



PREVALENCE OF ANTI-TOXOPLASMA GONDII IGG ANTIBODIES IN SHEEP SLAUGHTERED AT A FEDERALLY INSPECTED SLAUGHTERHOUSE IN THE STATE OF BAHIA

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ABSTRACT

Toxoplasma gondii is an obligate intracellular parasite with worldwide distribution. In several countries, this parasite causes reproductive disorders in sheep, resulting in economic losses in sheep farming. Additionally, it is a cosmopolitan zoonosis with significant public health implications. This study aimed to determine the seroprevalence of *T. gondii* in sheep slaughtered in federally inspected slaughterhouses in the state of Bahia and to associate risk factors with the disease's occurrence. Serum samples from 227 sheep, collected from a federally inspected slaughterhouse, were analyzed using the indirect hemagglutination assay (IHA). Titers ranged from 1:32 to 1:4096, with an overall seropositivity of 40.53% (92/227). The highest frequencies were observed at titers of 1:32 (33.34%) and 1:64 (35.9%). No positive correlation was found between the studied variables and seropositivity; however, a high seroprevalence of anti-*Toxoplasma gondii* IgG antibodies was identified in sheep slaughtered for human consumption in federally inspected facilities.

Keywords: Antibodies; Indirect Hemagglutination; Toxoplasmosis.

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan of the phylum Apicomplexa, whose infection in humans and animals is prevalent worldwide (Dubey, 2009). The parasite has felids as its definitive hosts and a variety of mammals and birds as intermediate hosts, including humans (Tenter et al., 2000; Yarovinsky, 2014).

Infection by *T. gondii* can occur through the consumption of food and water contaminated with sporulated oocysts, ingestion of meat and milk, as well as via transplacental transmission (Camossi et al., 2011; Dubey, 2008; Hunter & Sibley, 2012; Yarovinsky, 2014). Although the clinical importance of toxoplasmosis is widely perceived to be underestimated, Hoffmann et al. (2012) demonstrated that it has great relevance in the cost calculations of diseases caused by foodborne pathogens. In this regard, estimates suggest that 23% of adult adolescents are infected by *T. gondii* and that 24% of deaths caused by foodborne diseases in the United States are due to toxoplasmosis (Hussain et al., 2017; CDC, 2016). However, these estimates can vary greatly depending on the geographic region and the presence of different types of intermediate hosts, which impacts the abundance of the parasite in the environment (Hill & Dubey, 2016).

There are several routes through which oral contamination can occur, but high-risk foods for this condition include meat contaminated with oocysts, unpasteurized goat milk, fresh vegetables, and contaminated water. The consumption of undercooked sheep meat has been considered an important source of infection for humans (Dubey, 2009; Hussain et al., 2017). Thus, the aim of this study was to evaluate the prevalence of anti-*Toxoplasma gondii* IgG antibodies in sheep slaughtered at a federally inspected slaughterhouse in the state of Bahia.

MATERIALS AND METHODS

Blood samples were obtained from sheep slaughtered at a federally inspected slaughterhouse in the state of Bahia, located in the city of Feira de Santana, in the north-central region of Bahia, Brazil. Blood was collected in 9 mL vacutainer tubes without anticoagulant at the time of exsanguination, in accordance with the current animal welfare standards of Brazilian legislation (Ethics Committee on Animal Use, Federal University of Recôncavo da Bahia, Protocol No. 23007.001511/2016-50). Subsequently, the samples were properly identified and then stored in a thermal box at 4°C and transported to the Laboratory of Immunology and Veterinary

Biochemistry at the Federal University of Recôncavo da Bahia. The sera were obtained by centrifugation at 675 x G for 10 minutes, transferred to polypropylene tubes, identified, and stored at -20°C until the serological test was performed.

The detection of anti-*T. gondii* IgG antibodies was performed using the commercial kit Toxotest HAI (Wiener Lab, Argentina), following the manufacturer's instructions. The serum samples were initially screened in singlicate at a 1:32 dilution. Those showing a positive reaction at the cut-off point were serially diluted at a twofold ratio until no further reaction was observed, to determine their titers. The titer was determined by the last positive reaction.

