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Case Report

Outbreak of Ovine Abortion by Toxoplasmosis in Southeastern Brazil

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Abstract

This study describes an outbreak of *Toxoplasma gondii* abortions in a sheep herd in January/February 2010. An aborted fetus was submitted to the Veterinary Diagnostic Laboratory for post mortem evaluation. Tissue samples were collected and processed by routine histological methods, stained by hematoxylin-eosin and by immunohistochemistry using anti-*T. gondii* and *Neospora caninum* specific antibodies. The 250 sheep herd was reared in a semi-intensive production system and one month before delivery the ewes were confined. Forty out of 100 pregnant ewes aborted in the last month of pregnancy or had stillborns. Serum samples previously collected from some ewes were positive for *T. gondii* by serological test. Histologically, the fetal central nervous system had multifocal necrotic areas surrounded by inflammatory cells, with intralesional cysts positive for *T. gondii* by immunohistochemistry. Based on clinical signs, serology, histopathology and immunohistochemistry results a diagnosis of *T. gondii* abortion was firmed.

Key Words: *Toxoplasma gondii*, abortion, encephalitis, histopathology, immunohistochemistry.

Introduction

Infections by Apicomplexa protozoans have been reported as a common cause of reproductive problems in ovine, particularly those related to *Toxoplasma gondii* and *Neospora caninum* (14, 20). These infections cause great losses in ovine herds (24, 11), and infected sheep can transmit them to humans (12, 9). Toxoplasmosis can be highly prevalent in ovine herds due to sheep low resistance to infection and type of production systems that allow sheep to have access to oocysts eliminated by infected cats (25). Infection by *T. gondii* is generally subclinical or may cause fever in adult non-pregnant ewes (3). Clinical toxoplasmosis occurs when a pregnant ewe is primarily infected, and the tachyzoites enter the bloodstream, multiply in the placentoma, and then infect the adjacent trophoblastic fetal cells. Placental tissue may present characteristic gross lesions that include multifocal areas of white discoloration that represent necrosis (1, 2). Typical clinical signs include abortions, stillbirths, mummified fetuses or delivery of underweight lambs, causing economic losses (11, 28). Abortions may occur sporadically or epidemically within herds, depending on herd immunological status (3, 4, 5). Infected fetuses do not show pathognomonic gross lesions. The brain is the most commonly affected organ, presenting nonsuppurative necrotizing encephalitis. Grossly, multifocal white discoloration areas can be seen in lungs, liver, heart and kidneys. These areas are histologically characterized by necrosis and nonsuppurative inflammatory infiltrates (2, 5). Diagnosis is performed by serology tests, *post mortem* examination of aborted fetuses, histopathology, immunohistochemistry and polymerase chain reaction (PCR) (27, 23). Histologic diagnosis is based on multifocal necrosis and nonsuppurative inflammation on various organs. Cysts in brain and placenta sections are more likely to be seen in aborted fetuses (22), and are often located within areas of necrosis, what makes it difficult to identify (23).

Considering that abortion due to toxoplasmosis is underdiagnosed (7), this study aimed to describe an

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abortion outbreak caused by *T. gondii* in an ovine herd in Minas Gerais State, Brazil.

Case report

One aborted ovine fetus was sent to the Veterinary Pathology Laboratory at the Universidade Federal de Minas Gerais in February, 2010 for *post mortem* examination. The submitted fetus came from a farm located in Serro, Minas Gerais State. The city is located in the Brazil Southeastern region, with altitude of 781m, latitude 18 ° 36 '17 "S and longitude 43 ° 22 '46" W. The Dorper-Santa Inês mixed breed ovine herd had experienced an outbreak of reproductive problems. There were approximately 250 animals of various ages and both sexes, all vaccinated for clostridial diseases, rabies and leptospirosis, raised in a semi-intensive production system, with overnight housing. Ewes were confined 30 days prior to delivery. In January and February 2010, 50% of the 200 ewes were pregnant and confined, 40 of which had abortions or delivered stillborn lambs. At necropsy, the submitted fetus did not have any gross lesions. There was no bacterial growth on the samples, of ruminal contents, liver and lungs, collected for bacteriological tests.

