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# SQUAMOUS CELL CARCINOMA IN THE FOOT OF A COCKATIEL (Nymphicus hollandicus) FROM NORTHEASTERN BRAZIL

#### E.P.F. Souto<sup>1\*</sup>, A.M. Oliveira<sup>1</sup>, D.F. Cardoso<sup>1</sup>, F.N.L. Oliveira<sup>2</sup>, G.J.N. Galiza<sup>1</sup>, A.F.M. Dantas<sup>1</sup>

<sup>1</sup>Laboratory of Animal Pathology, Veterinary Teaching Hospital, Federal University of Campina Grande, Patos, Paraiba, Brazil; <sup>2</sup>Centro Educacional de Ensino Superior de Patos, UNIFIP, Patos, Paraiba, Brazil

ARTICLE INFO	ABSTRACT
Article History: Received 01 <sup>st</sup> December, 2020 Received in revised form 11 <sup>th</sup> December, 2020 Accepted 16 <sup>th</sup> January, 2021 Published online 28 <sup>th</sup> February, 2021	A 16-year-old male cockatiel ( <i>Nymphicushollandicus</i> ) was presented with a multinodular mass at the base of the second and third digits of the right foot. Manipulation resulted in amputation of the toes. The bird showed emaciation, respiratory signs, and died. Histology revealed an infiltrative neoplasm in the deep dermis and underlying bone tissue composed of epithelial cells arranged in cords and nests. Neoplastic cells demonstrated intense immunolabeling for the antipan cytokeratin antibody. A severe necroheterophilic bronchopneumonia was also observed. The diagnosis of squamous cell carcinoma was based on the epidemiological, clinical, anatomopathological, and immunohistochemical findings. The late diagnosis of squamous cell carcinomas precludes the adoption of suitable treatment and determines the incidence of local and systemic complications. Overexposure to ultraviolet light should be investigated as a possible risk factor for the incidence of these neoplasms in birds.
Key Words:	
Avian disease, cutaneous neoplasm, Bone invasion.	
*Corresponding author: E.P.F. Souto,	

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# **INTRODUCTION**

Squamous cell carcinoma (SCC) is a malignant neoplasm of epidermal cells in which the cells show differentiation to keratinocytes. It is one of the most common malignant skin tumors of all domestic animals,<sup>1</sup> including avian species.<sup>2</sup> In birds, SCC typically arise from single or multiple sites in the skin, mucocutaneous junctions, uropygial gland, and upper gastrointestinal tract.<sup>2,3</sup> Regarding the biological behavior of these tumors, the incidence of metastasis is extremely low but the capacity of invasion into the surrounding tissue is generally very high, which results in a guarded prognosis.4,5,6 Therefore, the present report describes the epidemiological, clinical. anatomopathological, and immunohistochemical findings of a SCC in the foot of a cockatiel (Nymphicus hollandicus) from northeastern Brazil.

## RESULTS

A 16-year-old male cockatiel (*Nymphicus hollandicus*) was presented with a 5-month history of apathy, weight loss, and a multinodular mass at the base of the toes (digits II and III) of the right foot. The cockatiel was kept in a large metal cage and sunbathed every morning. Its lifelong diet consisted of a seed mixture (birdseed, millet, sunflower), often supplemented with cabbage, coriander, and green corn. Water *ad libitum* and vitamin complex twice a year. On physical examination, manipulation of the mass resulted in bleeding and amputation of the affected toes. Cytological examination by fineneedle aspiration was performed but considered inconclusive. The area was treated with a topical 0.5% chlorhexidine solution and bandage. The bird was treated orally with enrofloxacin (10 mg/kg q24h) and dipyrone (25 mg/kg q24h) for two weeks. About a month later, no response to treatment was noted and the mass continued to grow involving the whole right foot. The bird presented sneezing and dyspnea, and general condition progressively worsened. The animal died and was referred for necropsy. At postmortem examination, the bird was emaciated (body condition score 1 of 5) and weighed 70g. A yellowish, roughened, thickened, irregular, multinodular mass, measuring 4 x 3 x 3 cm, was observed in the right foot (Fig. 1A). At cut, the mass was firm, compact, off-white to yellow, and ill-defined. At the longitudinal section of the foot, a segmental area of loss of distinction between the mass and the underlying bone tissue was observed. Gross findings also included severe pectoral muscle atrophy and no fat stores. The lungs had yellowish, firm, slightly elevated, multifocal 1-4 mm nodules, often surrounded by a reddish rim, and randomly distributed throughout the pleural surface. Samples of the mass, skin, central nervous system, and organs of the coelomic cavity were fixed in 10% neutral buffered formalin. Additionally, the entire foot was decalcified in 10% nitric acid. All these tissues were routinely processed for histology, embedded in paraffin wax, cut at 3 µm sections, and stained by hematoxylin and eosin (HE). Immunohistochemistry (IHC) was performed to confirm the diagnosis. Histological sections 4µm in thickness were deparaffinized, rehydrated in a graded series of ethanol, and rinsed in distilled water.

