

## Ocurrence and characterization of bovine abortion caused by *Brucella abortus* infection in southern Brazil

### Ocurrencia y caracterización del aborto bovino causada por *Brucella abortus* infección en el sur de Brasil

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#### RESUMEN

La brucelosis causada por *Brucella abortus* es una enfermedad zoonótica contagiosa que causa abortos en las vacas, así como alteraciones articulares y reproductivos en los toros. En 469 fetos bovinos abortados analizados en un período de nueve años, nueve (1,92%) se debieron a *B. abortus* infección. El diagnóstico se basó en los estudios de lesiones macroscópicas, histológicas, microbiológicas, serológicas, inmunohistoquímicas y hallazgos moleculares. La edad gestacional de los fetos osciló de 6 a 9 meses. Los principales hallazgos macroscópicos se caracterizaron por una descarga de pus en la cavidad nasal, tráquea y bronquios, y depósitos de fibrina pleural y pericárdico, además de los pulmones se han observado manchas blancas en el parénquima y las zonas rojas en los lóbulos apicales. En la región intercotiledonaria de tres placentas examinadas, hubo áreas enrojecidas intercaladas con zonas redondas de color amarillo-blanco bien definidos, con paredes engrosadas en la superficie de los cotiledones. Microscópicamente se observó bronconeumonía necrosupurativa, neumonía intersticial y placentitis necrosupurativa con áreas de vasculitis, la mineralización, y la trombosis. *Brucella abortus* fue aislado de los tejidos fetales de siete de los nueve casos investigados. La prueba de inmunohistoquímica fue positiva en cuatro casos, y la PCR amplifica el material genético de las bacterias en tres. La infección con *B. abortus* tiene que ser considerado como una posible causa de aborto en el ganado en el sur de Brasil.

*Palabras clave:* brucelosis, diagnóstico, ganado, fetos bovinos.

#### SUMMARY

Brucellosis caused by *Brucella abortus* infection is a contagious zoonotic disease that causes abortions in cows, as well as joint and reproductive alterations in bulls. This study reports that eight (1.92%) out of a total of 469 aborted bovine fetuses analysed in a time frame of nine years were caused by *B. abortus* infection. The diagnosis was based on macroscopic, histological, microbiological, serological, immunohistochemical, and molecular findings. The gestational age of the fetuses studied ranged from 6 to 9 months. The main gross findings were purulent discharge from the nasal cavity, trachea and bronchi; pleural and pericardial fibrin deposits; and non-collapsed lungs with white spots in the parenchyma, and red areas in the apical lobes. Intercotyledonary areas of three placentas examined exhibited reddened areas, and the cotyledons exhibited well-defined, yellowish-white round areas with thickened walls on their surface. Microscopically, the most frequently observed lesions were necrosuppurative bronchopneumonia, interstitial pneumonia, and necrosuppurative placentitis with areas of vasculitis, mineralization, and thrombosis. *Brucella abortus* was isolated from fetal tissues of seven of the nine cases investigated. Immunohistochemical tests were positive in four cases, and PCR detected *B. abortus* genetic material in three cases. Infection by *B. abortus* should be considered a possible cause of abortion in cattle in southern Brazil.

*Key words:* brucellosis, diagnosis, cattle, bovine fetuses.

#### INTRODUCTION

Brucellosis is a contagious and generally chronic zoonosis from the mononuclear phagocyte system; it causes abortion in the last trimester of pregnancy as well as articular and reproductive changes in males (Paulin 2003). In bovines, this disease is caused by *Brucella abortus*, a Gram-negative, non-spore-forming, non-encapsulating rod or coccoid rod that is preferentially intracellular (Paulin

2003). Brucellosis has considerable socioeconomic importance owing to its implications for public health, and also because of economic losses caused by reproduction problems or imposition of sanitary barriers (Brasil 2001, Paulin 2003). Brucellosis is reported to have low prevalence in cattle herds worldwide (Godfroid *et al* 2004, Schlafer *et al* 2007). However, in some countries where brucellosis is endemic and not controlled, prevalence may vary between 20% and 30% (Godfroid *et al* 2004). In Brazil, the endemic status of this disease is stable, with higher prevalence in regions where animal density is higher (Brasil 2001).

Environmental contamination with membranes and fetal fluids as well as vaginal discharges of infected

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females is the main infection source of transmission for bovines (Godfroid *et al* 2004, Schlafer *et al* 2007). In pregnant females, *B. abortus* reaches the endometrium and the placenta through the blood during bacteremia, and reproduces in trophoblastic cells (Meador and Deyoe 1989), causing necrotic pancreatitis which eventually leads to abortion (Paulin 2003, Godfroid *et al* 2004, Schlafer *et al* 2007). Fetal alterations, when present, may include bronchopneumonia (Lopez *et al* 1984, Schlafer *et al* 2007), meningitis (Hong *et al* 1991), vasculitis (Schlafer *et al* 2007), thrombosis in lymph and blood vessels (Lopez *et al* 1984), and formation of giant cell granulomas in several organs (Schlafer *et al* 2007).

