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Squamous cell carcinoma in raptors: literature review and a case report

Carcinoma de células escamosas em aves de rapina: revisão da literatura e um caso clínico

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Área científica: Medicina e cirurgia de animais exóticos e selváticos

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ABSTRACT

Neoplastic disorders are showing an increase in avian patients, partly due to the prolonged life expectancy. Among avian patients, raptors demonstrate a higher prevalence of neoplasms than previously thought, although reports remain scarce. Squamous cell carcinomas (SCC) are among the most common neoplasms found in exotic veterinary practice and appear to be one of the most prevalent types of epithelial tumours in raptors. SCC is a malignant epithelial neoplasm that often presents without distinct characteristics. In raptors, this condition is frequently associated with chronic ulcerative dermatitis and may be misdiagnosed if biopsies and histopathological examinations are not performed. Surgical excision is the primary treatment modality, offering the most favorable results in terms of complete response. When complete surgical excision is not possible or the surgical margins are inadequate, adjuvant therapies are recommended. Chemotherapy, radiation therapy, cryosurgery, and non-steroidal anti-inflammatory drugs, have been employed in avian medicine, each yielding different outcomes. Electrochemotherapy has emerged as a promising adjuvant therapy, demonstrating success across various domestic and exotic species. Since there is insufficient information regarding etiology, diagnosis and treatment modalities in raptors, as well as a defined standard of care for the generality of avian species, this report aims to compile the existing information regarding SCCs in birds and raptors, extrapolating from small animal and human medicine when applicable. Additionally, a case of cutaneous SCC in a Harris' hawk (*Parabuteo unicinctus*), treated with a combination of surgery, cryosurgery, and electrochemotherapy is also presented . This case illustrates the importance of multidisciplinary approaches and innovative techniques in the management of avian SCC. Despite this, the long-term prognosis for these patients is generally poor, with recurrence being a common outcome. In conclusion, these findings highlight the necessity of advancing avian-specific oncology protocols to enhance diagnostic accuracy and therapeutic outcomes for raptors.

KEYWORDS: Squamous cell carcinoma; Raptors; Avian oncology; Electrochemotherapy.

RESUMO

As doenças neoplásicas têm verificado um aumento em aves, parcialmente devido ao prolongamento da esperança média de vida. Entre as diferentes aves, as aves de rapina aparentam ter uma prevalência de neoplasias superior ao expectável, embora os estudos permaneçam limitados. Os carcinomas de células escamosas (CCE) são frequentemente encontrados em animais exóticos e são, presumivelmente, uma das neoplasias epiteliais mais prevalentes em aves de rapina. CCE é uma neoplasia epitelial maligna sem uma apresentação clínica característica. Em aves de rapina, ocorre frequentemente associado a dermatite ulcerativa crónica e poderá ser diagnosticado incorretamente caso não seja realizada biópsia e histopatologia. A ressecção cirúrgica representa a primeira linha de tratamento, proporcionando uma maior probabilidade de resposta completa. Nos casos em que esta não é possível ou as margens cirúrgicas são inadequadas, recomendam-se terapias adjuvantes como quimioterapia, radioterapia, crioterapia e agentes anti-inflamatórios não esteróides. A eletroquimioterapia tem demonstrado resultados promissores em espécies domésticas e exóticas. Uma vez que não existe informação suficiente sobre a etiologia, o diagnóstico e as modalidades de tratamento em aves de rapina, bem como sobre protocolos específicos para aves em geral, o presente relatório tem por objetivo compilar a informação existente sobre os CCE em aves e aves de rapina, extrapolando da medicina humana e de pequenos animais quando necessário. Adicionalmente, será abordado um caso de CCE numa águia de Harris (*Parabuteo unicinctus*), cujo tratamento envolveu o uso de cirurgia, crioterapia e eletroquimioterapia. Este caso ilustra a importância de abordagens multidisciplinares e de técnicas inovadoras no tratamento de CCE em aves. No entanto, o prognóstico a longo prazo é desfavorável devido à elevada taxa de recorrência. Estes resultados reforçam a necessidade de desenvolver protocolos oncológicos específicos para aves, de modo a obter diagnósticos mais precisos e melhores resultados terapêuticos em aves de rapina.

PALAVRAS-CHAVE: Carcinoma de células escamosas; Aves de rapina; Oncologia em aves; Eletroquimioterapia.

CASE REGISTRY AND ACTIVITIES DEVELOPED DURING THE EXTERNSHIP

Over the course of 4 months, I had the opportunity of undertaking an externship at Oasis Wildlife Fuerteventura. This institution is home to over 3000 animals and is also involved in dromedary farming, with a total of over 400 dromedarius (*Camelus dromedarius*). The veterinary hospital provides comprehensive care for all animals within the zoo, as well as those that are transferred to the facility as part of rescue operations.

The majority of primates currently residing in the zoo have been rescued from situations involving traffic and/or abuse. Additionally, some parrots and birds of prey were rescued from circumstances of neglect. At the time of the externship, a sterilisation programme for free-ranging cats was in progress, in which I was able to participate and perform some surgical procedures. The presence of cats in zoos has a detrimental effect on the well-being of the animals in these facilities, due to their capacity of hunting smaller animals and transmitting diseases.

During this period, I was able to observe the routine activities of the veterinary team, which included the performance of general and diagnostic examinations, surgical and anaesthetic procedures, treatments, and necropsies on a variety of captive species. Diagnostic techniques included imaging modalities such as radiography and ecography, as well as haematological and biochemical analyses. I was responsible for the care of animals in the hospital, ensuring that they received the correct daily medications and maintaining the hygiene and organisation of the clinic. Furthermore, I was able to perform control coprologic examinations on varied species and observe the daily routine of all departments of the zoo. I was also able to participate in diverse vaccination routines (**Table 1**). The clinical cases observed during the externship are listed in **Table 2**.

In addition to this, I was afforded the chance to engage with a number of distinguished international veterinary specialists. In particular, I had the opportunity to learn with Dr. Sara Peña Santana, a veterinary dermatologist and oncologist, whose expertise proved invaluable in the development of this case study. Additionally, I had the privilege of learning from Dr. Paola Lozano, a renowned specialist in fauna rehabilitation and physiotherapy, with whom I had the opportunity to work for a period of 2 months. I observed a number of laser therapy sessions being conducted on a variety of animals, including giraffes (*Giraffa camelopardalis*), giant elands (*Taurotragus derbianus*), dromedaries (*Camelus dromedarius*) and birds of prey. The work of Dr. Paola Lozano yielded significant outcomes in the treatment and management of diverse diseases and conditions, including osteoarthrosis, feather picking, pododermatitis, and wounds in general. Dr. Alicia Angosto, a globally renowned ecographist, was also observed conducting examinations on south american sea lions

(*Otaria byronia*), a black-and-white ruffed lemur (*Varecia variegata*), an asian otter (*Aonyx cinereus*), american alligators (*Alligator mississippiensis*) and dromedaries (*Camelus dromedarius*).

Finally, I participated in a course on "Well-being and Training of Animals under Professional Care" presented by Proactive Animal Consulting. The course provided an opportunity to gain further insight into the training of animals in captivity, with a particular focus on facilitating medical procedures and assisting veterinarians.

Table 1. Activities performed during the externship.

	Procedures	Avian	Mammalia	Reptilia	Total
Imaging Techniques	Ecography	1	7	3	**11**
	Endoscopy	1	NA	NA	**1**
	Radiography	5	20	3	**27**
Laboratory Work	Coprology	32	21	40	**93**
	Diff-Quik™ stain	5	5	NA	**10**
	Hematocrit	5	13	NA	**18**
	Hematology and biochemistry	3	7	1	**11**
Administration	Endovenous	NA	1	NA	**1**
	Intramuscular	1	14	12	**27**
	Oral	NA	81	1	**82**
	Subcutaneous	NA	9	2	**11**
Surgery	Anesthesia and Monitoring	23	25	1	**49**
	Surgery	NA	11	NA	**11**
	Catheterization	NA	1	NA	**1**
Others	Blood draw	1	1	1	**3**
	Vaccination	NA	101*	NA	**101**
	Wound cleaning	NA	24	4	**28**
	Necropsy	7	5	5	**17**
	Euthanasia	NA	4	1	**5**
	Microchip	NA	4	NA	**4**

*Myxomatosis and Rabbit Hemorrhagic Disease in *Oryctolagus cuniculus* = 75
Corynebacterium pseudotuberculosis in *Camelus dromedarius* = 26

Table 2. Clinical cases observed during the externship.

System	Cases	Species	Number of cases	Total
Cardiorespiratory	Fungal pneumonia	*Aonyx cinereus*	1	**2**
	Aspiration pneumonia	*Camelus dromedarius*	1	
Gastrointestinal	Amebiasis	*Chelonoidis carbonaria*	1	**1**
Urogenital	Cloacal prolapse	*Struthio camelus*	1	**3**
	Dystocia	*Canis lupus familiaris*	1	
	Chronic kidney disease	*Acinonyx jubatus*	1	
Oftalmology	Corneal ulcer	*Lemur catta*	1	**5**
		Camelus dromedarius	1	
		Bubo bubo	1	
	Iris prolapse	*Lama glama*	1	
	Third eyelid gland prolapse	*Salvator merianae*	1	
Oncology	Lipoma	*Canis lupus familiaris*	1	**3**
	Squamous cell carcinoma	*Parabuteo unicinctus*	1	
	Hepatic mass	*Varecia Variegata*	1	
Musculoskeletal System	Degenerative joint disease, Osteoarthritis	*Camelus dromedarius*	2	**13**
		Struthio camelus	1	
		Lama glama	1	
		Giraffa camelopardalis	1	
		Taurotragus derbianus	1	
		Otaria byronia	1	
	Intervertebral disc herniation	*Mephitis mephitis*	2	
		Equus africanus asinus	1	
	Complete femoral fracture	*Ovis aries*	1	
	Hoof abcess	*Equus africanus asinus*	1	
	Scapulohumeral luxation	*Gyps africanus*	1	
Infectious Diseases	Serpentovirus (Nidovirus)	*Python bivittatus*	2	**24**
	Feline Calicivirus	*Acinonyx jubatus*	1	
	Corynebacterium pseudotuberculosis	*Camelus dromedarius*	21	
Dermatology	Pododermatitis	*Chelydra serpentina*	1	**5**
		Parabuteo unicinctus	3	
		Bubo bubo	1	
Metabolic Disorders	Visceral gout (Urate deposition)	*Phoenicopterus roseus*	1	**2**
	Metabolic bone disease	*Iguana iguana*	1	

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ABBREVIATIONS

%	Percent
<	Less than
>	Greater than
AWAG	Animal Welfare Assessment Grid
BID	“*Bis in die*” - Twice a day
cGy	Centigray
cm	Centimeter(s)
COX	Cyclooxygenase
CT	Computed tomography
DNA	Deoxyribonucleic acid
ECT	Electrochemotherapy
FAS	Fear, anxiety and stress
Gy	Gray
IM	Intramuscularly
IU/cm³	International units per cubic centimeter
IU/ml	International units per millilitre
kg	Kilogram(s)
L/min	Litre(s) per minute
mg/kg	Miligram(s) per kilogram
mm²	Square millimetre(s)
MRI	Magnetic resonance imaging
NSAIDs	Non steroidal anti inflammatory drugs
PO	*“Per os”* - orally
RT	Radiation therapy
SCC	Squamous cell carcinoma
SID	“*Semel in die*” - Once a day
UV	Ultraviolet

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1. INTRODUCTION

The remarkable advancements of medicine resulted in an exponential increase in average life expectancy, with an unprecedented growth in the global population. Nevertheless, this has also given rise to new challenges and obstacles. Cancer is currently the leading cause of death in developed countries, a trend largely attributed to increased longevity (Bray *et al.*, 2012; Townsend *et al.*, 2016; Torre *et al.*, 2015). Similarly, a parallel phenomenon can be observed in veterinary medicine.

In companion animals, as has been demonstrated in humans, recent dramatic changes in healthcare and social structures have allowed them to far exceed species-adapted longevity without providing the time necessary for compensatory natural selection (Sarver *et al.*, 2022).

Significant developments have been made in many fields of veterinary medicine, including surgical techniques, anesthesia, pain management, nutrition, and treatment of infectious diseases affecting a wide range of animals. Due to the increase in life expectancy and the decline in mortality rates from infectious diseases, our patients are now increasingly presenting with neoplastic diseases. This is not exclusive to small animals; it also applies to exotics and zoological medicine (Guzman, 2017; Pesavento *et al.*, 2018; Zehnder *et al.*, 2016; Zehnder *et al.*, 2018a).

With regard to the field of zoological medicine, birds represent one of the most significant groups of animals exhibiting neoplastic alterations. Avian species, including raptors, have higher basal metabolic rates in comparison to mammals (Gavrilov *et al.*, 2022). Consequently, this indicates that some pathogenic processes may manifest at a more accelerated rate in these species, including tumours (Dang, 2015). While the majority of available studies and reports were conducted on orders *Galliformes*, *Psittaciformes* and *Passeriformes*, Forbes *et al.* (2000) concluded that neoplastic processes may have a greater impact on raptors than was previously assumed. Raptors include all species within the order *Accipitriformes*, *Cathartiformes*, *Falconiformes*, and *Strigiformes* (McClure *et al.*, 2019).

The number of studies and case reports conducted in relation to oncology in birds of prey remains very limited, and there is a paucity of epidemiological studies on avian cancer. In fact, the diagnostic procedures and therapeutic options available for these species are not yet well established, when compared to those for small animals. In addition, avian patients present some particular challenges in regard to veterinary practice. Due to their excellent ability to hide signs of illness, they often present with advanced disease. Additionally, the small size of these patients may render certain therapeutic options impractical (Zehnder *et al.*, 2016).

