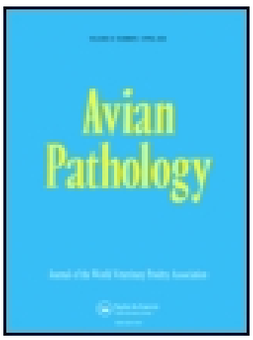

Avian Pathology

ISSN: 0307-9457 (Print) 1465-3338 (Online) Journal homepage: http://www.tandfonline.com/loi/cavp20

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To cite this article: Stefano Pesaro , Barbara Biancani , Gabriele Fabbrizi & Giacomo Rossi (2009) Squamous cell carcinoma with presence of poxvirus-like inclusions in the foot of a pink-backed pelican (Pelecanus rufescens), Avian Pathology, 38:3, 229-231, DOI: 10.1080/03079450902912176

To link to this article: http://dx.doi.org/10.1080/03079450902912176

Published online: 29 May 2009.

Avian Pathology (June 2009) **38(3),** 229–231

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Squamous cell carcinoma with presence of poxvirus-like inclusions in the foot of a pink-backed pelican (*Pelecanus rufescens*)

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Squamous cell carcinoma (SCC) or avian keratoacanthoma is a neoplastic skin lesion of unknown aetiology that has been well described in birds. Some studies have reported that poxviruses may contribute to the onset of SCC. Here we describe a case of SCC on the underside of a pelican's foot. Histologically, the tumour consisted of irregular cords of pleomorphic epithelial cells that invaded the adjacent tissues. Additionally, keratinized epithelial cells and moderate numbers of keratin pearls were observed. Intracytoplasmic inclusions, a characteristic of this virus, were observed in some of these cells, and viral particles were characterized by electron microscopy. Although the aetiology of the carcinoma in this case may have been secondary to chronic focal trauma, the possibility of a latent or chronic form of fowlpox should be considered in the pathogenesis of the lesion.

Introduction

Pox is a common viral disease in birds caused by avian poxviruses of the family *Poxviridae* (Tripathy & Reed, 1997). Among the 9000 bird species, only about 232 species have reportedly acquired a natural poxvirus infection, but it is probable that more birds are susceptible to these viruses (Bolte *et al.*, 1999). The disease is usually characterized by cutaneous lesions, but a diphtheritic or wet form, with mucosal lesions within the digestive and upper-respiratory tracts, can occur (Gerlach, 1999). Members of the *Poxviridae* family present oncogenic properties (Porter *et al.*, 1988; Schafer, 1998; Bolte *et al.*, 1999). Tumour-like lesions have been reported in different domestic and wild bird varieties (Mohanty & Dutta, 1981; Gerlach, 1984; Harrison & Harrison, 1986; Arai *et al.*, 1991; Ritchie & Carter, 1995; Tsai *et al.*, 1997; Bolte *et al.*, 1999; Hernandez *et al.*, 2001; Mete *et al.*, 2001). Avipoxvirus has also been associated with a dermal squamous cell carcinoma (DSCC), or avian keratoacanthoma—terms used for neoplastic skin lesions usually found in broiler chicken carcasses (Fallavena *et al.*, 1993; Back *et al.*, 1995; Fallavena *et al.*, 1997; Hafner & Goodwin, 1997). The aetiology of naturally occurring cases of DSCC is unknown.

Materials and Methods

History of the affected bird. An adult male pink-backed pelican (*Pelecanus rufescens*) maintained in captivity was examined in December 2006 after being observed to have difficulty in walking. Loss of weight and emaciation were noted during the clinical examination. A cauliflower-like mass was also found on the plantar surface of the right foot. Radiographs did not reveal abnormalities, but blood values indicated anaemia and a mild leukocytosis. Following examination, the animal was kept in the clinic for monitoring and supportive care. Despite supportive care consisting of forced feeding, the administration of Duphalyte (Fort Dodge Animal Health) subcutaneously and antibiotic (Enrofloxacin, 10 mg/kg intramuscularly, twice daily) therapy, the animal died after 3 weeks.

Diagnostic procedures. Necropsy was performed within hours after death and samples were taken of all organs and of the foot lesions. Samples were fixed in a 10% formalin-buffered solution, embedded in paraffin wax, and stained with haematoxylin–eosin for routine histopathologic examination and to grade and stage the carcinoma. Formalin-fixed, paraffin-embedded sections (3 to 4 μm) were de-waxed in xylene for 5 min before staged re-hydration through graded alcohols (100%, 90%, then 70%) to water. Antigen retrieval was performed on the slides by placing them in a bath of 10 mmol/l citric acid (pH 6) and boiling for 16 min using an 800-W microwave oven (2450 MHz; Panasonic NN-6453BBPQ; John Lewis, Watford, UK). The volume of fluid was topped up and the slides were then left to stand for 20 min at room temperature before being washed well in running tap water. After peroxidase block, the sections were incubated with the following primary antibodies: anti-cytokeratin 6 monoclonal antibody (NCL-LHK6B; Novocastra Labs Ltd, Peterborough, UK), anti-cytokeratin 8/18 monoclonal antibody (NCL-5D3; Novocastra Labs Ltd), anti-proliferating cell nuclear antigen monoclonal antibody (Clone PC10; Zymed Laboratories, California, USA), all diluted 1:20 in a buffer solution. Antibody binding was revealed with the ABC-peroxidase (Vector Laboratories, Inc., Burlingame, California, USA) procedure using 1:200 diluted biotinylated goat-anti mouse immunoglobulin (AO433; DAKO, Glostrup, Denmark), applied for 45 min at room temperature as secondary antibody. The enzymatic reaction was developed with 3,1-diaminobenzydine (Sigma Chemical Co., St Louis, Missouri, USA) as substrate. Stained slides were counterstained in