The diagnosis of abortion by *B. abortus* may be based on the presence of macroscopic and histological lesions, bacterial isolation (mainly from abomasal contents), immunofluorescence, immunohistochemistry (Schlafer *et al* 2007), or polymerase chain reaction (PCR) (Cortez *et al* 2006).

The objectives of the present study were to estimate the occurrence of abortions caused by *B. abortus* in bovines in southern Brazil, and to characterize macroscopic, histopathological, bacteriological, immunohistochemical, and PCR findings in the aborted fetuses.

## MATERIAL AND METHODS

In a period of eight years, 469 aborted bovine fetuses were submitted for diagnosis to the Veterinary Pathology Service (SPV), Federal University of Rio Grande do Sul (UFRGS), Brazil. All fetuses were from southern Brazil. Abortion was defined as spontaneous termination of pregnancy between 42 and 260 days. Fetuses were necropsied, and age was estimated based on crown-rump length (Barr *et al* 1990). Tissue fragments from fetuses and placentas, when available, were collected and fixed in 10% buffered formalin, processed for histological examination, and stained with hematoxylin and eosin following standard procedures. Kidney sections were analyzed by direct immunofluorescence to detect *Leptospira* spp. using multivalent fluorescent antibody conjugates at a dilution of 1:20 (Miller *et al* 1989). Liver and lung specimens and abomasal contents were collected for aerobic bacterial culture and isolation of *B. abortus*. Microaerophilic growth was attained by culture in blood agar supplemented with 7% sheep blood under incubation for 48 h. *Brucella abortus* was isolated using Brucella Agar supplemented with specific antimicrobial agents. The genus was identified based on the characteristics of culture morphology, biochemistry and color (Bathke 1988).

Immunohistochemical analysis to detect *B. abortus* was carried out whenever fetuses exhibited signs of pneumonia, meningitis and placentitis. The polyclonal antibodies used for immunohistochemical assays were produced at SPV-UFRGS by inoculation of the antigen in rabbits. To this end, a 1 mL dose of live *Brucella abortus* (B19)

commercial vaccine was subcutaneously administered in rabbits. Forty days later, sera were collected, and the IgG fraction was purified using an affinity chromatography column (GE Healthcare). For immunohistochemistry, the primary antibody was diluted 1:500, added to the specimens, and incubated for 60 min at 37 °C. A proteinase K solution (Dako) was used to retrieve the antigen (10 min), and non-specific nuclear staining was blocked by using 5% (w/v) nonfat dry Milk (Molico™) (15 min). Samples were incubated with a biotinylated secondary antibody for 15 min at room temperature. An avidin-phosphatase conjugate solution (LSAB + AP kit) (Dakocytomation) was added to the samples, which were then incubated for 15 min at room temperature. Bio Red (Biopath) was used as the chromogen; samples were incubated with it for 5 to 10 minutes, and counterstained with hematoxylin for 1 min. Thymus and central nervous system fragments were subjected to immunohistochemical analysis for bovine viral diarrhea (BVD) using the anti-BVDV monoclonal antibody (15C5 Syracuse, USA) as described by Schmitz (2006).

PCR was used to detect *B. abortus* in samples of fetuses with lesions indicative of infection by this bacterium, or in samples from which *B. abortus* isolation was successful. DNA was extracted from 25 mg samples from 10 mm paraffin-embedded tissue specimens (Garmatz *et al* 2004). PCR was performed using specific primers (5' -GAC GAA CGG AAT TTT TCC AAT CCC-3' and 5' -TGC CGA TCA CTT AAG GGC CTT CAT TGC CAG-3') as described by Baily *et al.* (1992). The PCR protocol used consisted of an initial denaturation step at 94 °C for 5 min, 30 cycles of denaturation (94 °C, 30 s), annealing (53 °C, 30 s) and extension (72 °C, 5 s), and a final extension step at 72 °C for 5 min. The reaction amplified 498 bp fragments, which were electrophoresed in 1% agarose gels. The gels were electrophoresed in TBE 0.5 X buffer (0.045 M TRIS/Borate and 1 mM EDTA pH 8.0). The amplified fragments were observed under UV light, and then photographed for subsequent examination. A suspension of the B19 vaccine was used as a positive control in PCR reactions; negative controls consisted of PCR reactions without any DNA, but with ultrapure water instead.

Abortion was defined as caused by *B. abortus* infection when fetuses exhibited lesions compatible with those caused by this pathogen, and results of at least one of the assays carried out to detect this agent (bacterial isolation, immunohistochemistry, and PCR) were positive.

## RESULTS

Macroscopic, histological, microbiological, serological, immunohistochemical, and molecular findings revealed that, of the 469 aborted fetuses examined in a period of eight years, nine (1.96%) were consequent to *B. abortus* infection (Table 1). Gestational age of aborted fetuses varied from 6 to 9 months. All fetuses were from nine farms located in southern Brazil. The history of previous vaccination

**Table 1.** Microaerophilic growth (isolation), immunohistochemistry, and PCR results of *Brucella abortus* in aborted bovine fetuses. Resultados del crecimiento en condiciones de microaerofilia, inmunohistoquímica y PCR para *Brucella abortus* en fetos bovinos abortados.

