Dynamics of natural infection by *Toxoplasma gondii* in goat herds in the semiarid region of northeastern Brazil

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Abstract

This study aimed to describe the transmission of *T. gondii* in naturally infected goats in the semiarid region of northeastern Brazil, through evaluating the serological status of these animals from one to 12 months of age. Seven goat farms were selected. During the first visit, an epidemiological questionnaire was applied. Blood samples were collected from pregnant goats and from their female offspring every month, to monitor them for anti-*T. gondii* antibodies, using the indirect fluorescence antibody test (IFAT). Out of 66 pregnant goats evaluated, 47% (31/66) (95% CI: 34.6% – 59.7%) were positive and out of 93 female offspring, 61.3% (57/93) (95% CI: 52.4% – 69.2%) were positive, in at least one month during the evaluation period. Among the positive female offspring, 43.3% (27/57) (95% CI: 39.7% – 56.9%) presented environmental infection and 56.7% (30/57) (95% CI: 43.4% – 66.3 %) probably had only colostral antibodies or vertical transmission. It was observed that 92,6% (25/27) from environmental infections occurred until reproductive age (six months) ($p < 0.0001$). It was also noted that there is a correlation between the titration of mothers and offspring, until four months of age ($p < 0.0001$). Large fluctuations in the presence of antibodies were observed among the animals over the months monitored. It can be concluded that the prevalence of anti-*T. gondii* antibodies within the herds was greater than what has been expressed in specific prevalence studies and that most sheep become infected up to the six months of life in the studied area.

1. Introduction

Raising goats for meat, leather and milk production is an important global economic activity (Dubey 2020). Thus, diseases that cause reproductive problems in goats generate significant economic losses for producers and have a negative impact on productivity (Pinheiro et al. 2000). In this way, the diseases that traditionally form part of health management for goat herds join together with emerging diseases, among which toxoplasmosis stands out, mainly due to the reproductive disorders that it can cause (Lucio et al. 2016).

Toxoplasma gondii, a protozoon that forms tissue cysts, is considered to be one of the main causes of reproductive disease in goats and gives rise to large economic losses (Dubey 2021). Reproductive failure is one of the important clinical consequences of primary *T. gondii* infection acquired during pregnancy. The main problems observed comprise embryo resorption, fetal death, mummification, abortion and birth of weakened offspring (Blewett 1983; Buxton 1998; Dubey 2021; Wanderley et al. 2013).

Transmission of *T. gondii* can occur through ingestion of sporulated oocysts present in contaminated water or food; through ingestion of tissue cysts present in the muscles of the definitive host; or transplacentally. The latter is a possible route for transmission to goats when the mother is infected during the gestational period (Dubey 2021). According to Dubey et al. (2020), reproductive losses in goats are much more serious than in sheep. In addition, goats can transmit toxoplasmosis to humans (Orlando et al. 2017), such that this disease can become a global anthroponosis, with great importance with regard to public health, due to the high risk that the disease poses for immunocompromised people (Belluco et al. 2016; Wang et al. 2017; Dubey et al. 2020).

In goats, the diseases can be silent (Belluco et al. 2016) but the prevalence of antibodies anti-*T. gondii* can be very high. According to Dong (2018), the following prevalences of anti-*T. gondii* antibodies have been found in goats around the world: 19.4% (393/2026) in North America; 35.9% (2878/8027) in Africa; 30.1% (2107/6988) in Europe; 25.2% (75/298) in Oceania; and 21.3% (1346/6311) in Asia. In Brazil, however, high variability in prevalence rates has been observed, ranging from 3.47–81.8% (Costa et al. 2012; Medeiros et al. 2014; Santos et al. 2018).

Occurrences of *T. gondii* infection in aborted goat fetuses have been reported in several countries around the world (Charleston 1994; Navarro et al. 2009; Caldeira et al. 2011; Albu-Dalbouch et al. 2012; Unzaga et al. 2014). In a retrospective study on goat abortions in California, USA, 211 cases that occurred between 1991 and 1998 were analyzed, and the result showed that only 4% of these were of protozoan origin (Moeller 2001). In the state of Paraná, Brazil, in a study on 179 goats, among which 33 had had abortions, 137 (76.53%) were serologically positive and 11 (33%) of the 33 goats with symptoms were positive in PCR (Ferreira Neto et al. 2018). Mesquita et al. (2019) reported on an outbreak that occurred in Alagoas in 80 animals, in which, among 27 placenta samples analyzed, 23 (85.2%) were positive in immunohistochemical tests.

