



Toxoplasma gondii infection in goats: serological, pathological, and clinical monitoring during gestation

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Abstract

This study aimed to evaluate *Toxoplasma gondii* infection in pregnant goats. The goats were divided into two groups: group one (G1) comprised of 31 pregnant goats naturally infected with *T. gondii*, and group two (G2) comprised of seven uninfected pregnant goats from a flock with a history of abortion due to toxoplasmosis. Serological investigation, ultrasonography, and clinical testing were performed on all goats during gestation. Serum samples from goats and their offspring (precolostral) were collected to evaluate the vertical transmission of *T. gondii*. Samples from placentas and aborted fetuses were also collected for molecular and histopathological analysis. Results showed that in G1, estrus recurrence occurred in 22.6% (7/31) of the goats, embryonic death in 3.3% (1/31), and abortion in 19.4% (6/31). An increase in anti-*T. gondii* antibodies was observed in G1 goats at day 150 of pregnancy. *T. gondii* DNA was detected in 42.8% (3/7) of aborted fetuses and was associated with histopathological lesions caused by this parasite. Moreover, toxoplasmosis in field conditions caused by genotype ToxoDB #1 in pregnant goats resulted in severe reproductive loss in the flock.

Keywords Caprine · Toxoplasmosis · Reproductive loss · Abortion · Pregnant goats

Introduction

Toxoplasmosis is caused by the obligate intracellular protozoan *Toxoplasma gondii* that infects a wide variety of warm-blooded animals. *T. gondii* infection in goats is highly prevalent worldwide and is considered an important cause of reproductive disorders (Dubey 2010). The main route of *T. gondii* transmission in goats is horizontal and occurs through the ingestion of food or water contaminated with sporulated

oocysts of the parasite (Tzanidakis et al. 2012). However, congenital transplacental transmission may also contribute to the maintenance and dissemination of the protozoan infection in flocks (Dubey 2010).

Transplacental transmission can occur endogenously when there is a reactivation of the parasite in chronically infected animals due to changes in immune response patterns during pregnancy, or exogenously, when animals become infected with sporulated oocysts during pregnancy (Trees and Williams 2005). *T. gondii* causes tissue lesions in infected animals (Uggla and Buxton 1990). These lesions are characterized microscopically by areas of necrosis and inflammation, and the presence of mononuclear cells has been observed in the central nervous system, liver, and lungs of aborted fetuses (Dubey 1989). In the placenta of infected animals, areas of necrosis and calcification of the cotyledonary mesenchymal cells have been observed (Unzaga et al. 2014), and in cases of acute infection, thrombosis and infarct areas have been observed in the caruncles (Castaño et al. 2014).

Although previous studies have shown transplacental vertical transmission in goats experimentally infected with *T. gondii* oocysts (Lafi et al. 2014) and in naturally infected

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goats (Misurová et al. 2009), few studies have focused on the importance of this type of transmission in goat herds naturally infected with the protozoan.

Due to the paucity of data on the dynamics of natural *T. gondii* infection in pregnant goats, the objective of this study was to describe the clinical, serological, molecular, and histopathological findings in aborted fetuses and placentas of goats naturally infected with *T. gondii*.

Materials and methods

Ethical approval

This study was approved by the Ethics Committee on Animal Use of *Universidade Federal Rural de Pernambuco* (license number 122/2015) and was conducted following the ethical principles of animal experimentation adopted by the Brazilian College of Animal Experimentation.

Experimental design: sampling and clinical monitoring

Initially, 52 adult goats were selected for inclusion in this study. The goats were divided into two groups: group 1 (G1) comprised of 41 multiparous goats positive in Enzyme-linked immunosorbent assay (ELISA) for *T. gondii*, and group 2 (G2) or the control group comprised of 11 goats serologically negative for *T. gondii* in the ELISA test. The reproductive management of all goats followed a breeding season using the male effect to induce estrus. Pregnancy was confirmed by transrectal ultrasound (Ultrason model Mindray Dp-2200) 30 days after natural mating. Ultrasonography was performed every month to monitor fetal viability until delivery. After the pregnancy was confirmed, 31 goats were maintained in G1 and 7 were maintained in G2. Clinical and serological parameters were evaluated in all goats along with the analysis of the aborted fetuses and placentas.

