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PATHOLOGY

Bovine abortion due to *Bacillus cereus* in Midwest Brazil

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ABSTRACT: *Bacillus cereus* is a Gram-positive bacterium commonly reported in soils and plants that occupy various ecological habitats, and the main source of contamination for cattle is silage. This report described a case of fetal loss associated with *B. cereus* infection in a cow. An 8-month-old, Nelore female bovine fetus from a beef farm was submitted for necropsy. A gross examination revealed fibrinous pleuropneumonia and fibrin exudation on the liver surface. The morphological diagnosis was restricted to the lungs and liver. In the lungs there was fibrinosuppurative pleuropneumonia associated with numerous aggregates of rod-shaped bacteria. In the liver there was moderate focally extensive fibrinous peri hepatitis.The lungs, liver, thoracic, and abomasal fluid cultures yielded pure cultures of *B. cereus,* indicating that these bacteria should be recognized as a cause of bovine abortion in fetuses that macroscopically present fibrin in the abdominal and thoracic cavity.
Key words: cattle, bacteria, fetal loss, placentitis.

Aborto bovino por *Bacillus cereus* no Centro-Oeste do Brasil

RESUMO: *Bacillus cereus* é uma bactéria Gram-positiva, comumente encontrada em solos e plantas que ocupam diversos habitats ecológicos sendo a silagem a principal fonte de contaminação para bovinos. Este relato descreve um caso de perda fetal associada à infecção por *B. cereus* em uma vaca. Um feto bovino fêmea da raça Nelore, de oito meses de idade, procedente de uma fazenda de corte, foi submetido à necropsia. Ao exame macroscópico observou-se pleuropneumonia fibrinosa e exsudação de fibrina na superfície do fígado. Histologicamente, as lesões estavam restritas aos pulmões e fígado. Nos pulmões havia pleuropneumonia fibrinosupurativa associado a numerosos agregados de bactérias em forma de bastonete. No fígado haviaperi hepatitefibrinosa focalmente extensa moderada. As culturas de pulmão, fígado, líquido torácico e abomasal produziram cultura pura de *B. cereus* indicando que esta bactéria deve ser reconhecida como causa de aborto bovino em fetos que apresentem macroscopicamente fibrina nas cavidades abdominal e torácica.
Palavra-chave: bovino, bactéria, perda fetal, placentite.

INTRODUCTION

Bacillus cereus consists of large, sporulating Gram-positive, rod-shaped aerobic or facultative anaerobic bacteria that are widespread in the environment (LOTTE et al., 2022). Microorganisms are ubiquitous and can contaminate various food products, leading to transient colonization of the human gut (GROSH, 1978). Localized or systemic infections may occur in immunocompromised patients or newborns (premature or full-term), particularly in nosocomial environments. In these groups, *B. cereus* causes various types of infections, including sepsis, septic shock, central nervous system infections, and eye infections (BOTTONE, 2010).

In production animals, *Bacillus* spp. has been considered abortogenic in cattle worldwide (AGERHOLM et al., 1995; SCHUH & WEINSTOCK, 1985; ANDERSON et al., 1990; KIRKBRIDE, 1993; REICHEL et al., 2018); the incidence varies between 1–10%, especially in Europe (AGERHOLM et al., 1995). The ability of *Bacillus* spp. to infect the bovine placenta, resulting in fetal loss, has been studied only for *B. cereus* (WOHLGEMUTH et al., 1972) and *B. licheniformis* (AGERHOLM et al., 1999), which placentitis and abortion can be established following the hematogenous spread of bacteria to the pregnant uterus.

Although, reproductive problems in cows associated with infection of *B. cereus* have been

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reported in several countries (AGERHOLM et al., 1995; SCHUH &WEINSTOCK, 1985, ANDERSON et al., 1990; KIRKBRIDE, 1993; REICHEL et al., 2018), in Brazil, only one recent case of bovine necrotizing placentitis (ROCHA et al., 2021) was reported without fetal assessment. We described the anatomopathologicaland microbiological findings of bovine abortion due to *B. cereus* in Mato Grosso, Brazil, alerting veterinarians to the importance of analyzing fetal tissue.

