Outbreak of caprine abortion by *Toxoplasma gondii* in Midwest Brazil[1]

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ABSTRACT.- Caldeira F.B., Ubiali D.G., Godoy I., Dutra V., Aguiar D.M., Melo A.L.T., Riet-Correa F., Colodel E.M. & Pescador C.A. 2011. **Outbreak of caprine abortion by *Toxoplasma gondii* in Midwest Brazil**. *Pesquisa Veterinária Brasileira 31(11):933-937*. Laboratório de Patologia Veterinária, Faculdade de Agronomia e Medicina Veterinária, Universidade Federal de Mato Grosso, Av. Fernando Corrêa da Costa 2367, Boa Esperança, Cuiabá, MT 78069-900, Brazil. E-mail: capescador@ufmt.br

An outbreak of abortion by *Toxoplasma gondii* in goats on a farm in the Brazilian Midwest is reported. Gross lesions were not observed in seven aborted fetuses submitted to the Veterinary Pathology Laboratory, Federal University of Mato Grosso, for necropsy investigation. The main histologic lesions were mononuclear cell pneumonia and necrotizing encephalitis in varying degrees of intensity. PCR for *Brucella abortus* and *Neospora caninum* and aerobic cultures were negative in all cases. Antibody titles against *T. gondii* varying from 1:1024 to 1:32.768 were detected in serum samples from four aborted goats. Nested-PCR assay for *T. gondii* were positive in brain samples of all cases submitted. These findings indicate that *T. gondii* infection should be considered in the diagnosis of abortion in goats in Midwest Brazil.

INDEX TERMS: Encephalitis, PCR, protozoan, *Toxoplasma gondii*, goats, abortion.

RESUMO.- [**Surto de aborto por *Toxoplasma gondii* em caprinos na região Centro-Oeste do Brasil.**] Descreve-se um surto de aborto em caprinos por *T. gondii* em uma propriedade situada na região Centro-Oeste do Brasil. Não foram observadas lesões macroscópicas em sete fetos abortados encaminhados ao Laboratório de Patologia Veterinária da Universidade Federal de Mato Grosso para necropsia. As principais lesões histopatológicas foram encefalite necrosante e pneumonia mononuclear em variados graus de intensidade. PCR para *Brucella abortus* e *Neospora caninum* e cultivos em aerobiose obtiveram resultado negativo em todos os casos. Anticorpos anti-*T. gondii* em titulações de 1:1024 a 1:32.768 foram detectados no soro de quatro cabras que abortaram. Amostras de todos os cérebros enviados foram positivos para *T. gondii* pela técnica de *Nested*-PCR. Estes achados indicam que a infecção por *T. gondii* deve ser considerada no diagnóstico de aborto em caprinos na região Centro-Oeste do Brasil.

TERMOS DE INDEXAÇÃO: Encefalite, PCR, protozoário, *Toxoplasma gondii*, caprinos, aborto.

INTRODUCTION

Toxoplasma gondii infection is widely prevalent in humans and animals worldwide, and has been recognized as one of the main causes of abortion in small ruminants causing significant losses (Dubey 2010). Furthermore, goats infected by *T. gondii* represent an important source of human infection due to ingestion of meat and milk from infected animals (Garcia et al. 2002).

Cats are considered the definitive host and other animal species can be infected by ingestion of sporulated oocysts or cyst contaminated meats, by contact with free tachyzoites or congenitally through transplacental passage (Pepin et al. 1997, Dubey 2010).

Since goat breeding represents a major source of economic resources in Brazil (IBGE 2006), it is important to understand and monitor the diseases that occur in these

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animals (Silva et al. 2009). While the cause of abortion in goats has been studied in some countries (Buxton 1998a, Moeller 2001, Szeredi & Bacsadi 2002, Masala et al. 2003, Szeredi et al. 2006), the etiology of caprine abortion and stillbirth in Brazil has not been fully investigated, which explains the paucity of information on this subject (Pescador et al. 2007).

The prevalence of *T. gondii* antibodies in goats varies, depending on the geographical area and the type of serologic test used (Dubey & Kirkbride 1990). Seroprevalence rate of toxoplasmosis in northern and southern parts of Iran has been reported to be 55% and 22%, respectively (Assmar et al. 1997). In Brazil, studies in flocks of small ruminants found prevalence rates of 21.4 to 92.4% of anti-*T. gondii* antibodies (Amaral et al. 1978, Chiari et al. 1987, Sella et al. 1994, Gondim et al. 1999, Silva et al. 2003, Maciel & Araújo 2004, Motta et al. 2008, Modolo et al. 2008, Varaschin et al. 2011). Despite the numerous publications on prevalence of antibodies against *T. gondii* in goats in different Brazilian *regions* there is only one report in Brazil reporting abortion by this parasite in goats, in the state of Rio Grande do Sul (Pescador et al. 2007).