In avian practice, neoplasias commonly observed include tumours of the integument, oral cavity, sinuses, liver, kidney, reproductive organs, bones, brain, vascular structures as well as connective tissue (Lightfoot, 2006). A survey of 1539 cases of neoplasia in pet birds over a 10-year period, revealed the most common tumour locations were the integument (31.7%), followed by the urinary system (25.1%) and the reproductive system (17.3%) (Leach, 1992). Comparably, in a different retrospective study, the most common tumour locations were found to be the integument (21.5%), alimentary (12%) and reproductive tract (11.5%). In terms of epithelial, gonadal and bimorphic neoplasms, the most prevalent tumours were squamous cell carcinoma, biliary adenocarcinoma and ovarian/oviduct adenocarcinoma, followed by papilloma. In terms of mesenchymal neoplasms, the most common tumours were lymphoma, soft tissue sarcoma and fibrosarcoma, followed by lipoma (Garner, 2006).

An hereditary predisposition for certain types of neoplasias may be present, as some avian species exhibit a disproportionate prevalence of specific neoplastic diseases. To illustrate, African grey parrots (*Psittacus erithacus*) often exhibit cutaneous papillomas and squamous cell carcinomas, while Amazon parrots (*Amazona spp.*) frequently manifest gastrointestinal papillomas and adenocarcinomas, bile duct carcinomas, and lipomas. Other avian species with a high prevalence of specific neoplasias include budgerigars (*Melopsittacus undulatus*), cockatiels (*Nymphicus hollandicus*), cockatoos (*Cacatuidae spp.*), lovebirds (*Agapornis spp.*), macaws (*Ara*, *Anodorhynchus*, *Cyanopsitta*, *Primolius*, *Orthopsittaca*, and *Diopsittaca spp.*) and Galah or rose-breasted cockatoos (*Eolophus roseicapilla*) (Doneley, 2016).

Squamous cell carcinomas (SCC), therefore, represent one of the most prevalent forms of epithelial neoplasia observed in avian species. However, in the specific case of raptors, no apparent trends in neoplastic diseases have yet been observed, with only a few singular reports (Cooper, 1978, 1979; Cooper & Pugsley, 1984; Cooper *et al.*, 1978; Cooper *et al.*,1993; Forbes *et al.*, 2000; Garner, 2006; Ramis *et al.*, 1999; Rocha *et al.*, 2020; Romairone-Duarte *et al.*, 2016). *Strigiformes* appeared to have a higher overall prevalence of neoplasias (9.9% prevalence based on 131 post-mortem cases) when compared to other avian orders in a retrospective study of case submissions (Garner, 2006).

The main goal of this report is to present a comprehensive analysis of squamous cell carcinomas in birds in general, with particular focus on raptors. It draws upon the existing literature on avian oncology and, when necessary, extrapolates from the findings in companion animals and human medicine. Furthermore, a case report that I had the privilege to follow up during my externship will also be described. To the best of our knowledge, this represents the first documented treatment of a cutaneous squamous cell carcinoma in a Harris' hawk (*Parabuteo unicinctus*) using a combination of surgery, cryosurgery and electrochemotherapy.

2. SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma is a malignant epidermal neoplasm in which the cells demonstrate differentiation into squamous cells/keratinocytes (Goldschmidt & Shofer, 1998). Of the pet birds diagnosed with a neoplastic process, it is estimated that 1.7% suffer from SCC (Malka *et al.*, 2005). In a retrospective study, SCCs had a prevalence of 10% in Psittacine and 12.1% in Galliform birds, being one of the most prevalent forms of neoplasm (Garner, 2006). As reported by Forbes *et al.* (2000), of the 122 neoplasms observed in birds of prey, 13 were identified as SCCs (10.65%).

This neoplasia has its origin in squamous cells, which are located in the thickest layer of the epidermis. Consequently, it can manifest in any location. SCCs can be classified according to their origin in the integument (including the skin, mucocutaneous junctions, beak, legs and uropygial gland) or upper digestive tract (including oral cavity, esophagus, crop and proventriculus) (Gonzalez-Astudillo *et al.*, 2021; Shivaprasad & Barnes, 2016). Integumentary SCC can be the result of a prolonged intraepidermal dysplasia process, as it originates from an uncontrolled proliferation of atypical epidermal keratinocytes (Fania *et al.*, 2021). A further form of SCC, dermal squamous cell carcinoma (also known as avian keratoacanthoma) is not considered a true SCC, because it is a benign epithelial neoplasm and spontaneous regression occurs in many birds following ulceration of the overlying epidermis (Hafner *et al.*, 1991; Yildirim *et al.*, 2010). This condition is most frequently observed in the context of broiler chickens during the process of slaughtering (Turnquest, 1979).

In a multi-institutional survey of SCC in pet birds, the location of SCC was evaluated in a sample of 87 cases, with the following rates observed: 31% occurred in single dermal sites, 25% in oral cavity excluding the choana, 20% in the uropygial gland, 6% in multiple dermal sites, 6% in the choana, 3% in the beak, 1% in the digit, and 7% in other sites (Zehnder *et al.*, 2018b). In terms of integumentary system, the most common locations are mucocutaneous junctions of the head, distal wing and phalanges (Doneley, 2016). Specifically in raptors, the predilection sites are pre-femoral or the ventral aspect of the wing (Forbes, 2008).

2.1. Etiology

The etiology of squamous cell carcinomas in avian species remains to be clarified, and different species may present different predisposing factors (Jones *et al.*, 2020). It is hypothesised that the potential risk factors for SCC include chronic wounds or infections, exposure to tobacco smoke and hypovitaminosis A (Miesle, 2022; Schmidt & Reavill, 2024).

Neoplasias may develop as a consequence of genetically induced mutations in the DNA of somatic cells, which can remain undetected in healthy tissues until a stimulus triggers the "promotion" stage (Balkwill & Mantovani, 2012). This stage can be triggered through exposure to chemical irritants, substances released at the site of injury, organ resection, hormones, or chronic irritation and inflammation. Oncoproteins have the capacity to activate pathways that enhance the production of inflammatory cytokines and chemokines, which in turn facilitate the growth of tumour cells (Mantovani *et al.*, 2008). The initial phase of cancer is characterised by increased cell proliferation and reduced apoptosis, driven by inflammatory mechanisms (Karin, 2006). Inflammatory mediators have a great influence during the promotion stage, facilitating tumour growth through processes such as proliferation, survival, angiogenesis and metastasis. The impact of inflammation on cancer is multifaceted, exhibiting both pro and anti-tumour effects. Acute inflammation may possess anticancer properties, whereas chronic inflammation within the tumour microenvironment typically promotes cancer cell survival and proliferation (Okada *et al.*, 2000; Korniluk *et al.*, 2017). Controlled acute inflammation is even used in certain cancer therapies, while chronic inflammation usually favours tumour development (Korniluk *et al.*, 2017). Chronic inflammation caused by persistent infections of microbial, viral or fungal agents, as well as constant exposure to non-infectious factors such as smoke, may ultimately lead to the development of carcinogenesis (Khandia & Munjal, 2020).

As previously explained, one of the possible predisposing factors for SCC in birds is the presence of a chronic non-healing wound, a chronic skin disease, or any area subject to repeated trauma, such as chronic feather picking. Regions prone to chronic inflammatory conditions may also be a contributing factor to the development of integumentary SCC (Zehnder *et al.*, 2016; Klaphake *et al.*, 2006; Koski, 2002; Reavill, 2004). Regarding human literature, Jean Nicolas Marjolin observed that squamous cell carcinoma developed around post-traumatic scar tissue (Sharma *et al.*, 2011). In Harris' hawks (*Parabuteo unicinctus*) and peregrine falcons (*Falco peregrinus*), superficial chronic ulcerative dermatitis lesions have been reported to undergo neoplastic transformation ultimately leading to the development of SCC in the affected areas (Forbes & Guzman, 2017; Forbes *et al.*, 2000).

In mammals, one of the primary contributing factors to the development of SCC is exposure to sunlight and the subsequent effect of ultraviolet radiation, which is classified as physical carcinogenesis. In canines, felines and humans, SCC predominantly manifests in regions with reduced pigmentation and low fur density (Modiano & Kim, 2020). Long exposure to sunlight can induce chronic keratosis and culminate in invasive SCCs. Chronic keratosis is characterised by thickening and induration, which are the result of varying degrees of dermal scarring and acanthosis. In dogs, microscopic observation revealed the presence of fibrosis throughout the dermis, starting in the papillary layer with capillary-endothelial proliferation, vascular ectasia, and a cellular infiltrate of lymphocytes, plasma cells, and occasional neutrophils. Irregular acanthosis was often accompanied by varying degrees of hyperkeratosis and parakeratosis. Rete ridges ranged from small buds to broad, elongated cords designated pseudoepitheliomatous hyperplasia. Vacuolated cells containing glycogen were often prominent. Lesions were classified as invasive carcinomas when the epidermal basement membrane was disrupted and individual keratinocytes extended into the dermis (Hargis & Thomassen, 1979).

Although this is the case in mammals, ultraviolet light exposure does not appear to be a common mechanism in avian patients for the occurrence of SCC, because the majority of avian cutaneous tumours are located in areas that are not typically exposed to sunlight (Reavill, 2004).

The genetic abnormalities that underpin the development of cutaneous SCC remain incompletely understood within the field of human oncology, although one common finding is mutations in the p53 gene (Hauck & Oblak, 2020). The p53 gene is a tumour suppressor gene and its mutation and further dysregulation can culminate in a rapid and uncontrolled division of cells. Mutation of p53 can be induced by solar exposure (Park *et al.*, 1996) and its occurrence has been documented in feline SCCs (Blackwood, 2011). A higher prevalence of p53 dysregulation has been observed in feline oral SCC when compared with normal oral mucosa and inflammatory lesions. The immunohistochemistry detection of p53 expression appeared to reflect the presence of p53 mutations in the majority of the evaluated feline cases (Renzi *et al.*, 2019). Disruptions of the gene p53 have also been studied in cats exposed to tobacco smoke. The overexpression of gene p53 is also found in human squamous cell carcinomas associated with carcinogens present in tobacco products (Snyder *et al*., 2004). Similarly, chronic exposure to cigarette smoke has been identified as a potential contributing factor in the development of SCC in birds (Miesle, 2022).

Vitamin A deficiency has been associated with epithelial disorders according to human literature and studies in mice (Everts & Akuailou, 2021; Lotan, 1994). Vitamin A is obtained from the diet and plays a role in regulating cell growth and differentiation. Consequently, a deficient diet may result in malignant squamous metaplasia if not corrected in time. However, the role of hypovitaminosis A as a risk factor for the development of SCC in avian species remains to be fully elucidated (Zehnder *et al.*, 2016).

2.2. Presentation and clinical signs

Squamous cell carcinomas lack a distinctive, characteristic presentation. Their appearance is highly variable, ranging from plaquelike to papillary and from crateriform to fungiform (Gross *et al*., 2005). They may also present as an erythemic, ulcerated, or crusted lesion as well as proliferative, irregular and broad-based masses (Goldschmidt & Hendrick, 2002; Reavill, 2004). As previously mentioned, cutaneous SCC may be accompanied with feather-damaging behaviour and automutilation. Therefore, it is important for veterinary practitioners to avoid excluding this diagnosis based on the characteristics of a lesion alone.

SCCs can also visually resemble bacterial or fungal dermal infections (Reavill, 2004). It is important to exercise caution when interpreting cytological results, as it is not uncommon for secondary infections to develop as a consequence of this neoplasm (Friedrichs & Young, 2019; Zehnder *et al.*, 2016). Consequently, SCC should be included in the differential diagnosis for persisting cutaneous lesions, and a biopsy should be performed in order to exclude or confirm this diagnosis (Rocha *et al.*, 2020; Sojka *et al.*, 2020).

Clinical signs may differ according to the location of the lesion and presence of metastasis. In avian species, metastasis of SCCs are mostly found in the lungs, skin, spleen, cortex of the bones and liver (Abu *et al*., 2009; Zehnder *et al.*, 2016). It is important to mention that birds, with the exception of certain waterfowl, lack discrete lymph nodes. Lymphoid tissue is distributed throughout numerous tissues, including the liver, spleen, kidneys, and gastrointestinal tract (Doneley, 2016). Although the likelihood of metastasising is reduced, SCC have a great capacity to invade surrounding tissue, which results in a guarded prognosis (Abu *et al.*, 2009; Klaphake *et al.*, 2006; Koski, 2002; Lightfoot, 2010; Malka *et al.*, 2005; Manucy *et al.*, 1998; Reavill, 2004).

In a documented case, an adult crowned solitary eagle (*Buteogallus coronatus*) was treated for ulcerative pododermatitis on the footpad for a period of 3 months, but the condition persisted. Upon necropsy, metastatic nodules were identified throughout the coelomic cavity and determined to be multifocal to coalescent metastatic nests and cords of neoplastic squamous cells. Additionally, vascular invasion, tumour emboli, and keratin pearls were observed in the kidneys, liver, lungs, spleen, oviducts, air sacs, and sciatic nerves. The heart and pericardium exhibited multifocal metastasis and marked infiltration by neoplastic cells as well. Consequently, the initial diagnosis of ulcerative pododermatitis was ultimately determined to be a SCC with systemic metastasis (Rocha *et al.*, 2020).

In another reported case, a 25-year-old African grey parrot (*Psittacus erithacus*) was presented with progressive bilateral pelvic limb paresis and upper motor neuron signs. Five months prior to presentation, the bird underwent surgical amputation of the right humerus to remove a highly proliferative superficial chronic ulcerative dermatitis that had persisted for 2.5 years. Histopathology of the lesion revealed an incompletely excised SCC. CT scan was performed, showing a mass in the coelomic cavity that extended into the vertebral canal, compressing the spinal cord. The hepatic parenchyma was also affected, and additional metastases were suspected in the pneumatized bones and air sacs. Upon necropsy, metastatic SCC was confirmed, with metastasis present in the lungs, liver, ribs, and spine (Sojka *et al.*,2020).

