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Visceral Botryomycosis by *Pseudomonas aeruginosa* in a bovine - case report. Botriomicose visceral por *Pseudomonas aeruginosa* em um bovino - relato de caso.

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Abstract

Botriomycosis is a rare and chronic granulomatous disease, whose main etiologic agents are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Aimed to describe a case of visceral botriomycosis in a bovine attended at the Garanhuns Cattle Clinic, *Campus* of the Federal Rural University of Pernambuco (CBG), with clinical signs of chronic evolution. Rectal palpation displayed nodular structures of firm consistency in organs of abdomen. Ultrasonography and blood count revealed changes. The anatomopathological findings revealed nodular lesions in the gastrointestinal tract. Histopathology shows the presence of the Splendori-Hoepli phenomenon. Microbiological analysis isolated *Pseudomonas aeruginosa*. It was concluded that it is necessary to include this disease as differential diagnosis of granulomatous lesions in ruminants.

Keywords: Digestive system. Cow. Splendore-Hoepli. Intestinal granuloma.

Resumo

A botriomicose é uma doença granulomatosa crônica e rara, cujos principais agentes etiológicos são *Staphylococcus aureus* e *Pseudomonas aeruginosa*. Objetivou-se descrever um caso de botriomicose visceral em um bovino atendido na Clínica de Bovinos de Garanhuns, *Campus* da Universidade Federal Rural de Pernambuco (CBG), com sinais clínicos de evolução crônica. Na palpação retal constatou-se estruturas nodulares de consistência firme em órgãos do abdômen. A ultrassonografia e o hemograma revelaram alterações. Os achados anatomopatológicos evidenciaram lesões nodulares no trato gastrointestinal. A histopatologia revelou a presença do fenômeno de Splendori-Hoepli. A análise microbiológica isolou *Pseudomonas aeruginosa*. Concluiu-se que é necessário incluir essa enfermidade como diagnóstico diferencial das lesões granulomatosas em ruminantes.

Palavras-chave: Sistema digestório. Vaca. Splendori-Hoepli. Granuloma intestinal.

Introduction

Botryomycosis is a suppurative granulomatous bacterial disease, with a chronic and rare character. It has as synonyms staphylococcal actinophytosis, bacterial pseudomycosis, or granular bacteriosis 12. The term botryomycosis (from Greek - *botrys*: grape clusters, *mycosis*: of fungal origin) was coined by Rivolta in 1984 due to the clinical and histological similarity with granulomatous fungal lesions (BONIFAZ; CARRASCO, 1996; RIVOLTA, 1884; SCOTT, 2007).

Botryomycosis can be caused by anaerobic or aerobic bacteria, of which *Staphylococcus aureus* is the most frequently isolated, accounting for 42.9% of cases, followed by *Pseudomonas aeruginosa* which corresponds to 19.9% of cases, and, to a lesser extent, the disease can be caused by other agents such as bacteria of the genera *Proteus*, *Micrococcus*, and *Escherichia*, among others (BONIFAZ; CARRASCO, 1996).

The first reported case of botryomycosis was in an equine after a post-orchietomy complication (BOLLINGER, 1870). Only 43 years later, in 1913, was the first case of visceral human botryomycosis reported in the United States, by three American researchers: Lignieres, Spitz and Archbald (WINSLOW, 1959).

This disease, in humans, occurs in cutaneous, affecting muscles and bones, and visceral forms, affecting mainly lung, liver and gastrointestinal tract (BONIFAZ; CARRASCO, 1996). The lesions may be single or multiple with the presence of ulcers or micro abscesses that can fistulate and suppurate white-yellow purulent material (CUDMORE, 2012; SCOTT, 1988). Histopathology shows lesions containing non-filamentous granules and bacterial colonies surrounded by eosinophilic material, denominated the Splendore-Hoeppli phenomenon (BONIFAZ; CARRASCO, 1996; THOMPSON et al., 2001). This phenomenon is able to prevent phagocytosis and intracellular bacterial destruction, functioning as a defense mechanism of the microorganism (BERSOFF-MATCHA et al. 1998; HUSSEIN, 2008; PADILLA-DESGARENNE, 2012). Although most cases report visceral involvement, it is believed that the cutaneous form of the disease is much more frequent (HACKER, 1983).

