SCIENTIFIC COMMUNICATION

SCREENING FOR *TOXOPLASMA GONDII* IN ABORTED BOVINE FETUSES IN BRAZIL

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ABSTRACT

The aim of this study was to determine if *Toxoplasma gondii* was present in aborted bovine fetuses in Brazil. Histopathology of 105 cases with suspected infectious abortion, analyzed during the period from 2006 to 2008 at Centro de Pesquisa e Desenvolvimento de Sanidade Animal of Instituto Biológico, São Paulo, showed 75 cases with indications of abortion due to apicomplexan protozoa. These cases were submitted to PCR for verification of the laboratory diagnosis. Fetal DNA was extracted from central nervous system, heart, liver, muscle, and/or placenta samples to obtain a 529 bp DNA fragment. *T. gondii* DNA was not detected in any of the bovine fetuses analyzed, suggesting that it may not be a frequent cause of bovine abortion.

KEY WORDS: *Toxoplasma gondii*, abortion, cattle, Brazil, Apicomplexa.

RESUMO

PESQUISA DE *TOXOPLASMA GONDII* EM FETOS BOVINOS ABORTADOS NO BRASIL. O objetivo do presente trabalho foi avaliar a presença de *Toxoplasma gondii* em fetos bovinos abortados no Brasil. Com base em laudos histopatológicos, de um total de 105 casos com suspeita de aborto infeccioso, recebidos no período de 2006 a 2008, no Centro de Pesquisa e Desenvolvimento de Sanidade Animal do Instituto Biológico, São Paulo, 75 casos foram sugestivos de abortamento por protozoário Apicomplexa e foram submetidos à técnica de PCR para confirmação do diagnóstico laboratorial. O DNA foi extraído a partir de amostras de sistema nervoso central, coração, músculo, fígado e/ou placenta dos fetos para obtenção de um fragmento de DNA de 529pb. Não foi detectada a presença de DNA de *T. gondii* em qualquer dos fetos bovinos analisados, não sendo este agente infeccioso uma causa frequente de abortamentos.

PALAVRAS-CHAVE: *Toxoplasma gondii*, aborto, bovino, Brasil, Apicomplexa.

Toxoplasma gondii induces serious disease in humans and abortion in sheep and some animals (Tenter et al., 2000). While *Neospora caninum* frequently causes abortion in cattle (Dubey, 2003), *Toxoplasma gondii* is not commonly associated with disease in this animal, but consumption of infected beef may cause infection in humans and other animals (Tenter et al., 2000).

In many countries, the rate of bovine fetus infection is low and *T. gondii* is not an relevantause of abortion in cattle (Dubey, 1986). Gottstein et al. (1998) detected *T. gondii* DNA in 5% of aborted bovine fetuses in Switzerland. These authors showed that 24 out of 83 bovine fetuses analyzed for *N. caninum* and *T. gondii* were PCR positive for *N. caninum* DNA and four to *T. gondii* DNA. One fetus contained DNA of both parasites. They also found antibodies to *T. gondii* in 2 dead calves, suggesting transplacental infection. In another study from the same laboratory, *N. caninum* DNA was found in 50 (21%) and *T. gondii* DNA in 1 (< 1%) of 242 aborted fetuses (Sager et al., 2001). In Australia, Ellis (1998), using PCR, found that *N. caninum*, 40% (16/40), was more common than *T. gondii*, 5% (40), verifying infection from sections of brains of aborted bovine fetuses showing nonsuppurative encephalitis.

T. gondii was isolated from 2 aborted bovine fetuses, 1 from Portugal and 1 from the United States. Both isolates were made by bioassay of fetal brains in mice (Canada et al., 2002). The fetus from Portugal was about 5 months. in gestational age, and the fetus from the United States was a full-term stillborn.

In Paraná State, Brazil, Garcia et al. (1999) reported 25.8% (103/400) of cattle seropositive to *T. gondii* by IFAT, with reactive titers equal to or higher than 1:64. Santos et al. (2009) investigated infection rates caused

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by *N. caninum, Hammondia* sp., and *T. gondii* in brain and heart from 100 beef cattle in a slaughterhouse in Bahia, Brazil, by a nested PCR for Toxoplasmatinae rDNA, followed by sequencing of the PCR products. Seven brain samples tested positive for Toxoplasmatinae DNA, but no heart tissue tested positive. Sequencing analysis of the PCR products showed that five sequences matched *N. caninum* and two *T. gondii*. Antibodies to *N. caninum* and *T. gondii* were found in 20% and 26% of the animals, respectively.

