

MULTICENTRIC MALIGNANT LYMPHOMA OF IDIOPATHIC ETIOLOGY IN A CAPTIVE PINK-BACKED PELICAN (*PELECANUS RUFESCENS*)

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Summary: A mature female pink-backed pelican (*Pelecanus rufescens*), kept at the Al Wabra Wildlife Preservation in Qatar, was found dead. No clinical signs were observed before. Gross postmortem and histologic examination revealed multicentric malignant lymphoma involving liver, spleen, kidney, ventriculus, pancreas and small intestine. Immunohistochemistry results were consistent with T-cell lymphoma. As viral etiology of lymphoid neoplasia is confirmed in poultry and evident in several other avian species, virological examination was performed. Causative viruses of T-cell lymphoma include Marek's disease virus and reticuloendotheliosis virus. Polymerase chain reaction amplification and embryo culture were negative. Thus, the malignant lymphoma is considered idiopathic in this case. Additionally, a melanoma on the left pouch could be diagnosed.

Key words: malignant lymphoma, T-cell origin, polymerase chain reaction, embryo culture, melanoma, pink-backed pelican, *Pelecanus rufescens*

INTRODUCTION

Pink-backed pelicans (*Pelecanus rufescens*) are resident breeder in Africa, preferring lakes, rivers, swamps, seasonally flooded land, lagoons and estuaries (NELSON, 2005). The species is currently considered as "least concern" by the IUCN Red List due to its stable population size and its large distribution – from Egypt to Angola (BIRDLIFE INTERNATIONAL, 2009). According to the World Association of Zoos and Aquariums (WAZA) pink-backed pelicans are primarily kept in zoological facilities as ambassador species for wetland conservation and for educational reasons because of their evolutionary adaptation to fish catching in social groups. In 2008 the zoo population of pink-backed pelicans amounted to 255 birds as reported to ISIS – the International Species Information System (WAZA, 2009).

The malignant lymphoma is a frequently diagnosed condition in a wide range of avian species including poultry, companion and wild bird species, both captive and free-ranging (WADSWORTH et al., 1981; HIGGINS and HANNAM, 1985; NEWELL et al., 1991; COLEMAN and OLIVER, 1994; LATIMER, 1994; COLEMAN, 1995; DE WIT et al., 2003; KELLY et al., 2004; BURGOS-RODRÍGUEZ et al., 2007; MALKA et al., 2008; SOUZA et al., 2008; WILEY et al., 2009). It is defined as lymphoid neoplasm originating in peripheral lymphoid tissues and forming white-to-yellow tissue discolorations, diffuse enlargement of organs or solid tumors. Malignant lymphoma usually occur as a disseminated multisystemic disease that may involve all organs, especially liver, spleen and kidney (NEWELL et al., 1991; COLEMAN and OLIVER, 1994; LATIMER, 1994; COLEMAN, 1995; DE WIT et al., 2003; KELLY et al., 2004; BURGOS-RODRÍGUEZ et al., 2007). In addition, neoplastic lymphocytes can appear

in the peripheral circulation (NEWELL et al., 1991; COLEMAN, 1995; KELLY et al., 2004; BURGOS-RODRÍGUEZ et al., 2007).

Concerning the potential causes of malignant lymphoma in birds, a viral etiology is proven for poultry but also evident in other avian species. This type of neoplasia may be caused by the herpesvirus Marek's disease virus (MDV) and by retroviruses, such as reticuloendotheliosis virus (REV) and avian leukosis virus (ALV) (NEWELL et al., 1991; COLEMAN and OLIVER, 1994; LATIMER, 1994; COLEMAN, 1995; RITCHIE; 1995; WADE et al., 1999; CRESPO et al., 2002; KELLY et al., 2004; BURGOS-RODRÍGUEZ et al., 2007; MALKA et al., 2008; SANTOS et al., 2008; SOUZA et al., 2008; WILEY et al., 2009).

This report describes the necropsy and histopathologic findings of malignant lymphoma in a pink-backed pelican (*Pelecanus rufescens*) and illustrates the use of diagnostic tools to specify the type of neoplasia and to determine the potential cause.

CASE REPORT

History

A female adult pink-backed pelican, housed at the Al Wabra Wildlife Preservation (AWWP), Qatar, was found dead in July 2009. It was part of a group of pelicans which are kept outside throughout the year on a non-fenced lawn together with Greater flamingos (*Phoenicopterus roseus*), Saddle bill storks (*Ephippiorynchus senegalensis*) and Bewick's Swans (*Cygnus colombianus bewickii*), near to a free-roaming group of Marabou storks (*Leptotilos crumeniferus*). The pelicans are fed on pink ear emperor fish (5-8 kg per day), bought on local markets every day. The diet is supplemented with two fish-eater-tablets (Mazuri[®] Zoo Food, aleckwa Tiernahrung, Altrip, Germany) per week.