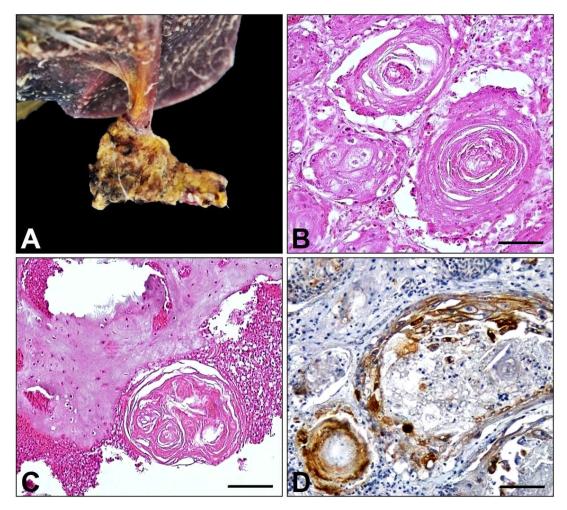


Figure 1. Squamous cell carcinoma in the foot of a cockatiel. A) Right foot, medial view. A yellowish irregular multinodular mass involving the whole foot. B) Dermis. Neoplastic epithelial cells arranged in nests, some with areas of central keratinization. HE. Obj. 40x. C) Underlying bone tissue. Neoplastic epithelial cells invading bony trabeculae. HE. Obj. 25x. D) Neoplastic epithelial cells showed intense immunolabeling for the anti-pan cytokeratin antibody in brown. IHC. Obj. 25x

Then, submitted to heat-induced antigen retrieval in 10mM citrate solution (pH 6.0) in the pressure cooker (PascalR, Dako). Slides were placed at room temperature for 20 minutes for cooling and washed with deionized water. After antigenic retrieval, endogenous peroxidase was blocked by immersing the slides in ready-to-use hydrogen peroxide (Peroxide Block, Cell Marque, 925B-09). Slides were washed in a tris solution (pH 7.4) and, then, the nonspecific sites were blocked with a nonspecific reaction blocking solution (Protein Block Serum-Free, DAKO ref. X0909). Incubation with primary anti-Pancytokeratin antibody (AE1/AE3) (Dako Agilent) diluted at 1:200 for 18 hours at 4° C. As an amplification and detection system, the standard ABC method (avidin-biotin complex, VECTASTAIN® Elite ABC kit, Vector Laboratories Inc., Burlingame, CA) and the chromogen diaminobenzidine (DAKO, K3468) were used. Sections were counterstained with Harris Hematoxylin. As a positive control, histological sections from a previous case of squamous cell carcinoma in a bird were used. Sections from the same bird were used as negative controls, with the primary antibody replaced by phosphate buffered saline. Histopathologic examination of the mass revealed a large poorly demarcated infiltrative neoplasm in the deep dermis, composed of epithelial cells arranged in cords and nests, often with brightly eosinophilic central accumulations of lamellations of keratin (keratin pearls), and supported by a moderate collagenous stroma (Fig. 1B). Polygonal neoplastic cells have distinct borders with prominent intercellular bridges, abundant eosinophilic cytoplasm, and an irregularly round to oval nucleus with sparse chromatin and 1-3 evident nucleoli. There is marked anisokaryosis and anisocytosis. Mitotic figures average 2 per 400x field. There are multifocal areas of intratumoral necrosis permeated by intense inflammatory infiltrate of heterophiles, lymphocytes and rare macrophages.