Fetuses	Isolation			Immunohistochemistry			PCR			
	Lung	Liver	AC	CNS	Lung	Placenta	Lung	Placenta	Liver, kidney, SM	CNS
1	N	N	P	NC	N	NC	N	NC	NC	NC
2	N	N	P	NC	P	NC	N	NC	NC	NC
3	N	N	P	NC	N	NC	N	NC	NC	NC
4	N	N	P	NC	P	NC	N	NC	NC	NC
5	N	N	P	NC	N	NC	P	NC	P	NC
6	N	N	N	NC	N	N	N	P	NC	NC
7	P	P	N	P	P	P	P	P	NC	P
8	N	N	N	NC	P	P	N	N	NC	NC
9	P	N	P	NC	N	N	N	N	NC	NC

N: Negative; P: Positive; AC: Abomasal content; CNS: Central nervous system; SM: Skeletal muscle; NC: Not conducted.  
N: Negativo; P: Positivo; AC: Contenido del abomaso; CNS: Sistema nervioso central; SM: Músculo esquelético; NC: No realizado.

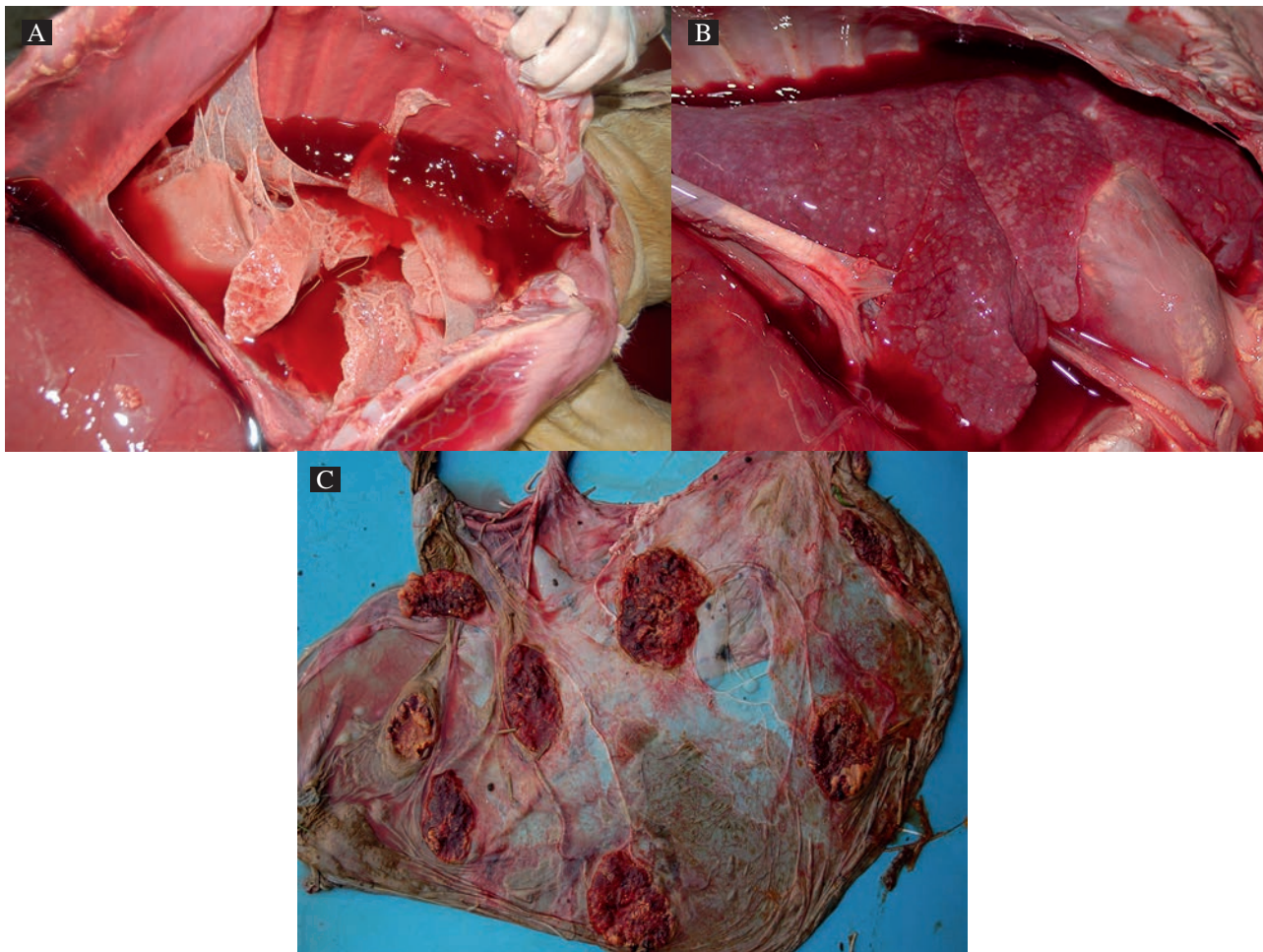
against brucellosis was analysed, and revealed that: (i) in three farms, vaccination was applied appropriately; (ii) in one farm, the age of animals at vaccination was unknown; (iii) in two farms, no vaccination protocol was followed; and (iv) in three farms, vaccination was not mentioned in the paperwork that accompanied the fetuses.

