

## CASE REPORT

## Conjunctival squamous cell carcinoma in a reindeer (*Rangifer tarandus tarandus*)

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### Abstract

An 8-year-old female adult reindeer (*Rangifer tarandus tarandus*) was referred to the Veterinary Hospital of Madrid for evaluation of a conjunctival mass on the left eye which had been present for about 2 months. A surgical excision was performed and biopsy material submitted for light microscopic evaluation which confirmed the diagnosis of conjunctival squamous cell carcinoma. Nuclear p53 immunolabeling was found in 52% of the neoplastic cells. Follow-up examination at 12 months postsurgery did not reveal recurrence of this neoplasm. Conjunctival squamous cell carcinoma has not been reported previously in reindeer and seems to have similar characteristics to the one existing in bovine species.

**Key Words:** conjunctiva, conjunctival squamous cell carcinoma, ki67, p53 protein, *Rangifer tarandus tarandus*, reindeer

### INTRODUCTION

Ocular squamous cell carcinoma (OSCC) is a common tumor of the eye in cattle. It affects the conjunctiva and ocular adnexa frequently. The lesion usually begins as preneoplastic epidermal plaques and papillomas and may progress to carcinoma *in situ* and invasive carcinoma.<sup>1</sup> Local invasion is common while systemic metastases are less frequent and occur late in the disease. Squamous cell carcinomas may respond to excision, cryotherapy, hyperthermia, radiation therapy, and immunotherapy, or a combination of these therapies. In advanced lesions with extension into orbit and ocular adnexa enucleation or exenteration are recommended.<sup>2</sup> Several factors seem to play important roles in OSCC development including heritability, sunlight, nutrition, eyelid pigmentation, and perhaps viral involvement.<sup>3</sup> This report describes a conjunctival squamous cell carcinoma in a reindeer. To the authors' knowledge, it has not been previously reported in this species.

### CASE REPORT

An 8-year-old female adult reindeer (*Rangifer tarandus tarandus*) from the Zoo–Aquarium of Madrid was referred to

the Veterinary Hospital of Madrid for evaluation of a conjunctival mass in the left eye. The mass had been noticed 2 months prior to presentation and was slow growing. No abnormalities were noted on routine physical examination.

Under manual restraint, ophthalmic examination revealed a papillomatous white lesion of approximately 5 mm of diameter affecting the lateral bulbar conjunctiva, which also showed hyperemic. Slit-lamp biomicroscopic (Kowa SL-14; Kowa Optimed, Inc., Torrance, CA, USA) evaluation of the cornea demonstrated superficial vascular infiltration from the lateral limbus (Fig. 1). Fluorescein staining was negative. The differential diagnosis for the conjunctival lesion included papilloma or squamous cell carcinoma. An impression smear of the lesion was taken after instillation of topical anesthetic drops on the ocular surface (Colir Cusi anestésico doble®; Alcon, Barcelona, Spain). The sample was stained following the May–Grunwald–Giemsa (Merck K GaA, Darmstadt, Germany) stain procedure. Cytologic examination of the impression smear revealed abundant neutrophils, macrophages, and bacteria. Bacteria were mostly extracellular although occasionally they could be observed inside the neutrophils. There were abundant mature cornifying squamous



**Figure 1.** Conjunctival squamous cell carcinoma with conjunctival hyperemia and superficial vascular infiltration of the cornea in an 8-year-old female reindeer.

epithelial cells. There were few squamous cells, with signs of asynchronous maturation of nucleus and cytoplasm (large cells with abundant cytoplasm that have retained a large functional nonpiknotic nucleus); being impossible to differ if the tissue cells are undergoing neoplasia or dysplasia in response to a local inflammatory process. As results were not conclusive, it was decided to excise the lesion.

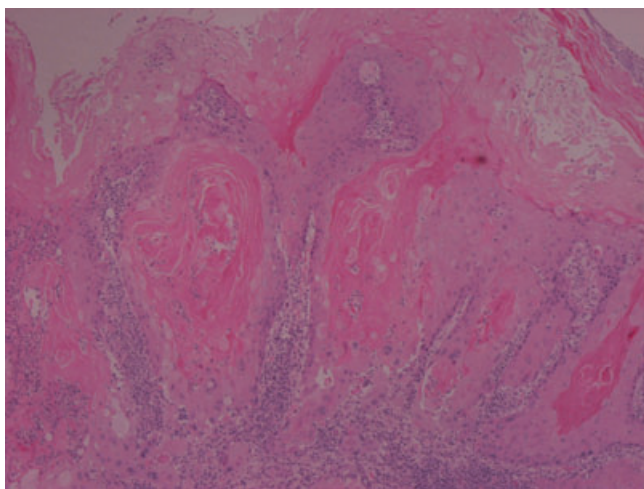
Preoperative complete blood count and serum chemistry were within normal ranges. Under general anesthesia, the mass was excised. The reindeer was premedicated in his stable with an intramuscular injection of ketamine (2.5 mg/kg; Imalgene<sup>®</sup> 1000, Merial, Toulouse, France) and medetomidine (0.1 mg/kg; Medenson<sup>®</sup>, Uranovet, Barcelona, Spain). The reindeer was then carried on a couch to the surgery room where inhalation anesthesia was maintained on 1% isoflurane (Isoflo<sup>®</sup> Esteve Abbot Laboratories Ltd., Berkshire, UK) with a mask. The conjunctival surfaces, including the fornices, were cleansed with sterile cotton-tipped applicators and 0.5% povidone-iodine solution. Initially, a bulbar conjunctival incision was practiced parallel to the corneoscleral limbus. Following a posterior episcleral dissection plane, the mass was completely removed. There was no apparent invasion of deeper layers. A 2–3 mm of normal conjunctiva around the mass was included in the excision. Three approximation sutures with 6/0 Vicryl<sup>®</sup> (Johnson & Johnson Medical Limited, Livingston, UK) were given to close the bulbar conjunctiva. Anesthesia was reversed with an intramuscular injection of atipamezol (0.5 mg/kg; Revazol<sup>®</sup>DFV Divasa Farmavic de Portugal, Vialonga, Portugal). After surgery, topical medication included triple antibiotic combination of gramicidin, neomycin sulfate, and polymyxin B sulfate (Oftalmowell<sup>®</sup>; UCB Pharma, Madrid, Spain) twice daily for 2 weeks. Systemic oxytetracycline