Microscopic examination of the brain, in sections of hippocampus and gray matter, showed multifocal areas of necrosis surrounded by lymphocytes and plasma cells infiltration and multifocal microglial proliferation (Figure 1A). There was deposition of a granular basophilic material interpreted as mineralization and numerous 50-60 μm-diameter cysts with bradyzoites associated with those previously described necrotic areas (Figures 1B and 1C). Some cysts were ruptured and bradyzoites could be seen in surrounding areas. Mild perivascular nonsuppurative cuffs were also observed. Unstained histological sections were submitted to the Animal Pathology Laboratory at UFRGS for immunohistochemistry for which primary polyclonal antibodies against both *T. gondii* and *N. caninum*, at a 1:1000 dilution were use. For immunohistochemistry staining, paraffin tissue slides were deparaffinized with xylene and rehydrated in a graded ethanol series. Endogenous peroxidase was blocked with hydrogen peroxide 10%, diluted in methanol, for 10 minutes. Trypsin 0.1% for 10 minutes at 37°C, followed by three washing with distilled water, and heated twice in microwave for 5 minutes at maximum power in citrate acetate solution, pH 6.0, were used for antigen retrieval. The tissues were incubated for 15 minutes in a blocking buffer of 5% skimmed milk and after washing were incubated overnight at 4°C with *T. gondii* or *N. caninum* primary antibody, diluted 1/1000 in PBS. In the following day the slides were washed and biotinylated secondary antibody (LSAB-HRP Kit, K0690, DakoCytomation®) was used for 20 minutes. All slides were washed and streptavidin-peroxidase complex (LSAB-HRP Kit, K0690, DakoCytomation®) was applied for 20 min at room temperature. After washing with PBS-T, DAB (diaminobenzidin) was used as chromogen for labeling. Brain tissue sections were strongly positive for *T. gondii* (Figure 1D) on immunohistochemistry and mildly positive for *N. caninum*. Samples of heart, lungs, liver, kidney did not have any detected lesions.
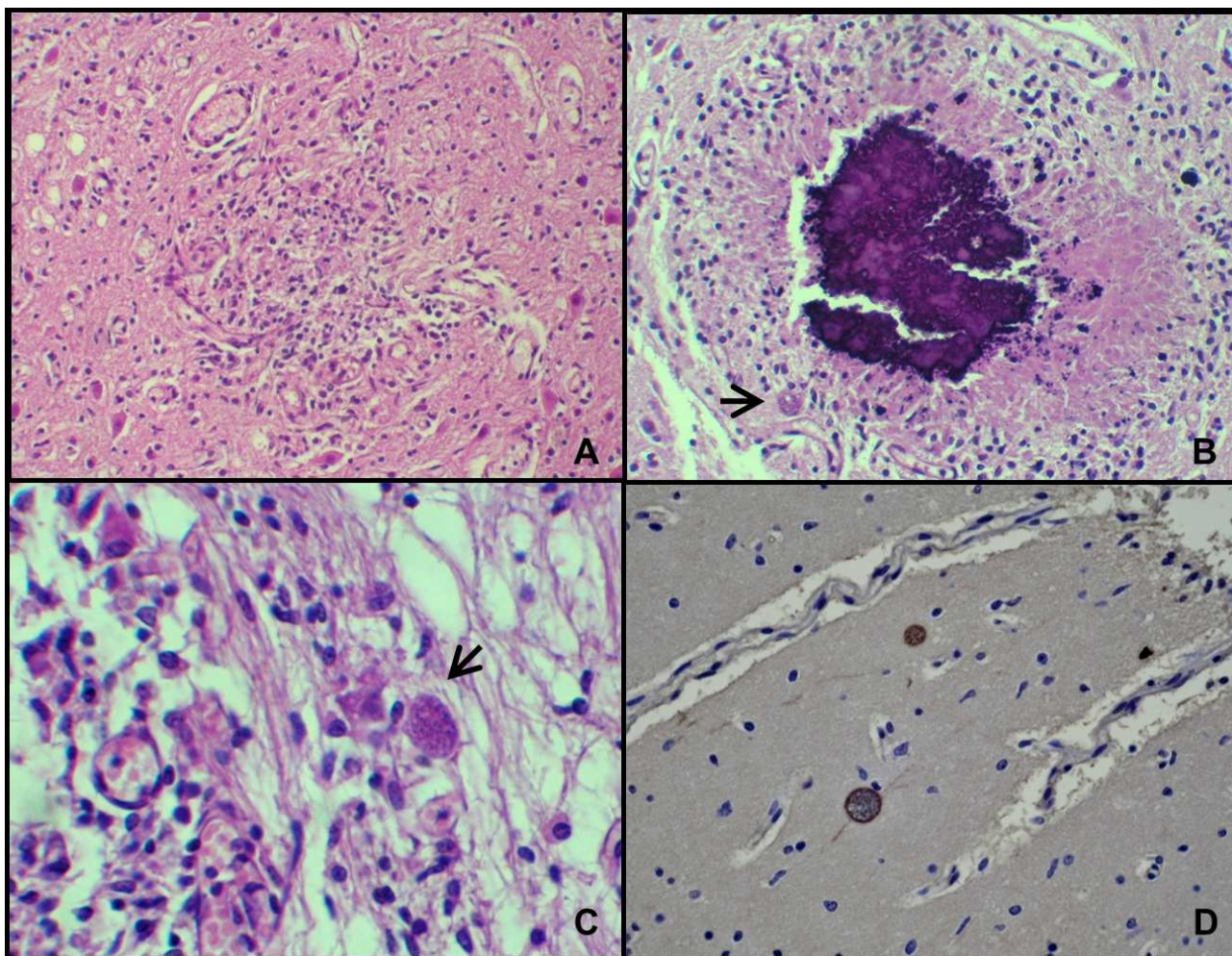
Some epidemiological data was obtained with the responsible veterinarian and the owner of the farm involved. None of the females had been introduced in the herd shortly before the outbreak and there were no relevant cases of reproductive problems in the property before these cases. There were feline in the property, but there was no information about contact between cats and the livestock. During the outbreak, animals were serologicaly tested for brucelosis, leptospirosis, toxoplasmosis and neosporosis, and showed positive results for toxoplasmosis only. The property owner was contacted one year after the diagnosis and did not report any more reproductive problems within the herd.