MATERIALS AND METHODS

In July 2021, a Nelore female bovine fetus was referred for post-mortem examination at the Veterinary Pathology Laboratory of the Federal University of Mato Grosso (UFMT). The herd was composed of 12,000 female beef cattle, grouped in batches of 120–150 animals. The farm had a reproductive loss rate of 14 losses. The farmer could not identify which bovine female the fetal product belonged to. Therefore, he was unable to inform whether the cow had any clinical changes other than the abortion. The animals were maintained on improved pastures (*Brachiaria brizantha*) and received mineral mixture supplementation. The animals in the referred farm have good overall health status and were vaccination against the main reproductive pathogens including bovine herpesvirus type I, bovine viral diarrhea virus, *Brucella abortus* and *Leptospira* spp. Reproductive management was performed using fixed-time artificial insemination, and natural services were used only in empty cows. Pregnancy diagnoses were based on ultrasound examinations performed 30 days after insemination.

The fetus's crown-rump length was 69 cm, consistent with 8 months of gestation. Samples from various organs, including the placenta, were collected and fixed in 10% formalin. Fixed tissues were routinely processed and embedded in paraffin wax, and sections (3–4 µm) were stained with hematoxylin and eosin. Furthermore, the lung, liver, and placental sections were stained using the Brown-Hopps and methenamine silver nitrate (Grocott) method.

Fresh samples were collected for microbiological diagnostic tests. The lung, liver, placental fragments, and abomasal fluid were inoculated with sheep blood on 5% agar and incubated at 37°C for 72 h in an aerobic atmosphere. Furthermore, fresh kidney and liver samples were tested for *Leptospira* spp. using PCR as previously described (AHMED et al., 2012), thymus and spleen samples were tested for pestivirus (BVDV) (VILCEK et al., 1994), lung, liver, spleen and placenta samples for herpes virus type 1 (SILVA et al., 2007) and lung and abomasum content for *Brucella* sp. (SILVA et al., 2009).

RESULTS

An external examination did not reveal abnormalities. At necropsy, gross lesions were observed in the lungs and liver and were characterized by a large amount of serosanguineous fluid in the thoracic cavity associated with moderate fibrin deposition covering the surface of the visceral pleura in the pulmonary cranioventral lobe and the surface of the liver (Figure 1A). Serohemorrhagic fluid was observed within the pericardial sac. The placenta had some cotyledons covered with mildly coagulated blood and grayish white foci in the central area of the surface of the cotyledon (Figure 1B). Histopathological exam of the liver showed moderate fibrin deposition around the Glisson's capsule (Figure 2A). In the lungs surface there is a mild fibrin deposition that covered the visceral pleura (Figure 2B). Moderate multifocal infiltration of neutrophils with fibrin exudate was observed in the alveolar space and interalveolar septa (Figure 2C). Few aggregates of 3–4 µm rod-shaped bacteria were observed in the alveolar space. Mild multifocal suppurative inflammation with necrosis was observed in the placenta. A myriad of extracellular and intracellular Gram-positive bacterial rods was observed in trophoblastic cells. Microscopic lesions were not detected in any other organ. Brown-Hopps staining showed that the bacterial aggregates observed in the lungs (Figure 2D) and placenta were Gram-positive bacilli.

Beta-hemolytic, gray and opaque, irregular, medium-sized colonies were subjected to biochemical tests to identify the microbial species. The isolates were motility +, catalase +, oxidase –, urease –, citrate +, gelatin +, nitrate reduction +, Voges proskauer +, lecithinase +, and esculin hydrolysis+. The isolated bacterium was identified by MALDI-TOF as *B. cereus* (ASSIS et al., 2017). The PCR results were negative for BVDV, *Leptospira* spp, bovine herpesvirus type I and *Brucella abortus*. Samples of placenta, lung and liver were negative in Grocott stain.

