

Abortion in association with transplacental *Theileria equi* infection in a mare from the State of Espírito Santo, southeast Brazil: case report

[Aborto equino associado à infecção transplacentária de *Theileria equi*, Espírito Santo, Brasil: relato de caso]

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ABSTRACT

Equine piroplasmosis is a tick-borne disease caused by the protozoan parasites *Babesia caballi* and *Theileria equi*. We report a case of abortion in association with transplacental *Theileria equi* infection in a mare from the State of Espírito Santo, southeast Brazil. An apparently healthy mare aborted at the tenth month of gestation. At necropsy, the subcutaneous tissue, skeletal muscles, and visible mucosae of the aborted fetus were pale, and there was moderate hydrothorax and marked splenomegaly. Microscopic findings included splenic lymphoid hyperplasia and nephrosis. Merozoite-infected erythrocytes were found within blood vessels of all organs examined and were most numerous in the brain. DNA extracted from the spleen, liver, kidney, and thymus was used as a template for PCR. Generic primers were employed for the detection of piroplasm 18S ribosomal gene. All samples were positive for piroplasm DNA by PCR. Amplicons were purified and then sequenced. Sequencing analysis of these amplicons revealed 98% identity to *T. equi* sequences. Based on our findings, we suggest that abortion in this case resulted from transplacental *Theileria* infection.

Keywords: piroplasmosis, theileriosis, mare, fetus

RESUMO

A piroplasmose equina é transmitida por carrapatos, sendo causada pelos protozoários *Babesia caballi* e *Theileria equi*. O objetivo deste trabalho foi relatar um caso de aborto equino associado à piroplasmose fetal. Uma égua gestante, aparentemente saudável, abortou no 10º mês de gestação. Na necropsia do feto, foi observada palidez de subcutâneo, da musculatura esquelética e das mucosas visíveis, moderado hidrotórax e acentuada esplenomegalia. Histologicamente, havia hiperplasia linfóide esplênica e nefrose. Muitos merozoítos foram observados em eritrócitos nos vasos sanguíneos de todos os órgãos examinados, com maior intensidade no encéfalo. Amostras de DNA do baço, fígado, rim e timo foram utilizadas para PCR com primers genéricos para detecção de sequências do gene 18S ribossomal de piroplasmas, com resultado positivo para todas as amostras. Em seguida, os amplicons das amostras de baço foram purificados, sequenciados e, após análise, mostraram 98% de identidade com a sequência de *T. equi*. Portanto, as alterações microscópicas, os resultados de PCR e sequenciamento confirmam aborto associado à *T. equi* por transmissão transplacentária.

Palavras-chave: piroplasmose, theileriose, égua, feto

INTRODUCTION

Theileria equi and *Babesia caballi* are tick-transmitted hemoparasitic protozoans that belong to the family Piroplasmidae within the phylum Apicomplexa. They are the causative agents of equine piroplasmosis (Wise *et al.*, 2013). Equine

piroplasmosis is endemic in tropical and subtropical areas. Animals are persistently infected and often asymptomatic. The introduction of the pathogen into non-endemic areas with large numbers of horses and increased densities of the vector (ticks) may result in disease outbreaks (Wise *et al.*, 2013).

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These piroplasms are usually transmitted by tick vectors but iatrogenic transmission may also occur (Wise *et al.*, 2013). There are some published reports of transplacental *T. equi* or *B. caballi* transmission (Phipps and Otter, 2004; Allsopp *et al.*, 2007; Georges *et al.*, 2011; Roncati *et al.*, 2011; Chhabra *et al.*, 2012; Sudan *et al.*, 2015; Sant *et al.*, 2016; Sousa *et al.*, 2017), a few of them associated with abortion (Sudan *et al.*, 2015; Sousa *et al.*, 2017). We report a case of transplacental *T. equi* fetal infection and abortion in an otherwise asymptomatic mare from the State of Espírito Santo (ES), southeastern Brazil.

CASE REPORT

In August 2016, a 10-month old equine aborted fetus was submitted to the Veterinary Pathology Laboratory at the Universidade Vila Velha (UVV) located in the city of Vila Velha, ES, Brazil, for necropsy and diagnostic workup. The pregnant mare did not show any clinical signs prior to abortion. All animals from this farm were raised in a semi-extensive system, and there was no history of ectoparasite (tick) infestation.