Macroscopic lesions were observed in six of the nine fetuses identified as positive for *B. abortus* infection (Fetuses 2, 4, 5, 7, 8, and 9). These lesions included mucopurulent nasal discharge (also present in the tracheal lumen and bronchi of fetus 8) as well as fibrin deposition on pleural surfaces (Fetuses 7, 8, and 9) and pericardium (Fetus 7) (figure 1A). All fetuses exhibited white spots in the lung parenchyma (figure 1B). Fetuses 1 and 3 exhibited non-collapsed lungs, and reddish areas were seen in the apical lobes of Fetus 9. The liver of Fetus 4 exhibited white areas interspersed with red areas. The liver of Fetus 5 was enlarged and presented a red surface. Hemorrhagic foci were observed on the sublingual surface of the tongue of Fetus 1, in the kidneys of Fetus 2, on the skin and in the spleen of Fetus 4, and in the skeletal muscle tissue of the hindlimbs of Fetus 9. The placentas of only three fetuses (6, 7, and 9) were submitted for analysis. The alterations observed in these placentas included red areas in the intercotyledonary regions (Fetuses 6 and 7), well-outlined yellowish-white circular areas (Fetus 7) (figure 1C), and thickened wall with intensely red, poorly defined areas (fetus 9).

Microscopic changes were present in seven fetuses (1, 2, 3, 4, 5, 7, and 9) and included various degrees of subacute fibrinopurulent pleuritis (Fetuses 1, 2, 3, 4, 5, and 7), severe diffuse fibrinopurulent pericarditis (Fetus 7), subacute necrosuppurative bronchopneumonia (Fetuses 4, 5, and 9) (figure 2A), fibrinopurulent bronchopneumonia (Fetus 8), and subacute mononuclear interstitial pneumonia (Fetuses 1 and 2). Mononuclear vasculitis was diagnosed in the lungs (Fetuses 3, 4, and 5) and skeletal muscle (Fetus

4). Mononuclear hepatitis was present in Fetuses 3, 4, and 9 (in the latter, it was accompanied by necrotic foci). Severe multifocal mononuclear meningitis accompanied by multifocal perivascular cuffing was detected in the brain of Fetus 7 (figure 2B). In Fetus 8, the thymus exhibited a diffuse mononuclear inflammatory infiltrate which was more pronounced in trabeculae, and fibrin thrombi. Mild focal mononuclear myositis and multifocal areas with marked hyaline degeneration were detected in the skeletal muscle of Fetus 9. Focal hemorrhagic infarction was observed in the spleen of Fetus 3. Mononuclear and polymorphonuclear inflammatory cells were observed in vessels of organs such as brain and lungs as well as in skeletal muscles of all fetuses, except in Fetuses 6 and 8. Congestion areas were noticed in kidneys, liver, spleen (Fetus 8) and thymus (Fetuses 8 and 9). Placental lesions included subacute mononuclear placentitis accompanied by necrosuppurative vasculitis (figure 2C), areas of mineralization, thrombosis, presence of structures compatible with bacteria inside of trophoblasts (Fetus 6), mild suppurative perivascularitis (Fetus 7), and moderate necrosuppurative placentitis (Fetus 9).

*Brucella abortus* was isolated from seven fetuses (1, 2, 3, 4, 5, 7, and 9). This bacterium was isolated from abomasal contents in five fetuses (Fetuses 1, 2, 3, 4, and 5), from lung and liver fragments in one fetus (Fetus 7), and from abomasal contents and lung fragments in one fetus (Fetus 9). Positive immunohistochemical staining for *B. abortus* was observed in Fetuses 2, 4, 7, and 8. Bacterial antigens were detected inside mononuclear inflammatory cells in lung alveoli and cell debris in Fetus 2, and in necrotic foci in the lungs of Fetuses 4, 7, and 8 (figure 2A). Positive staining was also observed in mononuclear inflammatory cells and in the lumen of blood vessels of the meninges (Fetus 7) and placenta (Fetuses 7 and 8) (figure 2B and 2C). PCR detected *Brucella abortus* genetic material in tissues of Fetuses 5 and 7, and in the placenta of Fetus



**Figure 1.** Aborted bovine fetuses infected with *Brucella abortus*. A. Intense fibrin deposition on the pleural and pericardial surfaces, and purulent fluid in the thoracic cavity. B. White spots in the lung parenchyma measuring between 0.1 mm and 0.5 mm in diameter, and purulent fluid in the thoracic cavity. C. Placenta exhibiting red areas in the intercotyledonary regions, and well-defined yellowish-white round areas on the cotyledonary surface.