DISCUSSION

The etiological diagnosis of bovine abortion is complex. Even in laboratories with experienced

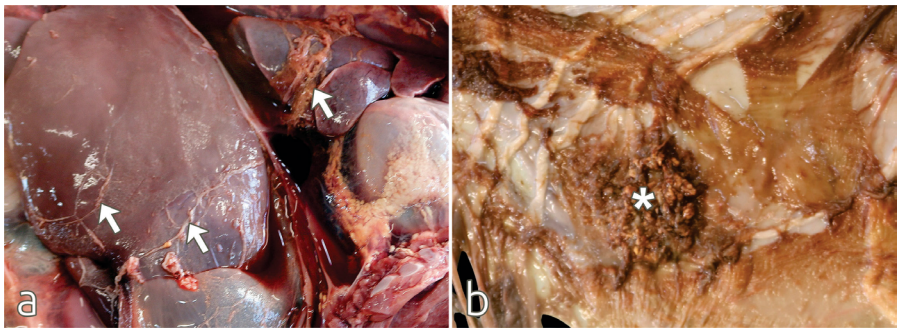

Figure 1 - Bovine abortion is associated with *Bacillus cereus* infection. (A) Gross appearance. The liver and lungs present moderate fibrin deposition (arrow) covering the visceral and pleural surface. (B) Placenta. On the surface of the cotyledon, there are grayish-white foci (asterisk) in the central area.

pathologists that routinely apply a broad panel of ancillary tests, mostly to detect infectious agents, the cause remains undetermined in a large proportion of cases (MEE, 2020). The present diagnosis was made based on bacterial isolation associated with pathological findings and a lack of evidence for other abortogenic agents. *Bacillus cereus* is a public health problem and represents a major economic risk to the food industry (RAMARAO et al., 2020).

In humans the major symptoms of food-poisoning caused by *B. cereus* are divided into either diarrhea or emesis (VIDIC et al., 2020; GRANUM, 2017). Diarrhea is mainly induced by three enterotoxins that belong to the family of pore-forming toxins (PFTs) (DAL PERARO et al., 2016), including non-hemolytic enterotoxin (Nhe) (ZHU et al., 2016; FAGERLUND et al., 2008), hemolysin BL (Hbl) (JESSBERGER et al., 2019) and cytolysin K (CytK) (FAGERLUND et al., 2010), while the emetic syndrome is tightly connected to a lethal toxin known as "cereulide", which is synthesized by a non-ribosomal peptide synthetase (NRPS) encoded by a *ces* gene (EHLING-SCHULZ et al., 2005; LUCKING et al., 2015).

The role of all these toxins in newborn neonate infections has not been described. The potential roles of these toxins have been studied in vitro and in animal models, but they cannot be considered alone as markers of pathogenicity, and the virulence potential of a strain is likely unknown, it is difficult to speculate on the impact of one specific toxin on the outcome of the disease (CORMONTAGNE et al., 2021).

In addition to soil, which is its primary reservoir, *B. cereus* can be isolated from vegetation and water (EHLING-SCHULZ et al., 2019) and can colonize insects and mammals (STENFORS et al., 2008). In the present study, it was not possible to identify the source of contamination in bovine females. However, the farm raises female beef cattle on pasture and provides mineral supplementation, suggesting that the host could be contaminated by spores present in the environment through the ingested pasture. After colonizing the placenta, microorganisms enter the fetus through two routes (MILLER, 1977). The first occurs after microorganisms spread to the amniotic fluid, where fungal or bacterial agents may be inhaled, ingested, or colonized within the skin of the fetus. A second route occurs in specific infections, in which, after colonizing the placenta, microorganisms enter the fetus through the umbilical cord circulation, leading to systemic lesions (MILLER, 1977).

Bacillus cereus may have gained access to the fetus predominantly through the first route, associated with the ingestion of amniotic fluid-containing bacteria after placental dissemination. This is consistent with the fact that fibrinosuppurative bronchopneumonia was a major and consistent finding observed in this fetus associated with mild changes in other organs, suggesting systemic dissemination due to disease progression (CLOTHIER & ANDERSON, 2016). However, some fetuses presented mild changes in other organs, which could indicate that systemic dissemination may occur with disease progression, as previously reported (MILLER, 1977).