The number of animals was determined using the sample size calculation for an infinite population, with a 95% confidence interval and a maximum error of 5%. An expected prevalence value of 18.75% was adopted, in accordance with epidemiological studies conducted in the state of Bahia by Gondim et al. (1999), resulting in a total of 227 animals, based on the following formula:

$$n = \left(\frac{Z_{\alpha/2}}{e_0} \right)^2 \cdot p \cdot (1 - p')$$

Where:

- n = number of individuals in the sample
- $Z_{\alpha/2}$ = Critical value corresponding to the desired confidence level
- P = Population proportion of individuals belonging to the category of interest

RESULTS AND DISCUSSION

Anti-*T. gondii* IgG antibodies were detected in 40.53% (92/227) of the analyzed samples, with titers ranging from 1:32 to 1:4096, as shown in Table 1.

Table 1: IgG antibody titers against *Toxoplasma gondii* in blood samples from sheep obtained at a slaughterhouse under Federal Inspection in the municipality of Feira de Santana, Bahia.

Titers	Prevalence
1:32	33,34% (31/227)
1:64	35,9% (33/227)
1:128	19,6% (18/227)
1:256	7,6% (7/227)
1:512	1,1% (1/227)
1:1024	1,1% (1/227)
1:4096	1,1% (1/227)



Titers	Prevalence
1:32	33,34% (31/227)
Total	40,52% (92/227)

Studies conducted in Brazil have reported prevalence rates of infection in sheep ranging from 18% to 61% (Braga-Filho et al., 2010; Guimarães et al., 2013; Tesolini et al., 2012; Luciano et al., 2011; Rossi et al., 2011; Gondim et al., 1999). High prevalence rates have also been found in other countries, such as Italy, 59.3% (Gazzonis et al., 2015), England, 54.2% (Hutchinson & Smith, 2015), and Spain, 48% (Díaz et al., 2016).

There is no reference serological method with 100% specificity (the ability to identify true negatives) and 100% sensitivity (the ability to identify true positives) for *T. gondii* (Olsen et al., 2019). Thus, several different tests have been used for this purpose. The different diagnostic methods and cut-off points used by each author are some of the factors influencing this wide variability in prevalence rates (Dubey, 2009). Although various diagnostic methods are widely used, Casartelli-Alves et al. (2014) found higher sensitivity for the ELISA test and greater specificity for the indirect hemagglutination test for toxoplasmosis diagnosis. Other important factors influencing prevalence variability are the different climatic conditions, which affect the maintenance and viability of infectious oocysts in the environment, and the varied types of sanitary management (Hill & Dubey, 2016).

CONCLUSIONS

According to the results found, sheep slaughtered for human consumption in the state of Bahia showed a high prevalence of anti-*Toxoplasma gondii* IgG antibodies.

REFERENCES

Amdouni Y, et al. Molecular detection of *Toxoplasma gondii* infection in slaughtered ruminants (sheep, goats and cattle) in Northwest Tunisia. *Meat Sci.* 2017;133:180-4.

Andrade M, et al. Seroprevalence and risk factors associated with ovine toxoplasmosis in Northeast Brazil. *Parasite.* 2013;20(20):1-5.

Braga-Filho E, Ramos OS, Freitas JA. Inquérito sorológico de *Toxoplasma gondii* em ovinos na microrregião Castanhal, Pará, Brasil. *Rev Arq Inst Biol.* 2010;77(4):707-10.

Camossi LG, et al. Detection of *Toxoplasma gondii* DNA in the milk of naturally infected ewes. *Vet Parasitol.* 2011;177:256-61. doi: 10.1016/j.vetpar.2010.12.007.

Casartelli-Alves L, et al. Sensitivity and specificity of serological tests, histopathology and immunohistochemistry for detection of *Toxoplasma gondii* infection in domestic chickens. *Vet Parasitol.* 2014;204:346-51.