Fetos bovinos abortados infectados con *Brucella abortus*. A. Intenso depósito de fibrina en la superficie pleural y pericárdico, y líquido supurativo en la cavidad torácica. B. Manchas blancas en el parénquima pulmonar entre 0,1 mm y 0,5 mm de diámetro, y fluido supurativo en la cavidad torácica. C. Placenta mostrando zonas rojas de la región intercotiledonaria y zonas bien delimitadas circunscritas amarillo pálido en la superficie cotiledonaria.

6. Direct immunofluorescence for *Leptospira* spp. and immunohistochemistry for bovine viral diarrhoea (BVD) yielded negative results". Table 1 shows a summary of the results of microbiological, immunohistochemical, and PCR analyses.

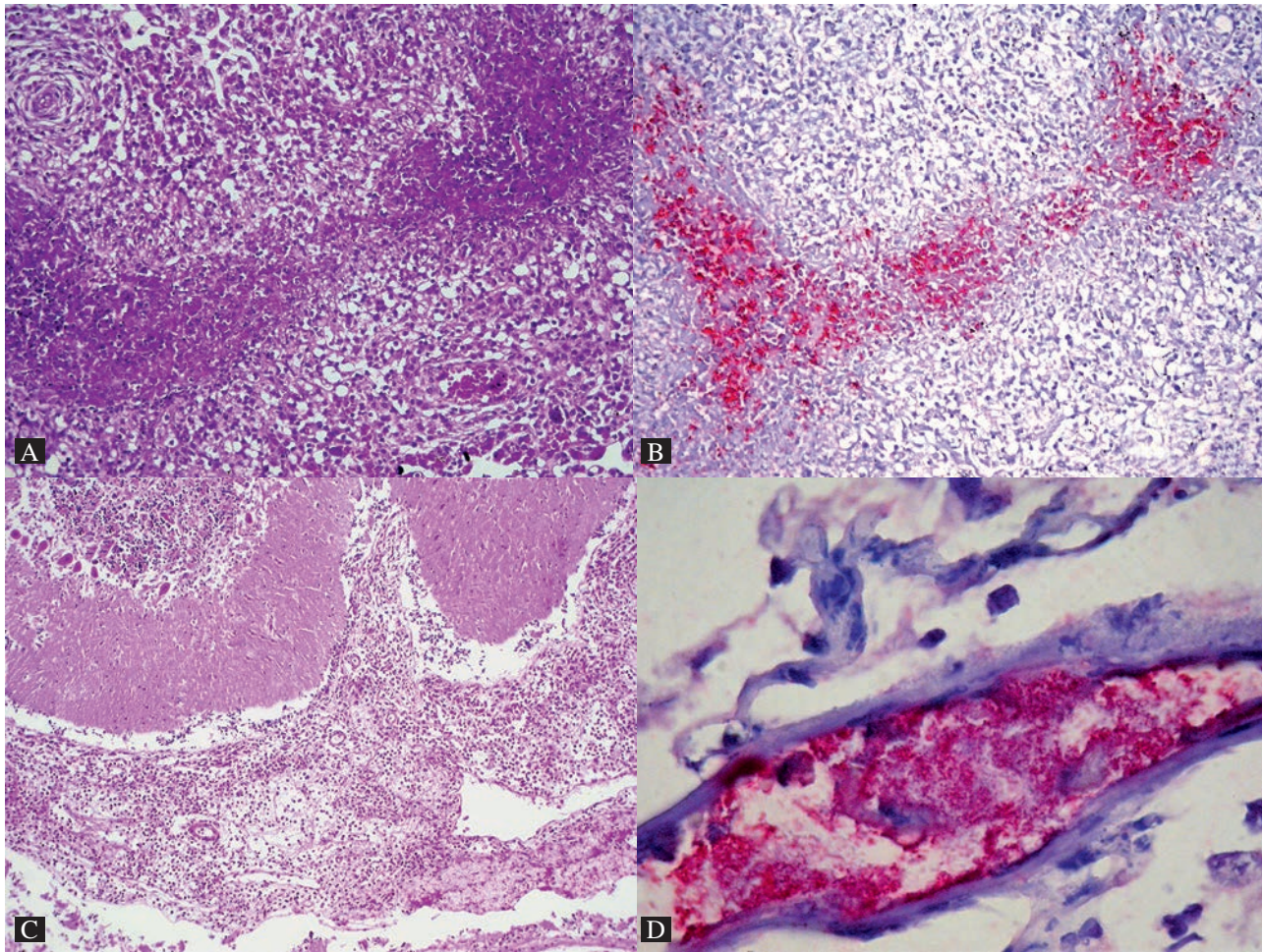
## DISCUSSION

The prevalence of *B. abortus* infection in aborted bovine fetuses analysed at SPV-UFRGS in a period of eight years was 1.92%. Data regarding the prevalence of this pathogen in aborted fetuses are scarce in the literature, and the variation in published results can be a result of the different diagnostic methods used across studies. In Argentina, Campero *et al* (2003) diagnosed infection by *B. abortus* in 7.9% of 354 fetuses. In Brazil, Cortez *et al*

(2006) detected the presence of *Brucella* spp. DNA in 13.7% of 124 samples analyzed using PCR. Likewise, *B. abortus* was isolated from 70 (5.56%) of 1,259 aborted fetuses using bacteriological methods (Genovez *et al* 2006).