In addition to humans, botryomycosis can affect several species of animals without predisposition in relation to race, age, or sex (SCOTT, 1988). The cutaneous form of the disease has been reported in guinea pigs (BOSTROM et al., 1969), dogs (SCOTT, 2007; WALTON; SCOTT; MANNING, 1982), cats (WALTON; SCOTT; MANNING, 1982), hamsters (GROSSET et al., 2014), horses (SCOTT, 1988) and zebra (PANDEY, 1998). The visceral form is rarer and has been described involving hamster lungs (BOSTROM et al., 1969), dog pericardium (CASAMIÁN-SORROSAL, 2008), and the mammary glands, uterus, cerebellum, and lungs of horses (ELLENBERGER; SCHOON; SCHOON, 2006; JOHNS et al., 2014; MOREIRA, 2018; SMIET et al., 2012).

In bovines, there are descriptions of the two forms of the disease, but the literature data are very scarce. There are reports of the cutaneous form (DONOVAN; GROSS, 1984; SPAGNOLI et al., 2011), and the visceral form in this species has been described by Thompson et al. (2001), Miller et al. (2001), and Sartelet et al. (2015), describing a case of botryomycosis in the nasopharynx of a cow, in the lungs of a bullock, and in the uterus of cows post-surgical caesarean section, respectively (MILLER, 2001; SARTELET, 2015; THOMPSON et al., 2001).

To date, the pathogenesis of the disease has not been fully elucidated. However, for infection to occur, a high infective dose, low agent virulence, and decrease in host immunity is required. The predisposing factors for the occurrence include trauma or solution of continuity for

the cutaneous form, and postoperative complications for the visceral cases (BONIFAZ; CARRASCO, 1996).

Considering that there are few reports in the literature on the occurrence of botryomycosis, this article aimed to report a case of visceral botryomycosis in bovines caused by *Pseudomonas aeruginosa*.

Materials and methods

The findings were obtained from the clinical care of a bovine, female, Dutch half-breed, seven years old and weighing 500 kg was attended at the Bovine Clinic of Garanhuns, *Campus* of the Federal Rural University of Pernambuco (CBG). The owner reported that for about a month the animal had shown signs of apathy, hyporexia, and reduced milk production. The owner suspected that the bovine was affected by hemoparasitosis and had therefore used oxytetracycline and diminazene diaceturate based drugs, but no improvement was reported to the institution.

The animal was examined clinically following the recommendations of Dirksen et al. (1993). The internal organs of the abdominal and thoracic cavities were evaluated through transabdominal and transrectal techniques with convex transducers, at 5MHz, and the transrectal linear method, at the frequency of 7.5 MHz, respectively. The device used was the Z6 Vet (Mindray Bio-Medical Eletronics Co. Ltd., Shenzhen China). Blood samples were collected for complete blood count, which was performed following the recommendations of Jain et al. (1993)

Due to the suspicion of incurable granulomatous disease, the animal was euthanized using a protocol with 2% xylazine hydrochloride (0.05 mg / kg), 10% ketamine hydrochloride (2mg / kg) and potassium chloride (1ml / kg). Was submitted to necropsy and, during the procedure, the material was collected from the mesentery, great omentum and liver, some fragments were fixed in 10% buffered formalin and later stained with hematoxylin and eosin and others kept cooled to 2 ° C for subsequently sent to the laboratory for histopathological and microbiological analysis.

In the laboratory microbiologic, the biological sample was seeded on a base agar plate with addition of 7% (v/v) of defibrinated sheep blood and incubated in a microbiological oven at 37°C, with readings after 24, 48, and 72 hours to observe the morphological characteristics of the colonies. The microscopy showed the arrangement of the cells and the morphotintorial characteristics through the Gram staining technique (CARTER et al., 1991). An inoculum was prepared for identification of the bacterial species in the VITEK®2Compact™ automated system according to the manufacturer's instructions for reading and interpretation.