Since *T. gondii* has not been regularly associated with bovine abortion in Brazil, the aim of this study was to search for the presence of *T. gondii* DNA in aborted bovine fetuses.

Samples of bovine aborted fetuses (n = 105) from various regions of Brazil were sent to Centro de Pesquisa e Desenvolvimento de Sanidade Animal do Instituto Biológico for differential diagnosis of infectious abortion, from January 2006 to May 2008. Samples were screened for *T. gondii* in brain, heart, kidney, liver, lung, spleen, thymus and placenta, using histology and PCR. Samples did not include all organs for each fetus.

Tissues were fixed in 10% buffered formalin, embedded in paraffin, cut at 4 µm, and stained with hematoxylin and eosin for routine screening.

The genomic DNA was extracted from fresh and frozen tissues using a commercial kit (Wizard Genomic DNA Purification Kit - Promega®), according to the manufacturer's instructions. Each sample was eluted in 100 mL of DNA hydration solution and heated for 1h at 64° C. The samples were stored at -20° C until the completion of PCR.

Amplification of the 529 bp DNA fragment was performed using primers Tox4/Tox5 (5′ CGC TGC AGG GAG GAA GAC GAA AGT TG3′/5′ CGC TGC AGA CAC AGT GCA TCT GGA TT 3′) (Homan et al., 2000). Five mL of the DNA sample were added to the PCR mix to a final volume of 20 mL with commercial buffer from the Promega PCR Master Mix ™ kit (M7502) and 0.2 mM of each primer. The amplification was 1 cycle at 94° C for 7 min, 35 cycles at 94° C for 1 min, 55° C for 1 min, 72° C for 1 min, a final extension of 72° C for 10 min, and maintenance at 4 °C.

Positive (purified *T. gondii* tachyzoite DNA) and negative controls (double-distilled water) were included in each PCR reaction. Amplification products were visualized by 2% agarose gel electrophoresis containing ethidium bromide staining under ultraviolet light. The DNA fragment size was compared with a standard molecular weight (100 bp DNA ladder - Fermentas ®).

Histology was used as an initial screen for infectious abortion. A mononuclear inflammatory infiltrate was predominant in the heart, kidney, liver, lung, brain and placenta in 75 fetuses, suggesting abortion due to protozoa, but none was positive to *T. gondii* by PCR. These findings are in agreement with a study in Argentina (Moore *et al*., 2008) where none of the 70 fetuses were positive by nested-PCR, although low rates of *T. gondii* infection have been reported in other countries (Ellis, 1998; Gottstein et al., 1998; Sager et al., 2001; Canada et al., 2002; Reitt et al., 2007).

Cabral et al. (2009), using the same 105 aborted fetuses of this study, confirmed the presence of *N. caninum* in 24.8% by IHC and nested PCR. The specificity of the nested PCR was showed by a BLAST analysis, that confirmed the primers of the ITS1 region were specific to this parasite, so no cross reaction occurred with other coccidians. The sequence of primers from the *N. caninum* Nc5 region are not found in the genomes of *T. gondii*, *Sarcocystis capracanis*, *S. cruzi*, *S. miescheliana*, *S. moulei*, *S. tenella* or *Hammondia hammondi* (Yamage et al., 1996; Hughes et al., 2006).

Despite the presence of *T. gondii* has been confirmed in beef cattle in Brazil by serological studies (Garcia et al., 1999), PCR and sequencing (Santos et al., 2010), and isolation by bioassay (Macedo et al., 2012), the present investigation performed in aborted fetuses with lesions indicative of protozoal infection were negative for *T. gondii* DNA, in accordance to studies performed in many countries, where the rate of infected bovine fetus with *T. gondii* was low (Dubey, 1986).

The results suggest that *T. gondii* may not be a frequent cause of bovine abortion but their investigation is necessary as much as the other infectious agents of bovine abortion.

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