Discussion

The histological findings in the brain of the fetus were compatible with infection by *T. gondii* or *N. caninum*. Both protozoan species present similar microscopic characteristics in brain sections stained by hematoxylin and eosin (16, 20). Moreno et al. (20) reported prevalence of 6.8% and 5.4% of *N. caninum* and *T. gondii*, respectively, in sheep fetuses with protozoan characteristic lesions, demonstrating the importance of making the differential diagnosis between these two agents. Although both protozoan species cause very similar histologic lesions, they can be easily differentiated antigenically (13, 26). In the present study, sections were markedly stained for *T. gondii* and mildly stained for *N. caninum* on immunohistochemistry, which confirmed *T. gondii* infection. The mild *N. caninum* labeling was considered as cross reaction, since both species are from the same phylum Apicomplexa, and polyclonal antibodies were used for the immunohistochemistry staining (26, 27). In addition, the positive serology for toxoplasmosis and negative serological results for brucellosis, leptospirosis and neosporosis also supported the diagnosis of toxoplasmosis.

Actual losses in lambs due to toxoplasmosis are difficult to estimate because usually few of aborted lambs are submitted to the laboratory, unsuitable materials are sent for diagnosis and toxoplasmosis does not produce clinical disease in ewes (7). In Brazil, seroepidemiological surveys have been conducted on ovine toxoplasmosis (6, 19, 24), but there are few reports using serological analysis with association between histology and immunohistochemistry as a diagnostic tool in aborted fetuses (5, 23). Nevertheless, this disease can be diagnosed more frequently if more suitable samples from aborted lambs are sent for histopathology.

