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SHORT COMMUNICATION

Fibro-Purulent Bronchopneumonia and Chronic Kidney Disease (CKD) in the Antillean Manatee (*Trichechus manatus manatus* L. 1758)

J Klećkowska-Nawrot[1*], K Goździewska-Harłajczuk[1*], K Barszcz[2], S Dzimira[3], M Janeczek[1], W Paszta[4], K Zagórski[4] and L von Fersen[5]

[1]Department of Animal Physiology and Biostructure, Faculty of Veterinary Medicine, University of Environmental and Life Sciences in Wroclaw, Kozuchowska 1/3, 51-631 Wroclaw, Poland; [2]Department of Morphological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland; [3]Department of Pathology, Wroclaw University of Environmental and Life Sciences, C. K. Norwida 25/27, 50-375 Wroclaw, Poland; [4]Wroclaw Zoological Garden, Wróblewskiego 1-5, 51-618 Wroclaw, Poland; [5]Tiergarten Nuernberg, Am Tiergarten 30, 90480 Nuernberg, Germany
*Corresponding author: lestat_v@poczta.onet.pl; k.gozdziewska.wroc@gmail.com

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ABSTRACT

The aim of this study was to present a first case of fibro-purulent pneumonia and chronic kidney disease in the Antillean manatee. The 23-yr-old female of Antillean manatee (Therese von Bayern) was moved from the Tierpark Berlin (Germany) to the Wroclaw Zoological Garden (Poland), on the 15th of December 2014. The manatee died on the 4th of April 2015. Postmortem examination revealed the right lung dark red lesions indicative of pulmonary congestion and extravasations. There was a fist-sized cavity filled with a bloody fluid and fragments of lung tissue at one-third of the length of that lung. Both kidneys with no pathologic changes, pale pink, with fragile structure. Histopathologic examination revealed fibro-purulent bronchopneumonia, interstitial renal fibrosis, nephritis and glomerulonephritis with protein deposits and acute tubular necrosis (ATN), hepatic fibrosis and hepatocellular degeneration. The lung tissue expressed cytokeratin, vimentin and a CD68 macrophage marker.

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INTRODUCTION

Pneumonia is a type of inflammatory reaction, which may involve the alveolar wall, bronchi and bronchioles, leading to the formation of bronchopneumonia. Pneumonia is frequently secondary to an underlying disease that compromises the lungs and airways and disrupts their function. Chronic kidney diseases (CKD) is the disorders which affecting kidney structure and function, as a results of decreased of glomerular filtration rate (glomerulosclerosis, atrophy, interstitial fibrosis) (Levey, 2012). Lung diseases (infectious, inflammatory or neoplastic) and CKD have been extensively studied in mammals. However, there are few studies focusing on lung diseases and CKD in the Cetaceans, Sirenia and Pinnipeds marine mammals (Dennison *et al*., 2007). There are no previous reports of fibrinous-purulent broncho-pneumonia and CKD in aquatic mammals. Ours is the first report to describe fibrinous-purulent bronchopneumonia and CKD in an Antillean manatee. The aim of this study was also to determine the expression of cytokeratin, vimentin and CD68 in fibrinous-purulent broncho-pneumonia in the Antillean manatee.

MATERIALS AND METHODS

Animal: On recommendation of the Manatee EEP a female Antillean manatee (Therese von Bayern) was moved from the Tierpark Berlin (Germany) to the Wroclaw Zoological Garden (Poland), on the 15th of December 2014. The female was born on the 4th of November 1992 in Nuremberg (Germany). The animal did not have any history of disease in the Tierpark in Berlin (Germany). The animal was not treated while at the Wroclaw Zoo. The manatee died on the 4th of April 2015. A post-mortem examination was carried out 6-8 hours after death (no signs of rigor mortis). The examination was compatible with the Polish law, and the use of post

mortem tissues in this study did not require the permission of the Ethics Commission (Parliament of the Republic of Poland, 2012) (Act of Animal Protection passed on August 21, 1997 by the Parliament of the Republic of Poland; No. 111/724).

Morphological study: The right and the left lung with the tracheal bifurcation were taken. The morphological and morphometric measurements of the right and left lung with the tracheal bifurcation were carried out using an electronic slide caliper accurate to the nearest 0.01 mm (Digitronic Caliper, Moore & Wright). Both lungs with the tracheal bifurcation were weighed using an electronic scale (AXIS B15 M, Gdańsk, Poland). The terminology used in this manuscript is in accordance with the prevailing veterinary nomenclature.

