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An abortion storm in a goat farm in the Northeast Region of Brazil was caused by the atypical *Toxoplasma gondii* genotype #13

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ABSTRACT

The objective of this study was to characterise a *Toxoplasma gondii*-induced abortion outbreak on a goat farm in the State of Paraíba, Northeast Region of Brazil. From a herd of 10 does, seven experienced abortions and one gave birth to twins (one stillborn and the other weak and underdeveloped). Serum samples from all of the does were analysed by indirect fluorescent antibody test (IFAT). Samples of colostrum and placenta from two does, along with lung, heart, brain and umbilical cord samples from four of the foetuses, were screened by nested ITS1 PCR specific for *T. gondii*. The positive samples were then analysed by multiplex nested PCR-RFLP. All ten does tested positive by IFAT for anti-*T. gondii* IgG (titrations ranging from 1:4096 to 1:65,536). The ITS1 PCR screening revealed *T. gondii* DNA in the placenta (2/2), colostrum (2/2), umbilical cord (2/4), lung (1/4), heart (1/4), and brain (1/4). Four samples produced complete RFLP genotyping results, identifying a single genotype, ToxoDB #13. In conclusion, we demonstrated a high rate of abortion caused by *T. gondii* in a goat herd, highlighting the pathogenicity of genotype #13, one of the most prevalent genotypes of *T. gondii* in Brazil.

1. Introduction

Abortion is a significant challenge affecting goat farming globally, with *Toxoplasma gondii*, the protozoan responsible for toxoplasmosis, being a key agent linked to outbreaks of abortions in goat herds. The primary mode of *T. gondii* transmission in goats is horizontal, occurring through the ingestion of food or water contaminated with sporulated oocysts (Tenter et al., 2000; Dubey et al., 2020). Additionally, exogenous transplacental transmission can play a role in perpetuating and spreading the protozoan infection within flocks (Dubey, 2010).

Toxoplasma gondii can also impair embryonic development, leading to foetal death and increased rates of embryonic resorption in goats (Wanderley et al., 2013). Furthermore, *T. gondii* can also result in foetal mummification, abortions (Buxton, 1998), and the birth of weakened offspring (Blewett and Watson, 1983).

In Brazil, high levels of genetic diversity of *T. gondii* are recorded, likely due to the predominance of non-archetypal genotypes (Ragozo et al., 2010). These non-archetypal genotypes may exhibit distinct biological behaviors regarding transmission modes, virulence, and their propensity to cause abortions (Boothroyd and Grigg, 2002). Despite there being studies confirming abortions caused by *T. gondii* in goats in Brazil (Mesquita et al., 2019; Pereira et al., 2021; Oliveira et al., 2022), considering its continental size and national goat herd, the molecular confirmation of reproductive losses due to toxoplasmosis remains relatively scarce.

In the Northeast Region of Brazil, a majority of goat herds are raised semi-intensively with inadequate facilities and in the absence of estrus synchronization (Riet-Correa et al., 2013). Producers often do not notice abortions, or do not submit samples for diagnosis, making such reports rare.

The objective of this study was to provide a detailed account of a toxoplasmosis-associated abortion outbreak that occurred on a goat farm in the Northeast Region of Brazil, including the genetic characterization of *T. gondii* through PCR-RFLP.

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2. Materials and methods

2.1. Region characterization

The Brejo Paraibano, Northeast Region of Brazil, falls within the domain of the Atlantic Forest biome. The topography varies from undulating to strongly undulating, with altitudes ranging between 400 and 600 m. According to Köppen's classification, the regional climate is categorized as type As', characterized by hot and humid conditions (Santos et al., 2010). The region maintains an average relative humidity of approximately 85%, with an average annual temperature of 25.1 °C (INMET, 2010). The yearly average rainfall stands at 1500 mm, exhibiting a uniform distribution throughout the year (Becker et al., 2011).