ELISA revealed that all goats included in the experiment were negative for *Neospora caninum*, *Brucella* spp., *Chlamydomphila abortus*, *Coxiella burnetii*, and *Campylobacter* spp.

Goats were kept in an intensive system, in their original farm that is located in Northeast Brazil, and fed with corn silage (*Zea mays*), elephant grass (*Pennisetum purpureum*), and concentrated feed twice daily. They had free access to water and mineral supplements ad libitum.

IgG response in pregnant goats

To study the kinetics of anti-*T. gondii* antibodies during pregnancy, 31 pregnant goats from G1 and seven from G2 were monitored serologically using ELISA at 30-day

intervals during the gestation period. Blood samples were collected by puncturing the jugular vein in tubes without anticoagulant (Vacutainer® type), and the samples were kept at room temperature until clot retraction occurred. Subsequently, the samples were centrifuged at $1090 \times g$ for 10 min to separate the serum and stored at $-20\text{ }^{\circ}\text{C}$ until serological processing.

Evaluation of the vertical transmission rate (precolostral serology)

To evaluate the vertical transmission rate of *T. gondii* in G1 goats, precolostral serum samples were collected from all kids after parturition but before colostrum ingestion to investigate the presence of antibodies using the indirect immunofluorescence assay (IFA).

Monitoring abortion cases

Goats from G1 that aborted during the experiment underwent clinical examination. Blood was collected from these goats to investigate the presence of antibodies against *T. gondii* at the time of abortion using ELISA. Aborted fetuses were inspected for macroscopic lesions, and the brain, thymus, lungs, heart, liver, and skeletal muscle were collected and stored in 10% buffered formalin for histopathological processing. Brain samples were also collected for extraction of the genomic DNA and PCR analysis.

Analysis of *T. gondii* DNA and placental histopathology

Sixteen placentas from G1 goats and seven placentas from G2 goats were subjected to molecular and histopathological evaluation to identify the lesions associated with *T. gondii*.

Laboratory analysis

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed to detect IgG antibodies against *T. gondii* in G1 and G2 goats. A soluble antigen from the *T. gondii* RH strain was used to sensitize the microplates at a concentration of 10^5 tachyzoites/well in carbonate-bicarbonate-buffered solution (0.1 M, pH = 9.6) with a volume of 100 μL per well.

The plates were read using a spectrophotometer (Model Multiskan GO N/S 1510–01,885; Fisher Scientific®) at a wavelength of 405 nm (OD405). Optical density values were converted to relative index percent (RIPC) using the formula: $\text{RIPC} = \frac{(\text{OD}_{405} \text{ sample} - \text{OD}_{405} \text{ negative control})}{(\text{OD}_{405} \text{ positive control} - \text{OD}_{405} \text{ negative control})} \times 100$. An RIPC score of ≥ 10 indicated a positive result. Sera

previously tested and known to be positive and negative for *T. gondii* were included as controls (Álvarez-García et al. 2003).

Indirect immunofluorescence assay (IFA)

The IFA was used to detect anti-*T. gondii* IgG antibodies in the precolostral serum samples from kids. *T. gondii* RH strain tachyzoites were used as antigen. Positive and negative controls were included on all slides. Samples were considered positive when tachyzoites exhibited total peripheral fluorescence and titers ≥ 16 (Seefeldt et al. 1989).