2.3. Complementary Diagnostic Tests

2.3.1. Cytology

Cytology is a commonly used exam to perform on unknown lesions and nodules, as it is relatively simple and inexpensive. One of the most significant advantages of this method is the rapid assessment of the morphological appearance of individual cells. Cytology can provide valuable information, but it is essential to be aware of its limitations to ensure effective utilisation in veterinary oncology (Friedrichs & Young, 2019).

The choice of cytological technique employed is determined by the relief of the lesion and its general appearance. In the case of flat or ulcerative lesions, the apposition cytology technique may be used, while in the case of masses and nodules, either aspiration or non-aspiration cytology may be performed (Bajwa, 2017).

Cytology, however, does not allow to evaluate architectural relationships among cells in cytologic specimens, which results in the inability to differentiate between reactive and neoplastic processes, as well as between benign and malignant tumours. In the event that atypia is identified within the cells aspirated, it is imperative to exercise caution to avoid misinterpreting this as malignant neoplasia. This is due to the fact that primary inflammatory lesions with dysplastic squamous epithelium can also present cells with criteria of malignancy and asynchronous maturation. Consequently, this gives rise to a cytological dilemma concerning the differentiation of SCC from a primary inflammatory lesion with dysplastic epithelium (Friedrichs & Young, 2019).

Neutrophilic inflammation is often associated with the presence of tumours, particularly SCCs and large tumours with necrotic centres. However, it should be noted that inflammation can also induce criteria of malignancy in non-neoplastic populations, including fibroblasts and squamous cells. In such cases, surgical biopsy is required to confirm suspected neoplasia when inflammation is prominent (Friedrichs & Young, 2019).

2.3.2. Biopsy and histopathology

Biopsy, either excisional or incisional, followed by histopathology, represents the gold standard for the diagnosis of SCCs (Chhabra *et al.*, 2015; Razi *et al.*, 2023). The removal and submission of an entire mass with indication of its borders will facilitate subsequent follow-up and enable the implementation of additional therapies, if necessary (Zehnder *et al.*, 2016). A biopsy should be followed by histopathological analysis, which should include a relevant and succinct history provided to the pathologist. Furthermore, bacterial and fungal cultures should be conducted, given the propensity of SCC to be accompanied by secondary infections. In the event of extensive inflammation and ulceration, it may be advisable to repeat the biopsy in any masses that may have been previously diagnosed as inflammatory lesions, as this can obscure the tumour itself (Zehnder *et al.*, 2016). This is also applicable to any lesion that recurs despite treatment with an antimicrobial agent that has been demonstrated to be effective. Complete removal is a viable option for many lesions when other therapies have failed to provide adequate control (Guthrie *et al.*, 2010).

The histological characteristics and appearance of SCC are variable. Squamous epithelial cells present a polygonal morphology and can be arranged as infiltrative nests, cords, and trabeculae. This neoplasia is generally locally invasive, with invasion below the basement membrane being the most common manifestation of malignancy (Goldschmidt & Goldschmidt, 2016; Shivaprasad & Barnes, 2016). The presence of cells forming nests and cords as well as producing whorls of cells with central keratinization (also known as keratin pearls) is associated with well differentiated SCC (Raidal, 2024). Keratin pearls are composed of central cores of compressed and laminated keratin (Reavill, 2004). Keratin can be observed as intracytoplasmic, eosinophilic fibrillar material (Goldschmidt & Goldschmidt, 2016). The absence of formation of keratin pearls as well as the absence of stratified layers of epithelial cells undergoing progressive keratinization, is associated with poorly differentiated SCCs (Goldschmidt & Goldschmidt, 2016; Raidal, 2024). Severe concomitant secondary infections are often reported (Sidor, 2024), and inflammation may also be present, with the potential for a granulomatous reaction if a foreign body reaction against tumour-related free keratin occurs (Raidal, 2024).

In histopathological evaluation of SCCs, pleomorphism can be observed as moderate-to-marked anisocytosis and anisokaryosis, hyperchromasia, and marked nuclear atypia (Friedrichs & Young, 2019). The assessment of mitotic count can also be taken into account, however, it is not uncommon to encounter cases with comparable mitotic indices that exhibit markedly disparate outcomes. Some cases manifest systemic metastasis, while others do not. Consequently, the mitotic index is an unreliable criterion for differentiating metastatic from non-metastatic SCCs in birds (Rocha *et al.*, 2020).

In canine oncology, oral and cutaneous SCC are graded on a scale of 1 to 4 according to degree of keratinization (with less keratinization being associated with a higher grade SCC), pattern of invasion (with increased invasiveness correlating with a higher grade SCC), host response (with responses decreasing with higher grades), nuclear pleomorphism (which increases with higher grades) and mitoses per high power field (which also increases with higher grades) (Nagamine *et al.*, 2017). Nonetheless, grading systems for avian tumours are not yet established, due to the lack of information relating tumour grade with patient outcome (Zehnder *et al.*, 2016).

2.4. Staging

Staging is crucial in the context of neoplasia, as it offers a standardised framework for characterising the extent and severity of the disease. Furthermore, it serves as a valuable tool for guiding treatment decisions and predicting the prognosis. In mammalia, the tumour-node-metastasis (TNM) approach, developed by the World Health Organization (WHO), is used to stage solid tumours. It evaluates the primary tumour (T), presence or absence of metastatic disease in local, regional, and distant lymph nodes (N), and the presence or absence of metastatic disease within the rest of the body (M) (Rosen & Sapra, 2023; Zehnder *et al.*, 2016; Zehnder *et al.*, 2018a).

In small animal oncology, the specific staging of SCC follows the guidelines for epidermal and dermal tumors. The primary tumour (T) can be classified as Tis (carcinoma in situ); T0 (no evidence of tumor); T1 (superficial tumour <2 cm maximum diameter); T2 (tumour 2–5 cm maximum diameter, or with minimal invasion irrespective of size); T3 (tumour >5 cm maximum diameter, or with invasion of subcutis irrespective of size); and T4 (tumour invading other structures such as fascia, bone, muscle and cartilage). Regional lymph nodes are classified according to nodule invasion (N0, N1, N1a, N1b, N2, N2a, N2b, N3) and distant metastasis are also evaluated (M0, M1) (MacDonald *et al.*, 2009). The majority of birds lack lymph nodes, therefore an adapted "TM" approach should be considered in these species. There is no clinical evidence that supports the necessity for a more detailed and precise tumour scale in avian species, in contrast to those employed in human medicine and, in certain instances, in companion animals (Zehnder *et al.*, 2016).

In light of the aforementioned considerations, Zehnder *et al.* (2016) propose a more general staging technique that assesses overall health by evaluating physical examination findings, nutritional status, complete blood count, biochemistry, viral testing, paraneoplastic diseases, tumor complications; and details regarding the primary tumor and metastatic lesions. The primary tumour must be evaluated in terms of its size and invasiveness, therefore specific location and measurements in 3 planes should be documented. The extent of the tumour as well as the presence of metastasis can be evaluated through diagnostic imaging techniques. Increased data collection on tumor characteristics and outcomes will facilitate more accurate prognostic predictions at initial presentation and improve staging systems. (Zehnder *et al.*, 2016).

In the context of small animal oncology, staging of SCC includes a complete blood count, biochemistry and urinalysis, in order to to establish the health status of the patient and organ function prior to treatment; a fine needle aspiration of the regional lymph nodes; thoracic radiographs (3 projections) or CT scan (preferred); abdominal ultrasound and fine needle aspiration of the findings; and CT scan of the lesion site (Biller *et al.*, 2016; MacDonald *et al.*, 2009). Ultrasonography has limited use in avian medicine, because ultrasound waves are unable to penetrate the gas-filled air sac system, resulting in a poor organ visualization. Alterations in size of the liver are more commonly diagnosed by radiography, but ultrasonography can be useful in guided hepatic fine needle aspiration (Helmer, 2006). Another option for the evaluation of organ alterations in avian species is endoscopic biopsy. Despite this and the absence of lymph nodes, the remaining procedures can also be employed in avian species (Zehnder *et al.*, 2016; Zehnder *et al.*, 2018a).

2.4.1. Imaging Modalities

Imaging modalities do not provide a definitive diagnosis of squamous cell carcinomas. Nevertheless, they are of significant value in terms of staging, as previously mentioned. In small animal oncology, the utilisation of advanced local imaging techniques has been demonstrated to lead to an increase in the stage of the primary tumour in 69% of patients (Hahn *et al.*, 1990).

In the context of SCC, imaging modalities have the capacity to evaluate the extent and invasiveness of the tumour, as well as the identification of metastasis. With regard to SCC metastasis, the most common sites include the lungs, skin, spleen, bones and liver (Abu *et al*., 2009; Zehnder *et al.*, 2016). Consequently, a comprehensive evaluation necessitates the acquisition of images regarding coelomic cavity (thoracic and abdominal cavities) and skeletal system.

Radiology is often the initial imaging modality employed in the assessment of oncological patients, largely due to its relatively low cost and simplicity (Nykamp & Randall, 2019; Zehnder *et al.*, 2016). In avian species, in order to properly evaluate the lungs, spleen and liver, both latero-lateral and ventrodorsal views should be performed (Crosta *et al.*, 2018). Whilst multiple radiographic views may help in the detection of metastasis, computed tomography is a more sensitive imaging modality (Zehnder *et al.*, 2016).

Computed tomography (CT) has become an invaluable diagnostic tool in the field of avian and has been employed in various avian species, including raptors. In the context of SCC, this imaging modality can be useful in determining size, extension and invasiveness of the tumour. This in turn facilitates the determination and planning of the possibilities for therapeutic intervention and the establishment of associated prognoses (Zeeland *et al.*, 2016). Furthermore, in veterinary medicine, CT demonstrates superior sensitivity in the detection of pulmonary metastasis, enabling the identification of nodules as small as 1 mm in diameter, a resolution that surpasses that of radiography, which fails to detect lesions smaller than 3 mm (Armbrust *et al.*, 2012; Eberle *et al.*, 2011). CT is also regarded as the most accurate modality for identifying bone invasion and nodal metastasis (Libson *et al.*, 2024).

Magnetic resonance imaging (MRI) has also been employed in avian medicine. This modality is considered the most effective method for diagnosing soft tissue changes, such as perineural spread. MRI also allows visualisation of the gastrointestinal tract, liver, spleen and urogenital tract (Zeeland *et al.*, 2016) and is often considered more sensitive in detecting tumor margins in soft tissues (Zehnder *et al.*, 2018a). However, its usefulness in imaging the heart and lungs of birds is limited due to the presence of artefacts caused by cardiac motion, which obscures the heart and adjacent lungs (Zeeland *et al.*, 2016).

Nonetheless, CT is employed more frequently in the field of avian medicine than MRI. MRI has limited use in birds due to the amount of time needed to perform the exam, which implies a prolonged anaesthesia. This can have detrimental effects on the patient due to limitations with regard to patient access and the fact that it is impossible to place the anaesthetic machine inside the MRI, which complicates monitoring (Crosta *et al.*, 2018; Zeeland *et al.*, 2016). Furthermore, a lot of birds have a metal leg ring for identification purposes, rendering the procedure unfeasible (Crosta *et al.*, 2018).

2.5. Prognostic Factors

In the context of small animal oncology, prognostic factors of SCC include: location (oral SCCs tend to be more aggressive), clinical stage and histological features (grade of differentiation, mitotic index, nuclear pleomorphism, invasion beyond basement membrane, vascular invasion and stromal reaction) (Biller *et al.*, 2016; Corrêa *et al.*, 2021; Nagamine *et al.*, 2017). Nevertheless, the connection between tumoral morphology, mitotic index, aggressive behaviour and metastasis remains unclear in avian species (Jones *et al.*, 2020). There is currently no definitive evidence in the avian literature to suggest that mitotic index is a prognostic indicator (Zehnder *et al.*, 2016).

In human medicine, the loss of E-cadherin has been utilised in the diagnosis and prognosis of epithelial cancers (Van Roy, 2014). E-cadherin is a protein that plays a key role in mediating cellular adhesion, influencing cell polarity, maintaining tissue cohesion, and preventing invasive cellular behavior (Braga, 2016; Niño *et al.*, 2019; Santarosa & Maestro, 2021). Similarly, a study evaluating E-cadherin expression in canine SCC verified a significant association between the intensity of E-cadherin expression and the histological grade of malignancy. The decreased expression of E-cadherin is linked to larger tumor size, higher histological grade, invasion, metastasis, increased mitotic index and, consequently, increased malignancy (Files *et al.*, 2024; Van Roy, 2014).

Furthermore, research in human medicine has demonstrated that the overexpression of COX-2 is associated with survival rates in SCCs of the head and neck. To illustrate, COX-2 overexpression correlates with an increased risk of lymph node involvement and is present in cells that exhibit more invasive and aggressive behaviour (Gallo *et al.*, 2002). Overexpression of COX-2 alters cell phenotype from benign to malignant by disrupting growth and proliferation, increasing the ability of cells to evade apoptosis and immune responses, promoting angiogenesis and increasing invasive potential (Rizzo, 2011). In domestic felines and canines, COX-2 expression was associated with progression of SCCs, however, it was not linked to lymphatic invasion, tumor grading, or tumor classification in cutaneous tumours (Millanta *et al.*, 2016).

