**INTRODUCTION**

*Clostridium perfringens* is a well-known strict anaerobic bacterium that may be found as a normal inhabitant of the intestine among healthy animals (Niilo 1980; Rogstad et al., 1993, Radosits, et al., 2007). Alterations in the intestinal environment caused by sudden changes in diet (Riet Correa et al., 2001), mainly those animals that ingest high fermentable carbohydrates contents are associated with enteric proliferation by *C. perfringens*. This organism is able to produces anepsilon toxin, which is activated by intestinal trypsin and other proteases (Myashiro et al., 2007, Palmacci et al., 2009). Epsilon toxin is responsible for the clinical and pathologic findings of the disease in sheep, cattle, dogs, horses, humans, and wild ruminant (Niilo 1980, Boersma et al., 1994, Mc Gavins and Zachary 2007). The clostridia species are uncommon causes of pleuropneumonia in human patients (Boersma et al., 1994, Palmacci et al. 2009). In livestock, companion animals, and wildlife, clostridia may affect the pulmonary structures causing a necrotizing pneumonia with involvement of the pleura (McGavin and Zachary, 2007, Palmaci et al., 2009).

In humans, the pathogen may be introduced into the pleural space secondary to invasive procedures, such as thoracentesis or thoracotomy, or by traumatic percutaneous injuries (Buxton and Morgan 1976, Palmacci et al., 2009, Hendrix et al., 2011). In addition, this condition in human is often associated with chronic disease, such as diabetes or cirrhosis, and underlying pulmonary disorders (Boersma 1994, Palmacci et al., 2009). The clostridia pneumonia in domestic animals have been associated with aspiration of oropharyngeal or gastric contents (Jones, Hunt and King, 2000, Myashiro et al., 2007) and pulmonary embolism with infarction (Baldassi et al., 1995, Smith et al. 2009). Reported predisposing factors for animal systemic clostridia dissemination include intraoral and intrabdominal pathology such as malignancy and enteric vascular malformation, or diaphragmatic infections (Miserez et al., 1998, Jones, Hunt e King 2000). The clinical course of pulmonary clostridial infections in livestock usually is associated with acute evolution and high mortality (Lobato et al., 2006, Myashiro et al., 2007, Radosits et al., 2007). The present report describes an unusual case of sepsis and necrotizing pneumonia caused by *C. perfringens* in *Cervus timorensis* with emphasis on the clinical and pathological aspects of the disease.

**Case Report**

Clinical examination was conducted in a *Cervus timorensis*, male, 4-years-old, weight 65 kg, with a history of sudden...
anorexia, difficulty breathing, and isolation of the lot, belonging to the Union of the Teaching Zoo's Southwestern, UNISEP, Campus DoisVizinhos, State of Paraná, Brazil. The food of animal consisted of native pasture and concentrate for cattle with 18% protein (1.8 kg/day).

At the clinical examination was identified depression, congestion of mucous membranes, hyperthermia (40.1°C), rumen stasis, apathy, tachycardia (120 strokes per minute), tachypnea (65 breaths per minute) and dyspnea. Auscultation showed abnormal heart and bilateral pulmonary sounds expiratory grunting, and serous and bloody nasal discharge (Figure 1, 2).

Hypertrophied heart, presence of hemopericardium, and thrombus adhered to the left cardiac ventricle were observed (Figure 4). Congestion was also evidenced in the serous rumen. The small intestine was diffusely necrotized with presence of gas. The suprascapular lymph node was enlarged and congested. The liver shows enlargement and irregular areas (Figure 5). The kidneys were congested, whereas only the left one was edematous. Both kidneys presented loss of areas in the cortical and medullar zones.

Clinical and epidemiological findings of animal were suggestive of acute respiratory failure. Due to the severity of the clinical condition, the animal died a few minutes after clinical evaluation and was immediately submitted to necropsy. Fragments of lung, liver, kidneys and lymph nodes were collected and kept in formol (10%) for histopathological examination of tissues. Simultaneously, the same samples were collected and kept under refrigeration for the microbiological culture. The samples were plated on sheep blood agar (5%) and incubated under both aerobic and anaerobic atmospheres at 37°C for 96 hours. Fragments of organs were also cultured in MacConkey agar under same aerobic conditions described above. The microorganisms isolated were classified based on conventional phenotypic tests. Isolated colonies that had hemolysis and other compatible characteristics of the genus Clostridia were subjected to specific biochemical tests (Quinn et al., 2011).