DNA extraction and PCR

DNA was extracted from tissue samples (brain and placental cotyledons) obtained from the aborted fetuses of G1 goats using the Wizard Genomic DNA Purification System Kit (Promega®, Madison, WI, USA) according to the manufacturer's protocol. The concentration of DNA in all samples was determined using spectrophotometry (Multiskan GO, Thermo Scientific) and adjusted to 100 ng/ μ L. *T. gondii* DNA was detected using a single-tube nested PCR with the external primers TgNN1-TgNN2 and internal primers TgNP1-TgNP2. These primers amplify a 227-bp fragment within the ITS1 region of the parasite DNA (Hurtado et al. 2001).

A suspension of *T. gondii* tachyzoites (RH strain, 10^4 tachyzoites/mL) and ultrapure water was used as positive or negative control, respectively. The amplified PCR products were separated by electrophoresis on a 1.5% agarose gel stained with BlueGreen (LGC® Biotecnologia, Cotia, São Paulo, Brazil) and visualized under UV light.

Histopathological analysis

Samples of the brain, thymus, lungs, heart, liver, and muscles of the aborted fetuses and placental cotyledons were processed following the routine steps of dehydration, diaphanization, and paraffin inclusion. The tissue blocks were cut into 5- μ m-thick slices using a microtome and mounted on slides. Subsequently, the tissue sections were stained with hematoxylin and eosin (HE) according to the protocol described by Luna (1968) and examined under an optical microscope to determine the presence of lesions compatible with *T. gondii* infection (Pereira-Bueno et al. 2004).

Statistical analysis

The relative index percent (RIPC) values were expressed as mean \pm standard deviation and were analyzed for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests. The analysis of variance (F-test) was used to compare the

RIPC values, and Tukey's HSD test was used to compare the mean IgG concentration. In addition, Kruskal–Wallis test was used to evaluate whether there was a difference between the mean IgG concentration of the goats that aborted during pregnancy. IBM SPSS Statistics software (version 23.0) was used for data analysis and a p -value < 0.05 was considered statistically significant.

Results

Clinical monitoring of the pregnant goats

Thirty-eight pregnant goats (G1 = 31 and G2 = 7) were evaluated in this study. In G1, estrus recurrence occurred in 22.6% (7/31), embryonic death in 3.3% (1/31), and abortion in 19.4% (6/31) of the goats. The clinical findings were as follows: dehydration in 66.7% (4/6), hyperthermia and congestion of episcleral vessels in 50.0% (3/6), and placenta retention in 33.3% (2/6). In G2, no clinical signs were observed.

IgG response to *T. gondii* in pregnant goats

Serology results are shown in Fig. 1. In G1, an increase in the mean antibody concentration was observed at day 150 of gestation compared to that at day 90 and day 120. There was no statistical difference ($p > 0.05$) in the mean antibody concentration during pregnancy in G2 goats.

Evaluation of the vertical transmission rate (precolostral serology)

Precolostral serum samples were collected from 32 kids of G1 goats. The vertical transmission rate observed in these goats was 34.3% (11/32). Two precolostral serum samples had titers of 1:16, four had 1:32 titers, and five had titers of 1:64. IFA revealed that anti-*T. gondii* antibodies were not present in all seven offspring of G2 goats.

Monitoring abortion cases

Seven aborted fetuses from six G1 goats were collected during the study. Serology results of the goats that aborted showed an increase in RIPC values at the time of abortion; however, there was no significant difference ($p > 0.05$) between the analyzed means (Table 1).

Histopathological findings compatible with *T. gondii* infection were observed in the brain and liver of the aborted fetuses. Gliosis and neuronal necrosis were observed in 28.5% (2/7) and mononuclear cell infiltration in the hepatic parenchyma was observed in 71.4% (5/7) of the analyzed samples. Brain samples from three aborted fetuses (42.8%;

Fig. 1 Mean and standard deviation of antibody concentration (RIPC) during gestational monitoring of goats that calved. Different letters on the same line indicate statistical difference ($p < 0.05$) between the moments