In avian species, an immunohistochemical study was conducted to examine and demonstrate the expression of COX-2 and E-cadherin in avian papillomas and squamous cell carcinomas. The results indicated that COX-2 expression was elevated, while E-cadherin membranous expression was significantly decreased in SCCs when compared to papillomas. These findings could prove invaluable in the diagnosis of these conditions, particularly when sample sizes are limited. A higher mitotic count was observed in tumours exhibiting membranous COX-2 expression, though no association was identified with any other feature. In addition to this, no notable correlation was identified between the COX-2 total score and the signalment, location or histopathological features of SCC. Similarly, no significant correlation was observed between E-cadherin expression and the aforementioned variables. Consequently, these elements are unable to differentiate between well and poorly-differentiated SCCs in avian species (Jones *et al.*, 2020).

2.6. Treatment Modalities

In the context of oncology, the specific treatment plan for a patient is determined not only by the type of neoplasia, but also by the characteristics of the patient. As many treatments necessitate repeated visits to the veterinary, some animals may experience considerable stress, which may render them ineligible for certain therapies. Consequently, the specifics of a treatment plan are not predetermined and are dependent on a number of variables. It is imperative to consider the advantages and disadvantages of any proposed treatment, as well as the potential stress and quality of life implications, before undertaking any course of action (Rohrer, 2018).

It is important to highlight the significant differences between companion animals/pet birds and zoo avian species in the context of veterinary oncology. Companion animals and, to a lesser extent, pet birds are generally accustomed to the human presence and touch, often allowing thorough physical examinations, diagnostic procedures, and treatments. In contrast, zoo animals are only accustomed to their caretakers, making any veterinarian procedure challenging and stressful (Brando & Norman, 2023).

The necessity for repeated treatments to achieve tumor remission can negatively affect these animals in the short term, due to stress, altered social settings, hospitalization, confinement, or painful/unpleasant procedures (Campbell-Ward, 2023). Specifically in wild/zoo avian species that are not used to veterinary handling, stress can have profound impacts on the general health and wellbeing (Kujiraoka *et al.*, 2024). However, these treatments might improve welfare in the long term, therefore a balanced evaluation must be continuously performed (Campbell-Ward, 2023). Furthermore, avian species require repeated anesthetic procedures for chemotherapy treatments, unlike companion animals, which also has a detrimental effect on their general health (Zehnder *et al.*, 2018a). As a result, quality of life assessments should be conducted for every oncological patient (Belshaw *et al.*, 2015). These assessments can aid in decision-making related to treatment options and ultimately help determine the appropriate timing for euthanasia (Campbell-Ward, 2023).

2.6.1. Surgery

Surgical excision represents the first-choice therapeutic option for the treatment of SCCs (Filippich, 2004; Klaphake *et al.*, 2006; Koski, 2002; Malka *et al.*, 2005; Reavill, 2004). Surgery should be meticulously planned, as the first surgery is frequently the surgeon's best opportunity for achieving a favourable outcome (Orencole & Butler, 2013).

Regarding anaesthesia in avian species, the reduction of anaesthetic times and the minimisation of the duration of surgical procedures will often result in improved surgical outcomes (Jolly, 2022). The crop must be completely empty prior to anaesthesia, in order to prevent regurgitation and aspiration. General guidelines recommend a fasting of 2-4 hours for birds <300 g body weight; 5-8 hours for birds >300 g body weight; 2-4 hours for frugivores; and 24-36 hours for larger raptor species weighing 2-4 kg. Water should be available until 1 hour prior to anesthesia (Bennett, 1994). Hypothermia is of paramount importance in avian species, particularly in small birds, therefore a heating system (circulating water heating pads, electric heat pads, heated forced air systems or overhead heat sources) should be available throughout the procedure in order to provide adequate supplemental heat to the patient (Bennett, 1994; Phalen *et al.*, 1996).

The surgical recommendations for the integument and excision of neoplasms in birds are limited due to the anatomical structure of these animals (Maxwell, 2022). In avian species, the epidermis consists of a thin stratum of only 10 cell layers deep (Doneley, 2016), often reaching 3 to 5 cells of thickness in most parts of the body (Shivaprasad & Barnes, 2016). This results in a very thin structure in comparison to that of mammals. Furthermore, the dermis is loosely attached to the underlying tissues, with exception to the distal extremities; and the skin is generally dry and inelastic, with reduced collagen content (Altman, 1997; Stettenheim, 1972). However, anatomic limitations can be countered by employing multimodal treatment options and a comprehensive understanding of tumour type, tumour behaviour, anatomic location, and surrounding tissues (Maxwell, 2022).

In avian species, hemostasis is of critical importance, as minor bleedings can have severe consequences for smaller birds. A number of instruments and methodologies are available to reduce and prevent haemorrhage, including chemical cautery agents, metal clips, radiosurgery, electrocautery and lasers (Altman, 1997; Bennett, 1994). Radiosurgery and laser surgery are frequently employed for both cutting and coagulation during avian surgery (Maxwell, 2022).

Avian species generally tolerate surgical wounds well, as long as discomfort is minimized. Effective post-operative analgesia is essential for promoting healing and recovery. The choice of analgesic, including dosage and administration route, depends on the species and individual factors. Inadequate pain management can lead to self-trauma, which can complicate recovery. Whilst the utilisation of bandaging with "distractor tabs" is a possible option, addressing both pain and structural issues is typically more effective for optimal healing (Jolly, 2022).

When excising a neoplasia, special considerations should be taken into account such as careful tumour handling, in order to avoid exfoliation of cells and local recurrence (Withrow, 2001; Soderstrom & Gilson, 1995). Furthermore, direct handling of the tumour should be avoided and surgical gloves as well as instrument packs should be changed between tumour excision and closure, in order to minimize seeding of neoplastic cells (Orencole & Butler, 2013). In the context of tumour resection, it is paramount that the surgical technique is executed in an aseptic manner, particularly in cases where adjunct chemotherapy is employed, as the majority of avian patients will often be debilitated, immunocompromised or geriatric (Filippich, 2004).

In accordance with current knowledge in the field of tumour biology, tumours that are locally invasive or have microscopic local extension require wide surgical margins and a deep resection of at least 1 fascial plane, in order to achieve complete/clean margins. In the event that these requirements are not met, adjunctive therapies should be employed in the postoperative period (Maxwell, 2022).

In small animals, most malignant carcinomas can be fully resected with lateral margins of 1 cm and deep resection of 1 fascial plane (Orencole & Butler, 2013). In the field of human medicine, the recommended surgical margins for cutaneous SCC are 4-6 mm (Stewart & Saunders, 2018). In the majority of exotic species, adequate margins are recommended for surgical excision of tumours, yet no specific measurements are provided (Maxwell, 2022). Despite the absence of a defined standard of care for avian patients and insufficient data on surgical margins for the excision of SCCs (Maxwell, 2022), some authors recommend surgical margins of 1 cm (Forbes, 2008; Lightfoot, 2010). Complete excision is infrequently achieved, as SCCs tend to be locally invasive and neoplastic processes in birds are often presented in an advanced stage (Lightfoot, 2010).

The most recent multi-institutional survey of 87 cases of avian SCC revealed that the unadjusted odds ratio for partial or complete response following complete surgical excision (compared to other treatment approaches) was 6.9 (Zehnder *et al.*, 2018b).

An informal survey of 39 single case reports of various neoplasias with different treatments revealed that surgical resection alone resulted in complete response or a greater than 6-month survival in 7 out of 9 (78%) cases. Conversely, 16 out of 30 (54%) cases that resorted to adjunctive therapies (with or without surgery) reported complete responses or long-term survival. Although the differences were not statistically significant according to Chi-square analysis, this may be attributed to the underreporting of surgical resection cases, as clinicians tend to publish cases utilising novel treatment strategies (Zehnder *et al.*, 2016).

A review of 13 SCC cases in *Falconiformes* revealed that 2 were fully cured by surgical excision, while 8 either died or were euthanised as a consequence of the neoplasia (Forbes *et al*., 2000).

Considering the aforementioned information, it can be reasonably concluded that complete surgical excision with wide surgical margins yields the best results in terms of complete response. However, it must be acknowledged that complete surgical excision can be challenging to achieve, given the locally invasive nature of SCCs (Lightfoot, 2006).

2.6.2. Chemotherapy

Chemotherapy can be employed to control tumours that are unresectable or that were resected with incomplete margins, for patients with distant and disseminated disease, as well as in a neoadjuvant treatment to reduce tumours and render them amenable for subsequent resection (Zehnder *et al.*, 2016).

The protocols for chemotherapy in avian species are still largely defined empirically, with extrapolations from other companion animals or humans (Graham *et al.*, 2004). The dosage of the chemotherapeutic agent should be based on the maximum dose that has a low probability of causing severe toxicity (Kent, 2004). In exotic species, the dosage of chemotherapeutic agents is more often calculated using the body surface area of the animal, instead of the body weight, as body surface area is considered a better indicator of the metabolic mass (Hahn, 2005). There have been reports of using the formula for the area of a cylinder to estimate the body surface area of birds. Another formula that has been proposed for use in avian species is $M^2 = Body\ weight\ (g)^{2/3} \times K \times 10^{-4}$, where K is equivalent to 10.0 (Schmidt-Nielsen, 1984). However, the use of body surface area for dosing has not been validated in birds and may not offer advantages over milligrams per kilogram (mg/kg) dosing (Zehnder *et al.*, 2016).

Avian species have a renal portal system, therefore, chemotherapeutic agents should not be injected into the leg vasculature, as this can cause them to be excreted before entering the full circulatory system. The same applies to any nephrotoxic agent (Zehnder *et al.*, 2018a).

Platinum-based antineoplastic agents, such as cisplatin and carboplatin, can be employed in the treatment of integumentary neoplasias via both intravenous and intratumoral routes. Side effects of cisplatin include myelosuppression, gastrointestinal toxicity, nephrotoxicity, ototoxicity and pulmonary edema. Carboplatin is considered less neurotoxic and ototoxic than cisplatin, but side effects also include myelosuppression and gastrointestinal toxicity (Zehnder *et al.*, 2018a).

Antitumor antibiotics, such as doxorubicin and bleomycin, represent a further option for the treatment of SCC, with bleomycin able to be administered intratumorally. Side effects of doxorubicin include cardiotoxicity, myelosuppression, gastrointestinal toxicity, renal toxicity, skin or gastrointestinal hypersensitivity, alopecia and perivascular damage with extravasation. Side effects of bleomycin include pulmonary toxicity, stomatitis, hyperpigmentation of the skin and hypersensitivity (Zehnder *et al.*, 2018a).

Paterson (1997) has described the use of topical 5-fluorouracil for the effective control of superficial ulcerative SCC in horses. Furthermore, in 4 raptors with cutaneous SCCs, surgical excision was performed in addition to the topical application of 5-fluorouracil SID to any regrowths (typically for a period of 5-7 days), until no signs of neoplasia were observed. This treatment proved curative, with no recurrences observed for a period of over 2 years (Filippich, 2004).

A review of the literature reveals a variety of outcomes associated with the use of chemotherapy in the treatment of SCC in avian species. In an Yellow-naped amazon (*Amazona auropalliata*) with a SCC located in choana, carboplatin (17.2 mg/kg) was administered for 4 sessions from 3 to 10 weeks apart. The tumour slowly progressed and the patient died 9 months following therapy (Zehnder *et al.*, 2010).

In a cockatiel (*Nymphicus hollandicus*) with a multicentric cutaneous SCC, carboplatin (27 mg/kg) was administered for 4 doses every 4 weeks, after 7 failed treatments of cryotherapy. The patient was euthanized 3 months after due to progression of the neoplasia despite treatment (Zehnder *et al.*, 2010). In another cockatiel, presented with integumentary SCC, the tumour recurred after surgical removal and thus intratumoral carboplatin (1.5 mg/cm^3) was administered once a week. The tumour continued to grow, and resection of the mass was performed, resulting in the patient's demise during anaesthesia. However, despite an increase in tumour size, histopathological and immunohistochemical evaluations demonstrated degeneration of the tumour with intercellular edema and vacuolization of the tumour cells, which were presumed to have resulted from carboplatin administration (Van Hecke *et al.*, 2018).

An African grey parrot (*Psittacus erithacus*) with a SCC in the plantar surface of the right tarsometatarsus underwent electrosurgery, followed by injections of carboplatin/sesame oil emulsion in the adjacent tissues and intravenous carboplatin chemotherapy (5 mg/kg) after 2 and 3 weeks. A complete remission was achieved, and no further symptoms were reported over a 12-month period (Ledwoń *et al.*, 2013).

It is essential that complete blood counts are conducted and monitored on a regular basis, with a frequency of at least once a week or twice a month. In the event that the chemotherapy regimen includes a drug that is typically regarded as myelosuppressive, the administration of such therapy should be postponed if the heterophil count falls below 2000 cells/µL. If the drug in question is not considered myelosuppressive in other species, therapy may be maintained, as long as the heterophil count remains at 1500 cells/µL. Administration of antibiotics is recommended to any avian patient with a heterophil count below 2000 cells/µL (Zehnder *et al.*, 2016).

Intralesional chemotherapy offers a more accessible and safer alternative for avian species, with reduced systemic risks and toxicity in comparison to the intravenous route. The pharmacokinetics and cytotoxicity of the chemotherapeutic agents can be improved through the addition of slow-release formulations, which allow prolonged local exposure of the tissue to high drug concentrations. Collagen matrix has been employed in both companion animals and humans as a drug carrier. A water-in-sesame oil emulsion represents a cost-effective drug carrier for the administration of cisplatin, carboplatin, and other antineoplastic agents (Zehnder *et al.*, 2016).

The intratumoral chemotherapy dosage is calculated according to the tumoral volume using the formula: *Tumoral volume (cm^3)=* $A \times B \times C \times \frac{\pi}{6}$; where A, B and C represent the length, width and thickness of the tumour, respectively. In the case of flat neoplasias, biopsies should be conducted in order to evaluate the thickness, however, when this is not assessed, the formula can be adapted to $A \times B^2 \times \frac{\pi}{6}$ (Tellado *et al.*, 2022).