The lesions were observed in the brain,

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Figure 1. Brain, hippocampus, ovine fetus. A: Area of moderate infiltration of glial cells (HE, 40X). B: Area of liquefaction necrosis focally extensive accented with deposition of granular and strongly basophilic material (mineralization), in the middle, surrounded by lymphoplasmocytic infiltration and glial cells, associated with a parasitic cyst (arrow) (HE, 10X). C: Parasitic cyst (arrow) adjacent to areas of necrosis associated with a mild lymphoplasmocytic infiltrate (HE, 100X). D: Positive labelled (brown) parasitic cysts capsule by anti-*T. gondii* antibody immunohistochemistry staining. (DAB, 40X).

especially the hippocampus and cerebral cortex, in agreement with O'Donovan et al. (21). These authors found that lesions associated with *T. gondii* encephalitis occur in greatest numbers at the level of the optic tract, rostral margin of the pons, or 4 mm caudal to the ansate sulcus. The author also mentioned that lesions are absent from the caudal cerebellum. In two studies, Masala et al. (14, 15) reported that *T. gondii* was detected by PCR in tissues of 334 aborted sheep, and the protozoan DNA was more frequently found in muscle and brain samples than abomasum, liver, and spleen. Tachyzoites tropism for blood-brain barrier or blood–cerebrospinal fluid barrier endothelium is currently unknown. However, it has been demonstrated that tachyzoites have varying endothelial tropism depending on the tissue type (21). Important determinants may include local variations in hemodynamic factors, including vascular density and/or tissue-specific endothelial cell surface molecule expression (21), or tachyzoites may infect and exploit monocytes to reach the perivascular space in the brains of mice (4).

The necrosis associated with lymphocytes and plasma cells infiltration suggests that infection occurred after the fetus was able to respond immunologically to infection, which happens around 70 days pregnancy (2). Usually, fetuses get infected 10 to 15 days after the dam, and lesions are apparent 20 days after infection (2). Thus, it is suggested that ewes were infected prior to confinement. Savio and Nieto (24) showed that pregnant ewes in extensive systems had higher seroconversion rates than those in intensive systems.

There is an association between feed and pasture contamination with sporulated oocysts and infection in ovine (10, 25). Sporulation occurs in one to five days in the environment, depending on various humidity and temperature conditions (8). Experimental studies have shown that the minimal infecting dose for pregnant ewes is 200 oocysts (17), and feline feces may

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contain up to 10^5 oocysts per gram (18). There is proven association between ovine toxoplasmosis and the presence of feline in herds with clinical disease (25). According to the owner, there were cats in the farm, but they were not found in contact with females or animals` feed. Thus, the source of contamination may have occurred due to water intake and/or pasture contaminated by cat feces, since the flock has been extensively affected.

The high number of abortions in various aged ewes that were in the herd years before the outbreak suggested the lack of immune response of all animals prior to the outbreak. The positive serology results demonstrated that the infection occurred during pregnancy, particularly in the mid-pregnancy and/or late pregnancy. Ewes seroconverting during pregnancy had lower delivery rates compared to non-infected females or females that were infected prior to conception. In addition to abortion, mummified fetuses or underweight lambs, and infection in early pregnancy ewes cause lamb death and resorption (24). *T. gondii* is not the cause of reproduction problems in ewes after the first infection, and ovine develop long lasting immune response against the protozoan (2). The absence of new cases in the farm in the subsequent year (2010) could be related to solid immune developing. Vaccine that prevents the disease is available only for ewes and current licensed for some countries as New Zealand, France, Portugal, Ireland and the UK. The vaccination, like natural infection, produces a solid immunity (22). In areas where vaccination is not established, it is important to prevent infections, especially during pregnancy. The contact of cats with sheep environment should be avoided, preventing any contact of cats with pasture, feedstuffs and water exposed to herd.

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