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SYSTEMIC PATHOLOGIES IN A CAPTIVE HUMBOLDT PENGUIN (*Spheniscus humboldti*) - STUDY CASE

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Abstract

The present case study was represented by a captive, adult, female Spheniscus humboldti penguin submitted for post mortem investigations at the Pathological Anatomy Department, from the Faculty of Veterinary Medicine of Bucharest. The diagnostic methods included necropsy, microbiology, cytology, histopathology and PCR examination.
Necropsy revealed poor body condition and obvious thickened air sacs along with multifocal, coalescing, yellow nodules in multiple organs. Cytology revealed necrotic and inflammatory cells, detritus, bacteria and fungal hyphae and microbiologic examination isolated Aeromonas hydrophila and Candida krusei. Histopathology revealed old and developing multifocal granulomas with a central oxyphilic material and circular disposition surrounded at the periphery by multinucleated giant cells and cellular reactivity. Other lesions identified were interstitial nephritis with glomerulosclerosis, lymphohistiocytic hepatitis. and splenic lymphocyte depletion. Also, protozoan cysts (50-80µ in diameter) were identified in all major tissues, but PCR examination was negative for Toxoplasma gondii.
The case of the Humboldt penguin presented multifocal granulomatous inflammations, associated with emaciation, immunosuppression and parasitism and the cause of the death was respiratory insufficiency.

Key words*: Humboldt penguin, granulomatous systemic inflammation, respiratory insufficiency.*

INTRODUCTION

Penguins are highly specialized birds, with morphologic and functional particularities induced by living in, both terrestrial and aquatic habitats (Schneider et al., 2014). This is the reason why raising in captivity this type of birds represents a challenge and requires specific notions regarding environment, feeding, resting and reproduction (Schneider et al., 2014).
Pathologies diagnosed in captivity penguins are frequently associated with inappropriate housing and feeding conditions (Schneider et al., 2014). Also, it is taken into account the fact that penguins are prey animals and instinctively they will tend to mask signs of disease until it becomes severe or critical for the body (Schneider et al., 2014).
Post-mortem examination is a valuable tool for lesion evaluation, causes of morbidity and mortality and also, overall evaluation of general health status and group pathologies in both cases of captivity and wild penguins (Schneider et al., 2014).
The present paper presents a detailed evaluation of lesions, both macroscopic and microscopic, encountered in a Humboldt penguin kept in captivity, the first of this kind in Romania, regarding the species and pathological findings.

MATERIALS AND METHODS

The present case study involved an adult, female penguin, belonging to *Spheniscus humboldti,* owned and kept in captivity by a private owner. After death, the bird was submitted to multiple investigations at the Faculty of Veterinary Medicine of Bucharest in March, 2014. Little information regarding housing conditions and feeding were obtained, only that it was the only bird of this species and was kept in an individual enclosure. Regarding clinic evolution, the information provided was that during the last week the bird had poor appetite and was lethargic with no medication administered. The following examinations were performed in the study: necropsy, citopathology (M.G.G. stain), microbiology,

histopathology (H.E., H.E.A., PAS stain and Giemsa stain) and PCR testing for *Toxoplasma gondii.*

RESULTS AND DISCUSSIONS

Gross examination in the necropsy procedure revealed an adult *Spheniscus humboldti* penguin in cadaveric resolution. Examination of the exterior revealed full plumage with a dirty aspect, due to droppings from the cloaca up to neck and even wing region. This aspect is suggestive for prolonged decubitus, lack of bathing and possibly, presence of digestive disorders that affected the bird before the moment of death (Figure 1). The legs were affected by discrete lesions of plantar hyperkeratosis and medium to long, not-worn claws.

Figure 1. External examination: dirty plumage with greenish watery droppings on the entire ventral surface

These aspects were associated in penguins with improper floor or bedding and lack of extensive movement due to lack of enrichment in the environment (Blay & Cote, 2002; Clarke, 2003).

After skinning, pectoral and leg muscles were observed as reduced, with a prominent sternum keel and obvious subcutaneous highly fibrous conjunctive tissue.

The extended breastbone with a large basal plate and a membrane, is a normal feature in diving birds, including the present case, since these animals require protection of the intestines from high water pressure while diving (Johnsgard, 1987).

