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PARASITOLOGY

Toxoplasma gondii induced abortions in a goat herd in Rio de Janeiro, Brazil

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ABSTRACT: *Although, serological assays for detecting immune response against Toxoplasma gondii in goats and sheep are frequent in Brazil, studies showing lesions and confirmation of infectious causes of abortion in goats in the state of Rio de Janeiro have not been reported. This study aimed to describe an abortion outbreak caused by T. gondii in a goat herd in the state of Rio de Janeiro. At histological examination, necrotizing encephalitis and lymphoplasmacytic myositis were observed in goat fetuses submitted to necropsy. The diagnoses were confirmed by immunohistochemistry, which revealed positive marking structures compatible with T. gondii bradyzoites in the brain and skeletal muscle cysts. T. gondii should be included among the causes of abortion in goats in the Southeast region of Brazil.*
Key words: *toxoplasmosis, abortion, immunohistochemistry, encephalitis, myositis.*

Aborto por *Toxoplasma gondii* em um rebanho de caprinos no estado do Rio de Janeiro, Brasil

RESUMO: *Embora sejam frequentes as pesquisas sorológicas da toxoplasmose em caprinos e ovinos no Brasil, os estudos que apresentam as lesões e a confirmação das causas infecciosas de aborto em caprinos no estado do Rio de Janeiro não foram encontrados. Neste trabalho descreve-se Toxoplasma gondii como causa de aborto em uma criação de caprinos no estado do Rio de Janeiro. Ao exame histológico foi observada encefalite necrosante e miosite linfoplasmocítica nos fetos caprinos submetidos à necropsia. A confirmação do diagnóstico foi realizada com o exame de imuno-histoquímica, que revelou marcação positiva de cistos com bradizoítos de T. gondii no encéfalo e em músculo esquelético. T. gondii deve ser incluído entre as causas de aborto em cabras na região sudeste do Brasil.*
Palavra-chave: *toxoplasmose, aborto, imuno-histoquímica, encefalite, miosite.*

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*, an obligate intracellular coccidian protozoan that naturally infects humans and domestic and wild animals (DUBEY et al., 2012). *T. gondii* can infect all warm-blooded animals, including sheep and goats. In these species, the main clinical signs are fever, anorexia, dyspnea, embryonic resorption and mummification, abortion, and neurological signs (OIE, 2017).

Toxoplasmosis presents worldwide geographic distribution. In countries such as South Africa (TAGWIREYI et al., 2019), Argentina (UNZAGA et al., 2014), China (QIAN et al., 2020), and the USA (DUBEY & JONES, 2008), *T. gondii* has been identified as one of the most important causes of reproductive diseases in sheep and goats.

Reports of abortions showing lesions and diagnostic confirmation of reproductive losses in goats due to toxoplasmosis in Brazil are scarce. *T. gondii* infection was associated with reproductive losses, and abortions in goat herds in the states of Rio Grande do Sul (PESCADOR et al., 2007a), Mato Grosso (CALDEIRA et al., 2011), and Alagoas (MESQUITA et al., 2019).

Association between *T. gondii* infection and abortion can be established through clinical history, microscopic analysis of characteristic lesions, mainly in cotyledons and brains of fetuses or

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stillbirths, using complementary techniques such as immunohistochemistry (IHC) and polymerase chain reaction (PCR) (BUXTON, 1998; CALDEIRA et al., 2011). This study aimed to describe an abortion outbreak caused by *T. gondii* on a goat farm in the state of Rio de Janeiro, Brazil.

MATERIALS AND METHODS

In June 2019, two crossbred Saanen goat fetuses, from twin pregnancy, from the municipality of Petrópolis, located in the mountain region of the state of Rio de Janeiro, were sent under refrigeration to the Pathological Anatomy Sector of the Federal Rural University of Rio de Janeiro (SAP/UFRRJ) for diagnosis. Epidemiological data were obtained from the farm owner and the veterinarian responsible for clinical care, who sent the fetuses for diagnosis. At necropsy, fetal age was estimated (TAYLOR & NJAA, 2012), and fragments of the brain, skeletal muscle, heart, liver, kidneys, thymus, spleen, lungs, and eyelids tissues were collected, fixed in 10% buffered formalin solution, processed routinely for histology, and stained with hematoxylin and eosin (H&E).