(20 mg/kg, IM, once every 3 days; Terramicina LA<sup>®</sup>; Pfizer, Madrid, Spain) was administered once every 3 days for 2 weeks, and meloxicam (0.5 mg/kg, SC; Metacam<sup>®</sup>; Boehringer Ingelheim España S.A., Madrid, Spain) once a day for 2 days postsurgery. Once the conjunctiva was healed, topical dexamethasone (Maxidex<sup>®</sup>; Alcon) was applied twice daily for 3 weeks to control corneal inflammation.

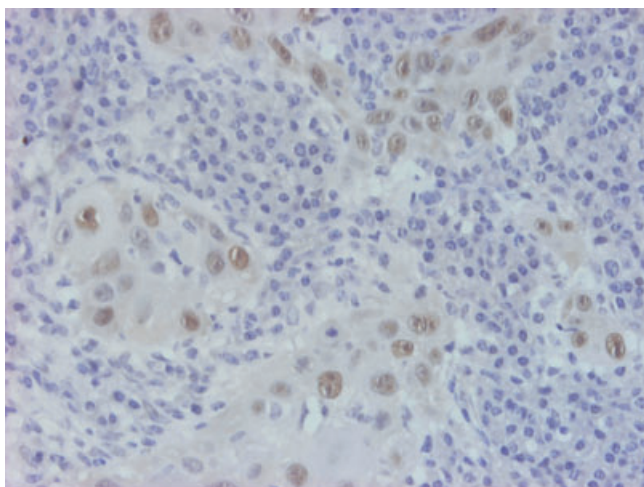
The removed mass, which included a 2–3 mm margin of normal tissue, was fixed in a 10% buffered formalin solution, routinely processed, and sections of about 5  $\mu$ m were stained with hematoxylin eosin. Immunohistochemical staining was performed using the streptavidin–biotin–peroxidase complex method. Tissue sections were processed with 10 mM citrate buffer (pH 6.0) in a pressure cooker for antigen retrieval. Endogenous peroxidase activity was inactivated by incubation with 3% hydrogen-peroxidase in methanol for 5 min at room temperature. Rabbit anti-human p53 polyclonal antibody (NCL-p53-CM1; Novocastra Laboratories Ltd, Newcastle, UK) diluted 1:150 in Tris-buffered saline (TBS) were incubated overnight at 4 °C in a humid chamber. Sections were also incubated with rabbit anti-human Ki-67 polyclonal antibody (Novocastra Laboratories Ltd) diluted 1:1000 in TBS for 1 h at 37 °C. A biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) diluted 1:400 in TBS were applied as secondary reagents for 30 min. After, tissue sections were incubated with streptavidin conjugated with peroxidase diluted 1:400 in TBS (Zymed Invitrogen Co., Carlsbad, CA) for 30 min at RT. The chromogen employed was 3-3'-diaminobenzidine tetrahydrochloride (Vector Laboratories). Immunoperoxidation of p53 protein and Ki-67 was evaluated in five representative areas 20 $\times$ .

Microscopic examination revealed a locally expansive mass arising from the conjunctival epithelium. The tumor was predominantly composed of polyhedral epithelial cells arranged to form either thick cords or nests which infiltrated the underlying connective tissue. Neoplastic cells were round to polygonal, pleomorphic with abundant eosinophilic cytoplasm, with irregularly shaped immature nuclei characterized by a high grade of anisokaryosis and multiple nucleoli. Prominent keratin pearls were seen. A mean of two mitotic figures per 40 $\times$  microscopic field was observed in the sections studied. The neoplasm was well vascularized as many lymphocytes and plasma cells and a few neutrophils were scattered throughout the neoplastic stroma and underneath it (Fig. 2). Nuclear p53 immunolabeling was found in 52% of the neoplastic cells (Fig. 3). The proliferation index, measured as the percentage of Ki-67-positive cells in three high-power fields, was 40%, suggesting that the neoplasm was malignant (Fig. 4). Based on the histological and immunohistochemical findings, a diagnosis of a conjunctival SCC was made.