In Brazil, it is likely that many cases of toxoplasmosis in goats go unreported (and thus that the number of cases is underreported), due to the scarcity of studies aimed at diagnosing the causes of abortion and the absence of a standard for analyses on placental and fetal tissues (Mesquita et al. 2019). Some authors have even emphasized that outbreaks may be really scarce, as shown in the

literature (Moeller 2001). There is no information on the age group most prone to primary infection among goats, or whether most animals become infected before entering reproductive age and would thus have some level of immunity against *T. gondii* infection.

Thus, the aim of the present study was to investigate the dynamics of anti-*T. gondii* antibodies in female goats aged one to 12 months, through evaluating the serological conditions of these animals and ascertaining the time at which the primary infection occurred.

2. Material And Methods

2.1 Study location

This study was carried out between December 2018 and April 2020, in the Sertão region of the state of Paraíba, northeastern Brazil. This is a semi-arid tropical area that is characterized by hot and dry landscapes. It has an average temperature of 26.6 °C, with low annual thermal variability (INMET 2010). Rainfall is irregular, but two annual climatic periods are distinctly marked out: rainy, between the months of January and May, with a maximum of 700 mm to 900 mm; and dry, between the months of June and December, marked by long periods of drought, with a mean of around 10 mm (Francisco and Santos 2017).

The herds in the region studied region are subjected to a semi-intensive rearing regime. They graze during the day in areas of native vegetation of the Caatinga biome, mainly composed of mata-pasto (*Senna obtusifolia* L.), jurema-preta (*Mimosa tenuiflora*), catingueira (*Caesalpinia pyramidalis*), quince (*Croton sonderianus*), Malva (*Malva* sp.), juazeiro (*Zizyphus joazeiro*), sabiá (*Mimosa caesalpinhiifolia*) and favela (*Cnidioscolus phyllacanthus*).

2.2 Experimental design and management of herds

Seven goat farms were selected, located in the municipalities of Pombal (P1), Nazarezinho (P2), Aparecida (P3 and P4), Marizópolis (P5) and Pedra Branca (P6 and P7) (Fig. 1). All the farms selected had at least 50 animals and included between three and 20 female goats that were in the final stage of pregnancy, in order to perform serological tests for *T. gondii*.

During the first visit to these farms, an epidemiological questionnaire was applied to the owner. This sought information relating to the physical structure of the farm, productive characteristics of the herd, purpose of rearing, type of food management, use of mineral supplementation, health care performed and its frequency and data on reproductive control, including crossbreeding, births and mortality. In addition, the presence of pre-existing diseases in the herd was investigated. In particular, it was sought to determine whether these were reproductive diseases.

2.3 Animals and sample collection

The female goats underwent abdominal palpation examination (Ishwar 1995), to identify final-stage pregnancy. Blood samples were collected for screening. Female offspring born from these goats were also identified and their anti-*T. gondii* antibodies levels were monitored monthly until 12 months of age. Pre-ingestion colostrum was not collected, but all the goat kids underwent their first blood collection before reaching one month of life (Valencio et al. 2020).

Blood samples were collected by puncturing the external jugular vein, using 5 ml disposable syringes. Subsequently, they were chilled and were sent to the Laboratory of Immunology and Infectious Diseases (LIDIC) of the Federal Institute of Paraíba (IFPB), Campus Sousa, Paraíba, for serum to be obtained. These serum samples were then stored at -20 °C, until the time when the analyses were performed.