In the present study, gestational age of fetuses varied between 6 and 9 months. This finding confirms previous reports on the occurrence of abortion at the end of pregnancy in bovines infected with *B. abortus* (Godfroid *et al* 2004, Schlafer *et al* 2007). Other cases of abortion caused by *B. abortus* had been reported in all farms where the fetuses analysed here had come from, showing that these were not isolated events, and that the cases investigated here may represent only a small fraction of the actual problem in a large-scale production system.

The diagnosis of *B. abortus* as the causative agent of abortions was based on macroscopic and histological



**Figure 2.** Histology sections from aborted bovine fetuses infected with *Brucella abortus*. A. Lung, acute multifocal necrosuppurative bronchopneumonia (100x magnification, HE). B. Lung, red immunohistochemical staining of *B. abortus* bacterial antigens in necrotic foci (100x magnification). C. Cerebellum, focally extensive granulomatous meningitis (100x magnification). D. Placenta, red immunohistochemical staining of *B. abortus* bacterial antigens in blood vessel (400x magnification, HE).

Secciones histológicas de bovinos abortados infectados con *Brucella abortus*. A. Pulmón, bronconeumonía multifocal aguda necrosupurativa (aumento de 100x, HE). B. Pulmón, mediante técnicas de inmunohistoquímica con la marca roja de antígenos bacterianos de *B. abortus* en zonas necróticas (aumento de 10x). C. Cerebelo, meningitis granulomatosa focalmente amplia (aumento de 10x, HE). D. Placenta, mediante técnicas de inmunohistoquímica con la marca roja de antígenos bacterianos de *B. abortus* en vasos sanguíneos.

findings combined with the isolation of the agent in fetal tissues, as well as on immunohistochemical and PCR analyses. The macroscopic and microscopic lesions reported here are compatible with those previously described for abortions caused by *B. abortus* (Lopez *et al* 1984, Meador *et al* 1986, Hong *et al* 1991, Godfroid *et al* 2004, Schlafer *et al* 2007), and are linked to the infection of the fetus by aspiration of contaminated amniotic fluid (Lopez *et al* 1984). However, in some cases, abortion may be a consequence of placentitis caused by brucellosis with or without aspiration of amniotic fluid, and the fetus may not exhibit typical lesions (Lopez *et al* 1984). In such cases, analysis of the placenta is essential for an accurate diagnosis. No alterations were observed in Fetus 6; however, its placenta exhibited not only lesions typically seen in abortion caused

by *B. abortus*, but also had genetic material from this bacterium amplified by PCR, proving that the pathogen was indeed present.

*Brucella abortus* was not isolated from tissues of Fetuses 6 and 8. It has been reported that, even when histological findings are present, the pathogen may not be isolated from fetal tissues owing to inappropriate isolation procedures; additionally, these lesions can be caused by bacteria other than *B. abortus* (Lopez *et al* 1984). In these two fetuses, diagnosis was confirmed by PCR of placental tissues (fetus 6) and immunohistochemical analysis of lung and placental tissues (Fetus 8).

Several authors have reported an association between *B. abortus* infection and macroscopic alterations such as subcutaneous and skeletal muscle edema, as well as

**Table 2.** Description of macroscopic and microscopic alterations observed in placentas and aborted fetuses with infection by *Brucella abortus*, examined at SPV-UFRGS.

Descripción de alteraciones macroscópicas y microscópicas observadas en placentas y fetos abortados infectados por *Brucella abortus*, examinados en SPV-UFRGS.