Furthermore, chemotherapy can be combined with electroporation, a procedure known as electrochemotherapy. Electroporation (also referred to as electropermeabilization) uses high-voltage, short and electric pulses, resulting in a transient elevation in the permeability of cellular membranes without causing irreversible damage to the cells. This process facilitates the penetration of chemotherapy agents into tumour cells (Neumann *et al.*, 1982) and increases cellular uptake, thereby enhancing their cytotoxic and anticancer efficacy by up to 1000-fold (Probst *et al.,* 2018). Bleomycin, cisplatin and calcium have been demonstrated to be effective agents, with bleomycin exhibiting superior outcomes (Mir, 2007; Račnik *et al.*, 2019a; Sersa & Miklavcic, 2008).

In a cockatiel (*Nymphicus hollandicus*) with a SCC in the uropygial gland, electrochemotherapy with intratumoral cisplatin was performed, but with no results. Intratumoral bleomycin was administered for the following sessions of electrochemotherapy, resulting in complete regression. The tumour mass reappeared after 3 months, necessitating a fourth electrochemotherapy procedure. No recurrences were observed following 18 months (Računik *et al.*, 2019a).

In a budgerigar parakeet (*Melopsittacus undulatus*) with an incompletely excised subcutaneous low-grade epithelioid hemangioendothelioma, electrochemotherapy with intralesional bleomycin was selected as the treatment modality. Two treatment sessions were performed with a 2-week interval and no tumour recurrence was observed in the 12 months following treatment (Lanza *et al.*, 2019).

In a study conducted on cats with superficial SCC treated with electrochemotherapy with intravenous bleomycin, a complete response was achieved for 81.8% cats and 87.5% nodules, with the longest lasting effect observed to be longer than 3 years (Tozon *et al.*, 2014).

As previously discussed, the efficacy of electrochemotherapy remains poorly established in the avian literature, with a limited number of reports. Additionally, reports in raptor patients are also particularly scarce, making it difficult to assess appropriately. Nevertheless, an examination of the data from both small animal and human medicine indicates that this approach may offer an alternative treatment option for SCC with a satisfactory response rate.

2.6.3. Radiation Therapy

Radiation therapy (RT) has been employed as a successful treatment modality for solid tumours in veterinary medicine. RT is not widely available due to a limited number of veterinary treatment centres (Larue & Gordon, 2020).

Radiation induces cell damage by generating highly reactive free radicals and interacting directly with DNA, causing double-strand breaks. Cells demonstrate increased sensitivity to radiation during the division phase, with radiation being most effective in the late G2 or M phase of cell cycle (Gunderson & Tepper, 2015; Ward, 1988). Normal tissue is susceptible to harm during radiotherapy; however, fractionating the total radiation dose enhances the treatment´s efficacy whilst sparing normal tissue. This is due to the fact that fractionated RT allows healthy tissues to heal between doses, given their enhanced ability to repair radiation-induced damage when compared to neoplastic tissues. Consequently, the employment of multiple low-dose radiation doses, which exert a cumulative effect, favours survival of healthy over neoplastic tissues (Ogilvie, 1988; Rancilio, 2024; Walker, 1997).

The combination of RT and surgery has been shown to result in superior local tumour control when compared to either modality alone, without a significant increase in patient morbidity. It is important to note that not all neoplasias are radiosensitive, and tumours demonstrate varying degrees of sensitivity according to the affected tissue. For instance, haematopoietic neoplasms present the highest radiosensitivity. Epithelial neoplasms, including squamous cell carcinomas, are considered moderately sensitive, while mesenchymal neoplasms demonstrate low radiosensitivity. Radiosensitivity is not directly related to the success of the therapy; however, it is evident that a higher cumulative dose of fractionated radiation therapy will be required to achieve local control in radioresistant tumours (Mauldin & Shiomitsu, 2005).

In avian species, brachytherapy (using Strontium-90) and external beam radiation (orthovoltage and megavoltage radiation therapy) have been used. RT can be employed as a sole method of treatment, adjunctive therapy or in a palliative setting (Zehnder *et al.*, 2016).

The use of Strontium-90 is limited to the treatment of very small or superficial tumours, such as uropygial gland tumours, or in cases where it is necessary to treat a surgical site following tumour removal. In cases of tumours with a depth greater than 2 mm, external beam radiotherapy is the preferred treatment modality (Zehnder *et al.*, 2016). Strontium-90 radiation has been employed as a sole treatment modality for SCC of the nasal planum in cats, with a study reporting a 98% response rate to treatment and a 88% complete response rate (Hammond *et al.*, 2007). A single irradiation fraction of 100 Gy/8.3 mm from a Strontium-90 ophthalmic applicator was used to treat a SCC of the uropygial gland following surgical excision in two budgerigars (*Melopsittacus undulatus*), with complete response (Nemetz & Broome, 2004). Similarly, an African grey parrot (*Psittacus erithacus*) with a SCC of the uropygial gland was administered two sessions of radiation therapy with a strontium probe (Sr-90 Ophthalmic Applicator) at 100 Gy following surgical excision, resulting in a complete response (Pignon *et al.*, 2011).

Orthovoltage and megavoltage radiation therapy provide different levels of energy and are used for distinct tumour types. Orthovoltage has a lower energy as well as penetration, being more appropriate for tumours of the skin and subcutaneous tissues. However, the interaction of megavoltage rays with tissue is more predictable, providing less skin damage and sparing more normal tissue (Zehnder *et al.*, 2018a).

In a 34-year-old Timneh grey parrot (*Psittacus timneh*) with a SCC of the rhinotheca, surgical debulking and palliative megavoltage radiation therapy were performed. Four radiation therapy sessions resulted in necrosis of the affected tissue; and healthy granulation tissue was revealed after debridement. The patient passed away 7 months following diagnosis and 4 months after cessation of radiation treatment. A minor scab lesion was observed at the left oral commissure; however, no visible tumour regrowth was detected at the time of death. No post mortem examination was performed (Swisher *et al.*, 2016).

In a 28.5-year-old Buffon's macaw (*Ara ambiguus*) with a SCC of the mandibular beak, debulking and Cobalt-60 radiation therapy were performed. The radiation dosage and approach were extrapolated from small animals' protocols. The total minimum tumour dose was established at 5,600 cGy, following an initial dose of 4,800 cGy that was augmented with an additional 800 cGy. Subsequent to the administration of radiation therapy, no observable effects or improvements were detected. The patient then began to lose weight and developed anorexia, with gavage feeding being necessary. Further surgical debulking, antifungal therapy and intralesional cisplatin were performed. The patient developed bilateral periorbital swelling and weight loss continued despite gavage feeding. Ataxia and hematological abnormalities were observed prior to the bird's demise. No evidence of metastasis was observed during necropsy (Manucy *et al.*, 1998).

It is interesting to note that certain species of birds appear to be less radiosensitive to the effects of whole body irradiation on bone marrow than mammals. A study evaluating tolerance doses of external beam megavoltage radiation in cutaneous and mucosal tissues of ring-necked parakeets (*Psittacula krameri*), showed minimal radiation-induced epidermal histologic changes in the high-dose group receiving 72 Gys in 4-Gy fractions (Barron *et al.*, 2009). Another study reported that the amount of radiation delivered to the choana of Military Macaws (*Ara militaris*) did not reach intended levels (Cutler *et al.*, 2016). These findings suggest the possibility that higher doses of radiation may be required in avian patients to elicit an equivalent response to that observed in mammalian models. Nonetheless, it should be noted that the radiosensitivity of avian tumours remains to be fully elucidated (Zehnder *et al.*, 2016).

2.6.3. Cryosurgery

Cryosurgery, also referred to as cryotherapy, employs the use of liquid nitrogen, liquid nitrous oxide or compressed argon gas in order to generate extreme cold and effectively destroy abnormal tissue. It represents a valuable treatment option, due its non-invasive nature and its potential for use in a palliative context (Farese *et al.*, 2020). The procedure is based on the principle of freeze-thaw cycles, which can result in tissue injury at any stage (Gage & Baust, 1998).

The most important factor in order to obtain cell death is tissue temperature, which should be less than or equal to −50 degrees Celsius in neoplastic tissue. The optimal duration of freezing time remains to be established, although prolonged freezing has been demonstrated to enhance cell destruction. Furthermore, the thawing rate represents a significant destructive factor and should be as slow as possible. Another important factor is the repetition of the freeze–thaw cycle, as multiple cycles have been shown to result in more efficient therapies. The cooling rate is not as essential as the aforementioned factors, but should be as fast as possible (Gage & Baust, 1998).

The use of cryosurgery in domestic cats to treat SCC has yielded some success, with tumours smaller than 1 cm^2 demonstrating the most favourable outcomes. In an evaluation study conducted on cats with SCCs, the response to cryosurgery was found to be complete with tumour remission in 38.5% and partial in 46.1% of patients (Prado *et al.*, 2017).

In the reported cases of cryotherapy in avian literature, a Mitred conure (*Aratinga mitrata mitrata*) was presented with a SCC of the lower beak and underwent surgical debridement of the wound followed by cryotherapy. The neoplasia recurred after the second session of cryosurgery (Jones, 2007). A cockatiel (*Nymphicus hollandicus*) presented with cutaneous SCC, underwent several surgical excisions and a total of 9 sessions of localized topical liquid nitrogen cryotherapy. Although the tumour recurred multiple times over a 13-month period, the patient was able to maintain a good quality of life (McLaughlin *et al.*, 2014). In an African penguin (*Spheniscus demersus*) with a SCC located in the choana, intralesional cisplatin was performed at 7 day intervals for a total of 3 treatments followed by cryotherapy with liquid nitrogen. It remained free of clinical disease for a period of 9 months, at which time a second cryotherapy was performed and remained in remission for a period of 13 months since then (Ferrell *et al.*, 2006).

Cryosurgery could represent a valuable adjunctive therapy for SCC. Further reports and studies are required to evaluate the efficacy of this treatment in avian species. The minimal secondary effects associated with the procedure make it a suitable option for the treatment of small, local lesions or residual disease following surgical resection. As was the case previously, no reports were identified that included raptors.

2.6.4.Non Steroidal Anti-inflammatory Drugs (NSAIDs)

Anti-inflammatory drugs have shown promising results in selected cases and animal model systems. NSAIDs can be cyclooxygenase selective or inhibit both isoforms, with COX-2 selective NSAIDs being a safer option with less gastrointestinal side effects. Asgari *et al.* (2004) referred that NSAIDs exhibited anti-tumour activity against SCCs in vitro and in animal models, as well as in precursor lesions of SCC.

Apoptosis represents the primary mechanism through which NSAIDs exert their antineoplastic properties, whereas the inhibition of cell proliferation has been predominantly observed in vitro or in experimental animal models (Gurpinar *et al.*, 2013). Eicosanoids such as prostaglandins, thromboxanes, and leukotrienes derive from COX-2 and contribute to tumour development through angiogenesis. COX-2 overexpression in tumour cells has been demonstrated to promote tumour vascularisation through the induction of vascular endothelial growth factor expression and the stimulation of endothelial cell proliferation and migration (Brown & DuBois, 2005; Gately & Li, 2004; Li *et al.*, 2002). Therefore, NSAIDs have been shown to inhibit tumour growth through anti-angiogenic mechanisms in experimental models (Gately & Li, 2004). The additional role of COX-2 in carcinogenesis includes its capacity to transform procarcinogens into carcinogens, regulate inflammation and immune responses, and enhance the invasiveness of tumor cells; although some studies suggest that NSAIDs may also exert COX-2-independent effects (Xu, 2002).

Immunohistochemical studies have revealed that COX-2 overexpression is present in avian SCCs. In view of these findings, it is suggested that the use of selective COX-2 inhibitors may have therapeutic value in the treatment of this neoplasia (Jones *et al.*, 2020).

It is necessary to emphasise that the concentration of these agents required to inhibit tumour cell proliferation in vitro is markedly greater than that necessary to inhibit COX-1 and/or COX-2 activity (Ahnen, 1998). To illustrate, in experimental studies conducted in rodents, the chemopreventive efficacy of NSAIDs was achieved at higher doses than those employed for anti-inflammatory effects (Gurpinar *et al.*, 2013).

Furthermore, the administration of piroxicam or meloxicam to canine patients has been observed to result in an enhanced quality of life, as evidenced by increased activity and alertness reported by owners (Argyle *et al.*, 2008). Although there is a paucity of literature on this approach in avian species, adjunctive NSAIDs therapy can provide benefits to oncologic patients, including analgesia and a reduction in discomfort.

2.7. Prognosis and Outcome

The prognosis for long-term recovery from squamous cell carcinoma in avian species is generally poor (Lightfoot, 2006; Gonzalez-Astudillo *et al.*, 2021). SCCs tend to be locally invasive and recurrence is a common outcome (Lightfoot, 2010). Necrosis can occur due to the development of the tumour or the treatment itself, which may lead to a more unfavourable prognosis due to the increased risk of developing septicemia following a secondary infection (Klaphake *et al.* 2006; Lightfoot 2010).

A study evaluating multiple cases of SCC in birds yielded the following results: of the 64 birds for whom complete outcome data were available, only 7 (11%) exhibited complete remission of SCC; the remaining 53 birds (83%) exhibited progressive disease, were euthanized or died of the disease. Eight birds underwent complete excision as a sole treatment, with 4 (50%) having complete remission, 2 (25%) presenting stable disease and 2 (25%) presenting progressive disease. Four birds underwent complete excision combined with another treatment modality and 2 (50%) presented complete response and another 2 (50%) presented progressive disease (Zehnder *et al.*, 2018b).

In the aforementioned study, with the exception of surgery involving complete excision, no other treatments have demonstrated significant efficacy. Overall, 1 of the 19 (5%) birds that received treatment other than complete excision presented complete remission of SCC, 5 (26%) had partial remission, 1 (5%) had stable disease, 6 (32%) had progressive disease, and 6 (32%) were euthanized or died due to disease progression. An overview illustrating the findings of this study is provided in **Table 1**, which summarises the outcomes of cases that were subjected to diverse treatment modalities (Zehnder *et al.*, 2018b).