2.4 Diagnosing of anti-*T. gondii* antibodies

To diagnose the presence of anti-*T. gondii* antibodies in goats, the indirect fluorescence antibody test (IFAT) was performed, using the cutoff titer of 1:64 (GARCIA et al. 1999). Serum dilution was performed in a 96-well microplate, using 3 µL of the serum to be tested and 189 µL of phosphate-buffered saline (PBS) solution (pH 7.2). After dilution, 20 µL were placed on a slide previously fixed with tachyzoites. This was then incubated in an oven (37°C for 30 minutes). After incubation, the slides were subjected to three washes by means of immersion in PBS, with 10 minutes for each wash. After drying, each slide was covered with 20 µL of goat anti-IgG conjugate (whole molecule, SIGMA, St. Louis, MO, USA). The material was incubated for another 30 minutes at 37°C in an oven. After the three washes and drying, the slides were evaluated by means of fluorescence microscopy with ultraviolet light emission. Serum samples that reacted at dilutions greater than or equal to 1:64 were considered positive, and these positive samples were then subjected to sequential dilutions at base two until negative.

2.5 Data analysis

To evaluate a possible correlation between antibody titers from mothers x offspring, Spearman's (ρ) correlation test was applied. To compare the proportion of offspring which seroconverted up to the 6th month of age with which seroconverted after the 6th month of age, a McNemar test for paired samples was used and, for this analysis, positive offspring in the first month of age were excluded.

To identify the factors associated with occurrences of *T. gondii*, the chi-square test was performed to select the variables that presented a statistically significant difference ($p \leq 0.2$) (Zar 1999). These variables were subjected to multivariate analysis, in which a multiple logistic regression test was used, with a significance level of 5% (Hosmer and Lemeshow 2000). Calculations were made using the Minitab 19 software (Minitab Inc. 2020).

3. Results

The seven farms evaluated had goats that were positive for anti-*T. gondii* antibodies during the experimental period. In total, 66 matrices goats were evaluated, of which 47% (31/66) (95% CI: 34.6% – 59.7%) were positive; and their 93 female offspring, among which 61,3% (57/93) (95% CI: 52.4% – 69.2%) were positive at some point during the evaluation period.

The titers presented by the positive matrices goats in the IFAT ranged from 1:64 to 1:1024. Among the 57 offspring, that were positive at some point during the experimental period, the titers ranged from 64 to 2048 over the study period, as described in Table 1. In the first month of life, it was observed that 52,6% (30/57) (95% CI: 39.4% -59.3%) of the offspring showed anti-*T. gondii* antibodies. It was found that 93,5% (29/31) (95% CI: 74.3% -97.3%) of the offspring that were seropositive in the first month of life were born from positive mothers. The mother's title has correlation with titration of offspring until four months of age (Fig. 2).

Table 1

Titers among female offspring that were positive for anti-*T. gondii* antibodies in the indirect fluorescence antibody test (IFAT), according to age in months, during the experimental period, on seven goat farms in the semiarid region of northeastern Brazil.