The isolation of *T. gondii* from aborted samples (fetuses and placentas) represents the gold standard for definitive diagnosis (Losson & Buxton 2007). However, isolation requires samples in optimal conditions containing a threshold number of living and viable microorganisms. In fact, contamination with other bacteria, inadequate transport conditions, autolysis, and other factors may all adversely affect isolation. DNA detection is more rapid than isolation and, associated with histopathological lesions, can be considered a useful technique for diagnosis of toxoplasmosis (Losson Buxton & 2007).

The aim of this paper is to report an outbreak of abortion in goats caused by *Toxoplasma gondii* in the Brazilian Midwest.

MATERIALS AND METHODS

Between June and September 2009, seven Saanen goat fetuses, from the municipality of Santo Antônio do Leverger, were sent to the Pathology Laboratory at the Federal University of Mato Grosso for diagnostic purposes.

The fetuses were necropsied and gestational age was estimated by crown-rump length. Samples of brain, heart, lung, liver, kidney, skeletal muscle, thymus and spleen collected from all fetuses were fixed in 10% buffered formalin, included in paraffin, cut on 4-µm thick sections and stained with hematoxylin and eosin (HE) for histologic examination. Samples of the lungs, liver and stomach contents of each fetus were cultured in sheep blood agar (5%) for aerobic bacteria. Additionally, brain samples were submitted to Nested-PCR for *Toxoplasma gondii* and *Neospora caninum*, and samples of stomach content to PCR for *Brucella abortus*. DNA extraction from samples of fresh brain, lung tissue and abomasum content of the fetuses was performed on proteinase K buffer, followed by phenol and chloroform treatment (Sambrook & Russel 2001). *Brucella abortus* (field strains), *N. caninum* and *T. gondii* were detected by PCR as described Bricker & Halling (1995), Burg et al. (1989) and Buxton et al. (1998). Negative control was ultrapure water and