Sections (3 µm thick) of the brain and skeletal muscle tissues of the two fetuses were dewaxed using xylol, submitted to successive alcoholic solution baths with decreasing concentrations, and washed with phosphate-buffered saline (PBS) solution. Subsequently, endogenous peroxidase was blocked using 3% hydrogen peroxide for 20 min, followed by blockingnon-specific reactions with 5% skim milk (Molico®). For the immunostaining of *T. gondii* and *Neospora caninum*, antigen retrieval was induced by three 2-min heating cycles in a microwave water bath of the sections in citrate buffer (pH 6.0) followed by a 2-min cycle in trypsin (0.1%) at room temperature. The sections were incubated overnight at 4ºC with primary anti-*T. gondii* (Dako, Carpinteria, California, USA) and anti-*N.caninum* (Dako) antibodies, both at a concentration of 1:1000. Streptavidin-biotin-peroxidase (LSAB + System HRP, Agilent Technologies, Santa Clara, CA, USA) was used as a secondary antibody, and the samples were incubated at room temperature for 20 min. Chromogen 3,3-diaminobenzidine (Liquid DAB + Substrate Chromogen System, Dako Cytomation, Carpinteria, California) was used to develop the antibodies. The sections were counterstained with Mayer's hematoxylin. During the test, previously positive controls were used simultaneously. Negative controls were established by replacing the primary antibody with PBS (PESCADOR et al., 2007a).

RESULTS

The fetuses were from a farm that housed approximately 30 dogs and 30 cats and six goats (five females and a male). The goats were raised for recreation under semi-extensive management, with no fold and drinking trough available in native pasture. The animals were regularly vaccinated against rabies and clostridium infection. Between June 2018 and June 2019, two goats aborted three fetuses, but only two underwent necropsy. On June 15, 2019, a 2-year-old primiparous goat, considered by the owners as a pet, aborted two fetuses measuring 22 and 27 cm from the nape to the tail base. Fetal age was estimated at 10-13 weeks (TAYLOR & NJAA, 2012). The doe had access to the domestic facilities and contact with cats, and the abortion occurred in the peridomicile. No significant gross lesions were observed at necropsy. One of the fetuses presented mild multifocal lymphoplasmacytic and histiocytic myositis and moderate multifocal lymphoplasmacytic necrotizing encephalitis with multifocal mineralization in the gray matter. The other fetus showed mild focal lymphoplasmacytic myositis and mild multifocal lymphoplasmacytic myocarditis, with a 22x17 µm tissue cyst containing numerous bradyzoites (Figure 1A). No significant histological changes were reported in the skin, liver, heart, thymus, spleen, lungs, and kidneys. IHC was positive for *Toxoplasma gondii* with marking of bradyzoite cysts in both fetuses' brain and skeletal muscle tissues (Figure 1B). Neither of the fetuses showed anti-*N.caninum* immunostaining.

DISCUSSION

The importance of toxoplasmosis as a cause of abortion in the state of Rio de Janeiro is highlighted. The lesions characterized by necrotizing lymphoplasmacytic encephalitis and the immunoblotting pattern used to detect *T. gondii* in goat fetuses in this study are compatible with the descriptions of toxoplasmosis as a cause of abortion in goats reported in the states of Rio Grande do Sul (PESCADOR et al., 2007a) and Mato Grosso (CALDEIRA et al., 2011). This classic presentation differs from the disease caused by *T. gondii* in sheep fetuses, which is characterized by early abortions and leukoencephalomalacia lesions (GUTIÉRREZ-EXPÓSITO et al., 2020).

The abortions caused by *T. gondii* in the present study had a fetal age of 10-13 weeks. In the abortions caused by *T. gondii* in the state of Mato

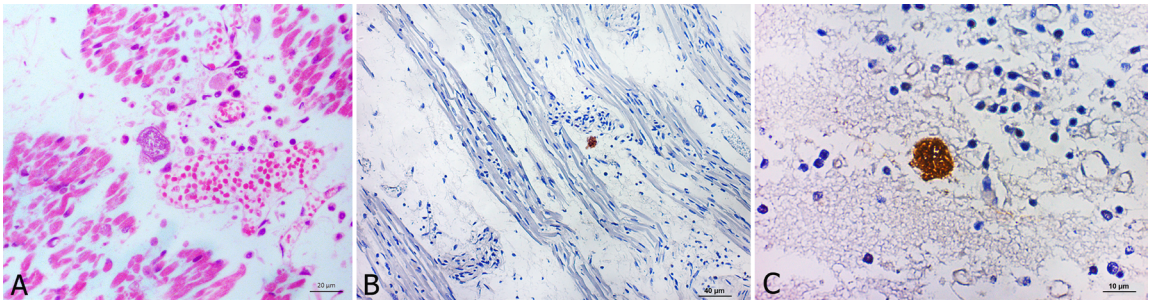

Figure 1 - *Toxoplasma gondii* induced abortions in goats in Rio de Janeiro, Brazil. A. Skeletal muscle of a goat fetus with a cyst containing numerous bradyzoites (arrow) and a mild infiltrate of macrophages and plasma cells. HE, Obj. 40x. B. Skeletal muscle of a goat fetus with positive marking of *T. gondii* cysts and lymphocyte infiltrate. IHC, Obj. 20x. C. Brain of goat fetus with positive marking of *T. gondii* cysts. IHQ, Obj. 63x.