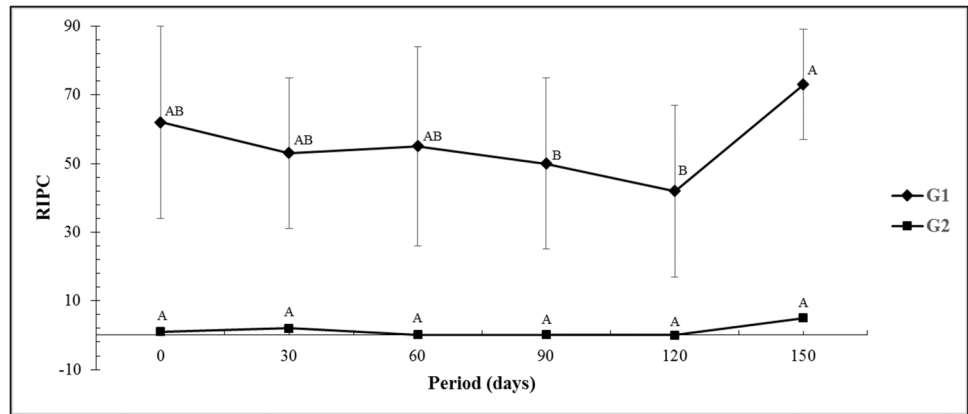


Table 1 Monitoring of serology (RIPC) of goats that aborted

Goat	Gestation (days)	Period (days)					
		0	30	60	90	120	135
Goat 1 ⁺	60	7787	3038	7988*			
Goat 2	72	63,266	86,448	52,125	82,079*		
Goat 3	123	88,011	87,030	86,048	40,502	78,068*	
Goat 4	109	50,725	52,509	67,634	46,527	51,869*	
Goat 5	116	23,125	25,194	6185	14,325	29,895*	
Goat 6	132	79,147	138,154	94,175	118,607	64,600	95,275*

*Sample obtained on abortion day; ⁺the goat seroconverted in the collection following the abortion and was included in the G1

Table 2 PCR results and histopathological examination for toxoplasmosis in aborted fetuses

Sample	PCR	Histopathological findings
Fetus 1	Positive	Gliosis, neuronal necrosis and mononuclear infiltrate in the hepatic parenchyma
Fetus 2	Negative	Mononuclear infiltrate in the hepatic parenchyma
Fetus 3	Negative	-
Fetus 4	Positive	Mononuclear infiltrate in the hepatic parenchyma
Fetus 5	Positive	Gliosis, neuronal necrosis and mononuclear infiltrate in the hepatic parenchyma
Fetus 6	Negative	-
Fetus 7	Negative	Mononuclear infiltrate in the hepatic parenchyma

3/7) were PCR-positive for *T. gondii*. A summary of the results of the diagnostic tests (PCR and histopathological analysis) for toxoplasmosis in aborted fetuses is given in Table 2.

Analysis of *T. gondii* DNA and placental histopathology

Two placentas (2/16) collected from G1 goats were PCR-positive for *T. gondii* DNA. One of the PCR-positive placentas had extensive areas of fibrinoid necrosis and calcification. The main histopathological findings of G1 placentas were