In a case series from New York State that investigated 947 cases of bovine abortion from 1978 to 1984, bacterial causes of abortion were diagnosed in 49 (5.6%) cases, of which eight were cases of *B. cereus*

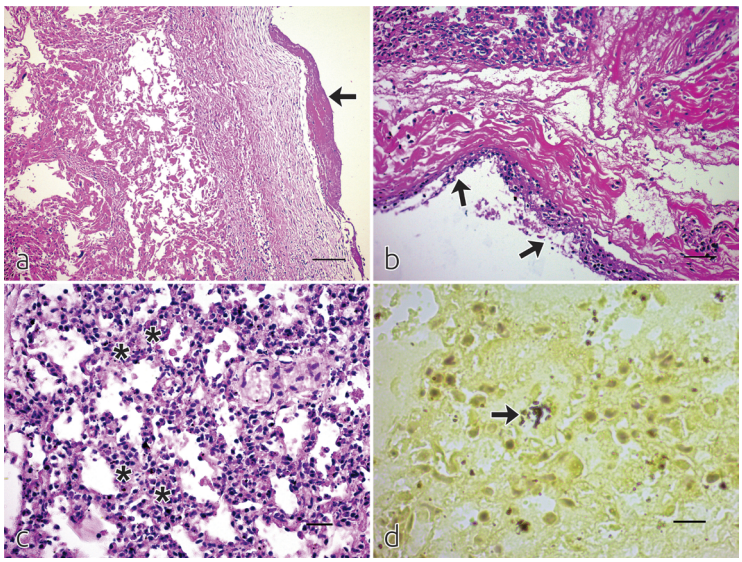

Figure 2 - Bovine abortion is associated with *Bacillus cereus* infection. (A) A moderate fibrin covering the Glisson capsule of the liver (arrow). (B) Lung with a mild fibrin exudation covering the pleura with an inflammatory infiltrate of neutrophils (arrow). HE: Bar, 50 µm. (C) Marked neutrophil inflammatory infiltrates in the interalveolar septa (asterisk). HE: Bar, 50 µm. (D) Few aggregates of Gram-positive rod-shaped bacteria in the alveolar space (arrow). Brown-Hopps staining: Bar, 20 µm.

(SCHUH &WEINSTOCK, 1985). In a case series from South America, including Uruguay (MACÍAS-RIOSECO et al., 2020), Brazil (ANTONIASSI et al., 2013), and Argentina (CAMPERO et al., 2003), which collectively evaluated several hundred laboratory submissions, *B. cereus* was not reported as a cause of bovine abortion, indicating that it is a rare and sporadic abortogenic agent among herds associated with fetal loss in the third trimester. The main fetal lesions included suppurative pneumonia and necrotizing suppurative placentitis (SCHUH &WEINSTOCK, 1985), representing histological findings consistent with our reported case. Interestingly, histological examination of the lungs of a neonate born at 26 weeks of gestation infected with *B. cereus* revealed necrotizing pneumonia following suspected nosocomial acquisition (VIEL-THÉRIAULT et al., 2019). *Bacillus cereus* is an opportunistic human pathogen associated with severe systemic infection among immunosuppressed premature infants due to prolonged invasive procedures, such as mechanical ventilation and central catheterization (RAMARAO et al., 2014). In veterinary medicine, viral-induced immunosuppression by BVDV has been suggested as a circumstance under which *B. cereus* becomes bacteremic and localizes to the placenta, with abortion as a consequence (REGGIARDO & KAEBERLE, 1981). However, the fetus in our case was PCR negative for BVDV as well as the bovine female had a history of vaccination against BVDV, excluding the possibility that the bacteremia was caused by viral immunosuppression. Due to inflammatory lesions in the placenta, a differential diagnosis for mycotic placentitis must be performed. Groccot staining was performed on the lung and placental fragments and the result was negative, which excluded this possibility.

CONCLUSION

This report described a case of abortion at 8 months of gestation in a Nelore bovine female caused by a *B. cereus* infection. In a diagnostic setting, *B. cereus* should be considered a pathogen that causes pneumonia and placentitis in aborted bovine fetuses.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This study was conducted according to the protocol approved by the Ethics Committee for Animal Experimentation (CEUA UFMT No. 23108.103506/2021-18).

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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