Histological study: The right and left lung with the tracheal bifurcation and middle tracheobronchial lymph node were directly fixed in 4% buffered formaldehyde for two weeks and washed in running water for 24 hours. The material was then processed in an ETP (RVG3, INTELSINT, Italy) vacuum tissue processor, embedded in paraffin and cut using a Slide 2003 (Pfm A.g., Germany) sliding microtome into 3-4 µm sections. The samples were stained with H&E, azan trichrome, Masson-Goldner trichrome, orcein and using Gomori's stain. All the obtained slides were examined using the Zeiss Axio Scope A1 light microscope (Carl Zeiss, Jena, Germany).

Immunohistochemical study: All the sections were prepared for the immunohistochemical staining using the same procedure as for the histological assessment. Then the sections were deparaffinized and inactivated in 3% hydrogen peroxide in PBS for 10 minutes at 23ºC temperature. Then samples were incubated with a primary antibody for 60 min and with a secondary antibody for half an hour. The sections were stained immunohisto-chemically using the following antibodies: cytokeratin (Abcam, cat. No. ab961), vimentin (Santa Cruz, cat. No. sc73259) and CD68 (Abcam, cat. No. ab955). Additionally, DAPI was used for fluorescent examination. The immunohistochemical studies were performed in the Institute of Animal Physiology and Genetics, v.v.i., Academy of Sciences of the Czech Republic, Brno.

RESULTS

The post-mortem examination showed some changes in the dorsal part and side of the body with the numerous abrasions and longitudinal skin lesions. Dorsal part and the vicinity of the tail, as well as the pectoral fin were covered with numerous fragments of exfoliation. In the subcutaneous tissue: firm consistency white fat which cover of 3-4 cm, uniformly thick. The deeper fat layer was divided by the skin muscles and with pale yellow colour, 10-12 cm and loose. The all layers contain clearly visible extravasations. Numerous extravasations in the deep fat layer were recognized, on both side of the chest, at a level of sternum ribs. The pericardial sac contains small amount of clear fluid. The heart was normal, with no pathologic changes noted, though the left ventricle wall was most probably too thin. The right lung had a dark red lesions, what indicative of pulmonary congestion and extravasations (Figs. 2A-B). There was a fist-sized cavity filled with a bloody fluid and fragments of lung tissue at one-third of the length of that lung. The length of each lung was 110 cm. Lobes of the liver were rounded, indicating their increase in size. The liver tissue was very loose, easily surrendering under finger pressure. The pancreas of 8 cm, was practically grown in to the left stomach area. Both kidneys were with no pathologic changes, pale pink, with fragile structure. Numerous adhesions of uterus and ovaries with peritoneum and bladder were present. The both ovaries showed features of polycystic ovary syndrome (PCOS) (Goździewska-Harłajczuk *et al*., 2017). The skin in abdominal surface was without changes. The gastrointestinal tract, peritoneal cave, pleural cave, pericardium, skeleton and muscles were without macroscopic pathological changes. The study of the liver, showed the diffuse swelling of cholestasis, hepatic fibrosis, hepatocellular degeneration. Additionally, interstitial renal fibrosis (Fig. 1A), nephritis and glomerulonephritis with protein deposits (Figs. 1B-D) and acute tubular necrosis (visible tubular epithelium and simple glomeruli necrosis (Figs. 1E-F) were recognized. A pink, amorphous deposition of fibrin and numerous neutrophils, macrophages were present in the lumina of the bronchioles and alveoli (Fig. 2D). Numerous clusters of lymphocytes were present in the alveolar cell wall (Fig. 2C). Dark-brown hemosiderin deposits were present in the thickened alveolar and bronchiolar wall (Fig. 2E). There were also sporadic clusters of bacteria (purple fine-grained deposits) surrounded by a neutrophil infiltrate and single hemosiderin-laden macrophages (Fig. 2F) in the alveolar lumina. Protein-rich filtrates indicating edema that contained single neutrophils and numerous small clusters of hemosiderin granules were present in the alveolar lumina. The cells of the alveolar epithelium positively expressed cytokeratin (Figs. 3A-B). The presence of numerous macrophages with granules that stained brown, were found in bronchial tissue using the CD68 marker (Figs. 3C-D). A positive vimentin expression was found in the lung interstitial tissue using immunofluorescence (Figs. 3E-F).