2.2. Farm history

On a small farm in the municipality of Alagoa Grande, Brejo Paraibano, a producer started goat farming by acquiring a male breeding Boer ram (18 months of age) and 10 Boer does during their first estrus cycle (6–8 months of age). For the initial 90 days following the purchase, the animals grazed on the farm's pasture comprised mainly of forage grasses (*Brachiaria* spp.), additionally, they received supplementation with balanced commercial goat feed and mineral salt. As the pasture diminished, the producer began offering a new pasture, located in the urban area of the same municipality and this new pasture was provided for about 30 days.

Approximately 15 days after introduction onto the new pasture and within a 30-day interval, starting in November 2022, of the ten does, seven experienced late-term abortions; one gave birth to twins (one stillborn and the other weak and underdeveloped, the doe still had retained placenta seven days after delivery). One doe gave birth to a healthy kid and it is uncertain whether the last doe became pregnant or experienced an unnoted abortion earlier in the pregnancy.

The new pasture was adjacent to an urban commercial property, with the pasture in the back garden area, covering roughly 0.5 ha. During a visit, around 15 cats, both young and adult, were noted to have unrestricted access to the pasture area. Furthermore, there were scattered feline faeces within the pasture (Fig. 1).

The goats had not received anthelmintic treatment for more than two months. Estrus synchronization was not performed, and the only vaccination protocol used was against clostridial diseases.

2.3. Serological and microbiological analyses

Serum samples from the 10 does, the breeding male, the two live offspring, and the colostrum from their mothers were collected and subjected to indirect fluorescent antibody test (IFAT) to detect anti-*T. gondii* antibodies, as described by Camargo (1974). In brief, tachyzoites from the ME49 strain of *T. gondii*, fixed on slides, were used as antigens. A goat anti-IgG conjugated to fluorescein isothiocyanate (whole molecule; Sigma, St. Louis, MO, USA), and a cut-off titer of 1:64 was used. The positive samples were subjected to sequential 2-fold dilutions until negative, according to Sousa et al. (2022).

The serum samples were also tested by IFAT for anti-*N. caninum* antibodies, with a cut-off titer of 1:50 (Bezerra et al., 2022), using NC-1 strain tachyzoites of *N. caninum* as the antigen. The sera were also evaluated for the presence of antibodies against *Leptospira* spp. by microscopic agglutination test (MAT) (Cole et al., 1973); *Brucella* spp. by buffered acid antigen test (AAT) (MAPA, 2016); caprine arthritis and encephalitis virus (CAEV) by the agar gel immunodiffusion (AGID) (Herrmann-Hoesing, 2010); *Chlamydophila abortus* (*Chlamydophila* total Ab Idexx ELISA); and *Coxiella burnetii* (*Coxiella burnetii* ELISA kit BIO-X, Rochefort, Belgium).

Microbiological culture of vaginal swabs and foetal organs were submitted to selective media (for *Salmonella* spp. and *Campylobacter* spp.) and non-selective media (Elvira-Partida et al., 2017).

2.4. Molecular analyses

DNA was extracted from samples of placenta and colostrum of two does, and samples of lung, heart, brain and umbilical cord of four foetuses using a commercially available kit (PureLink™ Genomic DNA Mini Kit, Invitrogen, Carlsbad, USA). The DNA was tested in the nested PCR protocol described by Burrells et al. (2013), which targets the multicopy 18S-5.8S rRNA internal transcribed spacer (ITS1) region of *T. gondii*. Each sample was tested in triplicate. A sample was considered positive if