Results

In the physical examination of the animal, the clinical signs that should be emphasized are, behavior in the period, with anterior kyphosis, abducted thoracic limbs, enlarged left precrucial lymph nodes, a temperature of 39.1°C, body score II, and hyporexia. The nostrils were dilated, presenting tachypnea respiration with polypnea intensity with expiratory dyspnea. Abdominal tension was slightly increased, with the sound of liquid present in the ballotement test; in the pain test, the percussion test was positive. The feces of the animal were diarrheal, overly digested, and brownish in color. When rectal palpation was performed, the presence of several nodular structures of firm consistency were found, located on the medial aspect of the rumen, peritoneum, dorsal aspect of the left kidney, and lateral aspect of the uterus. The hemogram revealed neutrophilic leukocytosis (Table 1).

Table 1 - Hemogram of a bovine attended at the Bovine Clinic of Garanhuns, *Campus* of the Federal Rural University of Pernambuco (CBG)

He: 6.32 x 10 ⁶ /μl	Leukocytes: 16,300/μl
Ht: 30%	Lymphocytes 34% ----- 5,542/μl
Hb: 9.45 g/dL	Segmented 66% ----- 10,758/μl
MCV: 47.46 fL	
CMCH: 31.5%	
TPP: 8.4 g/dL	
PF: 600 mg/dL	

He: red blood cells; Ht: hematocrit; Hb: hemoglobin; MCV: mean corpuscular volume; CMCH: concentration of mean corpuscular hemoglobin; TPP: total plasma protein; and PF: plasma fibrinogen

In ultrasonographic examination alterations were found in the reticulum, liver, rumen, greater omentum, and mesentery. In the reticulum, a reduction in the number and intensity of reticular contractions was observed (2 biphasic contractions in 3 minutes with maximum displacement of 14cm). The liver had a parenchyma with a heterogeneous echotexture, presenting nodular structures in large numbers, with sizes varying from 1 to 6cm in diameter in the ventral region (right lobe) at the height of the 9th and 10th intercostal spaces of the right antimer. The content of these nodules had an echotexture a little heterogeneous, but more echogenic than the normal hepatic parenchyma, and although suggestive of abscesses, these were not delimited by a well-defined capsule (Figure 1). Similar nodules were found in the greater omentum (Figure 2A), mesentery (Figure 2B), and adhered to the ruminal serosa.

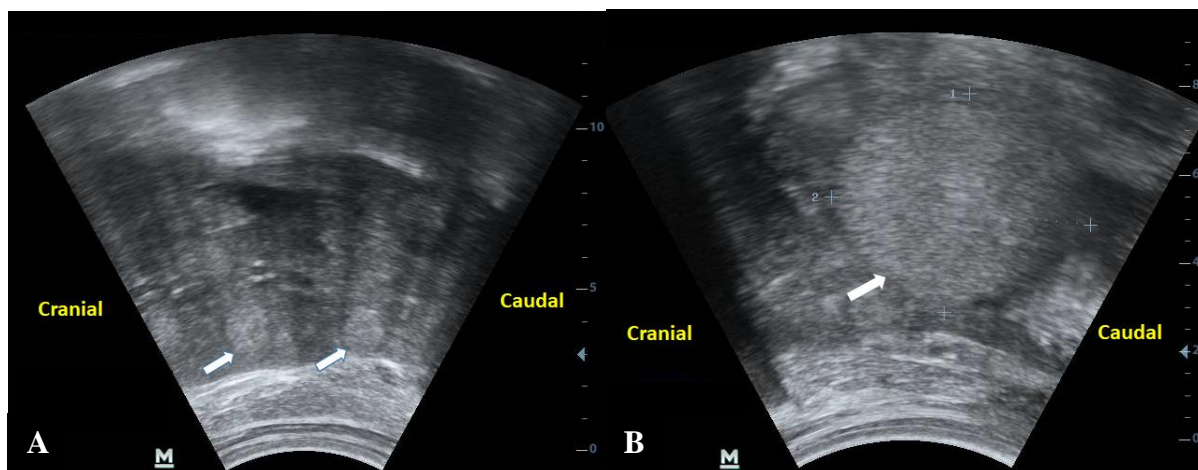


Figure 1 - Bovine botryomycosis. **A-** Liver presenting nodular structures (white arrows) with greater echotexture than the normal hepatic parenchyma, multifocal and sizes varying between 1 and 2 cm. **B-** Liver presenting nodular structures (white arrow), similar to those visualized in Figure 1A, but measuring 5x5.9cm.

