

Atypical Mannheimiosis in captive rheas (*Rhea americana*)

Mannheimiose atípica em emas (Rhea americana) cativas

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Abstract

Mannheimia haemolytica (MH) is a major cause of respiratory disease in bovine complex (BRDC) that causes great economic losses. To this day, there are no reports of birds affected by this bacterium. The present report describes an atypical outbreak of mannheimiosis in captive rheas (*Rhea americana*).

Keywords: BRDC. Birds. Brazil. *Mannheimia (Pasteurella) haemolytica*. *Rhea americana*.

Resumo

Mannheimia haemolytica (MH) é uma das principais causas da doença do complexo respiratório dos bovinos (DCRB) que causa grandes perdas econômicas. Até os dias atuais, não há relato de aves acometidas por tal bactéria. Relata-se um surto atípico de mannheimiose em emas em cativeiro (*Rhea americana*).

Palavras-chave: DCRB. Aves. Brazil. *Mannheimia (Pasteurella) haemolytica*. *Rhea americana*.

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Mannheimia haemolytica (MH) is a gram-negative opportunistic bacterium (SINGH et al., 2011), belonging to the family *Pasteurellaceae* (MUTTERS et al., 2005) associated with gangrenous mastitis in cows, sepsis in lambs and severe fibronectic pneumonia and acute hemorrhagic affecting cattle (SINGH et al., 2011) called: disease bovine respiratory complex (BRDC) (RICE et al., 2007).

Rheas (*Rhea americana*) and ostriches (*Struthio camelus*) are ratites found in different countries, being rheas typically found in South America. Ostrich and rhea farming has become a growing business in Brazil, serving as an alternative to agricultural activity in this

country. As this activity has grown, there has been a trend towards increasing health problems in these birds, including bird mortality and treatment expenses (FILHO; LUCIO, 2006). An overview of the diseases that affect rheas in Brazil described the presence of *T. gondii* antibodies (ALMEIDA et al., 2013), whereas a report referred pulmonary aspergillosis outbreak in commercial farms (COPETTI et al., 2004). The commercial raising of great rheas is expanding in Brazil and many diseases previously restricted to other avian species are emerging on rhea farms. Thus, to the best of our knowledge, the present report describes the first case of an outbreak of mannheimiosis among captive *R. americana*.

During the month of August of 2009, 21 (70%) of the 30 *R. americana* were referred for the Animal Pathology Laboratory of the Universidade Federal Rural do Semi-Árido – UFERSA, located in a semiarid area of Rio Grande do Norte, Brazil. The animals came from the Multiplication Center of Wild Animals (CEMAS), located in UFERSA and regarded as

scientific breeding, with registration at 12.492/0004 associated with the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA). The all rheas had acute history of dyspnea, diarrhea, upright feathers, decubitus (Figure 1) and subsequent death. All animals received feeding for rations, water *ad libitum*, had no contact with mammals and has no history of change in the management and or transport. Within the precincts of the rheas, specifically in food and water, the presence of various types of invasive birds like sparrow (*Passer domesticus*), black vulture (*Coragyps atratus*) and red-cowled cardinal (*Paroaria dominican*) were constantly observed. Due to the death of 21 animals, necropsy was carried out in all animals following histopathological examination. Lung, heart, liver, stomach muscle (ventricular), small intestine and large intestine fragments were fixed in buffered formaldehyde 10% solution. All this material was sent to routine histopathological examination with hematoxylin and eosin technique and analysis under light microscopy (JUNQUEIRA; CARNEIRO, 2008). Samples of above mentioned organs from the 21 necropsied birds were also subjected to bacterial culture. The material was plated on defibrinated sheep blood agar/MacConkey and incubated at 37°C for 48-72 hours in aerobic and microaerophilic condition. Subsequently, macroscopic and microscopic analysis of the colonies was performed by gram stain. The recovered colonies were plated on brain heart infusion (BHI) for phenotypic characterization, including catalase and oxidase tests, β -hemolysis, fermentation of some sugars (glucose, lactose, sucrose, maltose), indole production, reduction of urease, and use of methyl red and Voges-Proskauer tests.