Fetus	Macroscopic alterations	Microscopic alterations
1	Lung: not collapsed.	Lung: marked, diffuse, fibrinpurulent pleuritis. Interstitial mononuclear subacute pneumonia with the presence of fibrin; mononuclear inflammatory cells within the vessels. CNS: mononuclear inflammatory cells within the vessels.
2	Lung: diffuse white spots on the surface and parenchyma.	Lung: moderate subacute fibrinpurulent pleuritis; subacute mononuclear interstitial pneumonia with fibrin; cellular debris within bronchi. CNS: mononuclear inflammatory cells within the vessels.
3	Lung: not collapsed. Brain: Moderate congestion.	Lung: moderate multifocal fibrinpurulent pleuritis, severe multifocal mononuclear vasculitis; Liver: mild focal mononuclear hepatitis. CNS: mononuclear inflammatory cells within the vessels; moderate congestion. Spleen: acute, extensive, focal hemorrhagic infarction. Skeletal muscle: polymorphonuclear inflammatory cells within the vessels.
4	Lung: diffuse white spots on the surface and parenchyma. Skin: hemorrhagic areas. Liver: red areas interspersed with whitish areas. Spleen: diffuse bruising.	Lung: mild multifocal subacute fibrinpurulent pleuritis; severe multifocal subacute necrosuppurative bronchopneumonia, mild multifocal mononuclear vasculitis; mononuclear inflammatory cells within the vessels. Liver: moderate, focal, mononuclear hepatitis. Skeletal muscle: mild multifocal mononuclear vasculitis. CNS: inflammatory mononuclear cells in blood vessels.
5	Lung: whitish areas of firm consistency interspersed with reddish areas.	Lung: moderate diffuse subacute fibrinpurulent pleuritis; marked diffuse necro-suppurative bronchopneumonia; acute to subacute multifocal lymphocytic vasculitis; large amount of mononuclear inflammatory cells inside vessels. CNS: mononuclear inflammatory cells within the vessels.
6	Placenta: reddened areas on intercotyledonary surface.	Placenta: marked diffuse subacute mononuclear placentitis with areas of calcification and necro-moderate multifocal suppurative vasculitis; presence of similar structures bacteria inside the cells. trophoblast; thrombi in vessels.
7	Pericardium and pleura: marked fibrin deposition. Placenta: reddened areas on intercotyledonary surface with yellowish-white circumscribed areas.	Lung: marked diffuse purulent pleuritis; Presence of inflammatory cells within the vessels. CNS: multifocal perivascular cuffing; moderate extensive focal purulent meningitis. Placenta: mild purulent perivasculitis. Heart: marked diffuse fibrinpurulent pericarditis.
8	Nasal and tracheal mucopurulent secretion. Pleura: mild fibrin deposition. Lung: white spots randomly distributed throughout the surface and parenchyma.	Lung: marked fibrinpurulent bronchopneumonia and fibrin thrombi. Liver: marked diffuse congestion. Rim: severe congestion and diffuse hemorrhage. Thymus: diffuse acute mononuclear inflammatory infiltrate in trabeculae, fibrin, and moderate diffuse congestion. Spleen: marked diffuse congestion.
9	Lung: red areas in the apical lobes and white dots on the surface and parenchyma. Skeletal muscles of the right hind limb: hemorrhagic focus; Placenta: thick, intensely red.	Lung: marked diffuse necrosuppurative bronchopneumonia. Placenta: moderate necrosuppurative placentitis. Liver: moderate multifocal hepatitis and focus of necrosis. CNS: polymorphonuclear cells inside vessels and moderate congestion. Skeletal muscle: mild focal polymorphonuclear infiltrate and multifocal areas of severe hyaline degeneration. Thymus: Moderate congestion.

sero-hemorrhagic lesions in the thoracic cavity (Hong *et al* 1991, Godfroid *et al* 2004, Schlafer *et al* 2007). However, these alterations are observed in essentially all aborted fetuses, independent of cause of abortion, which indicates that they may be a consequence of *post mortem* processes. The fact that fetuses 1 and 3 exhibited non-collapsed lungs suggests increased fetal breathing owing to anoxia caused by necrotic placentitis. The hemorrhagic foci observed in

various organs were also attributed to blood stasis owing to placental lesions.

Mononuclear and polymorphonuclear inflammatory cells observed in blood vessels of various tissues are commonly diagnosed in abortions caused by infections, and were detected in almost all fetuses examined here, except for fetuses 6 and 8. These cells may be linked to a cell response of the fetus against infection.

Positive immunohistochemical staining for *Brucella abortus* in organs such as lung, and the meninges placenta was observed (fetal 2, 4, 7 and 8). Antigen labeling in the cytoplasm of chorionic epithelial cells and of phagocytic cells demonstrates the intracellular location of *Brucella* spp. in these host cells, although positive labeling is also observed inside vessels and cell debris of necrotic foci (Meador *et al* 1986, Perez *et al* 1998). Immunohistochemical assays are highly specific to detect *B. abortus* antigens, although the sensitivity of this technique is low (Meador *et al* 1986, Perez *et al* 1998). Negative staining, even in cases in which bacterial isolation from tissues is successful, may be a result of low concentration of microorganisms in these samples. A minimum number of colony forming units, or at least accumulation of microorganisms on the tissue section, are necessary for the chromogen to reveal the pathogen (Meador *et al* 1986).

*Brucella abortus* genetic material was amplified by PCR in samples of placenta and/or fetal tissues in three cases only. In spite of the high sensitivity of the technique, some factors may affect results, such as sample preservation and storage conditions. Also, false negative results may be due to the presence of genetic material below the detection limit of the PCR strategy used, or to prolonged sample storage in formaldehyde, which can damage DNA. Accordingly, PCR protocols may be used as a complementary diagnostic tool, since the concomitant use of several diagnostic methods increases the odds of effectively detecting a pathogen (Silva *et al* 2009). Immunohistochemistry and PCR may be useful for the diagnosis of *B. abortus* when bovine fetal tissues are not available for bacterial cultivation, especially when placental tissues are available, since the concentration of contaminating microorganisms in the placenta is higher. However, observation of histological alterations both in the fetus and in the placenta, in addition to detection of the presence of *B. abortus* by immunohistochemistry, bacterial isolation, or PCR, is essential for the diagnosis of brucellosis abortion.