Horses were vaccinated annually with anti-rabies and Lexington-8® vaccines (Eastern Equine Encephalomyelitis, Western Equine Encephalomyelitis, Equine Influenza strain A / equine 1 / Prague / 1/56, A / equine / 2 / Kentucky / 94, A / equine 2 / South Africa 4/03 and Equine Herpes Virus types 1 and 4 inactivated by beta-propylactone), and dewormed every 90 days.

Necropsy was performed five hours after the abortion. Grossly, the subcutaneous tissue, skeletal muscles and visible mucosae of the aborted fetus were diffusely pale. Pallor was also observed diffusely at heart and liver. There were also mild hydroperitoneum, moderate hydrothorax, and marked splenomegaly (Figure 1) with a prominent white pulp. Samples of thymus, heart, lung, liver, spleen, kidney, brain, and placenta were collected at necropsy and fixed in 10% buffered formalin for histopathology. Sections were stained with hematoxylin-eosin and Giemsa.



Figure 1. Aborted equine fetus, *Theileria equi* infection. The subcutaneous tissue and skeletal muscles are markedly pale, and there is severe splenomegaly and moderate hydrothorax (white arrow).

Microscopic findings included diffuse lymphoid hyperplasia (Figure 2a), moderate erythrophagocytosis in the spleen and thymus, and moderate multifocal nephrosis with the presence of small amounts of orange intracytoplasmic pigment in the renal

tubular epithelium. Variable numbers of intra-erythrocytic merozoites measuring approximately 1 μm in diameter (Figure 2b) were seen in Giemsa-stained sections (Figure 2c) of all fetal organs examined and the placenta.

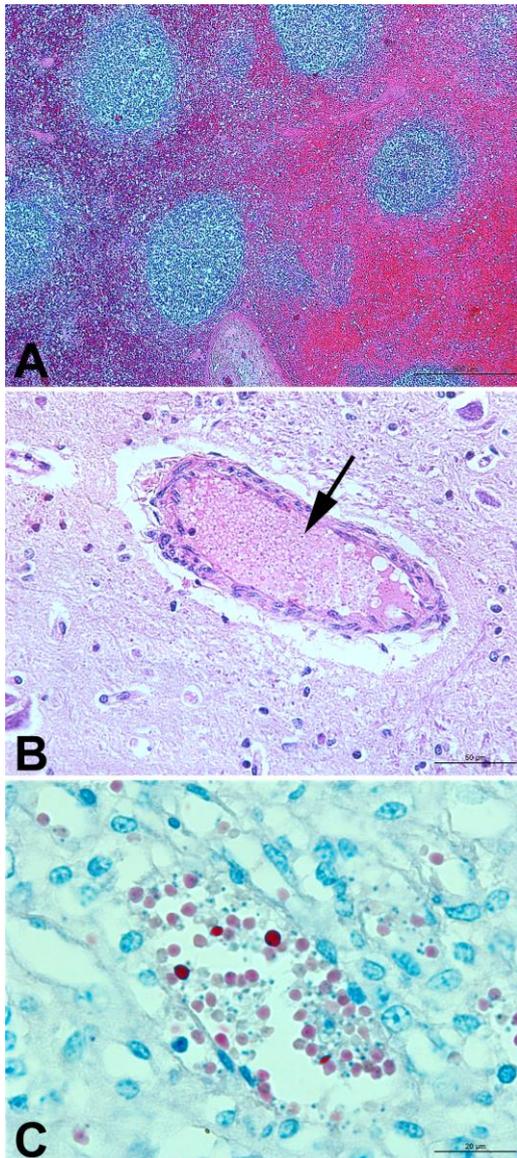


Figure 2. Aborted equine fetus, *Theileria equi* infection. A. Spleen: follicular lymphoid hyperplasia. HE, Bar = 500 µm. B. Brain cortex: a blood vessel is filled with erythrocytes containing merozoites (arrow). HE, Bar = 50 µm. C. Kidney: merozoite-infected erythrocytes within a blood vessel. Giemsa stain, Bar = 20 µm.

Additional samples of liver, spleen, kidney, and thymus were stored in microtubes at -80°C for further molecular analysis. Tissue grinding and DNA extraction for PCR analysis were performed according to the guanidine thiocyanate protocol (Pitcher *et al.*, 1989). Primers targeted the piroplasm 18S

ribosomal RNA gene region 5'-AATACCCAATCCTGACACAGGG-3' and anti-sense 5'-TTAAATACGAATGCCCCCAAC-3'. 1µL (10mM) of each primer was added to the reaction mixture containing 20µL of supermix (Invitrogen, Brazil) and 250ng of DNA. Amplification parameters were the following: 95°C for 10 minutes; 35 cycles of denaturation at 95°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds followed by a final extension step at 72°C for 7 minutes to generate a 400bp product.