The risk of death was 62% lower for birds that underwent complete excision when compared to conservative treatment. In addition, the median survival time (with 95% confidence interval) from initial evaluation (only birds with more than 6 months of documented follow-up information available and known survival status were evaluated) was 628 days (ranging from 210 to 1008 days) for patients undergoing complete excision, 357 days (ranging from 143 to 562 days) for patients receiving any other additional treatment, and 171 days (ranging from 89 to 286 days) for monitored patients with or without conservative treatment (Zehnder *et al.*, 2018b).

A different retrospective study of case submissions to a specialty diagnostic service revealed a total of 48 cases of avian SCC, with all exhibiting invasive behaviour. Additionally, 4 (8.3%) cases developed metastasis and 1 (2%) had lymphatic invasion. Excision was only feasible in 3 (6.2%) cases, and 16 (33.3%) deaths were attributed to the tumour (Garner, 2006).

In the context of birds of prey, case reports presenting different treatment modalities are insufficient; therefore, extrapolation from other avian species might be required. According to Forber *et al.* (2000), neoplasms are not uncommon in raptors and a lack of reporting is a more probable explanation for their apparent scarcity.

In conclusion, it is challenging to achieve complete SCC remission in the absence of surgical intervention. However, some alternative therapies are currently being evaluated in avian species, with some achieving partial remission and few achieving complete remission.

Table 1. Clinical outcomes following treatment modalities other than conservative treatment in a total of 31 birds with histologically confirmed SCCs (Zehnder *et al.*, 2018b)

			Outcomes				
Treatment 1	**Treatment 2**	**Total nº of birds**	**Complete Remission**	**Partial Remission**	**Stable Disease**	**Progressive Disease**	**Euthanized or died**
Complete excision	____________	**8**	4	–	2	2	–
Complete excision	Topical or nutraceutical treatment	**2**	1	–	–	1	–
Complete excision	External beam radiation	**1**	–	–	–	1	–
Complete excision	Systemic chemotherapy	**1**	1	–	–	–	–
Systemic chemotherapy	____________	**2**	–	–	–	1	1
Systemic chemotherapy	Cryotherapy	**3**	–	1	–	2	–
Systemic chemotherapy	Strontium radiation	**1**	–	–	–	–	1
Intralesional chemotherapy	____________	**1**	–	1	–	–	–
Intralesional chemotherapy	Cryotherapy	**1**	–	1	–	–	–
External beam radiation	Cryotherapy	**1**	–	1	–	–	–
Cryotherapy	____________	**3**	–	–	1	2	–
Strontium radiation	____________	**5**	1	1	–	–	3
Topical medication or nutraceutical	____________	**2**	–	–	–	1	1
Total		**31**	**7**	**5**	**3**	**10**	**6**

3. CASE REPORT

History

A 10-year-old adult male Harris' hawk (*Parabuteo unicinctus*) was presented with a brownish crusty lesion with a central nodule in the left lateral side of the ventral coelomic cavity, located under the wing and above the flank (**Figure 1 and 2**) (09/09/24). The caretakers noticed a peculiar smell while handling the bird and found the lesion. They also noticed that the bird would sometimes pick at it. The animal had previously been treated for a fungal skin infection in the same location in 2016. The pathogen was identified through biopsy as *Candida sp.* Topical and systemic treatments were administered. The infection recurred in 2017, 2018 and 2019, and the same treatment was applied each time. In 2019, the infection spread to other locations on the skin. The treatment was apparently successful and no further lesions have been described since 2019 until the present moment.

Figure 1 and 2. First clinical presentation on 09/09/2024 (Photographs by Mariana Lima with permission from Oasis Wildlife Fuerteventura).

Clinical findings

On physical examination, the bird showed an acceptable body (3/5) and general condition. The bird weighed 0.625 kg, which is consistent with the average weight observed for a male Harris hawk of a similar age (from 0.540 to 0.850 kg according to *BSAVA Manual of Avian Practice*, 2018). The skin surrounding the lesion was less densely feathered. The nodule measured approximately 1 cm x 0.4 cm and was located on the left lateral side of the ventral coelomic cavity, just above the coxofemoral joint and under the wing. It had an elliptical shape, with defined limits and was firm to the touch. The surrounding lesion extended to about 3 cm and presented light brown toned crusted plaques and tissue proliferation. It had an irregular shape and the limits were ill-defined. The skin also had a friable appearance with some erosion and ulceration. No other alterations were observed.

List of problems: Lesion with brown toned crusted plaques and tissue proliferation, presenting a central nodule, located in the left lateral side of the ventral coelomic cavity.

Differential diagnosis: The main differential diagnoses were malignant neoplasia (more specifically squamous cell carcinoma and soft tissue sarcoma), benign neoplasia (more specifically lipoma, fibroma and feather folliculoma), xanthoma, chronic ulcerative dermatitis, pyoderma (secondary bacterial or fungal infection), abscess and granuloma.

Complementary exams

Fine needle non-aspiration cytology of the nodule was performed (09/09/24). Some blood was present in the sample. Cytological examination after a Diff-Quik™ staining revealed some blood cells and multiple epithelial cells, with characteristics of dysplasia such as increased mitotic activity, anisokaryosis, elevated nuclear-to-cytoplasmic ratio and some cells also presented multinucleation (**Figure 3**).

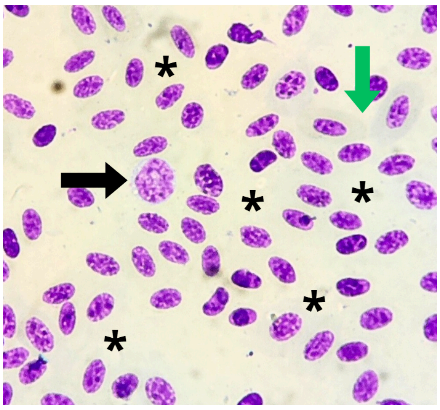

Figure 3. Cytological examination after Diff-Quik™ staining showing several scattered nuclei with signs of dysplasia, including elevated mitotic activity and anisokaryosis (asterisks). Note the presence of some erythrocytes (green arrow) and a lymphocyte (black arrow). (Photograph by Mariana Lima with permission from Oasis Wildlife Fuerteventura).

It was recommended that the lesion should be cleaned with a 1% chlorhexidine solution on a daily basis. Marbofloxacin 10 mg/kg BID PO was prescribed for 10 days. F10 germicidal barrier ointment cream was applied for 4 days SID.

Periodic examinations were carried out to check the general condition of the animal and the evolution of the lesion. Three days later, the nodule appeared to have grown in size, but no measurements were taken. Meloxicam 0.5 mg/kg SID PO was prescribed for 4 days to reduce the discomfort of the raptor.

An excisional biopsy was performed 10 days after the first examination (19/09/24) to provide further information. The raptor fasted for 12 hours. Butorphanol 0.5 mg/kg IM was administered as premedication and induction was achieved using 5% isoflurane in 2 L/min oxygen administered via mask. After induction, anaesthesia was maintained with 3% isoflurane in 2 L/min oxygen administered via mask. The nodule was surgically removed from the basis of the lesion and the small haemorrhage was controlled using a silver nitrate stick. There was no visible ulceration underneath the mass. The remainder of the lesion was not resectable, although some tissue proliferation did have some removal. Due to the location of the lesion in a very flexible area as well as the friability of the skin, it was not possible to do a complete excision. The nodule was fixed in a 10% formaldehyde container and was subsequently sent for histopathology. After the procedure, samples for bacterial and fungal culture were also collected from the lesion. Recovery was quick and without complications. Meloxicam 0.5 mg/kg was given by intramuscular injection. Meloxicam 0.5 mg/kg SID PO was given for 10 days following the surgery.

The histopathology results showed that the submitted sample corresponded to a poorly demarcated, unencapsulated neoplastic proliferation arising from the epidermis and infiltrating the deep dermis. The cells grew in bundles separated by thin trabeculae of fibrovascular stroma with central areas of keratin pearls. The neoplastic cells had a polygonal morphology, moderate pale eosinophilic cytoplasm, well-defined cytoplasmic borders and increasing amounts of keratin granules as they differentiate in the central portion. The nucleus was rounded, central, with lax, basophilic chromatin and one or two obvious nucleoli. The mitotic count was very high (66 mitotic figures/0.273 mm^2). The degree of anisocytosis and anisokaryosis was moderate. The overlying epidermis was almost completely ulcerated, with abundant fibrin, interspersed with cellular detritus and numerous aggregates of coccoid bacteria. In the interstitium there were few heterophils. The neoplastic growth extended to the surgical resection margins. The lesion was identified as a squamous cell carcinoma. The neoplastic tissue extended to the very edge of the biopsy in its deep portions, which would indicate that recurrences may occur.

The fungal culture was negative, while the bacterial culture was positive for *Enterococcus faecalis*.

Definitive diagnosis: Squamous cell carcinoma.

Treatment

Due to the incomplete excision of the neoplasia, cryosurgery was selected as a second-line therapy.

Amoxicillin with clavulanic acid 100 mg/kg PO BID was administered 1 day prior to cryosurgery for 15 days.

On the 22nd day after surgery and 1 day before cryosurgery (09/10/24), a new growth nodule was discovered by the caretakers during their daily cleaning routine. This nodule appeared in the same region as the excised one. Cryosurgery was performed the following day (10/10/24). The lesion exhibited a size of 2.4 cm x 1.5 cm and the nodule had a diameter of 3 mm (**Figure 4 and 5**).

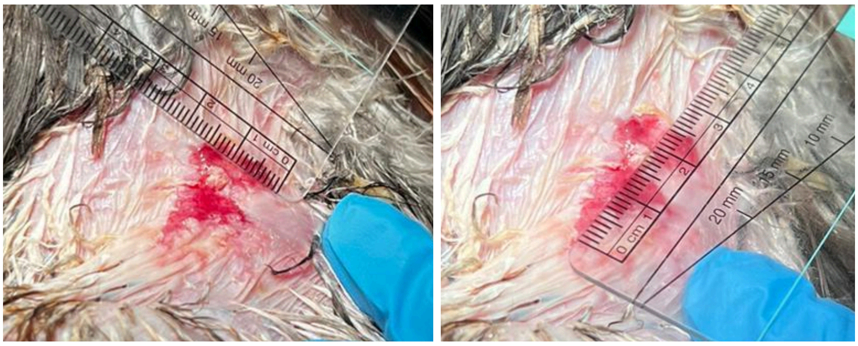

Figure 4 and 5. Measurements taken before cryosurgery. The presence of a new nodule can be observed in the centre of the lesion. (Photographs gently provided by Dr. Sara Peña Santana with permission from Oasis Wildlife Fuerteventura).

The raptor fasted for 12 hours. Induction was achieved with 5% isoflurane in 1.5 L/min oxygen administered via mask. After induction, anaesthesia was maintained with 3% isoflurane in 1.5 L/min oxygen administered via mask.

Cryosurgery was performed using Cryopen® with nitrous oxide gas containers. Three freeze-thaw cycles of approximately 20 seconds were performed. The freezing phase involves the formation of a small white layer of ice over the entire lesion. The temperature in the centre can reach up to -90 degrees Celsius. Thawing is not complete until the entire lesion has returned to its original colour and frost is no longer observed. The first two cycles were well tolerated by the raptor, with no significant increase in heart and respiratory rate. In the third cycle, the raptor’s heart and respiratory rate increased significantly and it was decided not to perform any more cycles this time. The lesion became evidently more erythematous after the cryosurgery. Vitamins A, D3 and E (ADEX-3-FORTE®) 0.5 mg/kg were administered intramuscularly. The raptor fully recovered in 2 minutes. No apparent signs of pain or stress were observed.

Following the procedure, robenacoxib (Onsior®) 4 mg/kg PO SID was prescribed to avoid discomfort as well as for its properties as a COX-2 selective inhibitor.

Periodic check-ups were also arranged, in order to closely monitor the development of the tumour. The following day the lesion was less erythematous and 3 days later the lesion appeared to return to its normal colour and less tissue proliferation was observed. Ten days following the initial cryosurgery session, the lesion exhibited a size of 2.4 cm x 1.5 cm (**Figure 6**).

The lesion remained unchanged in size. No further abnormal growths developed, though there was still visible tissue proliferation. In the light of these results, a new approach was taken. Electrochemotherapy was performed 20 days after cryosurgery (30/10/24).

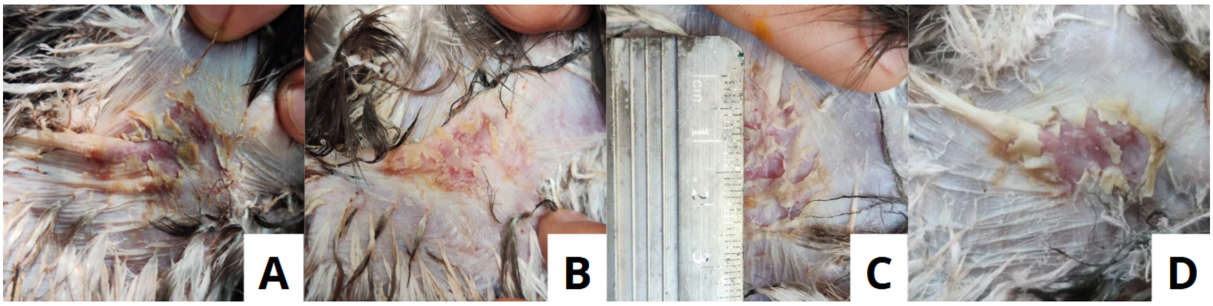

Figure 6. 24 hours (A), 5 days (B), 10 days (C) and 15 days (D) following cryosurgery (Photographs by Mariana Lima with permission from Oasis Wildlife Fuerteventura).