No signs of disease were discovered before in this bird and it never received any treatment. In the last 9 years there have not been any reports about other sudden deaths in pelicans at AWWP.

Material & Methods

Gross necropsy

Necropsy was performed after a standard protocol and impression smears were obtained from liver, spleen, lung, intestine and from an altered skin area located at the left pouch. Slides were stained with Diff-Quik (Diff-Quik[®], Dade Behring, Marburg, Germany).

Histologic examination

Representative tissue samples for histopathologic examination were fixed in 10 % neutral-buffered formalin and submitted to the Clinic for Birds and Reptiles, Faculty of Veterinary Medicine, University of Leipzig, Germany. Formalin-fixed samples were dehydrated, embedded in paraffin and sectioned at 4 µm. Tissue sections were stained with hematoxylin and eosin.

Immunohistochemistry for detecting CD (cluster of differentiation)-3, CD-45 and CD-79 antigens was performed applying the peroxidase anti-peroxidase (PAP) technique. Therefore, sections were dewaxed, rehydrated and treated with hydrogen peroxide 0,5 % in methanol for 30 minutes at room temperature to stop endogenous peroxidase activity. To detect CD-3, protease pre-digestion was required. 0,05 % protease XXIV (Sigma Aldrich Chemie, Munich, Germany, P8038) was applied for 5 minutes at 37 °C. For possible detection of CD-45 and CD-79 citrate pre-treatment with 10mM citrate buffer at pH 6.9 for 30 minutes at 96 °C was necessary. Using polyclonal primary antibodies, a blocking step with 50 % pig serum in Tris-buffered saline (TBS) for 10 minutes at room temperature was applied to the sections. The following primary antibodies, diluted in TBS at 4 °C over night, were used: rabbit anti-CD-3 at a 1:200 dilution in TBS (DAKO GmbH, Hamburg, Germany, A 0452), mouse anti-CD-45 at a 1:1000 dilution in TBS (Camon, Wiesbaden, Germany, B 220) and mouse-anti-CD-79 at a 1:10 dilution in TBS (DAKO, M 7051). The corresponding secondary antibodies used were rat anti-mouse IgG at a 1:100 dilution (Dianova GmbH, Hamburg, Germany, 415-005-166) and swine anti-rabbit IgG at a 1:100 dilution (DAKO, Z0196). Subsequently mouse PAP complex (1:500; Dianova, 223005025) and rabbit PAP complex (1:100; DAKO, Z0113) were applied respectively. As chromogen diaminobenzidinetetrahydrochloride (Fluka Feinchemikalien GmbH, Eschborn, Germany) was used and slides were counterstained with Papanicolaou's solution (Merck GmbH, Darmstadt, Germany). To establish negative control sections, primary antibodies were replaced by normal mouse (DAKO, X0931) or rabbit (DAKO, X0930) serum.

Virological examination

As T-cell lymphoma in birds can be induced by reticuloendotheliosis virus and Marek's disease virus frozen tissue samples of liver and spleen were submitted to the Clinic for Birds and Reptiles, Faculty of Veterinary Medicine, University of Leipzig, Germany for virus isolation.

Samples were homogenized and passed through 0.45 µm mesh size filters. Pathogen-free chicken embryos (Valo, Cuxhaven, Germany) were used to inoculate their chorioallantoic cavity at day 10 of incubation. After 7 days the chorioallantoic fluid was gained and passaged into fresh embryos for a total amount of 3 passages. In addition, monolayer cultures of liver cells of primary specific pathogen-free chicken embryos were inoculated and passaged once after 7 days of incubation. DNA extraction was performed using spin columns (NucleoSpin Tissue, Macherey-Nagel, Germany). For the detection of reticuloendotheliosis virus-DNA polymerase chain reaction (PCR) amplification was carried out as described in ALY et al., 1993. To detect Marek's Disease virus-DNA a real-time PCR protocol was applied as reported in PHILIPP et al., 2008.

Results

Gross pathologic findings

At necropsy, the pelican was in a poor body condition (3.5 kg). Gross abnormalities included a small dark, spherical mass on the left side of the throat pouch. The most significant changes could be observed in the liver, the spleen and the kidneys. An extremely enlarged and fragile liver with small areas of necrotic foci (0.2

cm diameter) embedded into the parenchyma was found. In addition, the spleen and both kidneys were profoundly enlarged, fragile and hemorrhagic. The digestive system was empty and the ventriculus showed petechial hemorrhages in the mucosa. The intestine contained dark red to blackish watery content. Other findings included an enlarged pancreas with vesicle-like structures containing clear fluid as well as hemorrhagic lungs containing froth.

The cytological impression smears revealed a liver with many lymphocytes among red blood cells. The spleen showed numerous lymphoid cells among red blood cells. Heterophil cells and red blood cells were found in the lung, epithelial cells, gram positive rods and cocci in the intestine as well as gram positive cocci and red blood cells in the skin mass.