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Received 31 October 2008
ISSN 0307-9457 (print)/ISSN 1465-3338 (online)/09/030229-03
DOI: 10.1080/03079450902912176

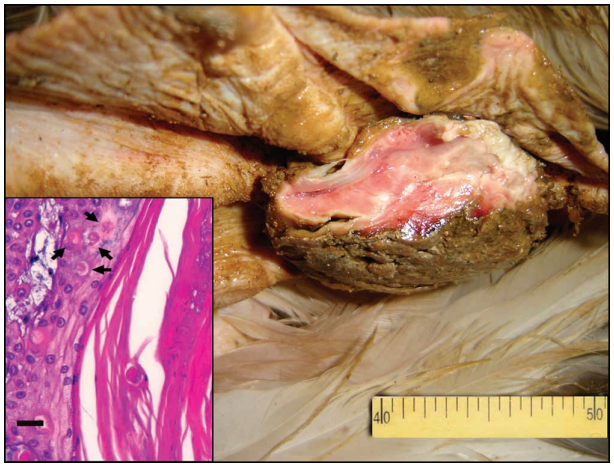

Figure 1. *Macroscopic view of cauliflower-like mass on ventral surfaces of a pelican foot. Note the histological aspect (inset) of the neoplastic tissue. A keratin pearl is evident and four well-defined intracytoplasmic Bollinger's inclusion bodies (arrows) are observed in the epidermal basal layer. (Haematoxylin and eosin, scale bar =25 μm.)*

haematoxylin for 30 sec, washed in tap water, dehydrated in graded alcohols (70%, 90%, and 100%), cleared in xylene, and mounted in DPX (08600E; Surgipath Europe Ltd). Positive control slides for cytokeratin 6 and cytokeratins 8/18 were represented by normal chicken skin and salivary glands, respectively, and a negative control for each antibody was used for this study—consisting of the substitution of the primary antibody for an isotype antibody control at the same protein concentration. In addition, portions of the lesion were immediately fixed in phosphate-buffered 0.1 M of 2% glutaraldehyde (pH 7.4), post-fixed in phosphate-buffered 1% OsO_4 and, after dehydratation, embedded in Epon/Araldite (Polyscience Inc., Warrington, Pennsylvania, USA). Semi-thin sections were stained with methylene blue and Azur II, while for immunoelectron microscopy ultrathin sections (70 nm) were placed on 200-mesh nickel grids supplied with formvar-carbon film (Agar Scientific Ltd, Stansted, UK). Grids were then stained with uranyl acetate and lead citrate and were examined with a JEOL 1200-EX transmission electron microscope (JEOL, Peabody, Massachusetts, USA).

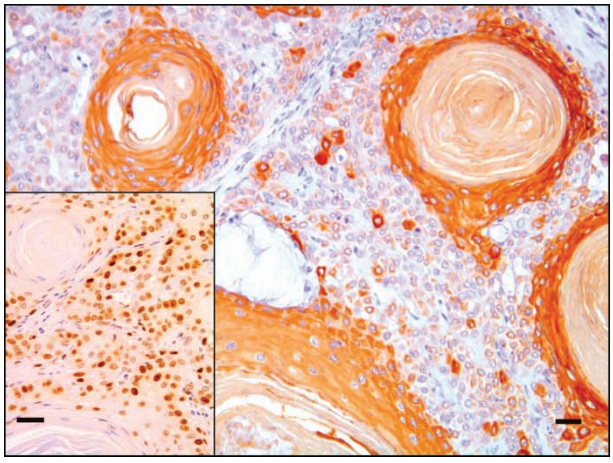

Figure 3. *Immunohistochemical staining of neoplasm. Strong and diffuse positive immunostaining for cytokeratin 6. Inset: Note the strong nuclear expression of proliferating cell nuclear antigen in proliferating neoplastic cells. (Scale bar =50 μm.)*

Results and Discussion

Necropsy examination of the bird indicated poor body condition characterized by severe pectoral muscle mass atrophy. An ulcerated cauliflower-like mass (approximately 6×12 cm^2) with irregular edges was present on the ventral surface of the right foot that, on sectioning, was composed of solid and fibrous inner tissues (Figure 1).