Due to the clinical condition of the patient, the extent of the lesions found, and the echotextural characteristics of the nodulations, which suggested abscess lesions and, therefore, an unfavorable prognosis, euthanasia of the animal was authorized by the owner in order to arrive at a definitive diagnosis through anatomopathological examination.

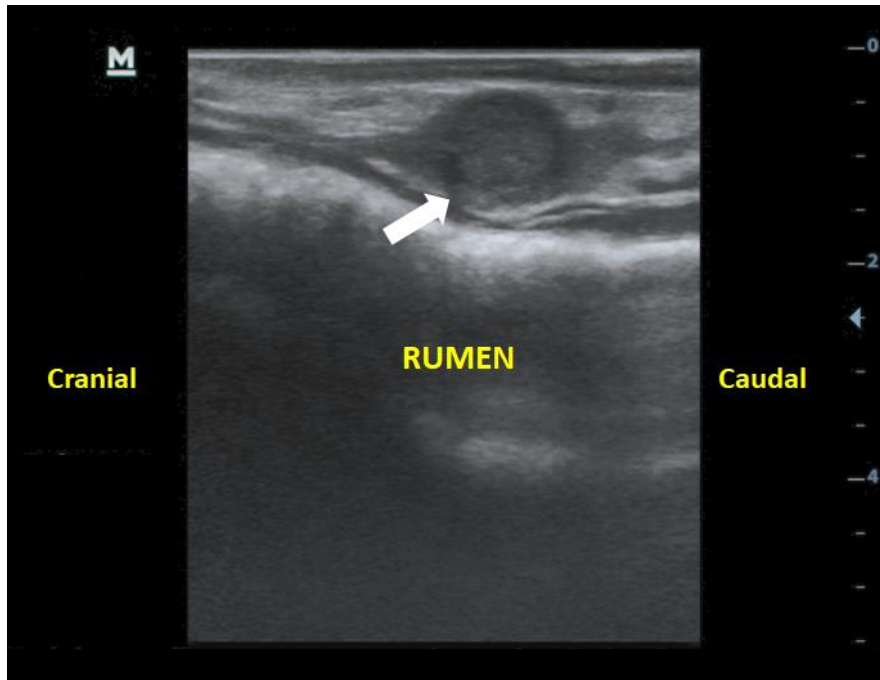


Figure 2 - Bovine botryomycosis. Greater omentum with nodular structure (white arrow) in a heterogeneous echotexture measuring 1.1x1.3cm suggestive of abscess.

In the macroscopic examination of the abdominal cavity, the most prominent features were; in the greater omentum and lymphatic chain of the mesentery, nodular structures of hardened consistency, multifocal and pleomorphic, which drained contents of caseous consistency and a yellowish color, characterized as abscesses (Figures 3A and 3B). There were, also, adhesions of the great omentum to the peritoneum and the serosa of the rumen. In the reticulum, a metallic foreign body (screw, approximately 10 cm) was found lodged in the mucosa, with adhesions between the inner surface of the left lobe of the liver, as well as the presence of abscesses on the surface of this organ that extended to the parenchyma. The bladder was empty and adherent to the intestine segment. The kidneys presented capsules adhered to and on the surface of the scar formation area, in addition to clear points distributed, mainly in the left kidney. The lungs were congested, with mild alveolar emphysema. The nodular structures present in the mesentery and greater omentum, in addition to the liver fragment, were collected for microbiological and histopathological analysis.