DISCUSSION

Pneumonia and chronic kidney diseases commonly occurs in humans and animals. The diagnosis of pneumonia and chronic kidney diseases is very difficult in marine mammals due to their living environment. Therefore, it is frequently diagnosed *post mortem* concomitantly to an underlying systemic disease. It rarely occurs as an independent disease entity in free-living and captive animals. Reports of fibro-purulent broncho-pneumonia and chronic kidney diseases in Antillean manatee are rare and poorly described. Keller *et al*. (2008) described the multifocal to diffuse pyelonephritis, interstitial nephritis and nephrocalcinosis in West Indian manatees. On the other hand, Deninson *et al*. (2007) showed urate nephrolithiasis in a northern elephant seal (*Mirounga angustirostris*) and a california sea lion (*Zalophus californianus*). The extensive emphysema was observed in a malignant lymphoma of the West Indian manatee of the lungs and pneumonia with multisystemic

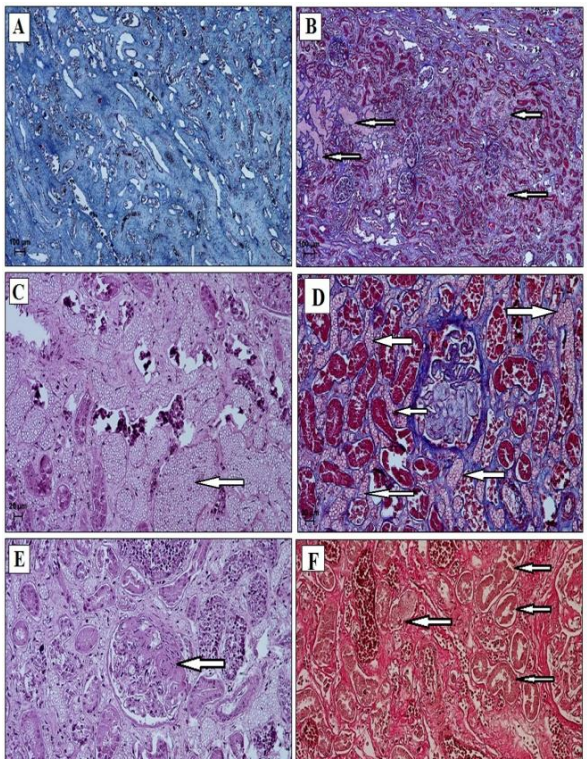

Fig. 1: The microscopic findings in the chronic kidney diseases of the Antillean manatee. A) interstitial renal fibrosis. Stain=azan trichrome, Bar=100 µm; B, C, D) nephritis and glomerulonephritis with protein deposits (black arrows). Stain=azan trichrome (B, D) and H&E (C), Bar=100 µm (B) and 20 µm (C, D); E, F) acute tubular necrosis and simple glomeruli necrosis (black arrows). Stain=H&E (E) and orcein (F), Bar=20 µm.