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## REFERENCES

Baily GG, JB Krahn, BS Drasar, NG Stoker. 1992. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *J Trop Med Hyg* 95, 271-275.

- Barr CB, ML Anderson, PC Blanchard, BM Daft, H Kinde, PA Conrad. 1990. Bovine fetal encephalitis and myocarditis associated with protozoal infections. *Vet Pathol* 27, 354-361.
- Brasil. 2001. Regulamento Técnico do Programa Nacional de Controle da Brucelose e da Tuberculose Animal Departamento de Defesa Animal, Secretaria de Defesa Agropecuária, Ministério da Agricultura e do Abastecimento, Brasília, Brasil.
- Bathke W. Brucellosis. 1988. *Doenças infecciosas em animais domésticos: doenças causadas por vírus, clamídias, rickettsiose, micoplasmose*. Roca, São Paulo, Brazil.
- Campero CM, DP Moore, AC Odeon, AL Cipolla, E Odriozola. 2003. Aetiology of bovine abortion in Argentina. *Vet Res Commun* 27, 259-269.
- Cortez A, AMG Castro, MB Heinemann, RC Soares, RC Leite, E Scarcelli, ME Genovez, AA Alfieri, LJ Richtzenhain. 2006. Detecção de ácidos nucléicos de *Brucella* spp, *Leptospira* spp, herpesvirus bovino e vírus da diarreia viral bovina, em fetos bovinos abortados e em animais mortos no perinatal. *Arq Bras Med Vet Zootec* 58, 1226-1228.
- Garmatz SL, LF Irigoyen, RR Rech, CC Brown, J Zhang, CSL Barros. 2004. Febre catarral maligna em bovinos no Rio Grande do Sul: transmissão experimental para bovinos e caracterização do agente etiológico. *Pesq Vet Bras* 24, 93-106.
- Genovez ME, E Scarcelli, MV Cardoso, RM Piatti, FR Campos, SR Teixeira, V Castro, TB Gotti, CA Batista. 2006. Main bacterial agents as cause of abortion in bovines in Brazil. *XXIV World Buiatrics Congress*, Nice, France.
- Godfroid J, PP Bosman, S Herr, GC Bishop. 2004. Bovine brucellosis. In: Coetzer JAW, Tustin RC (eds). *Infectious Diseases of Livestock*. Vol 3. 2<sup>nd</sup> ed. Oxford University Press, Cape Town, South Africa, Pp 1510-1527.
- Hong CB, JM Donahue, RC Giles, JKB Poonacha, PA Tuttle, NF Chevillie. 1991. *Brucella abortus*-associated meningitis in aborted bovine fetuses. *Vet Pathol* 28, 492-496.
- Lopez A, F Hitos, A Perez, RR Navarro-Fierro. 1984. Lung lesions in bovine fetuses aborted by *Brucella abortus*. *Can J Comp Med* 48, 275-277.
- Meador VP, LB Tabatabai, WA Hagemoser, BL Deyoe. 1986. Identification of *Brucella abortus* in formalin-fixed, paraffin-embedded tissues of cows, goats and mice with an avidin-biotin-peroxidase complex immunoenzymatic staining technique. *Am J Vet Res* 47, 2147-2150.
- Meador VP, BL Deyoe. 1989. Intracellular localization of *Brucella abortus* in bovine placenta *Vet Pathol* 26, 513-515.
- Miller DA, MA Wilson, CA Kirkbride. 1989. Evaluation of multivalent leptospira fluorescent antibody conjugates for general diagnostic use. *J Vet Diagn Invest* 1, 146-149.
- Paulin LM. 2003. Brucelose. *Arq Inst Biol* 70, 239-249.
- Perez J, M Quezada, J Lopez, O Casquet, MA Sierra, JM Mulas. 1998. Immunohistochemical detection of *Brucella abortus* antigens in tissues from aborted bovine fetuses using a commercially available polyclonal antibody. *J Vet Diagn Invest* 10, 17-21.
- Schlafer DH, RB Miller. 2007. Female genital system. In: Maxie MG, Jubb KV, Kennedy PC, Palmer N (eds). *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 3. 5<sup>th</sup> ed. Saunders Elsevier, Philadelphia, USA, Pp 429-564.
- Schmitz M. 2006. Caracterização patológica e imunoistoquímica da infecção pelo vírus da diarreia viral bovina. *MSc thesis*, Faculdade de Medicina Veterinária, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
- Silva TMA, RLG Oliveira, JPS Mol, MN Xavier, TA Paixão, A Cortez, MB Heinemann, LJ Richtzenhain, AP Lage, RL Santos. 2009. Etiologic diagnosis of bovine infectious abortion by PCR. *Cienc Rural* 39, 2563-2570.