Organs within the coelomic cavity did not show signs of metastasis or other histopathologic lesions. The tissues obtained from the foot lesions showed DSCC associated with the presence of intracytoplasmic Bollinger's inclusion bodies. Histologically, the tumour consisted of irregular cords of pleomorphic epithelial cells with a disorganized pattern of growth and invasion of the adjacent tissues. Dyskeratosis and moderate numbers of keratin pearls were frequently observed. The tumour was locally invasive, but no evidence of vascular invasion or metastasis was found. In contrast to other studies,

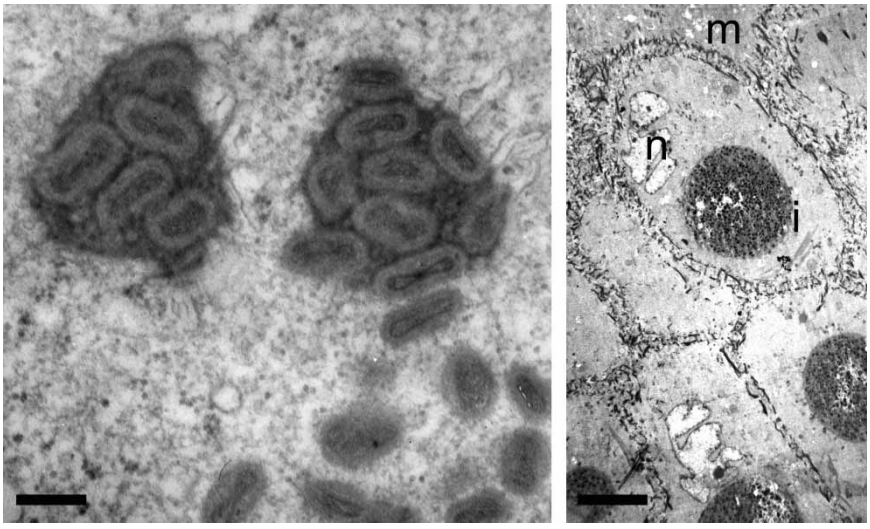

Figure 2. *Intracytoplasmic inclusion containing a large number of 320 to 350 nm brick-shaped viral particles (bar =350 nm). Inset: Ultrastructural image of keratinocytes showing intracytoplasmic inclusions; m, cellular membrane; n, nucleus; i, inclusion body (bar =10 μm).*

wherein the only evidence of poxvirus in the DSCC lesion was by polymerase chain reaction (Fallavena *et al.*, 2002), the haematoxylin and eosin staining showed pale eosinophilic intracytoplasmatic inclusion bodies, characteristic of avipoxvirus infection (Figure 1). Ultrastructural examination of these inclusions demonstrated aggregates of a great number of 320 to 360 nm brick-shaped viral particles (Figure 2). The neoplastic tissues showed strong and diffusely positive immunostaining for cytokeratins of different molecular weights and high nuclear expression of proliferating cell nuclear antigen, indicating a high rate of tumour cell turnover (Figure 3). These findings, in association with the presence of the Bollinger's inclusion bodies, may confirm the oncogenic properties of poxvirus discussed by different authors.

In avian species, the majority of data on neoplasms are reported in domesticated birds, and correlations between avipoxvirus and tumour-like lesions in domesticated and wild species have been reported only in few cases (Mohanty & Dutta, 1981; Harrison & Harrison, 1986; Arai *et al.*, 1991; Gerlach, 1984; Ritchie & Carter, 1995, Tsai *et al.*, 1997; Bolte *et al.*, 1999; Hernandez *et al.*, 2001; Mete *et al.*, 2001). The evidence of association of squamous cell carcinoma and poxvirus has only been described in poultry, suggesting that latent or chronic forms of fowlpox infection may be implicated in development of this particular neoplasm (Fallavena *et al.*, 1993, 1997; Back *et al.*, 1995; Hafner & Goodwin, 1997). To our knowledge, this is the first report of squamous cell carcinoma in a pelican. In contrast to other studies in poultry where the lesions were located in the feathered parts of the body and were characterized by crater-like ulcers (Turnquest, 1979; Riddel & Shettigara, 1980; Fallavena *et al.*, 1993, 2002; Hafner *et al.*, 1993; Weinstock *et al.*, 1995), the lesion in this pelican was found on the ventral surface of the foot and was characterized by a cauliflower-like mass. The aetiology of the squamous cell carcinoma in mammals is associated with physical, chemical and biological agents, but causes in birds have not been well defined. Experimentally, neoplastic lesions have been induced by repeated applications of a carcinogen (methylcholanthrene) to the skin of macroscopically healthy chickens (Duran-Reynals & Bryan, 1952; Rigdon & Brashear, 1954; Rigdon & Hooks, 1956; Rigdon, 1959). However, as recently reported (Fallavena *et al.*, 2002), the consistent detection of fowlpoxvirus DNA in a large number of macroscopically normal skin samples from vaccinated and non-vaccinated poultry corroborates the possibility that a latent or chronic form of fowlpox occurs concurrently with development of DSCC skin lesions. In this case, although chronic local trauma and inflammation may have contributed to development of this lesion, poxvirus infection should be considered in the pathogenesis.

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