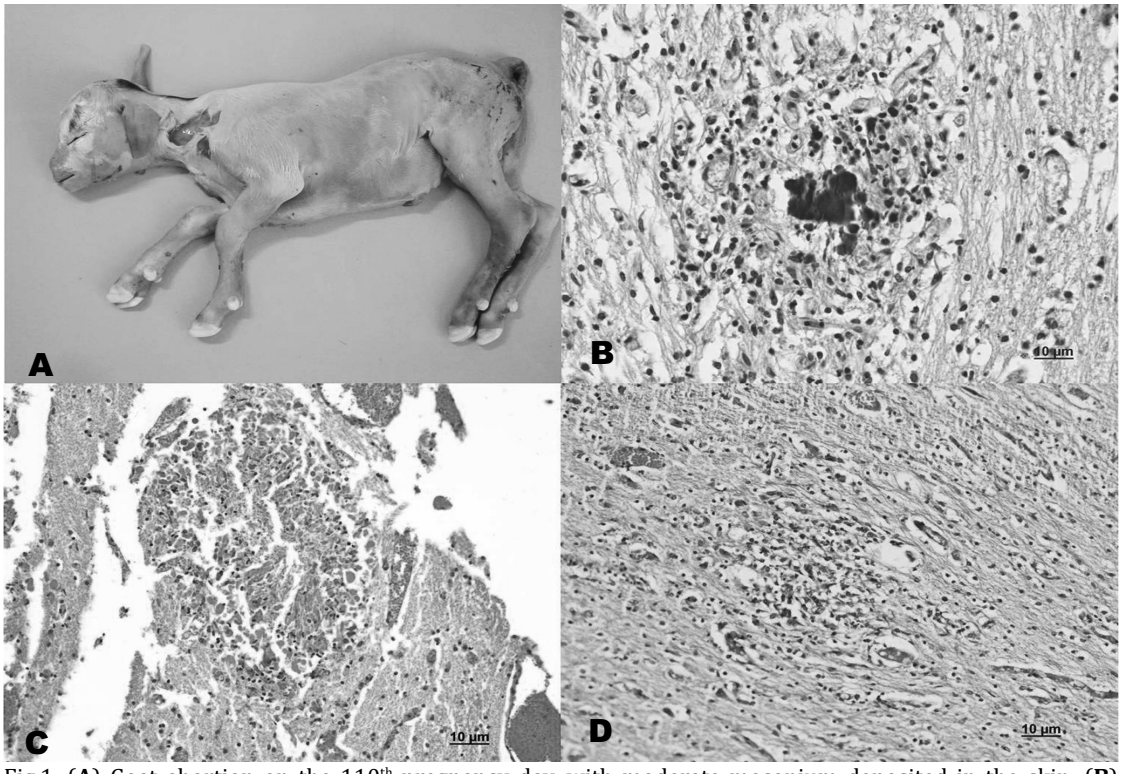

Fig.1. (**A**) Goat abortion on the 110th pregnancy day, with moderate meconium deposited in the skin. (**B**) Goat abortion. Brain. Mild mononuclear inflammation with mineralization at the centre of the lesion. HE, obj.40x. (**C**). Focal necrosis surrounded by a narrow zone of glial cells in the brain. HE, obj.20x. (**D**). Mild, focal non-suppurative encephalitis around small vessels. HE, obj.20x.

positive control was 50ng of genomic DNA from parasites or bacteria culture.

Serum samples from dams of fetuses collected during the visit to the farm were tested by the indirect immunofluorescence assay (IFA) using *Toxoplasma gondii* (RH strain) cultivated in Vero cells, with 1:64 dilution as cut-off value (Opel et al. 1991). All reactions were performed with fuorescein-conjugated anti--coat IgG (Sigma, St. Louis, MO) in a 1:400 dilution according to Figliuolo et al. (2004). In each slide, a serum previously shown to be nonreactive (negative control) and a known reactive serum (positive control; with titer of 16.384) were tested at 1:64 dilution. The positive reactions for *T. gondii* were considered only when tachyzoites surfaces were fluorescent, and samples that reacted at the screening dilution (1:64) were then titrated using serial two-fold dilutions to determine endpoint titers.

RESULTS

In 2009 the herd consisted of 73 Saanen goats, with 72 females and one male. Abortion and stillbirths had been observed since June 2009. The animals were treated periodically with anthelmintics. Vaccinations against infectious diseases were not performed. The flock was grazing on pastures during daytime, while at night they were kept, overcrowded, in unpaved stalls with dirt floor. Furthermore, a cat and chickens were commonly observed in the goat house. Of the 39 pregnant females, nine (23%) aborted in a thirteen days period.

Seven fetuses were sent for necropsies. No significant gross lesions were observed. In the seven cases, histological analysis of the brains revealed gliosis and moderate multifocal necrotizing encephalitis. In one case, mineralization was also observed in the centre of the necrotic area of the brain. In one fetus, pulmonary lesions characterized by moderate focal mononuclear pneumonia were also observed. Moderate keratin aspirations were seen within the pulmonary alveoli in four cases. No other significant microscopic changes were found in any of the other organs examined. No significant bacterial pathogens were isolated on aerobic cultures. The nested-PCR for detection of *Toxoplasma gondii* resulted in an amplification product of 194 bp in all cases, which was consistent with the expected product. *Neospora caninum and Brucella abortus* PCR were negative in all samples of fetuses analyzed. The microscopic lesions, PCR and serological results are summarized in Table 1.

Table1. Summary of histopathology, serology and PCR results on goat fetuses aborted by *Toxoplasma gondii*

Case	Fetus age*/sex	Dam antibody title (IFA)	Histopathology	PCR T. *gondii*
1	135/F**	1:16384	Mild, multifocal gliosis. Moderate, focal mononuclear pneumonia.	+
2	130/F	1:8192	Moderate, multifocal necrotizing encephalitis;	+
3	120/M***	ND	Moderate, multifocal necrotizing encephalitis.	+
4	130/M	1:32768	Mild, focal encephalitis.	+
5	110/M	1:1024	Accentuate focal necrotizing encephalitis.	+
6	135/F	ND	Mild, focal necrotizing encephalitis with mineralization areas.	+
7	140/M	ND	Mild, focal encephalitis.	+

* Days; ** F= female; *** M= male. ND= not determined.

DISCUSSION

Abortion by *Toxoplasma gondii* is relatively common in goats, which seem to be more susceptible than sheep (Tibary 2009). Although in Brazil it is difficult to accurately define the incidence of toxoplasmosis as the cause of abortion and stillbirth in goats, a study made in the UK suggested that the disease was responsible for 1-2% of neonatal losses per year (Blewett & Tress 1987) and if this incidence was extrapolated throughout Europe, this would mean a loss of around 1.5-2 million animals, suggesting that *T. gondii* is an important cause of reproductive losses (Innes et al. 2009).