RESULTS

Postmortem examination revealed serous to bloody effusion in thoracic cavity, adhesion between lung and pleura, congestion, pleurisy, blood clots throughout the chest cavity, and areas of pneumonia. Emphysema, petechiae, suffusion and ecchymosis lesions were also observed (Figure 3).

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Clostridium perfringens colony morphology, the organism was characterized as morphological and staining characteristics, biochemical and a large Gram positive rods, non-sporulate organisms. According to morphological and staining characteristics, biochemical and colony morphology, the organism was characterized as Clostridium perfringens (Quinn et al., 2011).

Microbiological culture of organ fragments revealed after 72 hours of incubation in anaerobic condition from lung and liver fragments, white-gray, hemolytic, with 2mm diameter colonies of typical double halo of hemolysis (Figure 7), compatible with Clostridium perfringens. Gram staining of colonies show a large Gram positive rods, non-sporulate organisms. According to morphological and staining characteristics, biochemical and colony morphology, the organism was characterized as Clostridium perfringens (Quinn et al., 2011).

DISCUSSION

Clostridia comprise a complex group of anaerobic spore-forming bacteria. C. perfringens produces one or more of four major toxins (α, β, ε and i), particularly in equines. In addition, the organism is able to express other virulent factors such as β2 toxin, associated with equine diarrhea, and NetB, a pore-forming toxin (Radostits et al., 2007, Quinn et al., 2011, Silva et al., 2013). C. perfringens is commonly associated with enteritis, pneumonia, gas gangrene, enterotoxaemia, dysentery, struck, pulpy kidney diseases in livestock (Radostits et al., 2007, Quinn et al., 2011). In Brazil, C. perfringens have been referred usually as causative agent of bovine, goat, and ovine enterotoxaemia (Baldassi et al., 2005, Lobato et al., 2006, Myashiro et al., 2007), foal enteritis (Silva et al., 2013), septicaemia in livestock (Riet Correa et al., 2001). Recently, a rare fatalcourse of infection by clostridia was reported in this country (Ribeiro et al., 2012). Nevertheless, C. pyogenes fatal infections in Cervus sp. are considered uncommon (Cubas et al., 2014). In the present report, clinical examination at admission of Cervus timorensis was suggestive of septicaemia and pneumonia. Hyperacute fatal course of our animal may be attributed to ability of C. perfringens to produce various hemolytic and necrotizing toxins (Radostits et al., 2007, Quinn et al., 2011, Silva et al., 2013). In livestock, the establishment of disease usually is associated with sudden changes of diet, particularly contents high fermentable carbohydrates, which favor enteric or tissue proliferation and toxin production by C. perfringens virulent strains (Myashiro et al., 2007, Palmacci et al., 2009).

Microbiological isolation of white-gray colonies with typical double halo of hemolysis under anaerobic atmospheres using sheep blood agar, presence of large gram-positive rods submitted to conventional phenotypic testing by clostridia, allowed C. perfringens diagnosis in the current report. In addition, necropsy findings and histopathological examination of our animal revealed typical lesions caused by C. perfringens, characterized by edema, congestion, necrosis, severe hemorrhage, intense infiltration of neutrophils, with presence of gram-positive rods (Zachary, 2007, Jones, Hunt and King, 2000).
The lung and liver isolation of *C. perfringens* in the present report is probably because to the spread of bacteria from the gastro-intestinal tract. This finding reinforces opportunistic behavior of microorganism, usually associated with extensive necrotic and hemorrhage processes in various tissues, characterized by sudden course, difficult therapeutic resolution, and poor prognosis (Lobato et al., 2006, Quinn et al., 2011, Hendrix et al., 2011).

**Conclusion**

The present report describes an uncommon case of necrotizing pneumonia and sepsis, with hyperacute fatal course, caused by *C. perfringens* in a *Cervus timorensis*. Likewise livestock, the present report was associated to extensive edema, hemorrhage, and necrosis lesions in diverse tissues, characterized by sudden occurrence, and fatal course. The association of diagnostic tools, such as clinical-epidemiological, microbiological, necropsy, and histopathological is valuable to confirm the diagnosis.

**REFERENCES**


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