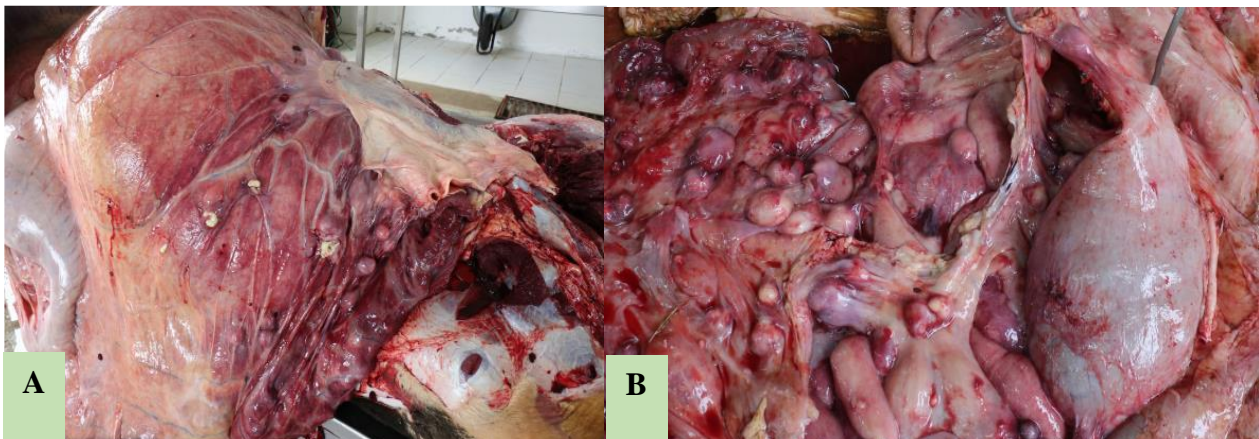


Figure 3 - Bovine botryomycosis. **A-** Greater omentum with the presence of nodular structures of hardened consistency, multifocal, and of differentiated sizes, which when cut drained content of yellowish color and creamy consistency (abscess), adhesions to the peritoneum. **B-** Mesentery: lymphatic chain with the presence of several distributed nodular structures of differentiated sizes, which, when cut drained yellowish caseous content (abscess).

The microbiological examination of the nodular structures, through microscopy, confirmed the presence of Gram-negative bacteria, where the isolated species was *Pseudomonas aeruginosa*.

In the histopathology, a lesion was observed with the characteristic of a pyogranulomatous process, associated with the presence of neutrophils and multinucleated giant cells surrounding bacteria, forming the phenomenon known as Splendore-Hoeppli (Figures 4A and 4B).

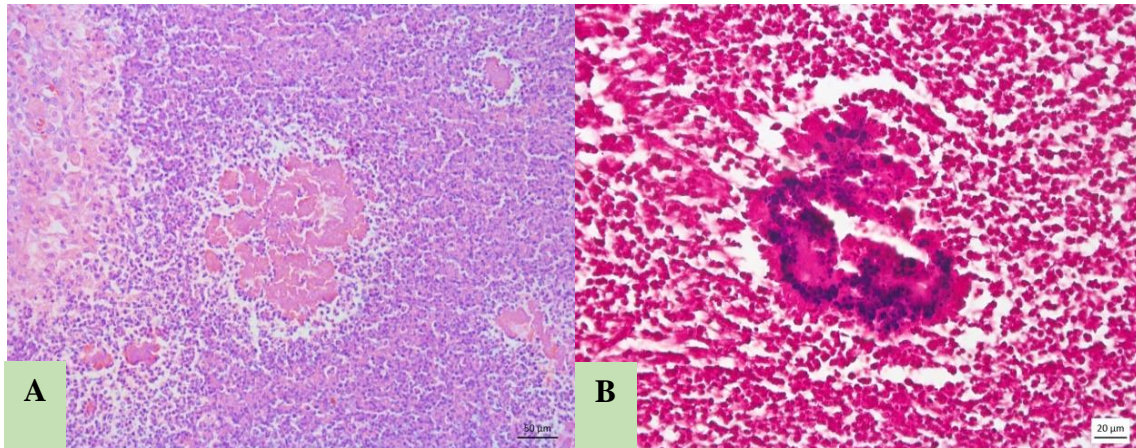


Figure 4 - Bovine botryomycosis. **A-** A marked inflammatory infiltrate of intact and degenerate neutrophils (asterisk) can be observed, associated with myriads of bacteria bordered by Splendore-Hoeppli material (black arrow). HE. Obj.20x. **B-** Gram staining demonstrating a myriad of Gram-positive coccoid bacteria amid the Splendore-Hoeppli material. Obj. 40x.