Centers for Disease Control and Prevention (CDC). Estimates of Foodborne Illness in the United States. 2016. Available from: <https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html> Accessed 2019 Apr 11.

Díaz P, et al. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in goats from north-western Spain. *Ann Agric Environ Med.* 2016;23(4):587-90.

Dong H, et al. Prevalence, risk factors, and genotypes of *Toxoplasma gondii* in food animals and humans (2000–2017) from China. *Front Microbiol.* 2018;9.



- Dubey JP. The history of *Toxoplasma gondii*—the first 100 years. *J Eukaryot Microbiol.* 2008;55(6):467-75. doi: 10.1111/j.1550-7408.2008.00345.x.
- Dubey JP. Toxoplasmosis in sheep - the last 20 years. *Vet Parasitol.* 2009;163:1-14. doi: 10.1016/j.vetpar.2009.02.026.
- Gazzonis AL, et al. *Toxoplasma gondii* in small ruminants in Northern Italy – prevalence and risk factors. *Ann Agric Environ Med.* 2015;22(1):62-8. doi: 10.5604/12321966.1141370.
- Gondim LFP, et al. Serological survey of antibodies to *Toxoplasma gondii* in goats, sheep, cattle, and water buffaloes in Bahia State, Brazil. *Vet Parasitol.* 1999;82:273-6.
- Gorji GRS, Rassouli M, Staji H. Prevalence of cerebral toxoplasmosis among slaughtered sheep in Semnan, Iran. *Ann Parasitol.* 2018;64(1):37-42.
- Guo M, et al. A systematic meta-analysis of *Toxoplasma gondii* prevalence in food animals in the United States. *Foodborne Pathog Dis.* 2016;13(3):109-18.
- Hill DE, Dubey J. *Toxoplasma gondii* as a Parasite in Food: Analysis and Control. *Microbiol Spectr.* 2016;4(4).
- Hoffmann SI, Batz MB, Morris JG Jr. Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. *J Food Prot.* 2012;75(7):1292-302.
- Hunter AC, Sibley LD. Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. *Microbiology.* 2012;10:766-78.
- Hutchinson JP, Smith RP. Seropositivity to *Toxoplasma* infection in Plant Health Agency laboratories between sheep samples submitted to Animal and 2005 and 2012. *Vet Rec.* 2015;176:573.
- Izadyar N, et al. A serologic study on *Toxoplasma gondii* infection in slaughtered sheep and goats in Qazvin Province, Iran. *Trop Anim Health Prod.* 2019;51(5):1289-93.
- Kalambhe D, Gill JPS, Singh BB. Molecular detection of *Toxoplasma gondii* in the slaughter sheep and goats from North India. *Vet Parasitol.* 2017;241:35-8.
- Luciano DM, et al. Soroepidemiologia da toxoplasmose em caprinos e ovinos de três municípios do estado do Rio de Janeiro. *Pesqui Vet Bras.* 2011;31(7):569-74.
- Mendonça CED, et al. Prevalence and risk factors associated with ovine toxoplasmosis in northeastern Brazil. *Rev Bras Parasitol Vet.* 2013;22(2):230-4.
- Olsen A, et al. Seroprevalence of *Toxoplasma gondii* in domestic pigs, sheep, cattle, wild boars, and moose in the Nordic-Baltic region: a systematic review and meta-analysis. *Parasite Epidemiol Control.* 2019;4:e00100.
- Robert-Gangneux F, Dardé M. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev.* 2012;25:264.
- Rossi GF, et al. Evaluation of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Uberlândia, Minas Gerais State, Brazil, by different serological methods. *Vet Parasitol.* 2011;175(3-4):252-9.
- Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol.* 2000;30(12-13):1217-58.
- Tesolini PMA, et al. Seroprevalence of *Toxoplasma gondii* antibodies in sheep Santa Ines in Big Vitória, the State of Espírito Santo. *Rev Bras Cienc Vet.* 2012;19(1):38-41.
- Yarovinsky F. Innate immunity to *Toxoplasma gondii* infection. *Immunology.* 2014;14:109-12.