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CASE REPORT

HYPERACUTE NECROTIZING PNEUMONIAAND SEPTICAEMIACAUSED BY TO *Clostridiumperfringens* IN*Cervus timorensis* – CASE REPORT

[1*]Motta, R. G., [2]Azollini, F., [3]Pachaly, J. R, [4]Paes, A. C. and [5]Ribeiro, M. G.

[1,4,5]Departamento de Higiene Veterinária e Saúde Pública. Faculdade de Medicina Veterinária e Zootecnia – FMVZ -UNESP – Caixa Postal 560, Cep: 18618-970 – Botucatu, SP. Brazil
[2]Curso de Medicina Veterinária. União de Ensino do Sudoeste do Paraná – UNISEP. Av. Presidente Kennedy, 2601 – Bairro Nossa Sra. Aparecida – Dois Vizinhos, PR. Brazil
[3]Curso de Medicina Veterinária. Universidade Paranaense. UNIPAR. Pç. Mascarenhas de Moraes, 4282 - Zona III. Umuarama, PR. Brazil

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ABSTRACT

Clostridia are uncommon causes of pleuropneumonia in wildlife In human and domestic animals,different *Clostridia* species may affect pulmonary structures causing a necrotizing and hemorrhagic pneumonia with involvement of the pleura. In livestock, most cases are associated with sudden changes of diet, iatrogenic lesionscaused by invasive procedures such as thoracentesis or thoracotomy, or traumatic percutaneous introduction of the microorganism.The clinical course of pleuropneumonia by clostridia infections may be very variable, although usually are associated with hyperacute or acute course and high mortality. The present report describes an uncommon case of necrotizing pneumonia and sepsis caused by *Clostridium perfringens* in *Cervus timorensis* with hyperacute fatal course, highlighting clinical, epidemiological, microbiological, and histopathological aspects.t

INTRODUCTION

Clostridium perfringens is a well-know strict anaerobic bacterium that may be found as a normal inhabitant of the intestine among healthy animals (Niilo 1980; Rogstad *et al*., 1993, Radostits, *et al*., 2007). Alterations in the intestinal environment caused by sudden changes in diet (Riet Correa *et al*., 2001), mainly those animals that ingest highfermentable carbohydrates contents are associated with enteric proliferation by*C. perfringens*. This organism is able to produces anepsilon toxin, which is activated by intestinal trypsine 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 Gavin and Zachary 2007).The clostridia species are uncommon causes of pleuropneumoniain 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).

Corresponding author: Motta, R. G.,
Departamento de Higiene Veterinária e Saúde Pública. Faculdade de Medicina Veterinária e Zootecnia – FMVZ -UNESP – Caixa Postal 560, Cep: 18618-970 – Botucatu, SP. Brazil

In humans, the pathogen may be introduced intothe 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 beenassociated 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 diaphragmaticinfections (Miserez *et al*., 1998, Jones, Hunt e King 2000). The clinical course of pulmonaryclostridiainfections in livestock usually is associated with acute evolution and high mortality (Lobato *et al*., 2006, Myashiro *et al*., 2007, Radostits *et al*., 2007). The present report describes an unusual case ofsepsis and necrotizing pneumonia caused by *C. perfringens* in *Cervustimorensis* with emphasis on the clinical and pathological aspects of the disease.

Case Report

Clinical examination was conducted in a *Cervustimorensis*, 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 with18% protein (1.8 kg/day).

Figure 1. Serous to bloody nasal discharge in *Cervustimorensis,* that died by hyperacute necrotizing pneumonia and sepsis due to *Clostridium perfringens*infection. Brazil, 2014

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).

Figure 2. Hiperacute necrotizing pneumonia and septicaemia caused by *Clostridium perfringens* infection in a *Cervus timorensis*. Detail ofbloody appearance of nasal discharge. Brazil, 2014

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 immediatelysubmitted 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 sampleswere collected and kept under refrigeration for the microbiological culture. The samples wereplated on sheep blood agar (5%) andincubated underboth 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 thathad hemolysis and other compatible characteristics of the genus *Clostridia* weresubjected to specificbiochemical 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).

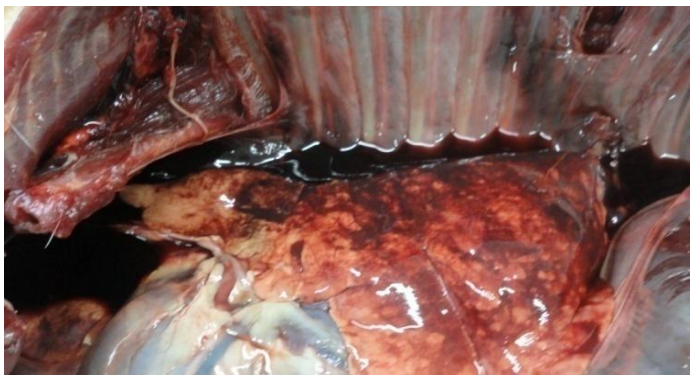

Figure 3. Hiperacute pneumonia and septicaemia in *Cervus timorensis* caused by *Clostridium perfringens.* Note haemothorax, pleural and lung congestion, red hepatic aspect of pulmonary lobes,diffuse petechiae, suffusion and ecchymosis lesions. Brazil, 2014

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.