Histopathologic findings

On histopathologic examination, liver, spleen, kidney, pancreas and the mucosa of ventriculus and small intestine were severely and diffusely infiltrated with a homogeneous population of lymphocytes with mild mitotic figures. Immunohistochemistry showed that the neoplastic lymphocytes were only markedly reactive to CD-3 antibody.

Histopathologic and immunohistochemical findings were consistent with multicentric malignant T-cell lymphoma involving liver, spleen, kidney, pancreas, ventriculus and small intestine.

Histologic evaluation of the altered skin region showed severe infiltrative and diffuse melanin-filled melanozytes in the subcutaneous tissue leading to the diagnosis of melanoma. Bleaching revealed good differentiation of melanocytes with only a few mitotic figures suggesting low malignity. Further more, hemorrhagic diathesis in the intestine was detected. All other organs seemed to be without abnormal findings.

Virological results

Neither DNA of MDV nor of REV could be amplified. Due to the negative virological results the lymphoma are considered as idiopathic in this pelican.

DISCUSSION

Multicentric malignant T-cell lymphoma was diagnosed in a pink-backed pelican. To the author's knowledge, it is the first report of this type of neoplasia in this species. Neoplastic infiltrations were discovered in liver, spleen, kidney, pancreas, ventriculus and small intestine. To differentiate between T-cell and B-cell lymphoma cell markers can be used (BURGOS-RODRÍGUEZ et al., 2007; SOUZA et al., 2008). In the pelican T-cell origin was proven immunohistochemically by positive reaction of lymphocytes to the cell marker CD-3, which is broadly reactive in a wide range of species (MALKA et al., 2008). T-cell lymphomas in non-gallinaceous avian species, which are confirmed by positive reaction to CD-3, have been reported in a great horned owl (MALKA et al., 2008) and in Amazon parrots (DE WIT et al., 2003; BURGOS-RODRÍGUEZ et al., 2007; SOUZA et al., 2008).

Further more, a melanoma on the skin of the left throat pouch was diagnosed. No metastases were found. An etiological connection between both neoplasias is fairly

unlikely as cutaneous melanoma are normally induced by high exposure to ultraviolet (UV) light which is not the case for lymphoma.

According to an evaluation of IPPEN et al. in 1987 (EULENBERGER, 1995) tumors are responsible for 2 % of deaths in pelicans. Neoplasms that have been described in pelicans so far include chondrosarcoma, squamous cell carcinoma, cholangiocarcinoma, bronchial carcinoma, phaeochromocytoma and ganglioneuroma (IPPEN et al., 1987; SEIDEL and SCHRÖDER, 1988; EULENBERGER, 1995; PAULY et al., 2009; PESARO et al., 2009).

Clinical signs of malignant lymphoma in birds are non-specific, depending on the organs involved (BURGOS-RODRÍGUEZ et al., 2007). Symptoms described range from sinus, cutaneous or abdominal swellings to depression, anorexia, weight loss, paresis, diarrhea and blindness (COLEMAN and OLIVER, 1994).

In this case, no clinical history was present. A complete blood count (CBC) and differential may have revealed a high white blood cell count and lymphocytosis with numerous immature lymphocytes in the blood smear ("leukemic blood picture") (LATIMER, 1994; WADE et al., 1999).

Regarding the etiology viral infections have been linked with lymphoid neoplasia in humans, nonhuman primates, cats and birds, among other vertebrate taxa, but not in pelicans so far. In avian species most research on virus-induced neoplasia has been done in poultry due to the high economic impact of this disease on the food industry (BURGOS-RODRÍGUEZ et al., 2007; COLEMAN, 1995; KELLY et al., 2004; LATIMER, 1994; WADE et al., 1999).

As causative agents for a T-cell lymphoma in bird species Marek's disease virus (MDV), a herpesvirus and reticuloendotheliosis virus (REV) can be considered. Therefore, PCR amplification and embryo culture were used to determine, if MDV or REV could be detected in the tumor material of the pelican, but no supporting sign for viral etiology could be found. As avian leukosis virus (ALV) usually induces B-cell lymphoma (MALKA et al., 2008), no test to detect ALV was performed.

Marek's disease-induced tumors are characterized by heterogeneous populations of lymphocytes and infiltration of neoplastic cells into nervous tissue (MALKA et al., 2008). In the case of the pelican homogeneous populations of lymphocytes were detected and nervous tissue was not examined.

In humans environmental contaminants, like pesticides, are considered as a possible contributing factor to the development of lymphoma (KELLY et al., 2004). Tissue concentrations of possible contaminants were not analysed in this pelican.

Due to gross post mortem and histological findings as well as the immunohistochemical staining a multicentric malignant T-cell lymphoma was diagnosed in this pink-backed pelican. As a viral etiology could not be detected, the pathogenesis of this neoplasia is considered as spontaneous.

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