The raptor fasted for 12 hours. Butorphanol 0.5 mg/kg IM was administered as premedication and induction was achieved using 5% isoflurane in 1.5 L/min oxygen administered via mask. After induction, anaesthesia was maintained with 3.5% isoflurane in 1.5 L/min oxygen administered via mask. During the electric pulse the anaesthesia was maintained with 5% isoflurane.

Electrochemotherapy was performed using bleomycin as intralesional chemotherapy. The tumoral volume was calculated in accordance with the formula $A \times B \times C \times \frac{\pi}{6}$ (where A, B and C are the length, width, and thickness of the tumour, respectively). In the case of flat neoplasias, biopsies should be performed in order to evaluate thickness; however, this was not done. The dimensions of the lesion were measured to be 2.1 cm (A) x 1.5 cm (B). Bleomycin, with a concentration of 1500 IU/ml, was administered intratumorally at a dosage of 250 IU/cm³ using a 25-gauge needle in multiple locations. Subsequently, electrostimulation was conducted in two cycles utilising 8-needle electrodes (**Figure 7**). An electrical pulse generator (ELECTROVET EZ® electroporator) was used to create the electrical pulses, with a voltage-to-distance ratio of 1000 volts/cm. A conductive gel (ultrasound gel) was applied to the lesion to facilitate contact between the electrodes and the treatment area, enhancing transmission of electrical impulses and tissue permeability. Following the application of the electrical pulses, mild muscle contractions were observed, accompanied by minor bleeding. The procedure was repeated until all affected areas were fully treated. The entire procedure lasted for 15 minutes, and the raptor demonstrated normal recovery without any complications. After the procedure some light to mild signs of pain were exhibited, such as slight trembling.

Figure 7. Electrochemotherapy procedure using 8 needle electrodes (Photograph by Mariana Lima with permission from Oasis Wildlife Fuerteventura).

The lesion was monitored following 24 and 72 hours. Brownish-to-yellowish crust began to form within the lesion. The administration of robenacoxib was maintained in conjunction with the cleaning of the lesion with a 1% chlorhexidine solution. The neoplasia was observed and measured at 3-day intervals. The bird was weighed twice a day, and no apparent weight loss was observed. No changes in behaviour, feeding, handling or activity. Nine days later the lesion measured 2.1 cm x 1.5 cm and 18 days later the lesion measured 1.6 cm x 1.5 cm (**Figure 8**).

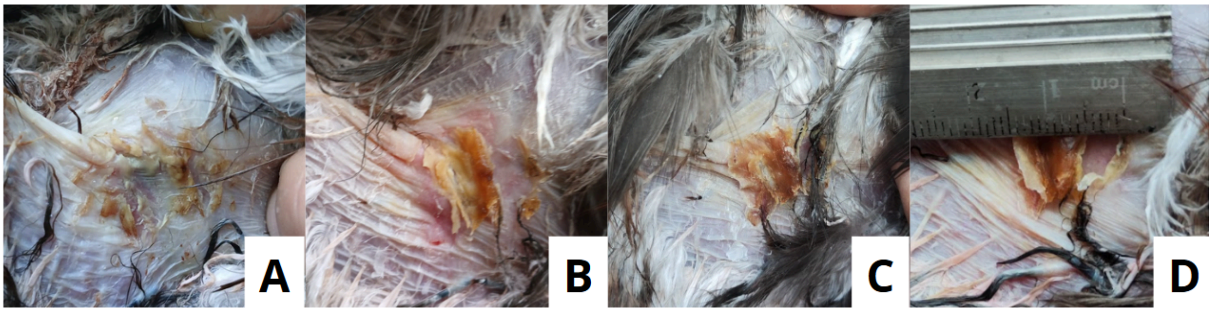

Figure 8. 48 hours (A), 9 days (B), 13 days (C) and 18 days (D) following the first electrochemotherapy session (Photographs by Mariana Lima with permission from Oasis Wildlife Fuerteventura).

A second electrochemotherapy procedure was performed with identical parameters to those employed in the initial procedure, following a 24-day interval (22/11/2024). After removal of the crust, the lesion was measured to be 1.2 cm (A) x 0.6 cm (B) (**Figure 9 and 10**). This indicates a reduction in size of approximately 77%. Following the removal of the crust and electrochemotherapy, a reduction in haemorrhage was observed when compared to the previous procedure. Given the decreased bleeding, we concluded that the thickness of the tumour (C) was significantly reduced, because only the dermis contains blood vessels. Consequently, we speculate that the healing process has occurred within the dermis, with the tumour now predominantly located in the epidermis. The raptor exhibited a normal recovery from anaesthesia without any complications and no signs of pain were observed. The lesion was monitored at 2-day intervals. Similarly to the first session, brown-to-yellow crusts began to form after 24 hours. No changes in weight, behaviour, feeding, handling or activity were reported. Feathers surrounding the lesion started to grow normally again. Twenty-six days following electrochemotherapy, the crust detached and the underlying lesion exhibited a reduction in size and decreased hyperemia (**Figure 11**).

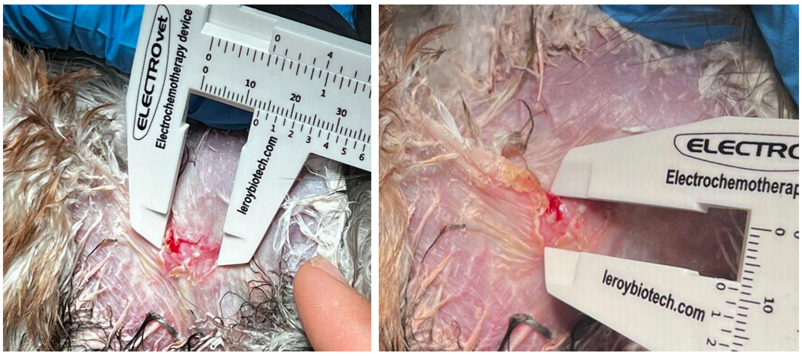

Figure 9 and 10. Measurements taken before the second electrochemotherapy session (Photographs gently provided by Dr. Sara Peña Santana with permission from Oasis Wildlife Fuerteventura).

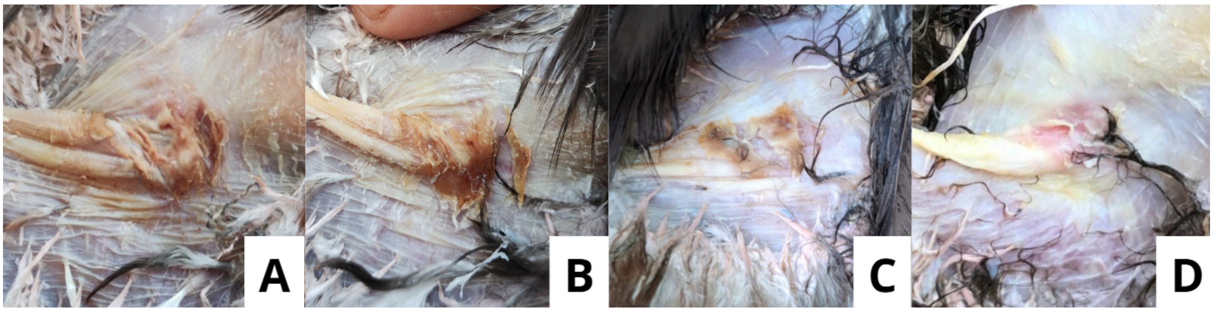

Figure 11. 48 hours (A), 4 days (B), 16 days (C) and 27 days (D) following the second electrochemotherapy session (Photographs by Mariana Lima with permission from Oasis Wildlife Fuerteventura).

A third electrochemotherapy session was performed following a 28-day interval, with identical parameters to those employed in the previous procedures (20/12/2024). The lesion was measured to be 1 cm (A) x 0.6 cm (B). This indicates a reduction in size of approximately 17% from the previous treatment and 81% in total from the first ECT. A smaller 4-needle electrolyte was used this time (**Figure 12**). The recovery was normal and mild signs of pain were observed following the procedure. The lesion developed similarly to the previous sessions, albeit with a lighter toned crust (**Figure 13**).

Figure 12. Electrochemotherapy procedure using 4 needle electrodes (Photograph by Mariana Lima with permission from Oasis Wildlife Fuerteventura).

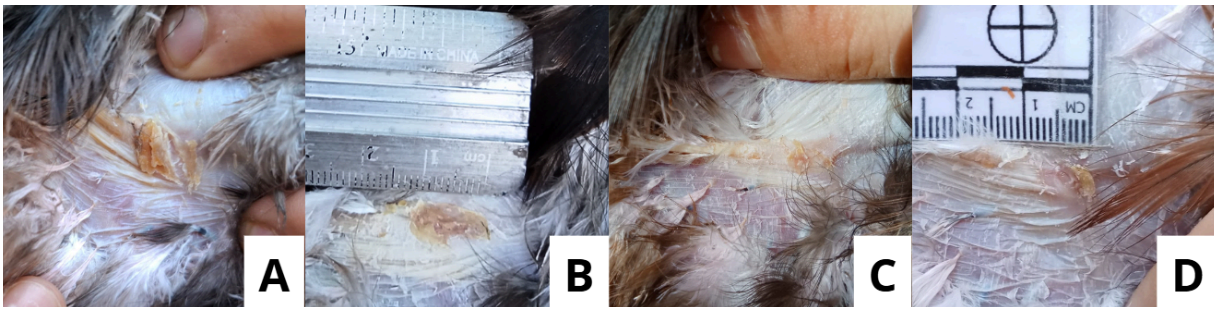

Figure 13. 9 days (A), 14 days (B), 19 days (C) and 24 days (D) following the third electrochemotherapy session (Photographs gently provided by the veterinarian team with permission from Oasis Wildlife Fuerteventura).

Follow up

Surgery was ultimately performed following 28 days (17/01/2025), as the lesion had reduced to a stage where complete excision was feasible without compromising the raptor's locomotion and general well-being. At the time of surgery the lesion measured 0.5 cm (A) x 0.4 cm (B) (**Figure 14 and 15**), which indicates a reduction in size of approximately 67% from the previous treatment and 94% in total from the first ECT.

Figure 14 and 15. Measurements taken before the surgical procedure (Photographs gently provided by the veterinarian team with permission from Oasis Wildlife Fuerteventura).

Induction and anaesthesia were achieved with isoflurane administered via mask, with similar parameters to those employed before. The excision of the lesion was performed using a scalpel, and no relevant bleeding occurred during the procedure. Surgical margins of 1 cm to 1.5 cm were performed in addition to the removal in depth of the epidermis, dermis and subcutaneous tissue (**Figure 16**). Interrupted sutures using absorbable, synthetic and multifilament suture (Novosyn® 3/0) were used to close the excision site (**Figure 17**). The articulation was left with sufficient skin to enable proper movement and function. The lesion was fixed in a 10% formaldehyde container and was subsequently sent for histopathology. The raptor exhibited a normal recovery without any complications. Marbofloxacin 10 mg/kg PO BID was prescribed for 7 days and robenacoxib was maintained in conjunction with daily cleaning with 1% chlorhexidine solution.

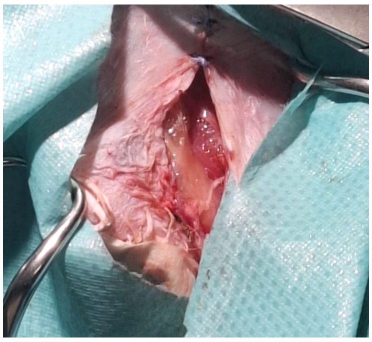

Figure 16 and 17. Excision of the tumour with exposure of the subcutaneous tissues and closure of the surgical site (Photographs gently provided by the veterinarian team with permission from Oasis Wildlife Fuerteventura).

The histopathological findings revealed a marked proliferation of mature connective tissue without cellular atypia. The overlying epidermis showed marked thickening with orthokeratotic hyperkeratosis, but no neoplastic growth was seen in the sample. Isolated hypereosinophilic epidermal cells with discernible borders (apoptosis) and multifocal distribution were occasionally seen. In the superficial dermis, multiple discrete cell aggregates composed of a lymphoplasmacytic infiltrate as well as few heterophils were identified. Therefore, no neoplastic cells were observed in any of the sections examined. The submitted sample was consistent with mild multifocal lymphoplasmacytic dermatitis as well as epidermal hyperplasia with orthokeratotic hyperkeratosis.

In view of the information provided, electrochemotherapy was able to achieve a complete response with no neoplastic cells being identified in the histopathology report. This results in a more favourable prognosis for the patient, although the long-term prognosis remains guarded, with a possibility of recurrence over time. Appropriate monitoring is recommended, with particular attention to any discernible integumentary infections or lesions.

Discussion

Harris' hawks belong to order *Accipitriformes* and have an average lifespan of 10 to 12 years in the wild, but can reach up to 25 years in captivity (Oregon Zoo, n.d.). Therefore, a captive breed 10-year-old raptor is considered an adult, but not geriatric.

These birds of prey can present with some skin problems associated with captivity. An elevated solar exposure, along with inappropriate humidity, temperature, ventilation and hygienic housing conditions, can increase the likelihood of dermal lesions. Picking and self-trauma can also cause and worsen any existing lesion. While an adaptation of Elizabethan collar can be used to avoid this in Psittacidae, it is not a viable option for raptors due to the excessive stress caused (Chitty, 2008).

The prevalence of dermal infections appears to be increasing among Harris' hawks. The propatagium and the precrural fold are the most common sites for the lesions, as it is exemplified by the present case. It usually presents as a secondary infection and primary causes are not easily identified. In addition, secondary infections are significantly more prevalent and are linked to a persistent or recurrent underlying issue that compromises the skin's ability to resist infection. In the absence of an identified and resolved underlying problem, the infection will typically respond only temporarily to treatment, before recurring (Chitty, 2008). In this case, the lesion recurred through the years, despite the fungal agent having been identified. No underlying pathology was investigated, and the biopsy did not include a histopathology report.