All tissue samples were positive for piroplasm DNA by PCR (Figure 3). DNA from a spleen sample was purified and then sequenced by capillary electrophoresis using an ABI3730 automatic sequencer (Applied Biosystems®). Sequencing analysis of the amplicon revealed 98% identity to *T. equi* sequences available in GenBank under Accession No. KX722520. A definitive diagnosis of theileriosis was made based on positive *T. equi* PCR results.

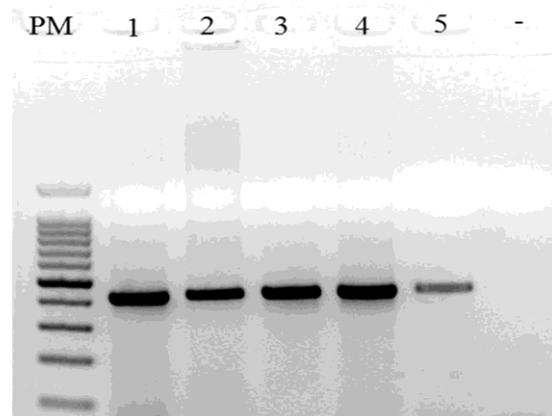


Figure 3. PCR for the detection of piroplasms in tissue samples of the equine aborted fetus. Generic primers targeted the piroplasm ribosomal 18S region. Products of the expected size of approximately 400 bp. (MW) Molecular weight marker; (1) Positive control (2) PCR product amplified from DNA of spleen from the equine aborted fetus; (3) PCR product amplified from DNA of liver from the equine aborted fetus; (4) PCR product amplified from DNA of kidney from the equine aborted fetus; (5) PCR product amplified from DNA of thymus from the equine aborted fetus; (-) Negative control.

DISCUSSION

Equine piroplasmosis is endemic in Brazil including in the State of Espírito Santo (Spolidorio *et al.*, 2010; Wise *et al.*, 2013). However, according to the owner, no tick vectors were found infesting animals from this farm. During acute theileriosis, affected horses may develop fever, anemia, jaundice, weakness, haemoglobinuria, and death. Animals that recover from an acute *T. equi* infection may become asymptomatic carriers for several years (Wise *et al.*, 2013), playing an important role in the maintenance of this pathogen in endemic areas (Roncati *et al.*, 2011).

Unfortunately, the mare of the present report was not tested for *T. equi* at the time of abortion, due to its asymptomatic state and no suspicion of any hematological disease at that time. Chronic theileriosis results in diverse and mild clinical signs and therefore it may not be clinically diagnosed (Waal, 1992). However, the presence of intraerythrocytic merozoites in placental blood vessels strongly suggests transplacental infection of the equine fetus during parasitemia.

The abortion occurred during the tenth month of gestation. Late-term abortions are uncommon in cases of *T. equi* infection (Sant *et al.*, 2016). Sant *et al.* (2016) reported an abortion rate of 2.7% in mares naturally infected with *T. equi* in Trinidad with abortions usually occurring during the fourth month of gestation.

Sousa *et al.* (2017) describes natural abortion associated with *T. equi* with pathological findings in aborted fetuses that are typical of equine piroplasmosis, including severe jaundice. This clinical sign is also seen in cases of equine neonatal isoerythrolysis and equine abortion due to leptospirosis. Therefore, these two diseases should be considered in the differential diagnosis of equine abortions due to *T. equi* or *B. caballi* in which marked icterus is observed at necropsy (Pescador *et al.*, 2004; Georges *et al.*, 2011; Sousa *et al.*, 2017).

In the present case, there was significant pallor of the carcass of the aborted fetus but no icterus was noted. However, microscopically small amounts of intracytoplasmic brownish pigment were present in the renal tubules and there was also moderate erythrophagocytosis in the spleen

and thymus. These findings are consistent with a hemolytic process.

In conclusion, the present report highlights the fact that laboratory testing for *T. equi* and *B. caballi* should be included in the standard diagnostic workup during the investigation of cases of abortion in horses including those that occur during the final third of pregnancy in regions endemic for piroplasmosis. Even if there is no jaundice in the aborted fetus at necropsy, equine piroplasmosis should still be suspected as a possible cause of abortion. Light microscopy is an efficient tool for the identification of piroplasms in tissues whereas PCR and sequencing are important ancillary tools to determine the protozoan species involved and reach a final etiological diagnosis.

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