Farms	Antibody titer for mother	Identification of offspring	Months of age											
			1	2	3	4	5	6	7	8	9	10	11	12
P1	256	102F1	64	256	64	0	0	0	0	0	0	0	0	0
P1	< 64	122F1	0	0	0	0	0	0	0	0	512	512	128	NC
P2	512	202F1	512	512	256	256	1024	512	256	0	0	NC	NC	NC
P2	< 64	206F1	0	0	0	0	0	0	0	128	64	0	NC	NC
P2	256**	207F2**	512	512	0	1024	512	512	128	64	64	0	0	0
P2	256	208F1	64	64	0	0	64	128	0	0	0	0	0	NC
P2	< 64	209F2	0	0	0	0	0	64	0	0	0	0	0	0
P2	512	210F1	1024	1024	128	64	0	0	0	0	128	64	0	0
P2	1024	211F1	1024	2048	512	128	64	64	512	128	128	NC	NC	NC
P3	< 64	304F1	0	0	0	0	0	64	0	0	0	0	0	0
P3	64	305F1	0	0	256	128	128	128	64	64	64	0	0	0
P3	< 64**	601F2**	0	0	512	256	64	64	64	0	0	0	0	0
P3	< 64	602F1	0	0	0	0	64	64	64	64	0	0	0	128
P3	< 64**	603F1**	0	0	512	512	256	128	64	0	0	0	0	0
P3	< 64	603F2**	0	0	0	0	64	0	0	0	0	0	0	0
P3	< 64	604F1	0	0	0	64	0	0	0	0	0	0	0	NC
P3	64***	606F1***	0	0	0	512	512	128	0	0	0	0	0	0
P3	64	606F2***	0	0	0	64	64	128	64	0	0	0	0	0
P3	512	26F1	256	256	128	64	64	0	0	0	0	0	0	0
P3	512	77F2	1024	1024	1024	512	64	64	0	0	0	0	0	0
P3	512	78F1	2048	1024	256	64	0	0	0	0	0	0	0	0
P4	< 64	850F1	0	128	256	0	0	0	0	0	0	0	0	0
P4	< 64	851F1	0	0	0	64	0	0	0	0	0	0	0	0
P4	64	852F1	0	0	256	256	256	256	64	0	0	0	0	0
P5	512***	24F3***	1024	1024	512	128	64	1024	512	128	64	64	64	0
P5	64**	32F2**	512	512	128	128	64	64	0	0	0	0	0	0
P5	512**	30F1**	512	128	128	128	64	64	0	0	0	0	0	0
P6	< 64	250F1	64	0	0	0	0	0	0	0	0	0	NC	NC
P6	64**	012F1**	64	0	0	0	1024	512	128	128	64	0	0	0
P6	64	012F2**	512	128	64	64	0	0	0	0	0	0	0	0
P6	< 64	07F1	512	128	128	64	64	0	0	0	0	0	0	0
P6	1024**	10F1**	512	64	64	0	0	0	0	0	0	0	0	0

** double birth; *** triple birth; NC: not collected.

Farms	Antibody titer for mother	Identification of offspring	Months of age											
			1	2	3	4	5	6	7	8	9	10	11	12
P6	1024	10F2**	1024	512	256	128	128	64	64	0	0	0	0	0
P6	512	09F1**	0	64	0	0	0	0	0	0	0	0	0	0
P6	512**	09F2**	64	64	64	64	0	0	0	0	0	NC	NC	NC
P6	1024**	43F1**	64	64	64	0	0	0	0	0	0	0	NC	NC
P6	1024	43F2**	1024	512	512	128	64	0	0	0	0	0	NC	NC
P6	< 64**	44F1**	0	0	0	0	0	128	128	64	64	0	0	0
P6	< 64	44F2**	0	0	0	64	64	0	256	64	64	0	0	0
P6	512	46F1	1024	1024	1024	512	256	256	128	128	0	0	0	NC
P7	128	59F1	64	64	64	0	0	0	0	0	0	0	0	0
P7	512	45F1	128	128	64	0	0	0	0	0	0	0	0	0
P7	128	48F1	128	64	64	0	0	0	0	0	0	0	0	0
P7	< 64	57F1	0	0	0	0	0	64	64	64	NC	NC	NC	NC
P7	< 64	50F1	0	128	128	64	64	0	0	0	0	NC	NC	NC
P7	512	38F1	128	128	64	64	0	0	0	0	0	0	0	NC
P7	< 64**	03F2**	0	0	128	64	0	0	0	0	0	0	0	0
P7	< 64	280F1	0	0	0	0	0	128	128	64	64	0	0	0
P7	< 64**	018F1**	256	0	0	0	0	0	0	0	0	0	0	0
P7	< 64	11GAF1	0	0	512	256	256	256	128	64	64	64	64	0
P7	512	16F1	1024	256	128	64	64	64	64	64	NC	NC	NC	NC
P7	64	17F1	0	0	0	0	0	0	1024	512	128	128	128	128
P7	< 64	056F1	0	0	0	0	0	128	0	0	0	0	0	0
P7	< 64	12F2**	0	0	0	0	0	128	64	64	64	0	0	0
P7	64	19F1	128	128	64	0	0	0	512	512	0	0	0	0
P7	512	41F1	64	64	64	0	0	0	0	0	0	0	0	0
P7	64**	01F1**	64	64	0	0	0	0	0	0	0	0	0	0
** double birth; *** triple birth; NC: not collected.														

