-*Toxoplasma gondii* antibodies in cattle and pigs slaughtered, State of Rio de Janeiro. Rev. Bras. Parasitol. Vet. Jaboticabal 20, 351–353. https://doi.org/10.1590/S1984-29612011000400018.

Rani, S., Cerqueira-Cézar, C.K., Murata, F.H.A., Kwok, O.C.H., Dubey, J.P., Pradhan, A. K., 2020. Distribution of *Toxoplasma gondii* tissue cysts in shoulder muscles of naturally infected goats and lambs. J. Food Prot. https://doi.org/10.4315/JFP-20-024.

Ribeiro, A.C., Mutis, M.S., Fernandes, O., 2008. Association of the presence of residual anti-*Toxoplasma gondii* IgM in pregnant women and their respective family groups in Miracema, Northwest Rio de Janeiro. Brazil. Mem. Inst. Oswaldo Cruz. 103, 591–594. https://doi.org/10.1590/S0074-02762008000600013.

Silva, R.C., Langoni, H., Su, C., Silva, A.V., 2011. Genotypic characterization of *Toxoplasma gondii* in sheep from Brazilian slaughterhouses: new atypical genotypes and the clonal type II strain identified. Vet Parasitol 175, 173–177. https://doi.org/10.1016/j.vetpar.2010.09.021.

Silva, A.F., Oliveira, F.C., Leite, J.S., Mello, M.F., Brandão, F.Z., Leite, R.I., Frazão-Teixeira, E., Lilenbaum, W., Fonseca, A.B., Ferreira, A.M., 2013. Immunohistochemical identification of *Toxoplasma gondii* in tissues from Modified Agglutination Test positive sheep. Vet Parasitol 191, 347–352. https://doi.org/10.1016/j.vetpar.2012.09.022.

Sousa, S., Thompson, G., Silva, E., Freire, L., Lopes, D., Correia Da Costa, J.M., Castro, A., Carvalheira, J., 2009. Determination of the more adequate modified agglutination test cut-off for serodiagnosis of *Toxoplasma gondii* infection in sheep. Canada Zoonoses Public Health 56 (5). https://doi.org/10.1111/j.1863-2378.2008.01187.x, 252-6. 2009.

Verhelst, D., de Craeye, S., Jennes, M., Dorny, P., Goddeeris, B., Cox, E., 2015. Interferon gamma expression and infectivity of *Toxoplasma* infected tissues in experimentally infected sheep in comparison with pigs. Vet. Parasitol. 207, 7–16. https://doi.org/10.1016/j.vetpar.2014.11.014.