Due to high mortality rates, all animals that remained in the enclosures were orally treated using enrofloxacin (10% solution). The antibiotic was diluted at a ratio of 1: 2, in the drinking water, with an interval of 24 hours for five days. Frequent cleaning of feeders and drinkers was also recommended, along with continuous disposal of waste soil from captivity, and measures to prevent the introduction of invasive species

of birds were applied. The mortality rate of the rheas was reduced after the introduction of antibiotics.



Figure 1 – *Rhea americana* showing respiratory distress (animal with open beak and upright feathers) – Mossoró, RN, Brazil – 2009

Hemorrhagic aspect, with rough and unsightly surface, were the major *post-mortem* lesions observed macroscopically in the lungs (Figure 2). The air sacs were opaque due to liquid accumulation. Hydropericardium was characterized by the presence of bright yellow fluid which, when drained, reached a volume up to 140 ml (Figure 3). Cardiac hypertrophy and hemorrhagic areas were also visualized. Viewed macroscopically, the intestine showed hemorrhagic enteritis. The intestinal mucosa revealed friable areas, with bleeding and exudate regions ranging from the fibrinous to purulent (Figure 4). The muscular stomach was swollen. The liver revealed pale and friable regions with firm macules on capsular surface and abscesses (Figure 5).

Histopathological examination of the lung revealed severe hemorrhage throughout the parenchyma, with the red cells filling all parabronchi, associated with the presence of amorphous eosinophilic material (pulmonary edema). The lungs revealed marked congestion with focal mononuclear cell infiltration that was classified as focal mononuclear pneumonia associated with severe pulmonary hemorrhage and mild edema. In ruminants affected by MH, similar

histopathological findings were found by Subramaniam et al. (2011) who observed severe interlobular edema, hemorrhagic and suppurative pneumonia characterized by the deposition of fibrin, edema and neutrophil infiltration in the bronchioles

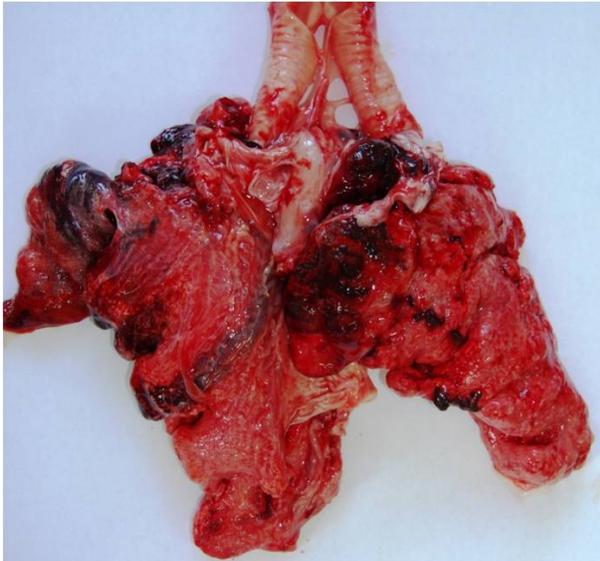


Figure 2 – Pulmonary parenchyma of captive rhea showing hemorrhagic areas, rough and unshapely aspect – Mossoró, RN, Brazil – 2009

and alveoli. In sheep, Dassanayake et al. (2009) found bronchiolar and alveolar walls occasionally interrupted by necrosis and hemorrhage.



Figure 4 – Intestinal mucosa of captive rhea showing hemorrhagic exudate and areas ranging from fibrinous to purulent – Mossoró, RN, Brazil – 2009



Figure 3 – Section of the pericardial sack of captive rhea showing light yellow fluid (hydropericardium) – Mossoró, RN, Brazil – 2009

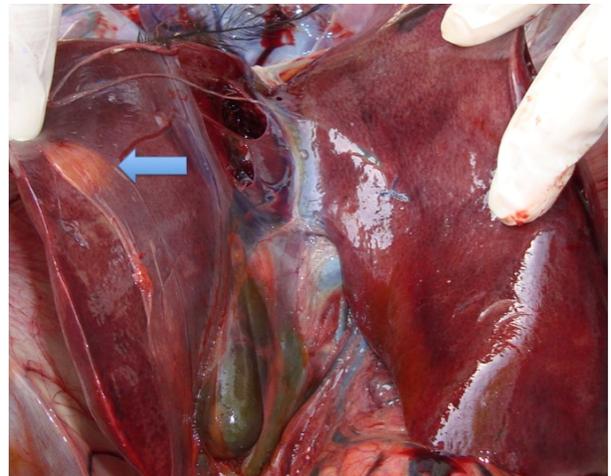


Figure 5 – The liver of captive rhea showing revealed pale and friable regions with abscesses (blue arrow) – Mossoró, RN, Brazil – 2009