The examination of coelomic cavity revealed white-yellow nodules, with variable dimensions, from 0.2-3 cm in the air sacs (Figure 2). In addition to the nodular structures, air sacs presented thick, white-yellow deposits, that resulted in a rigid structure of the wall. Similar structures were observed on the surface and in-depth in both lungs and surrounding serous structures. In addition, the lungs presented a red colour, increased volume and a foaming reddish fluid on fresh cut section, suggesting inflammatory pulmonary oedema.

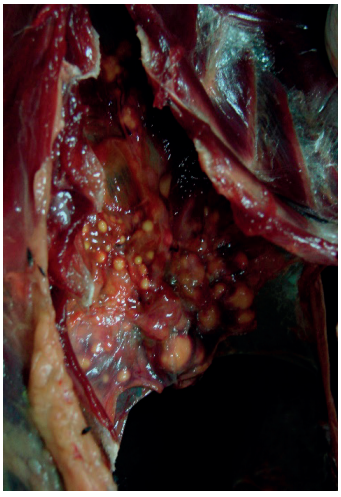

Figure 2. Abdominal air sac with thick walls and multiple yellow nodular structures with varying dimensions

Occasionally, white nodules were observed in the liver, kidney and spleen. On cut section, the nodules presented a dry, multilayer, concentric yellow material, suggestive for a granulomatous inflammation.

The digestive tract was generally empty and a white-green liquid content was observed in the large intestine.

The liver was large and black. The colour of the organ is specific (Hocken, 2002), but the size and the fact that dark, red blood was expressed on cut surface, sustained the diagnosis of stasis in the liver.

The fat tissue stored in the subcutaneous pelvic region and peritoneal cavity was reduced and also, the one from the base of the heart that had a transparent, slightly yellow colour, lesion diagnosed as serous atrophy of the fat tissue. It is known that penguins have particularities regarding lipid storage, appearing to be an obligatory prerequisite of the periods of fast,

mostly before molt and the breeding cycle (Cherel et al., 1993; Hocken, 2002; Schneider et al., 2014). Both, Blem (in 1990) and Lewden (in 2017), suggest that penguins have different body sites and mechanisms for body fat usage. While subcutaneous fat is the first to be deposited and last to be used, the peritoneal fat may be the first to be mobilized during stress. Another aspect regards the relationship between subcutaneous fat tissue deposits and thermoregulation. Less the fat tissue deposit, higher peripheral body temperatures in order to restore fat deposits and, in consequence, less capacity of insulation (Lewden et al., 2017)

In the present case, reduction of internal and subcutaneous fat tissue deposits, along with pectoral muscle emaciation, are strong indicators of poor body condition (Schneider et al., 2014).

Cytopathologic examination was performed by imprint and scraping from the nodular lesions previously observed and the internal layer of air sacs. The result obtained was a cellular population composed of macrophages and heterophils, erythrocytes, thrombocytes, cellular detritus, rare epithelial cells, along with intra and extracellular bacteria and fungus (Figure 3).

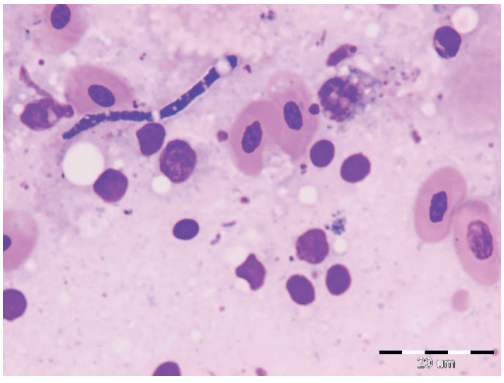

Figure 3. Air sac smear with a slightly eosinophilic background, bacillar and coco-bacillar bacteria extra and intracellular, nude nuclei, few erythrocytes and a fungus (M.G.G., 40x)