Goats have been reported to become infected by *T. gondii* oocysts following ingestion of feed and water contaminated by feces from shedding cats (Dubey 2010). In the present study, there was one cat in the farm that was in constant contact with pregnant goats, which could be a possible source of contamination in the flock. Cats can shed millions of oocysts that can survive for 12-18 months in the environment, depending on climatic conditions (Tender et al. 2000).

The most common lesion observed in abortion by *T. gondii* is necrotizing placentitis involving almost exclusively cotyledonary areas. This lesion is easily visualized as gray-white to yellow small areas of necrosis and calcification (Dubey & Kirkbride 1990). However, in our study the owner did not send the placenta for analysis, and the presumptive diagnosis was therefore based on microscopic lesion observed mainly in the brain of the goat fetuses. These lesions were non-suppurative encephalitis with necrotic areas and, in one case, with mineralized areas in the centre of the necrosis, as described by Uggla et al. (1987). These lesions represent a fetal immune response following direct damage by local parasite multiplication, and fetal anoxia in late gestation, caused by progressive multifocal necrosis in the placentome preventing sufficient oxygen transfer from mother to fetus (Buxton et al. 1982, Buxton & Finlayson 1986).

The definitive diagnosis of toxoplasma abortion is obtained by visualization of characteristic histological changes, mainly in the brain, heart and lung, by detection of *T. gondii* antibodies in fetal fluids, by detection of cysts and tachizoites of the protozoa by immunohistochemistry (Pescador et al. 2007), by polymerase chain reaction (PCR) assay, or by isolation of *T. gondii* in the fetuses (Losson & Buxton 2007). Still, isolation is considered the gold standard method for toxoplasma diagnosis, although it is costly and time-consuming, in comparison with other techniques (Losson & Buxton 2007). In all fetuses analyzed in the present study, the pathogen was detected by *Nested*-PCR associated with microscopic lesion observed in the brain. *Nested*-PCR is a reliable and sensitive alternative diagnostic tool; which is more sensitive than immunohistochemistry,

because it affords to detect small quantities of protozoa DNA (Owen et al. 1998a). Nonetheless, because of the similarities in the histopathology of *Neospora caninum* and *T. gondii* infections, a molecular test which can differentiate between them will be valuable (Owen et al. 1998).

The detection of *T. gondii* antibodies in fluids of fetuses has proved helpful in the diagnosis of small ruminant abortion (Dubey & Kirkbride 1990), because the maternal antibodies are not transmitted to the fetus and the presence of antibodies in immunocompetent fetuses is indicative of an infection (Agerholm et al. 2006, Broaddus et al. 2009). As goat fetuses become able to produce IgG around gestation day 80 (Nettleton 1990), all fetuses in the present study were considered immunocompetent. However, serological examination of fetuses is generally carried out on fluids from the thoracic cavity, and in all fetuses necropsied here thoracic effusions were absent, preventing the IFA test.

Serum samples from four female goats that aborted were positive for *T. gondii* antibodies, with titers above 1:1024, suggesting acute toxoplasmosis, which in pregnant animals is associated with abortion (Buxton 1990). However, in this study was not possible to test all female goats serologically to investigate the association between abortions and seroprevalence for *T. gondii* antibodies. During three goat reproductive seasons in the farm, from 2008 to 2010, abortions only occurred in 2009, probably because in goats *T. gondii* immunity is for the rest of the life and goats rarely abort twice (Dubey & Kirkbride 1990). Nevertheless, control measures to avoid the presence of cats in the stables and food warehouses should be taken. Additionally, serologic investigations should be done in the farm, because, if control measures are not efficient, recurrence of abortion in the farm is to be expected, since the immune does will be replaced by non-immune goats.

In toxoplasmosis the severity of disease is a function of the stage of gestation when infection occurs (Innes et al. 2009). In our study, the age of the fetuses was between 110 and 140 days, but it was not possible to know the time when infection was established. However, taking into account that when the infection occurs at 80-90 days of pregnancy there is no evidence of infection in the fetal tissues (Owen et al 1998b) it is suggested that, in the present case, the infection occurred around the 100 days of pregnancy.

In conclusion, *Toxoplasma gondii* infection could be an important cause of reproductive losses in goats in Midwest Brazil and in other Brazilian regions, where goats are raised in similar conditions than those observed in this outbreak, e.g. kept at least at night in stalls probably contaminated by cat feces. The potential zoonotic risk of the disease should be also taking into account.

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