Discussion

Several species of bacteria are included as causes of botryomycosis, among them *Staphylococcus aureus*, which corresponds to the most commonly isolated agent, however in the present case *Pseudomonas aeruginosa* was isolated as the agent responsible for the lesion. This finding corroborates with reports that described the involvement of this pathogen affecting other organs in cattle (DONAVAN; GROSS, 1984, THOMPSON et al. 2001, MILLER et al. 2001, SARTELET et al. 2015). Some fewer common pathogens such as *Escherichia coli*, *Proteus vulgaris*, *Streptococcus* spp., *Biberstenia trealosi* may be involved (WINSLOW et al, 1959, SPAGNOLI et al, 2011).

The etiopathogenesis of this disease is not well understood (SCOTT, 2007), but it is known that factors such as immunodepression of the host, low virulence of the pathogen, and a large number of inoculums are important to establish the infection (BONIFAZ & CARRASCO, 1996). Botryomycosis is defined as a chronic suppurative piogranulomatous inflammation caused by bacteria, which can occur in both cutaneous and visceral forms (WINSLOW, 1959). The latter form can be divided into primary disease, where the infection starts in the affected organ, or secondary, when the infection originates from an injury in one organ and spreads to others (PADILLA-DESGARENNE et al, 2012).

Predisposing factors that include trauma, presence of foreign bodies and postoperative complications may be involved (HACKER, 1983; CONSTABLE et al, 2017). In this study, the

condition of visceral botriomycosis diagnosed in the cow, probably originated from reticulitis traumatic peritonitis caused by the foreign body

This could justify the colonization by opportunistic bacteria of the species *Pseudomonas aeruginosa* and subsequent systemic spread, mainly affecting organs of the gastrointestinal tract, resulting in a framework of secondary visceral botryomycosis in the animal.

In the clinical examination, in addition to changes in the white blood cell, the animal in this report presented alterations indicative of a severe process, such as apathy, reduced milk production, impaired gastrointestinal tract function, and including slightly increased abdominal tension, diarrhea, and the presence of nodular structures on the surface of the rumen, kidneys, peritoneum and mesentery, which were evidenced by rectal palpation. These structures can be observed in some bovine diseases, among them, intestinal tuberculosis, enzootic bovine leukosis (lymphosarcoma), and less frequent actinobacillosis and actinomycosis (SILVA FILHO et al., 2011; CONSTABLE et al., 2017).

In the macroscopy, the nodular structures found, more frequently in the greater omentum, of hardened, multifocal consistency and of varying sizes, draining yellowish contents with a caseous consistency, resembled lesions commonly found in cases of intestinal tuberculosis (RIET-CORREA & GARCIA, 2007; PAES & FRANCO, 2016). However, to a lesser extent, similar lesions have been found in cases of subcutaneous, pulmonary botryomycosis, in the nasopharynx and uterus of cattle (MILLER, et al., 2001; THOMPSON et al, 2001; SPAGNOLI et al, 2011; SARTELET et al, 2015). Botryomycosis had not been raised as a possible diagnosis for the disease of this animal, since this disease presents nonspecific clinical signs and there are few descriptions in the literature, there being no reported cases of visceral botryomycosis visceral with involvement of the omentum, mesentery lymphonodes and liver of cow.

Histopathological examination showed a lesion characteristic of the disease, termed the Splendore-Hoeppli phenomenon, which corresponds to a pyogranulomatous reaction characterized by an eosinophilic matrix formed by an antigen-antibody complex, cellular debris, and fibrin disposed between bacterial granules. This phenomenon has frequently been described in cases of visceral and cutaneous botryomycosis (MILLER et al. 2001, THOMPSON et al. 2001, HUSSEIN, 2008).

The diagnosis of botryomycosis is performed through the association of cytological and histopathological findings (SCOTT, 2007; HUSSEIN, 2008), and isolation of the agent, the latter being considered the gold standard for diagnosis of the disease (PADILLA- DESGARENNES et al, 2012). In the present work, anatomopathological and histopathological findings associated with the isolation of the etiological agent were fundamental to confirm the diagnosis of this disease.

Conclusion

Botryomycosis is a chronic granulomatous disease, which can be confused with other diseases, especially due to the scarcity of studies on the disease in production animals in veterinary medicine. The present report has great relevance in the literature because it is the first record of the disease affecting the gastrointestinal tract of bovines, which demonstrates the need to include this disease as a differential diagnosis of granulomatous lesions in this specie, in addition to alerting the scientific community to the underreporting of this disease due to lack of diagnosis.

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