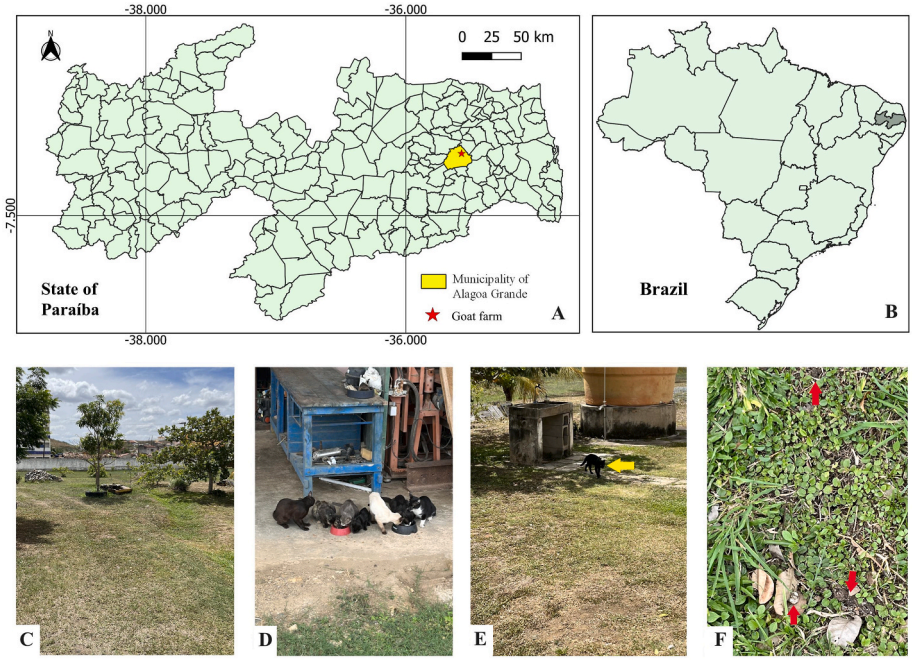

Fig. 1. A, **B** Geographical location of the goat farm in the municipality of Alagoa Grande, State of Paraíba, Brazil. **C** Pasture where the goats were kept. **D** Domestic cats raised on-site being fed. **E** Cat (*yellow arrow*) with unrestricted access to the pasture. **F** Scattered feline faeces within the pasture (*red arrows*).

a 227 bp amplicon was detectable across any of the triplicate reactions. To ensure accuracy, each PCR run incorporated multiple negative controls (dH_2O), positive control (M4 isolate tachyzoite DNA) along with DNA extraction controls.

The ITS1-positive samples were genetically characterized using the *T. gondii* multiplex nested (mnPCR-RFLP (restriction fragment length polymorphism)) method, with 10 markers: SAG1, SAG2 (5′ and 3′ SAG2, Alt. SAG2), SAG3, BTUB, GRA6, c22-8, c29-2, PK1, L358 and Apico). The mnPCR-RFLP method is capable of distinguishing the three clonal lineages (Types I, II and III) (Su et al., 2010). Genotypes were determined using RFLP banding profiles of reference strains S48 (Type I), M4 (Type II variant; used for all markers except Apico where Type II strain ME49 was used instead) and NED (Type III). The results were compared and classified according to the genotypes present in the ToxoDB database (http://toxodb.org/toxo/) and recently published papers.

3. Results

The IFAT analysis revealed positivity for anti-*T. gondii* antibodies in all samples. The titration in the serum samples of the does and the ram ranged from 1:4096 to 1:65,536; both colostrum samples had titration 1:131,072; and the titration in the serum samples of the live offspring ranged from 1:32,768 to 1:65,536 (Table 1).

All serum samples tested negative for antibodies against *N. caninum*, *Leptospira* spp., *Brucella* spp., CAEV, *C. abortus* and *C. burnetii*. The microbiological tests of the vaginal swabs did not show any growth of *Salmonella* spp. or *Campylobacter* spp.

The results of the nested PCR analyses performed on tissue samples collected from the foetuses and mothers are presented in Table 2. Nine out of the 20 individual tissue samples, analysed for the presence of *T. gondii* DNA, were positive (45%), being placenta (2/2), colostrum (2/2), umbilical cord (2/4), lung (1/4), heart (1/4) and brain (1/4) samples. At least one sample from each of the five does, their respective offspring or aborted fetuses tested positive for *T. gondii* in the PCR.

In four samples - lung and heart of the doe B's foetus and the placentas of does C and E – it was possible to determine the complete genotype using all 10 PCR-RFLP markers, resulting in a single genotype, #13 (Table 3).

4. Discussion

There was a strong suspicion that the abortion outbreak was likely due to toxoplasmosis. During the visit to the second pasture numerous cats were seen to have unrestricted access to the pasture, as was evident by the presence of large amounts of feline faeces. Toxoplasmosis is well-known as a significant abortion-causing disease in goats, being acquired through the ingestion of oocysts present in contaminated water or pasture (Dubey et al., 2020).