Grosso, the fetuses were 16-20 weeks old (CALDEIRA et al., 2011),whereas in those occurred in the state of Rio Grande do Sul, they did not have their age determined age or stillborn (PESCADOR et al., 2007a). A study reported that sheep experimentally infected with *T. gondii* oocysts during the 12th gestational week miscarried at the 14 weeks of gestation (GUTIÉRREZ-EXPÓSITO et al., 2020). Therefore, these findings suggested that abortion caused by *T. gondii* in small ruminants occurs predominantly between the middle and final third of gestation.

Although, there are no reports on reproductive losses resulting from *T. gondii* infection in goats in the state of Rio de Janeiro, a serum epidemiological survey was conducted with 206 goats from two herds in the municipality of Maricá showed 29.12% of the animals with the presence of anti-*T. gondii* Immunoglobulin G (IgG) antibody (LUCIANO et al., 2011), which reveals the circulation of this protozoan in rural production areas of this state.

Numerous studies have described the anti-*T. gondii* serological response in goats and sheep in different regions of the country. In the Northeast region, a study conducted in Pernambuco's state reported a prevalence rate of 40.4% for anti-*T. gondii* antibodies in goats (SILVA et al., 2003), whereas a research was carried out in Bahia using the latex fixation test, PITA GONDIM et al. (1999) found prevalence rates of 18.75 and 28.93% in sheep and goats, respectively. In the southern region, a survey conducted in Londrina, State of Paraná, showed 30.7% of goats positive for anti-*T.gondii* (SELLA et al., 1994). In the southeast region, research carried out with goats in the south region of the state of Minas Gerais reported 21.4% seropositivity (VARASCHIN et al., 2011). Also, in Minas Gerais, MACHADO & LIMA (1987) observed a prevalence rate of 36.8% in goats from 46 studied properties: 36.1 and 11.4% for dairy and meat herds, respectively. In São Paulo, the rate of positive goats was 14.5% (MAINARDI et al., 2003). It is worth emphasizing that serological results' interpretation determines only the immune response, not the infection. For the diagnosis of toxoplasmosis, serology is recommended only in association with other tests such as IHC and PCR (PESCADOR et al., 2007a; CALDEIRA et al., 2011).

In this study, infection in goats was probably associated with the high cat population density on the farm investigated. Domestic and wild felids are definitive hosts of *T. gondii*, and their importance in the epidemiology of toxoplasmosis is emphasized due to the excretion of environmentally resistant oocysts for extended periods (MONTAZERI et al., 2020). In addition to inadequate sanitary conditions, it is known that the free circulation of cats probably disperses feces contaminated with *T. gondii* in the pasture and water consumed by the goats, thus increasing the survival of oocysts in the environment (FIALHO et al., 2009). This epidemiological variable contributes as an important source of infection for goats. The cats on this farm were rescued from the streets and have had extensive contact with other species, including humans. The importance of not raising cats, together with goats, is emphasized.

The infection of goats by oocysts shed in the definitive host's feces in the environment is greater on farms under intensive management (SILVA et al., 2003). In contrast, extensive management

favors the habit of goats grazing on high grassland branches, thus decreasing the chance of infection by pathogenic agents such as *T. gondii* (MACHADO & LIMA, 1987). In this outbreak of abortion caused by *T. gondii* infection, the goats were raised under semi-extensive management, but they had close contact with other animals, including cats and humans.

Infections by other protozoa such as *N. caninum* (MESQUITA et al. 2018) and *Sarcocystis* spp. (PESCADOR et al., 2007b) should be considered among the differential diagnoses. The main microscopic lesion reported in aborted fetuses as a result of *T. gondii* infection is necrotizing encephalitis (BUXTON, 1998; HURTADO et al., 2001). This histological lesion pattern is similar to that of infections by *N. caninum*, and the distinction between these infections only through histological analysis is difficult (DUBEY, 2003). Thus, differentiation occurs only with the use of complementary techniques, such as IHC. The fetuses in this study were negative for *N. caninum* by IHC. Differentiation between *T. gondii* and *Sarcocystis* spp. lesions were based on the morphology of the parasitic agents. *Sarcocystis* spp. has a rosette shape, and the cysts tend to present a perivascular location (PESCADOR et al., 2007b).