The heart showed thickening of the epicardium due to severe hyperemia and focal edema, featuring a pattern of petechial hemorrhages subepicardial. The myocardial fibers showed apparent histologically

normal aspect. The liver revealed severe trabecular disorder of the hepatocyte with marked macro and micro degeneration. There was discreet multifocal mononuclear inflammatory infiltrate, mainly around

the vessels. These changes confirm the diagnosis of moderate to severe degenerative liver (Figure 6). Salerno and Paes (2016) corroborate these findings when mentioning the autopsy findings of primary epidemic form of pasteurellosis in ruminants. The muscular stomach (ventricle) showed organisms with aspect of bacilli and coccobacilli. In the subjacent mucosa, sharp diffuse mixed inflammatory infiltrate (diffuse ventriculitis) were observed. Edema of mucosa and submucosa was found in the intestines, with severe and diffuse character, concomitant with hyperemia and hemorrhagic foci. There was marked heterophilic inflammatory infiltrate related with hypertrophy and hyperplasia of lymphoid follicles in the submucosa. Necrosis in the intestinal villi was also observed. Microscopic findings were consistent with necrotic enteritis. The necropsy findings related with bowel is agree with Salerno and Paes (2016), that described hemorrhagic enteritis in animals affected by such bacteria.

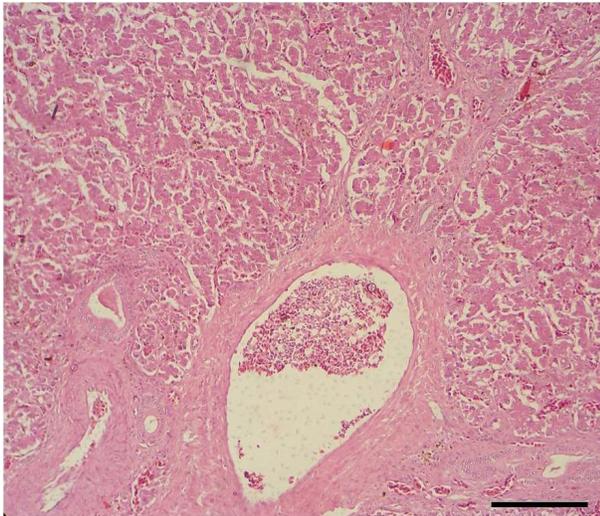


Figure 6 – Photomicrograph of the liver of captive rhea. Note severe trabecular disorder of the hepatocyte caused by *Mannheimia haemolytica* infection. (HE, obj.10x
b a r : 2 0 0 μ m, São Paulo, RN, Brazil – 2009

Small and convex colonies with grayish color were observed in microbiological culture of clinical specimens. Gram stains of colonies revealed gram negative rods, mainly grouped and less frequently in pairs or chains with bipolar aspect. Isolates were β -hemolytic and catalase, oxidase positive. There was uptake of glucose, lactose, sucrose and maltose. The isolate was negative for indole, urease, methyl red and Voges-Proskauer tests. These results are consistent with Katsuda et al. (2009) and Griffin et al. (2010) that referred to MH as gram-negative hemolytic bacillus, positive for oxidase/catalase and negative to indole. After 48 h the colonies on MacConkey agar had colonial and growth characteristics such as being odorless, convex, hemolytic, and in the form of small red/rosy points. The biochemical characteristics are compatible with MH according to the description of Salerno and Paes (2016). *M. haemolytica* is the most common bacterium isolated in cases of BRDC (FULTON et al., 2002) characterized by high morbidity and mortality in feedlot cattle (TAYLOR et al., 2010), and a major cause of the drastic decline of wild sheep (MILLER, 2001).

To the best of our knowledge, the current report describes for the first time manheimiosis in rheas based on macroscopic post-mortem lesions, histopathological and microbiological analysis. Therefore, *M. haemolytica* should be included among pathogens that also affect captive *R. americana*.

Conflict of Interest

The authors declare that there are no conflicts of interest associated with this paper. All authors of this manuscript state that it has not been published or submitted to another journal.

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