It was found that 47,3% (27/57) (95% CI: 39.7% – 56.9%) of the offspring became infected through horizontal transmission, excluding all positive animals in the first month, as it is not possible to assess with certainty whether the antibody is colostral or from a transplacental infection. About the period of infection, it was determined that most infections occurred up to six months of age ($p < 0.00001$).

During the experimental period, there was variation in the positivity of the goats, such that positive animals remained positive for short periods. In many cases, the same animal was positive and then negative during the first year of life (Table 1). It was seen that 68.4% (39/57) (95% CI: 53.3% – 79.3%) of the animals evaluated had detectable antibody titers for only up to four consecutive months, and then they became negative. In addition, 73% (14/19) (95% CI: 48.8% – 90.9%) of the animals that had antibodies for more than four consecutive months were positive from the first month of life onwards.

Among the mothers that were positive during pregnancy, 35.5% (11/31) (95% CI: 19.2% – 54.6%) had twin births and 6.5% (2/31) (95% CI: 0.79% – 21.4%) had triple births. It was observed that even among the positive mothers that had double and triple births, at least

one of their offspring was negative in the first month of life. Out of the 22 goats born from twin births, 31,8% were negative in the first month. Out of the six kids that were born from triple deliveries, only one (15.1%) had anti-*T. gondii* antibodies in the first month, and this animal (24F3) remained positive throughout the experimental period.

Regarding management, it was observed that the animals did not receive vaccination. Wild animals were present on all the farms, and on 42.8% (3/7) domestic cats that were in direct contact either with the herd or with the pasture were present. On four farms (P1, P2, P6 and P7), the animals received supplementation with corn-soy protein-energy concentrate (0.5% of live weight), in the late afternoons. The other farms (P3, P4 and P5) did not receive any protein-energy supplementation. Management practices such as navel disinfection, quarantining of newly acquired animals, proper disposal of carcasses and a place for an infirmary were not practiced on any of the farms evaluated. All the herds had the purpose of subsistence, without veterinary assistance, and the animals were being reared for their meat. Goat herding was the main activity of these farms

Comparison performed using multiple logistic regression, of infected animals on the farms between the categories of the variables analyzed (presence of cats, type of water supplied, type of enclosure, sanitary management and presence of sewage on the farm) showed that there were no statistically significant differences regarding these variables ($p > 0.05$).

4. Discussion

A total of 93 female offspring were serologically monitored, among which 61.3% had anti-*T. gondii* antibodies in at least one evaluation. During the experimental period, there were constant variations in antibodies among the female offspring, with animals showing different and variable periods of positivity and negativity for anti-*T. gondii* antibodies. Thus, some animals seroconverted before reaching six months of life and others only after six months of life. These differences in antibody behavior show the importance of periodic monitoring of these animals. In comparing the numbers of positive animals found in the present study, we observed that they were higher than those described in punctual studies carried out in northeastern Brazil (Garcia et al. 2012; Lucio et al. 2016). This result shows that punctual studies do not represent the real occurrence of anti-*T. gondii* antibodies in herds.

The sixth month of age was defined as the milestone for reproductive age, based on Fonseca (2011), who described the reproductive age of goats in northeastern Brazil. Among the offspring analyzed, 59.1% (55/93) had antibodies with titers ranging from 64 to 1024 until 6th month of age. Thus, they reached reproductive age with active immunity against *T. gondii*. This would reduce the risk of acquiring toxoplasmosis in the acute phase, a factor that could lead to reproductive problems such as congenital transmission, with the possibility of embryonic absorption, abortions or stillbirths caused by *T. gondii* in these future mothers (Nunes et al. 2013).

Note that the titles obtained from offspring up to four months of age have a direct correlation with the titration found in their mothers, probably due to passive colostral antibody. This idea is reinforced by the findings from a study by Dubey et al. (1987), among lambs: they found evidence that passive immunity to *T. gondii* remains present and regresses until the third or fourth month postpartum. The three offspring that had anti-*T. gondii* antibodies in the first months of their lives and were born from negative mothers, may have become infected in the environment, through ingestion of water and food contaminated with oocysts. It can be too suggested that the presence of antibodies in these offspring was due to goat kids' behavior of not feeding exclusively from their own progenitors. This gives rise to the possibility that goat kids can ingest colostrum from positive mothers and thus become positive for passive immunity.