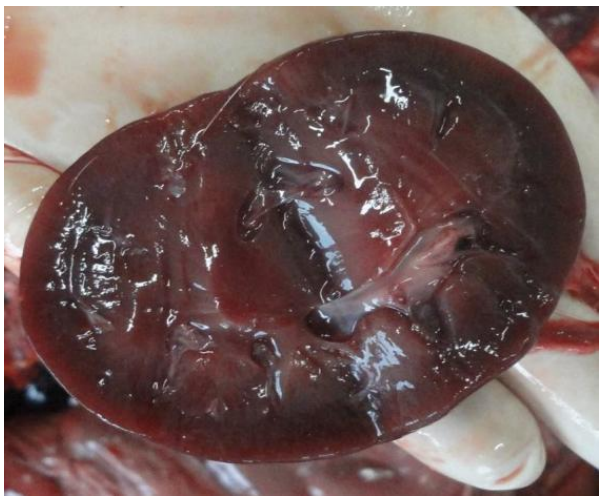

Figure 4. Detail of heart hypertrophy, presence of hemopericardium and thrombus adhered to the left cardiac ventricle in *Cervus timorensis* diedby hyperacute *Clostridium perfringens* infection. Brazil, 2014

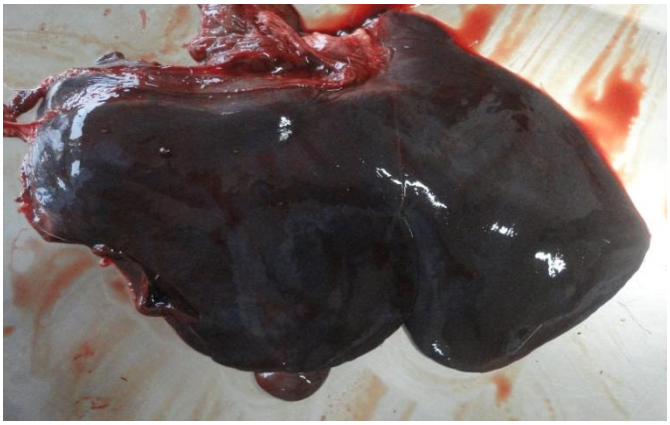

Figure 5. Hepatomegaly, tissue congestion, and rounded areas *Cervus timorensis* died by hiperacute *Clostridium perfringens*infection. Brazil, 2014

Fragments of organs were processed in paraffin inclusion. The histological sections (5µm thick) were stained with hematoxylin-eosin and subjected to microscopic examination. Both kidneys revealed several hemorrhagic foci in the renal parenchyma, large areas of coagulation necrosis in cortex and medulla, affecting glomeruli, renal tubules and collector ducts. The liver presented intense hyperemia, degeneration, necrosis of the centrilobular area, and presence of rods organisms. Necrotizing enteritis of the small intestine, severe pulmonary edema, and congestionof intra-alveolar septacontaining gram-positive rods were also observed. Severe disruption alveolar, edema, alveolar hemorrhage, occlusion of the bronchioles and alveoli by intense infiltration by neutrophils with fibrin were documented (Figure 6).

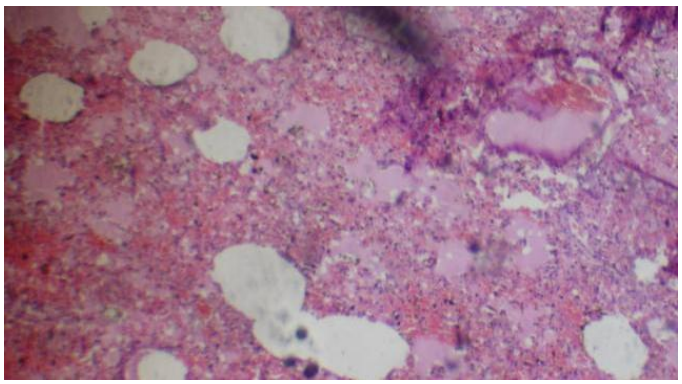

Figure 6. Histopathological findingof hyperacute *Clostridium perfringens* infection in a *Cervus timorensis.* Note edema, congestion, andnecrosis in intra-alveolar pulmonary septa, severe hemorrhage, alveolar edema, and occlusion of the bronchioles and alveoli by intense infiltration of neutrophils. Brazil, 2014

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 with typical double halo of hemolysis (Figure 7), compatible with *Clostridium perfringens*. Gram staining of colonies show a large Gram positive rods, non-sporulateorganisms. According to morphological and staining characteristics, biochemical and colony morphology, the organism was characterized as *Clostridium perfringens* (Quinn *et al*., 2011).

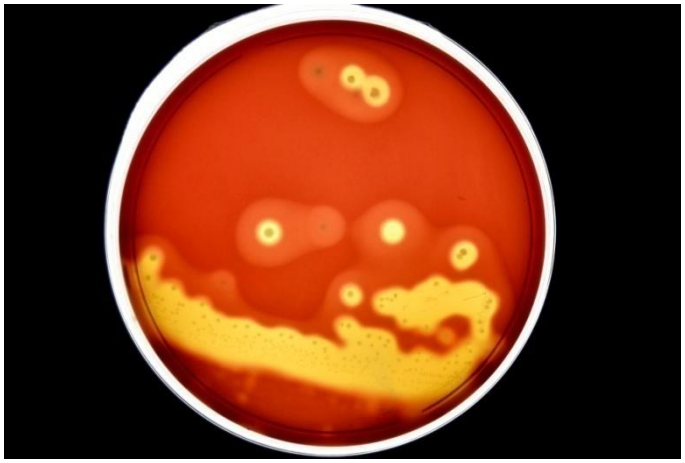

Figure 7. White-gray aspect and typical double halo of hemolysis of *Clostridium perfringens* colonies in sheep blood agar (5%) under anaerobic condition at 72 hours of incubation

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 findingreinforces opportunistic behavior of microorganism, usually associated with extensive necrotic and hemorrhageprocesses 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 histophatological is valuable to confirm the diagnosis.

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