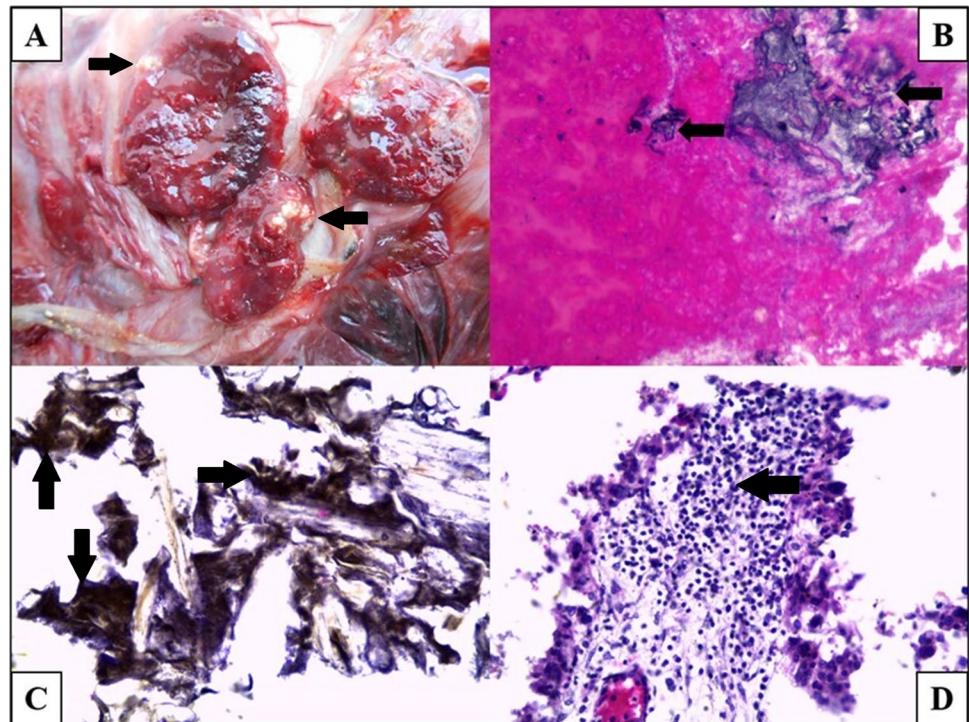
diffuse mononuclear infiltrate in 62.5% (10/16), necrosis in 43.7% (7/16), and calcification in 18.7% (3/16) (Fig. 2). Neither histological lesions nor parasite DNA was detected in the placentas of G2 goats.

Discussion

In our study, serological, histopathological, and molecular methods were used to monitor the aborted fetuses of goats naturally infected with *T. gondii* in Brazil. Despite the vast literature on toxoplasmosis in goats worldwide, there are few studies on clinical characteristics, serological monitoring, histopathological analysis, and vertical transmission in naturally infected flocks.

Serological investigation of G1 goats during pregnancy revealed that the levels of parasite-specific antibodies were increased at day 150 of gestation compared to that at day 90 and 120. This might be due to reduced innate immune response during gestation, which would have provided a favorable environment for reactivation and multiplication of the parasite in infected goats (Innes et al. 2005). One of the main immunological defense mechanisms triggered by *T. gondii* infection is the modulation of the cellular response through the production of proinflammatory cytokines, such as interferon- γ (IFN- γ) and interleukin-12 (IL-12) by Th1

Fig. 2 Placenta. **A** caseous lesion; **B** severe fibrinoid necrosis and calcification (HE, 10×); **C** calcification (dark areas; HE, 40×); **D** mononuclear infiltrate (HE, 40×)



lymphocytes (Tan et al. 2011; Dupont et al. 2012). However, during gestation, there is a decrease in the immune response by Th1 lymphocytes (cellular) and increase in the Th2 lymphocyte response (humoral), which favors infection by intracellular agents such as *T. gondii* (Menzies et al. 2008). This change in the immune response pattern during pregnancy may have facilitated the reactivation of the infection and explains the increase in mean IgG concentration close to parturition in G1 goats.

In G2, one seronegative goat aborted on day 60 of gestation and seroconverted to *T. gondii*. The fetus of this goat was negative for *T. gondii* on PCR; however, histopathological lesions compatible with toxoplasmosis, such as gliosis and hepatic mononuclear infiltrate, were observed. The data from this goat suggest that it was a case of exogenous toxoplasmosis that probably occurred due to the ingestion of sporulated oocysts. The negative serology of this goat may be explained by acute infection without sufficient humoral response at the time of abortion. In experimental studies, humoral response occurs after 14 days of infection depending on the serological technique and the *T. gondii* strain (Conde et al. 2001; Wanderley et al. 2013).