Multifocally, neoplastic cells invade the underlying bony trabeculae (Fig. 1C), which are lined by increased numbers of osteoclasts in Howship's lacunae (osteolysis). The overlying epidermis is ulcerated and replaced by a serocellular crust composed of fibrin, cellular debris, intact and degenerate heterophiles, and keratin. IHC revealed intense immunolabeling of neoplastic epithelial cells for the anti-pan cytokeratin antibody (Fig. 1D). Features consistent with a dermal well-differentiated SCC with bone invasion. There was no evidence of vascular invasion or metastasis. The pulmonary sections revealed severe multifocal subacute necroheterophilic bronchopneumonia characterized by well-demarcated central areas of necrosis surrounded by intact and degenerate heterophiles, lymphocytes, plasma cells and macrophages admixed with necrotic cellular debris, hemorrhage, fibrin and numerous basophilic coccobacilli.

### DISCUSSION

The diagnosis of SCC was based on the epidemiological, clinical, anatomopathological and immunohistochemical findings. The incidence of SCC in birds has been limited to isolated cases in aged individuals and young broiler chickens.<sup>7</sup> In all species SCCs may occur in young animals, but the incidence increases with age.<sup>1</sup> No species, breed, or sex predilection has been properly documented for SCCs in birds.<sup>7</sup> However, SCCs are commonly observed in cockatiels (*Nymphicus hollandicus*), Amazon parrots (*Amazona spp.*), and budgerigars (*Melopsittacus undulatus*).<sup>2,3</sup> For cockatiels, the skin is the most common location<sup>2</sup> but there are few case reports describing gastrointestinal tract<sup>4</sup> and uropygial gland involvement.<sup>8</sup> There are several factors that are associated with the development of SCC in mammals, including prolonged exposure to ultraviolet light, certain

chemicals, chronic skin diseases, radiation, among other.<sup>1,5</sup> Although these factors can be related to birds, the causes of SCCs in birds are not completely understood.<sup>5</sup> It has been suggested a relationship between the incidence of cutaneous SCCs in avian species and chronic exposure to chronic skin diseases, chronic feather picking, and a species-specific poxvirus in young chickens.<sup>6,9</sup> In this case, the advanced age associated with regular exposure to high levels of ultraviolet light, which is present throughout the year in northeastern Brazil, may have favored the development of the neoplasm. In fact, geographic location and climate, related to ultraviolet light exposure, greatly influence the incidence of SCCs.<sup>1</sup> The appearance of a cutaneous SCC can vary from a proliferative mass to a wound-like ulcer.<sup>5</sup> The SCC seen in this cockatiel had a proliferative appearance with an ulcerated surface; which may have created a portal of entry for bacteria and resulted in fatal bronchopneumonia. SCCs often have necrotic areas, therefore secondary infections are common, and up to 22% of pet birds treated for SCC have concurrent bacterial or fungal infections.<sup>2,10,11</sup> The biological behavior of cutaneous SCCs is also negatively influenced by neoplastic cells' ability to invade surrounding tissues.<sup>4,6</sup> In this case, invasion of the underlying bone tissue resulted in osteolysis and amputation of two toes. Despite the long clinical course of the disease, no evidence of metastasis was identified, which is consistent with previous reports,<sup>5,7,10</sup> and suggests that early detection and treatment may allow remission. Several treatment modalities for SCCs in avian species are described, including surgical excision, chemotherapy, cryosurgery, radiation therapy, and electrosurgery.<sup>6,12</sup> The efficacy of these treatment modalities varies with several limiting factors, presumably with regard to the location and tumor stage at the time of diagnosis. In conclusion, the late diagnosis of SCCs precludes the adoption of suitable treatment and determines the incidence of local and systemic complications. Overexposure to ultraviolet light should be investigated as a possible risk factor for the incidence of cutaneous SCCs in birds, as previously observed in mammals.

**Declaration of conflicting interests:** The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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