Microbiologic examination of samples of freshly cut granulomas from the lung and liver isolated *Aeromonas hydrophilia* and *Candida krusei. Aeromonas hydrophilia* is widely known as an opportunistic pathogen that affects debilitated organisms and has the capacity of infecting through aquatic mediums (Hazen et al., 1978). In 2005, a study concerning penguins belonging to species *Spheniscus demersus*, kept in a zoologic garden, identified a clinic episode of anorexia, lethargy, vomitus and diarrhoea with green droppings, that ended by death of four from the total of seven individuals. Microbiologic examination of affected tissues and the food, isolated a bacterial population among which *Aeromonas hydrophilia* had the pathogenic potential to cause the outbreak of the disease (Kim et al., 2005). In another case study, a captivity spectacled caiman which was exposed to stressful conditions, developed an infection with *Aeromonas hydrophilia* that was involved in the death mechanism. Captivity conditions are stressful events are involved in infections with potential pathogens for exotic animals (Kim and Kwak., 2013).

Mycologic examination isolated *Candida krusei,* a potential pathogen for immunosuppressed individuals. Donnelly et al. isolated and studied non-albicans Candida species in gastro-intestinal pathologies for birds demonstrating the possibility of transformation from a commensal species to a pathogenic one (Donnelly et al., 2019). Nevertheless, most studies indicate that Aspergillus spp. is the main fungus encountered in respiratory, chronic, granulomatous lesions in birds and, in particular, in penguins (Khan et al., 1977; Xavier et al., 2007; Beernaert et al., 2010). As a matter of fact, for wild Humboldt penguins (*Spheniscus humboldti*) as a part of ongoing ecological study, health surveys were carried and comprised blood tests, bacterial and virusologic tests and serology for Aspergillus sp. (Smith et al. 2008). This gives the fungus a central position in incidence and in screening health status for penguin population.

Cytopathology and histopathology using PAS stain, from respiratory tract lesions revealed long fungi with a septate hypha and sharp-angular branching suggestive for Aspergillus spp., although it was not positive for microbiologic examination.

Histopathology revealed mainly chronic lesions. Multiple granulomatous lesions, with different stages of evolution and extension were evident in lungs, air sacs, liver, spleen and kidneys.

The normal structure of the lungs and air sacs was severely compromised. The air sacs presented thick layers of amorphous material, formed by cellular detritus and fibrine along with granulomatous lesions (Figure 4).

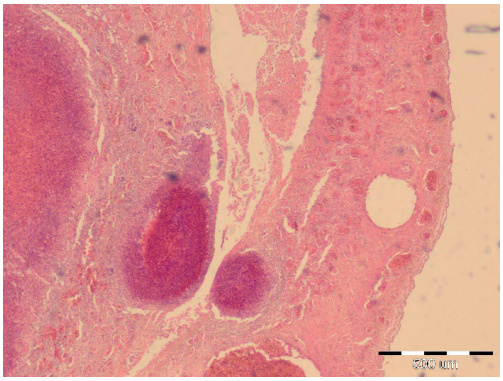

Figure 4. Air sac with a thickened wall due to inflammation, congestion and fibrin deposits and multiple granulomatous lesions (H.E., 4x)

The lungs were affected by focal to coalescing areas of chronic granulomatous inflammation, congestion and inflammatory oedema. The microscopic aspect of the granulomatous lesions revealed a central region, represented by an oxyphilic, unstructured necrosis surrounded by concentric layers of detritus and inflammatory cells, mainly lymphocytes and macrophages, epithelioid cells and, less frequent, gigantic multinucleated cells. Often, marginal hyperaemia and congestion was observed in lung and liver. Due to severe affection of the air sacs and the lungs, with acute and chronic lesions, respiratory insufficiency was the main cause of death for the present case of Humboldt penguin.

The liver presented a lympho-histiocytic hepatitis, with groups of inflammatory round cells frequently observed in perivascular areas, along with uneven distribution of hepatic stasis. Also, hepatocyte degeneration and necrosis, chronic inflammatory reaction and discrete proliferation of conjunctive tissue were observed in the liver and quantified as possible incipient granulomatous lesions.

The spleen was affected by lymphocytic depletion, represented by rare or degenerated lymphocytes in the white pulp and a histiocytic infiltrate in the red pulp along with circulatory disturbances suggestive for immune suppression (Figure 5).