The presence of necrotizing encephalitis in the fetuses of this study collaborated to guide the diagnosis. In general, toxoplasmosis in fetuses and stillborn goats can be characterized by foci of inflammation and necrosis in cotyledons, brain, lung, liver, kidney, and spleen (MORENO et al., 2012, PESCADOR et al., 2007a and MESQUITA et al ., 2019, BUXTON, 1998). No studies show skeletal muscle as one of the primary sites of inflammatory and necrotic lesions caused by *T. gondii* in goats. In bovine fetuses with neosporosis, skeletal fiber myositis is usually observed (ANTONIASSI et al. 2013). The same lesion also occurs in fetuses of sheep (DUBEY & LINDSAY, 1990) and goats (COSTA et al., 2014) due to infection caused by *N. caninum*.

Systemic toxoplasmosis in sheep fetuses was correlated with loss of gray matter (GUTIÉRREZ-EXPÓSITO et al., 2020). In that study, 32 brains of sheep in the fetal phase exposed to experimental infection with T. *gondii* were evaluated and presented fetal leukoencephalomalacia, with loss of oligodendrocytes without neuronal damage, and subsequent activation of the microglia. One of the hypotheses of the cause of this injury was the action of mediators of inflammation induced by infection with *T. gondii*.

Toxoplasmosis is a significant public health problem (DUBEY et al., 2005). It is known that *T. gondii* can be transmitted to humans through the consumption of improperly prepared pork (HILL et al., 2006). In this context, samples of goat meat obtained in supermarkets showed 17.9% positivity for *T. gondii* using a mouse bioassay (SURABHI et al., 2020). In an experimental study carried out with infected young goats, the dorsal region muscles showed a 32% positive reaction rate using the real-time PCR technique to detect *T. gondii* DNA (JURÁNKOVÁ et al., 2012). There are still no studies available in the literature correlating toxoplasmosis transmission through goat meat consumption.

PINTO-FERREIRA et al., (2019) demonstrated that human toxoplasmosis could be transmitted by eating or drinking contaminated raw food or water. *T. gondii* cysts, oocysts, or tachyzoites can contaminate food such as meat and meat products, milk, fruits and vegetables. A study addressing raw goat milk revealed that 90% of the samples had anti-*T. gondii* antibodies, and that 3.3% of them were positive using PCR (SAAD et al., 2018). Due to the risk of human contamination, health programs should be implemented on farms.

The diagnosis of infectious abortions in ruminants is difficult due to the need for specialized laboratory techniques (NAVARRO et al., 2009; ANTONIASSI et al., 2013). Stages of the investigative process, such as collecting various tissues, transport and packaging of the samples, and choice of tests to be performed, are fundamental for the diagnosis. For example, HURTADO et al. (2001) demonstrated that fetal brain samples were more sensitive in PCR than fetal placenta samples from sheep with toxoplasmosis. Formalin fixing and processing tissues enable visualization of histological lesions and IHC and PCR's performance with laboratory safety and good sensitivity. However, it is recommended that the tissues be kept in formalin for controlled periods (<72 h) for greater efficiency of the analyses (NAVARRO et al., 2009). In this study, IHC was effective for diagnosing *T. gondii* infection in goats, corroborating the studies conducted in the states of Rio Grande do Sul (PESCADOR et al., 2007a) and Mato Grosso (CALDEIRA et al., 2011).

However, IHC should be carefully carried out and diagnostically interpreted (NAVARRO et al., 2009). The cases reported in Alagoas were diagnosed from the collection of the placenta from goats during parturition, not from miscarrying goats (MESQUITA et al., 2019). Thus the authors would be investigating only the vertical transmission of the protozoan. Some important points to avoid interpretation errors should be highlighted: observation of the morphology of the researched agent, inflammatory lesions, and false-

positive immunostaining, such as those caused by peroxidase in erythrocytes or hemosiderin pigments, mainly in placentas.

CONCLUSION

Toxoplasmosis in goat fetuses causes encephalitis and myositis and should be included as a differential diagnosis of the causes of abortion in goats in the state of Rio de Janeiro. The importance of necropsy and immunohistochemistry to optimize diagnosis is emphasized.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare having no conflicts of interest. Funding agencies played no role in the study design, collection, analysis, and interpretation of data, writing of the manuscript, and in the decision to publish its results.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the study design and writing, critical review and final approval of the manuscript.

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