The presence and high titers of anti-*T. gondii* IgG antibodies (≥ 4096) in all the serum and colostrum samples evaluated is unusual but is indicative of acute infections within the herd. In cross-sectional studies of chronically infected herds, serological test positivity rates tend to be underestimated due to transient antibody production, where animals that test positive in some months may become negative in subsequent months (Valencio et al., 2020; Sousa et al., 2022).

Toxoplasma gondii DNA was detected in at least one sample of brain, heart, lung, and/or umbilical cord from three fetuses originating from does A, B, and D, strongly indicating that this was a *T. gondii*-induced abortion outbreak, particularly in the absence of any of the other common abortifacient agents. These findings represent the first detections of *T. gondii* DNA in the lungs and umbilical cords of naturally infected aborted goat fetuses. Notably, the umbilical cord showed the highest positivity rate (50%; 2/4). In several studies, only the brain was used to detect parasite DNA in aborted goat fetuses, through PCR (Giadinis et al., 2013; Partoandazanpoor et al., 2020) or bioassays in mice (Unzaga et al., 2014; Oliveira et al., 2018). Sah et al. (2019) also detected *T. gondii* in 33.3% (1/3) of heart and liver samples in addition to the brain. In the present study, only one out of four brain samples was positive, underscoring the importance of not relying solely on the brain for *T. gondii* diagnosis in aborted fetuses. Burrells et al. (2018) also advised this broader tissue testing approach, as complete reliance on the brain alone can significantly reduce diagnostic sensitivity, potentially leading to underestimated diagnoses.

Despite the samples from the foetus of doe C testing negative, her placenta tested positive for *T. gondii.* This may have been a case of 'early abortion' or 'sterile abortion' linked to foetal hypoxic damage, with no direct involvement of the parasite. However, the mechanism behind early abortion during the acute phase of the disease remains poorly understood. It appears likely that the acute inflammatory response and vascular lesions within the placenta disrupted nutrient and oxygen transport, ultimately resulting in fatal hypoxic damage to the foetus (Castaño et al., 2020).

This outbreak of caprine toxoplasmosis represents one of the most severe and extensively researched cases. It indicates that transmission through the ingestion of *T. gondii* oocysts from a contaminated environment has a greater impact, as previously established (Innes et al., 2009). Here, between 80% and 90% of goat kid foetus/pregnancy losses were likely attributed to toxoplasmosis. It should be noted that there is uncertainty regarding the pregnancy history or abortion of doe J and there might have been an embryonic loss that went unnoticed by the producer. This doe tested positive for anti-*T. gondii* antibodies in the IFAT, with a titer of 1:16,384. As per Buxton (1998), infection early in gestation in goats can result in foetal death and resorption/abortion,

Table 1
Titration of anti-*T. gondii* antibodies by indirect fluorescent antibody test (IFAT) in the serum of does (A-J), colostrum and offspring (D and E), and the male goat.

Goat ID	Titration IFAT		
	Serum	Colostrum	Offspring
A[a]	65,536	ns	ns
B[a]	65,536	ns	ns
C[a]	65,536	ns	ns
D[b]	65,536	131,072	32,768
E[c]	32,768	131,072	65,536
F[a]	8192	ns	ns
G[a]	65,536	ns	ns
H[a]	65,536	ns	ns
I[a]	4096	ns	ns
J[d]	16,384	ns	ns
Male	4096	na	na

Abbreviations: ns, no sample; na, not applicable.
[a] Pregnancy resulted in an abortion.
[b] Gave birth to twins, one stillborn kid and the other weak and underdeveloped.
[c] Gave birth to a healthy offspring.
[d] Unconfirmed pregnancy or possible reabsorption.

Table 2
Results of nested ITS1 PCR analyses on colostrum and placenta samples from does and lung, heart, brain and umbilical cord samples of the foetuses.