In our study, abortions and embryonic deaths resulted in a 22.6% decrease in the lambing rate of G1 goats. The presence of *T. gondii* DNA in 42.8% of the aborted fetuses was associated with histopathological lesions, which confirms that *T. gondii* was the primary cause of abortions that resulted in significant economic loss in this flock. In sheep and goats, primary maternal infections may cause embryonic

death and resorption, fetal death and mummification, abortion, stillbirth, and neonatal death. The disease is more severe in goats than in sheep (Lindsay and Dubey 2020).

There are few studies on the impact of *T. gondii* infection in goats during pregnancy. In our study, abortion occurred in the middle and final third of gestation. Congenital infection may occur following maternal infection during pregnancy, and the severity of the disease depends on the immune status of the mother, parasite genotype, and the stage of pregnancy at the time of infection (Lindsay and Dubey 2020). A wide spectrum of clinical signs occurs in congenitally infected goat's kids. Castaño et al. (2016) reported that the concentration and virulence of the protozoan strain affect pregnancy and multiplication of the parasite in fetal and placental tissues may result in abortion at any stage of gestation. *T. gondii* isolates of this study were previously characterized by Oliveira et al. (2018) as clonal lineage type II (genotype ToxoDB #1) and were shown to be intermediate virulence in mice.

We observe that transplacental transmission (34.3%) is an important route of infection of this protozoan to the fetus. In the present study, IFAT was used for evaluation of goat precolostral serum samples according to Seefeldt et al. (1989) who reported that IFAT was more effective than ELISA for detecting *T. gondii* antibodies in serum samples from aborted ovine fetuses. We believe that transplacental transmission in our study is of exogenous type because during the experiment, it was possible to observe that cats of different ages had free access to the facilities, feed deposits,

and drinking fountains. Cats are the definitive hosts of *T. gondii* (Dubey 2005) and are considered the main risk factor for toxoplasmosis in goats (Anderlini et al. 2011; Nunes et al. 2013; Liu et al. 2015).

Our findings agree with those of Rahman et al. (2015), who reported that exogenous transplacental transmission in goats is the most relevant in Bangladesh, indicating that oocysts ingestion plays a role in the maintenance of endemicity of *T. gondii* in goats. In our study, the presence of cats in the farm was a determining factor in the introduction of *T. gondii* to this flock.

Histopathological analysis of aborted fetuses revealed that areas of necrosis and non-suppurative inflammatory reactions in fetal tissues are frequently associated with *T. gondii* infection (Caldeira et al. 2011; Moreno et al. 2012). In placentas, the presence of mononuclear infiltrate, areas of necrosis and calcification, and detection of *T. gondii* DNA are indicators of active protozoan infection in the cotyledonary tissue (Pereira-Bueno et al. 2004; Van Den Brom et al. 2012). This active infection may result in decreased supply of oxygen to the fetus during gestation (Penning et al. 1994; Juan-Sallés et al. 2011; Castaño et al. 2014). Further research on the pathogenesis of lesions caused by *T. gondii* will aid in better understanding of the consequences of toxoplasmosis in pregnant goats.

Conclusion

Natural *T. gondii* infection in pregnant goats results in severe reproductive loss of the flock. Moreover, the infection severely compromises reproduction in goats producing serologically positive offspring through transplacental transmission. It is recommended to monitor the presence of felines close to the food and water source to avoid exogenous transmission of the parasite to pregnant goats.

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Author contribution Conceptualization and study design: RAM and AAFO. Investigation and sample collection: JMBO and BPS. Laboratorial analysis: JMBO, BPS, RPB, and MRA. Data analysis: RAM, JMBO, WJNP, and JWPI. Supervision: RAM, AAFO, and WJNP. Writing — original draft: RAM and JMBO. All authors reviewed the manuscript.

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Data availability All relevant data generated during this study are included in the article.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The study was approved by the Ethics Committee in Animal Use at *Universidade Federal Rural de Pernambuco* (UFRPE) (license number 122/2015).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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