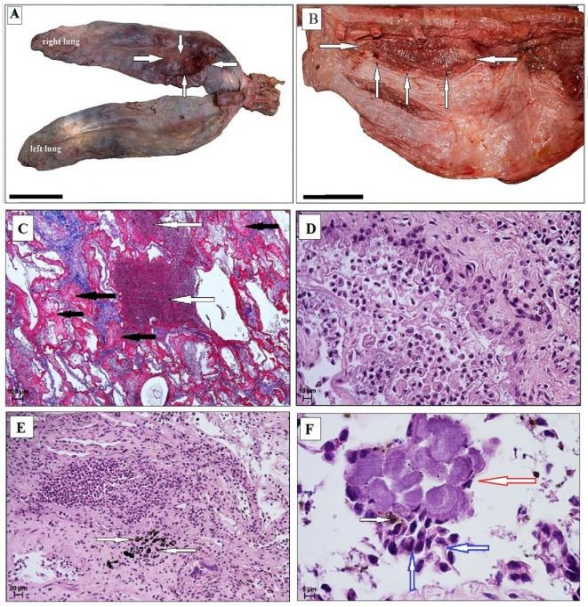

Fig. 2: The left and right lung and microscopic findings in the fibrinous-purulent bronchopneumonia in the Antillean manatee. A) The arrows point to the cavity at one-third of the height of the right lung, Bar=20 cm; B) The fist-sized cavity visible in the right lung (arrows), Bar=20 cm; C) Pink fibrin deposits visible in the lumina of the bronchioles (black arrows) and numerous clusters of lymphocytes in the peribronchial tissue (white arrows). Stain=azan trichrome, Bar=100 µm; D) The lumen of the bronchiole is filled with an exudate containing neutrophils, macrophages and sloughed epithelial cells. Stain=H&E, Bar=10 µm; E) Numerous hemosiderin granules are visible in the alveolar wall (white arrows). Stain=H&E, Bar=20 µm; F) A sporadic cluster of bacteria (red arrow) surrounded by a neutrophil infiltrate (blue arrows) and single hemosiderin-laden macrophages (white arrow). Stain= H&E, Bar=5 µm.

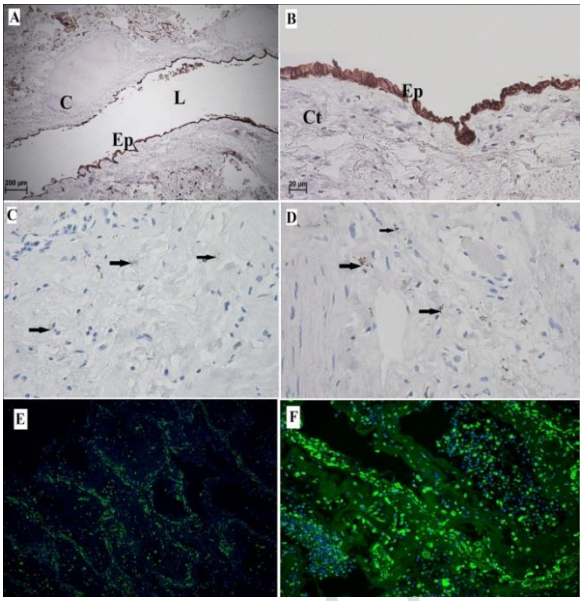

Fig. 3: The expression of cytokeratin, CD68 and vimentin in the lung of the Antillean manatee. A) Cytokeratin expression within the lung epithelium, Bar=200 µm; B) Cytokeratin, Bar=20 µm; C) CD 68 marker within macrophages (black arrows), Bar=40x; D) CD 68, Bar=40x; E) vimentin positive reaction (light green), Bar=10x; F) vimentin (light green), Nuclei stained with DAPI (blue), Bar=20x. Ep - epithelium, Bv - blood vessel, C - cartilage, L - lumen, Ct - connective tissue.

disease in Florida Manatees (*Trichechus manatus latirostris*) (Bossart *et al*., 2004; Hammer *et al*., 2005). Other authors found in 106 fresh dead Mexico common bottlenose dolphins (*Tursiops truncatus*) more likely to primary bacterial pneumonia (Venn-Watson *et al*., 2015). Jang *et al*. (2010) described fibrinous pleuritic, copious pus in the pleural cavity and suppurative broncho-pneumonia in the California sea lions (*Zalophus californianus*). Our study showed that cytokeratin, vimentin and CD68 were expressed in the bronchial, bronchiolar and alveolar wall, which may confirm pneumonia. A strong expression of cytokeratin, vimentin and CD68 in macrophages was described in lung adenocarcinoma, lung large cell carcinomas and non-small-cell lung cancer, pulmonary fibrotic diseases, e.g., organizing pneumonia, interstitial pneumonia, idiopathic pulmonary fibrosis, diffuse alveolar damage and the hepatopulmonary syndrome (Dauphin *et al*., 2013).

Conclusions: The pathological changes (within the lung) in examined manatee were rather as a consequence of the bacterial infection. Besides, clinical symptoms were evoked by oxygen deficiency together with bacterial toxemia. However, the kidney had a features of chronic disease (because of an immune complexes were present). Pathological changes in kidney can be associated with PCOS in ovaries (Goździewska-Harłajczuk *et al*., 2017) of the examined female of manatee, what showed similarity to the PCOS in women.

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Authors contribution: JKN collected material after post mortem and interpreted the findings of the gross macroscopic and histopathologic examined. KGH collected material after post mortem, macroscopic examination, immunohistochemical examination of the lung, drafted the manuscript. KB, MJ drafted the manuscript. SD interpreted the findings of the histopathologic examination. LF drafted the manuscript. WP, KZ collected material.

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