Goat ID	Mothers		Foetuses			
	Colostrum	Placenta	Lung	Heart	Brain	Umbilical cord
A[a]	a	nc	–	–	+	+
B[a]	a	nc	+[b]	+[b]	–	–
C[a]	a	+[b]	–	–	–	–
D[c]	+	nc	–	–	–	+
E[d]	+	+[b]	ns	ns	ns	ns

Abbreviations: ID, identification; +, positive sample; –, negative sample; nc, not collected; ns, no sample.
[a] Pregnancy resulted in an abortion.
[b] Complete genotyping (PCR-RFLP).
[c] Gave birth to twins, one stillborn and the other weak and underdeveloped.
[d] Gave birth to a healthy kid.

Table 3
Multilocus genotyping of *Toxoplasma gondii* from a goat abortion outbreak in the Northeast Region of Brazil, by PCR restriction fragment length polymorphism (RFLP) analysis.

Identification	PCR-RFLP markers											ToxoDB
	SAG1	(5'+3') SAG2	Alt. SAG2	SAG3	BTUB	GRA6	C22-8	C29-2	L358	PK1	Apico	RFLP-Genotype
S48-Type I[a]	I	I	I	I	I	I	I	I	I	I	I	#10
M4-Type II[a]	II or III	I or II	II	II	II	II	II	II	II	II	I	#1
ME49-Type II[a]	II or III	II	II	II	II	II	II	II	II	II	II	#1
NED-Type III[a]	II or III	I or III	III	III	III	III	III	III	III	III	III	#2
This study	I	I	I	I	I	III	II	III	III	I	III	#13[b]

[a] Reference strains.
[b] Genotype previously reported (Ajzenberg et al., 2004; Dubey et al., 2008, 2009; Ragozo et al., 2010; Pena et al., 2011; Rajendran et al., 2012; Clementino Andrade et al., 2013; Almeida et al., 2017; Feitosa et al., 2017a, 2017b; Hamilton et al., 2017).

potentially leading to an underestimation of the occurrence and reporting of abortion outbreaks.

Complete genetic characterization of primary samples is more challenging compared to isolates from mice because the quantity of *T. gondii* DNA in the tissues may be lower (Dubey et al., 2004). Nevertheless, it was possible to obtain a full PCR-RFLP genotype for four samples, revealing the ToxoDB-RFLP genotype #13, which is considered one of the common genotypes circulating in Central and South America (Shwab et al., 2014; Meireles et al., 2022). In Brazil, #13 has only been described in the Northeast Region (Ragozo et al., 2010; Clementino Andrade et al., 2013). In the State of Paraíba, genotype #13 was the most prevalent genotype in chickens, 48.3% (14/29) (Feitosa et al., 2017a) and pigs, 21% (4/19) (Feitosa et al., 2017b).

Regarding pathogenicity, mice inoculated with genotype #13 from chickens in St. Kitts, Caribbean, exhibited significantly higher levels of mortality ($P \leq 0.05$) than mice infected with some other *T. gondii* genotypes isolated from chicken (Hamilton et al., 2017). When assessing the virulence of genotype #13 (isolates TgCkStK9 and 11), Hamilton et al. (2019) described that mice infected with this genotype had a significantly lower survival rate compared to the other groups ($P < 0.001$), indicating that it proved to be highly virulent in mice.

5. Conclusion

In conclusion, we demonstrated a high rate of abortion caused by *T. gondii* in a goat herd from the State of Paraíba, Northeast Region of Brazil, with significant implications for the pathogenicity of genotype #13, one of the most prevalent genotypes in Brazil.

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Ethical approval

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 23000.999663.2022–61.

CRediT authorship contribution statement

Vinícius Longo Ribeiro Vilela: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. **Thais Ferreira Feitosa:** Conceptualization, Methodology, Visualization, Investigation. **Sara Vilar Dantas Simões:** Conceptualization, Methodology, Validation, Investigation. **Rinaldo Aparecido Mota:** Conceptualization, Methodology, Investigation. **Frank Katzer:** Investigation, Data curation, Supervision, Writing - review & editing. **Paul M. Bartley:** Investigation, Data curation, Supervision, Writing - review & editing. All authors read and approved the final manuscript.

Declaration of competing interests

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. Given their role as Co-Editor, Frank Katzer had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Editor-in-Chief Aneta Kostadinova.

Data availability

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

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