These lesions can present with crusts and ulceration as well as feather loss. Similarly, SCC may also manifest in a similar manner. Cytological samples and diagnostic cultures only allow to distinguish between fungal and bacterial infections, yet fail to provide insight into the underlying problem. Therefore, it is strongly recommended that a skin biopsy with following histopathology, culture and sensitivity testing are performed in all prolonged cases along with systemic investigations (Chitty, 2008). Although the lesion appeared to have resolved in 2019, the subsequent development of a squamous cell carcinoma in the same location gives rise to some uncertainty.

It is crucial to elucidate that, in this case, the squamous cell carcinoma extended beyond the hyperplastic well-defined nodule, spreading throughout the entire crusty lesion. The determination of the true extent of the tumour's invasion and its penetration into deeper tissues should be achieved through surgical excision and subsequent histopathological examination (Kamstock *et al.*, 2020).

Cytology is an easy, inexpensive and minimally invasive diagnostic procedure. Fine needle non-aspiration cytology was performed without the need for sedation, but the sample was contaminated with blood, indicating that the cytology should have been repeated. However, given the stressful nature of this procedure for raptors, a new cytology was not performed. Due to the contamination of the sample with blood, it was not possible to interpret the heterophils as part of the lesion, as they may have originated from the blood. The epithelial cells displayed classic signs of dysplasia, however, it is imperative not to conclude immediately that the diagnosis is malignant neoplasia. Primary inflammatory lesions with dysplastic squamous epithelium are particularly challenging to differentiate from a carcinoma through cytology, which may result in erroneous conclusions (Friedrichs & Young, 2019). Biopsy, either excisional or incisional, followed by histopathology represents the gold standard for the diagnosis of this neoplasia (Chhabra *et al.*, 2015; Razi *et al.*, 2023).

Excisional biopsy should achieve complete removal of the lesion and, in SCC, surgical margins of 1 cm are suggested in order to achieve a complete excision (Forbes, 2008; Lightfoot, 2010). After the excisional biopsy, there was a concern regarding the healing of the wound, given its considerable extent and friability. Daily cleansing with 1% chlorhexidine solution was maintained, but no other treatments were advised. Chlorhexidine is a disinfectant and antiseptic, effective against bacteria and fungus. Furthermore, it exhibits promising anti-oncogenic activity, as evidenced by select studies, which have demonstrated that it induces cell apoptosis through a number of mechanisms, including cytotoxicity, loss of cell membrane integrity and mitochondria dependent apoptotic pathways (Kalghatgi *et al.*, 2020). It is important to exercise caution when using creams and medications that enhance cicatrization, as these agents accelerate cellular production, proliferation, migration, and therefore tumorigenesis (Cangkrama *et al.*, 2020). As a result, they are unsuitable for use in integument neoplasias.

As mentioned above, the raptor showed no other alterations on physical examination. Although metastasis are uncommon in avian SCCs, further imaging exams, as well as complete blood count and biochemistry, are required to correctly stage the neoplasia. Staging is crucial in any neoplastic process, as it allows for a more precise prognosis. A CT scan of the coelomic cavity would have been the optimal imaging modality to search for metastasis and evaluate the extent of the tumour. However, radiography is usually the initial screening test for lung metastasis, because of its wide availability and lower cost (Nykamp & Randall, 2019). As none of these exams were performed, the neoplasia was not staged.

The histopathological results demonstrated that the tumour was not entirely removed, as initially thought. The fact that it infiltrated the deep dermis, along with poor demarcation and unencapsulated growth was consistent with a locally invasive neoplasia. Although keratin pearl formation is a hallmark feature of well-differentiated SCCs (Okawa *et al*., 2007), the elevated mitotic count is generally associated with a neoplasia with aggressive behaviour. The mitotic index may assist in the determination of tumour grade, however, there is currently no definitive evidence from the avian literature to suggest that the mitotic index is a prognostic indicator (Zehnder *et al.*, 2016).

The bacterial culture was positive for *Enterococcus faecalis*, which are usually a part of the natural intestinal flora of most animals. Therefore, their presence may indicate skin contamination (Freeman *et al.*, 2011). On the other hand, the histopathological findings of numerous coccoid bacterial aggregates and heterophils may be an indication of secondary bacterial infection. Heterophil is the avian equivalent to the mammalian neutrophil and shares many of the same functions. Elevations in heterophils can be caused by infections, inflammation, stress, trauma, and some toxins (de Matos & Morrisey, 2022).

Another surgical intervention was not a viable option, as it would have required an aggressive approach with wide margins in both length and depth. This is due to the infiltrative nature of the neoplasia and its tendency to recur. It was determined that some of the muscle would have to be removed, which would affect the raptor's ability to locomote and perch. Furthermore, the extensive nature of the lesion would necessitate a prolonged period of healing. Birds of prey are highly active and require regular exercise, which can render them potentially unsuitable for cage rest with restriction of movement. Consequently, in the authors' opinion, a second surgical procedure at this stage could have been detrimental to the raptor's overall health and well-being.

Cryosurgery was selected as it is a minimally invasive and low-pain procedure with few secondary effects. In the present case, cryosurgery was performed in three freeze-thaw cycles of approximately 20 seconds, although it has been demonstrated that longer freezing times (90 seconds) result in improved outcomes for cats (Lucas & Larson, 2006; Prado *et al.*, 2017). Smaller carcinomas (< $1cm^2$) are also associated with better results (Prado *et al.*, 2017; Queiroz *et al.*, 2008).

In studies carried out in cats with squamous cell carcinoma, the response to cryosurgery was classified as follows: complete response (total disappearance of the tumour with total reepithelization of the wound); partial response (reduction of the tumoral area above or equal to 50%); and absence of response (reduction of the tumoral area of less than 50% or tumoral progression) (Prado *et al.*, 2017). In this case, there was a clear absence of response 20 days after the procedure.

Although the preventive use of antibiotics is a very controversial issue, in the case of SCC it may offer some benefit to the patient. SCC causes constant necrosis and tissue damage, similar to that caused by chemotherapy and cryosurgery. This results in a fertile breeding ground for bacteria and fungus. Therefore, appropriate antimicrobial therapy should be continued throughout the duration of treatment to avoid septicemia (Lightfoot, 2010).

Given the absence of relevant changes in size, a decision was made to pursue a new treatment approach. Electrochemotherapy was performed 20 days after cryosurgery with the use of bleomycin as intralesional chemotherapy. Intralesional therapy represents a safer alternative with a localised effect, as opposed to the potential for systemic effects associated with intravenous chemotherapy (Tellado *et al.*, 2022). Nevertheless, it is recommended that complete blood counts and biochemistry parameters be evaluated on a regular basis in order to monitor the occurrence and progression of any adverse effects (Van Hecke *et al.*, 2018).

Although cisplatin is more often used and reported in exotic medicine literature (Račnik *et al.*, 2019b), bleomycin was chosen as the chemotherapeutic agent, due to superior results being reported by some authors (Račnik *et al.*, 2019a). Bleomycin is a glycopeptide antibiotic with cytotoxic effects, causing oxidative DNA damage by generating free radicals that break DNA strands, primarily inhibiting DNA synthesis. However, its high degree of toxicity is limited by its hydrophilic nature, which restricts its diffusion through cell membranes. Nonetheless, this can be enhanced by employing electroporation to facilitate cellular uptake (Mir, 2007; Neumann *et al.*, 1982).

Bleomycin's action is not immediate, as it does not manifest until cells begin the process of division. Therefore, the maximum effect may only be observed after a period of several weeks. In the case of companion animals, it is recommended that sessions should be conducted at intervals of at least 4 weeks (Tellado *et al.,* 2022). However, this may not be directly applicable to birds, given that they have a considerably higher metabolic rate, which can influence the speed at which their bodies process chemotherapy agents and the progression of the neoplasia.

Electrochemotherapy results in the necrosis of the target lesions, with considerable crusts beginning to form a few days after the treatment. Crusts should not be removed until the following treatment.

In order to evaluate the efficacy of electrochemotherapy, the Veterinary Comparative Oncology Group criteria for solid tumours may be employed (Nguyen *et al*., 2015). Complete response is defined as disappearance of all target lesions, while partial response consists of at least 30% reduction in the combined diameters of the target lesions. Progressive disease is defined as either the appearance of one or more new lesions or at least a 20% increase in the combined diameters of the lesions, taking as a reference the minimal sum on examination. Additionally, the sum must show an increase of 5 mm. Stable disease is characterised by tumours with less than 30% reduction or a maximum 20% increase in the sum of the diameters of the target tumours, compared to the smallest sum of diameters during monitoring (Rangel *et al.*, 2019). No neoplastic cells were identified in the histopathological evaluation of the lesion excised, which implies that electrochemotherapy achieved a complete response.

Regarding electrochemotherapy, no secondary effects were reported and pain management was efficient. The administration of robenacoxib was intended to provide analgesia in addition to its action as a selective COX-2 inhibitor. To date, there have been no studies conducted on the usage of this agent in birds of prey, although no adverse effects were observed.

Throughout treatment, no notable adverse effects on the Harris hawk's physical or psychological well-being were reported. No indications of high levels of pain, fear, anxiety, or stress were observed. The hawk's overall condition remained stable and consistent with its normal parameters. The bird’s behaviour within the enclosure remained consistent, exhibiting typical exploratory and perching behaviours. Additionally, there were no observable changes in his alimentary habits. However, further research is required to fully evaluate the potential adverse effects.

Electrochemotherapy has demonstrated a complete response, as confirmed by histopathological analysis showing an absence of neoplastic or dysplastic cells. The presence of cellular apoptosis could indicate that the chemotherapeutic agent is still exerting its effect on cells. Additionally, the surgical excision of the residual lesion, along with wide surgical margins, can help to ensure that no neoplastic cells remain in the surrounding tissue. This is particularly important given that SCCs are characterized by their infiltrative and locally invasive nature.

In conclusion, while complete excision of the tumour has been shown to yield the most favourable outcomes with a median survival time of 628 days, the long-term prognosis for SCC in avian species remains generally poor, with a high rate of recurrence (Lightfoot, 2006; Gonzalez-Astudillo *et al.*, 2021; Zehnder *et al.*, 2018b). Consequently, the administered treatments may only serve a palliative purpose (Stephens, 2019), with the neoplasia recurring within a period of a few months or years. The necessity for repeated therapies and anaesthesia must be evaluated, and euthanasia may be considered if the raptor's wellbeing is adversely affected (Föllmi, 2007). Caretakers and veterinarians should maintain a vigilant and comprehensive assessment of any indicators of distress or abnormal behaviour. To this end, the implementation of a systematic quality of life evaluation is recommended to facilitate an objective and systematic approach to the case (Föllmi, 2007; Jones *et al.*, 2022; Wolfensohn *et al.*, 2018). It is also essential that the animal is closely monitored, and routine appointments must be maintained to assess if the neoplasm has recurred, allowing for prompt action.

5. CONCLUSION

Neoplastic disorders are not uncommon among avian species, therefore appropriate diagnostic, treatment and prognosis should be evaluated in order to provide a standard of care (Zehnder *et al.*, 2018a). Particularly in raptors, options of treatments are lacking, with a limited number of reports (Cooper, 1978, 1979; Cooper & Pugsley, 1984; Cooper *et al*., 1978; Cooper *et al.*,1993; Forbes *et al.*, 2000; Garner, 2006; Ramis *et al.*, 1999; Rocha *et al.*, 2020; Romairone-Duarte *et al.*, 2016).

This report compiles the information available regarding squamous cell carcinomas in avian species, with a particular focus on birds of prey. SCCs are one of the most prevalent neoplasias in birds, affecting a wide range of avian species (Garner, 2006). Etiology of SCCs is not yet established, although chronic persistent inflammation appears to be related with the occurrence of this neoplasia (Zehnder *et al.*, 2016; Klaphake *et al.*, 2006; Koski, 2002; Reavill, 2004). Chronic ulcerative dermatitis can be challenging to differentiate from SCC based on a visual and cytological examination. Consequently, biopsy followed by histopathology is required to achieve a correct diagnosis (Rocha *et al.*, 2020; Sojka *et al.*,2020).

In the context of SCC treatment, various treatment modalities have been explored, each yielding different outcomes in terms of complete remission. Zehnder *et al.* (2018b) concluded that birds who underwent complete tumour excision were 6.9 times more likely to exhibit a partial or complete response compared to those who received alternative treatment approaches. Nonetheless, further research may be needed due to the limited number of reported cases.

The case report presented provides the first insight into various treatment modalities for SCC in a Harris' hawk. The case illustrates the challenges of reaching a SCC diagnosis, with recurrent chronic skin lesions sometimes being present for years before neoplasia is suspected. Furthermore, it explores different treatment options, each presenting its advantages, disadvantages and outcomes. Electrochemotherapy with intralesional bleomycin was proven to be an effective treatment in terms of achieving a complete response with no detectable neoplastic cells. This treatment did not exhibit any discernible adverse effects, although further research is needed.

The prognosis for SCC is generally considered poor, with a high rate of recurrence in the long term (Lightfoot, 2006; Gonzalez-Astudillo *et al.*, 2021). Patients may require multiple treatments to achieve complete or partial remission. In cases where this is not feasible, palliative treatments may be employed and quality of life should be evaluated. Quality of life assessments are of paramount importance in wild/zoo avian species and should be carried out frequently in oncologic patients (Belshaw *et al.*, 2015; Campbell-Ward, 2023).

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Squamous cell carcinoma in raptors: literature review and a case report

Carcinoma de células escamosas em aves de rapina: revisão da literatura e um caso clínico

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ICBAS