One offspring in the present study (# 211F1) had high titers, reaching 2048 in the second month and remaining positive until the ninth month of life. Considering that this animal's mother was positive with a titer of 1024, we can suggest that passive immunity was initially present and that environmental infection maintained this animal's positivity later on. Although no evaluation was done before ingestion of colostrum, we cannot rule out the possibility that transplacental transmission may have occurred in relation to this animal. Likewise, this may have occurred in relation to all the offspring that were evaluated as positive in their first month of life and presented persistence of antibodies.

In the cases of double and triple births from positive mothers, it was observed that at least one offspring was negative in the first month of life, and that the positive animals from the same goat had titers that differed from each other. Regarding these results, it can be suggested that competition for colostrum may have led to failure of passive immunity transfer (FPIT) between kids from multiple births. It has been report that the probability of FPIT among twins is greater than in single deliveries (McGuire et al. 1983; Christley et al. 2003; Tizard 2019). In addition, these data corroborate the findings of Gilbert et al. (1988), who demonstrated that the concentration of

IgG decreases linearly with increasing numbers of lambs born from the same mother, which indicates that natural competition between twins, and fiercer competition among triplets, may be an important factor in relation to FPIT.

Among all the offspring monitored, 38,7% (36/93) did not show anti-*T. gondii* antibodies at any time during the evaluation period and remained susceptible to infection throughout the experimental period. Considering the high environmental contamination of the region studied (Valêncio et al. 2020), these females are more susceptible to infection during the reproductive period, which considerably increases the risk of reproductive problems such as abortion, fetal malformation and stillbirths (Dubey 2021; Wanderley et al. 2013).

Regarding the form of rearing, all the herds evaluated here carried out semi-intensive rearing. This may explain the high prevalence of seroreactive animals. In a study by Djokic et al. (2014), rearing regimes that included grazing in fields imposed greater risk, since the animals could have access to contaminated pastures. In addition, the inadequate management of the herds that was practiced on the farms evaluated here can be considered to be an important factor in the animals' state of infection, given the scarcity of use of appropriate techniques for cleaning and sanitizing the environment, in the way suggested by Pereira et al. (2012) and Bawm et al (2016).

5. Conclusion

It was concluded that the level of positivity to anti-*T. gondii* antibodies in goats from the semiarid region of northeastern Brazil was high, the most goats become infected within the first six months of life in the studied area and that mother titles influence offspring titles up to four months of age. In addition, anti-*T. gondii* antibodies behave in a fluctuating manner, such that they persist in the animal's organism for a period of around three to four months. This demonstrates the importance of serological monitoring studies on goat herds, in order to better understand the epidemiology of *T. gondii* infections in goats.

Declarations

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Authors' contributions

LNS, VLRV and TFF designed the experiments; LNS, SPB, SSS, RFS, WFS and RAM performed the experiments; LNS, VLRV, TFF and MA analyzed the data; LNS, VLRV and TFF wrote and edited the manuscript.

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Data availability

All data generated or analyzed during this study are included in this published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement

The experiments conducted in this study were approved and performed according to the recommendations of the Ethics Committee on the Use of Animals of the Federal Institute of Paraíba, Campus Sousa (CEUA - IFPB), under protocol protocol 23000.001402.2018-64.

Consent to participate

Authors have permission to participate.

Consent for publication

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Figures

Figure 1

Geographical locations of the goat herds analyzed in the semiarid region of the state of Paraíba, northeastern Brazil.

Figure 2

Scatter plots of the correlation analysis between the antibody titers of mothers x offspring up to four months of age, demonstrating a significant correlation. A: First month ($R=0.645$; $p<0.0001$) B: Second month ($R = 0.592$; $p<0.0001$; C third month ($R = 0.359$; $p<0.0001$); and D: Fourth month ($R = 0.289$; $p=0.005$).