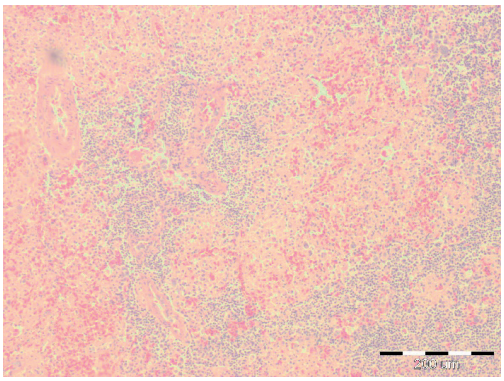

Figure 5. Lymphocytic depletion and hyperaemia in the spleen (H.E., 10x)

In birds, direct immune suppression can occur since fungal toxins interfere with protein synthesis, which affects both B and T-cell immunity. Secondary suppression can be encountered with high plasma corticosterone when birds have an acute feed restriction or are fastening. Nutrition plays a critical role in maintaining the immune system since poor protein and calorie diets supress antibody responses and enhance evolution of diseases (Schmidt et al. 2003). The penguin from the present study was affected by the presence of fungi, that could have delivered fungal toxins and produce primary suppression. The other mechanism was fastening before death and potentially inappropriate feeding during the time in captivity, that lead to lack of response of the immune system to immunogens.

Histopathology of the kidney revealed multiple lesions such as tubular degeneration and tubular necrosis, glomerular degeneration with reduced capillaries and few mesangial cells, along with interstitial mononuclear inflammatory cells. Some areas in the kidneys presented unstructured oxyphilic necrosis with granulomatous peripheral reaction, similar to those observed in the lungs and air sacs.

In addition, protozoan cysts were observed both in cytopathologic and histopathologic examinations, in all major tissues examined, including brain. They presented elongated form with a 50-80µ diameter, had multiple round structures inside, with discrete inflammatory or degerenative ractions in the tissues (Figure 6).

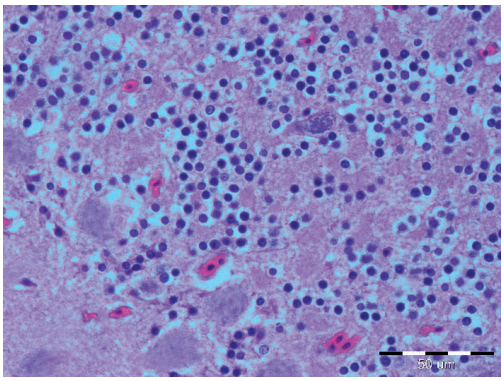

Figure 6. Cerebellum with necrotic neurons, mild lymphocytic inflammatory reaction and a parasitic cyst in the granular layer (H.E.A, 40x)

The morphologic aspects resemble for infection with *Toxoplasma gondii*. Still, PCR examination on paraffin embedded tissues gave a negative result, further studies being needed for sure exclusion of this parasite, such as immunohistochemistry. Several studies on wild and captivity penguins revealed the presence of *Toxoplasma gondii* in several organs, such as lung, liver and brain and was confirmed by PCR or immunohistochemistry or using antibody test surveillance (Ploeg et al., 2011; Bartova et al., 2018). In chronic cases, inflammation around the cysts is discrete in the tissues affected. In the brain, the inflammatory response consists of perivascular cuffing of blood vessels and mild lymphocytic reaction and gliosis (Zachary, 2017). This type of response was observed in the present case, with mild lymphocytic reaction and perivascular cuffing. The presence of the parasitic infection adds in the overall diagnosis of chronic, debilitating, systemic pathologies for the captive penguin.

CONCLUSIONS

The external gross examination in the necropsy procedure of the captive Humboldt penguin (*Spheniscus humboldti)* revealed a poor body condition.

Subcutaneous fat tissue can be used as a good indicator of body condition in penguins.

Necropsy of the coelomic cavity and organs revealed systemic granulomatous lesions on serous surfaces, air sacs, lungs, liver, spleen and kidneys.

Multiple investigations, such as microbiology, cytopathology, histopathology and PCR investigation indicated the presence of potentially pathogenic microorganisms represented by bacteria, fungi and parasitic cysts.

Poor body condition, the presence of granulomatous lesions and spleen lymphocytic depletion lead to the diagnosis of polyfactorial systemic disease in the case of the Humboldt penguin.

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