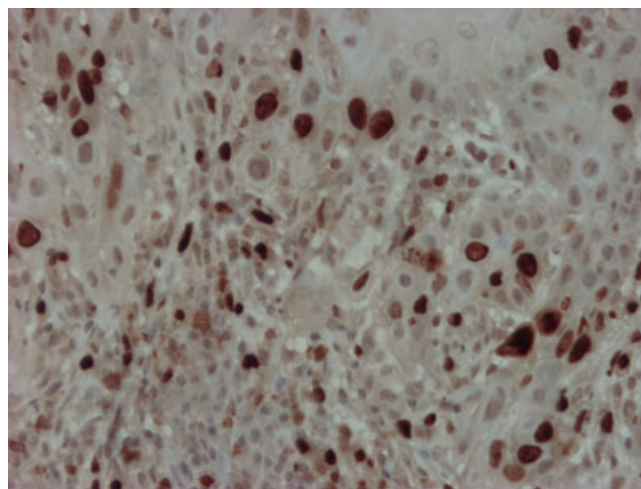
Twelve months after surgery, ophthalmic examination of the left eye was normal, and there was no evidence of tumor recurrence (Fig. 5).



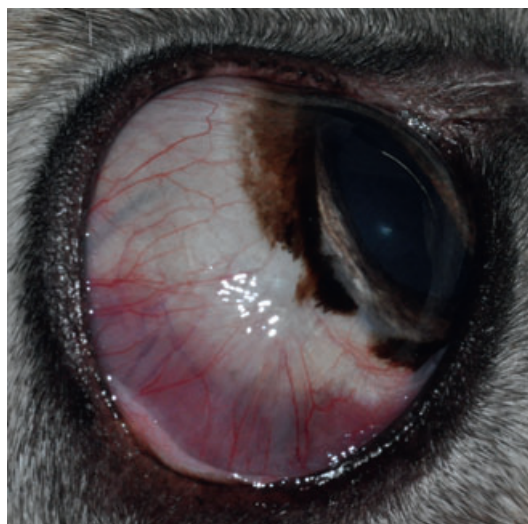
**Figure 2.** Neoplastic epithelial cells, prominent keratin pearls, and abundant inflammatory cell infiltrate. H&E staining 40 $\times$ .



**Figure 3.** Immunostaining for p53 protein. Nuclear immunoreactivity in the neoplastic cells. Streptavidin–biotin–peroxidase complex method 200 $\times$ .



**Figure 4.** Immunostaining for Ki 67. Intensive nuclear immunoreactivity in the neoplastic cells. Streptavidin–biotin–peroxidase complex method 200 $\times$ .



**Figure 5.** This is the surgical site of the left eye the reindeer previously diagnosed and treated for SCC. SCC, squamous cell carcinoma.

## DISCUSSION

Ocular squamous cell carcinoma is a primary neoplasm of epithelial origin which has been well characterized in the bovine species. The bulbar conjunctiva and limbus are most frequently affected (75%),<sup>4</sup> but the eyelids, palpebral conjunctiva, and nictitating membrane may also be affected. The lesions usually begin as benign, smooth, white plaques on the conjunctival surfaces; they may progress to a papilloma and then a squamous cell carcinoma, with or without local invasion or regression.<sup>1</sup> The malignant form may appear directly. OSCC has a multifactorial cause, but exposure to solar ultraviolet radiation is considered a major contributor to its development, probably being the initial cause in most cases.<sup>5</sup> Therefore, it would be advisable that

reindeer enclosures provide some kind of shade to the animals. In the Zoo–Aquarium of Madrid, the reindeers are kept in an enclosure where some shade is provided by trees but ultraviolet radiation is very high in Madrid, so it should still be considered as an important factor in this case. Some viruses, papillomavirus and herpes virus, may also contribute to the induction of precursor lesions and their progression to OSCC of cattle.<sup>5</sup> The same viruses have been identified in reindeer; therefore, their possible implication in the development of OSCC in this species should be purpose of future studies.<sup>6,7</sup> Several studies confirm the involvement of mutations of the p53 tumor suppressor gene in the development of squamous cell carcinomas of cattle, horses, cats, and dogs.<sup>8,9</sup>

In the present case, the OSCC diagnosed in a reindeer seems to keep the typical characteristics of conjunctival SCC found in cattle in terms of localization, macroscopic appearance, and histopathologic features. The conjunctival lesion had a papillomatous appearance, and the cornea showed a mild superficial neovascularization near the temporal limbus. The differential diagnosis after clinical assessment included papilloma plaque or squamous cell carcinoma. An impression smear of the lesion was inconclusive.

In the present case, after considering cytological diagnosis and localization of the lesion, a surgical excision was completed.<sup>2</sup> During surgery, no invasion of deeper layers was appreciated, and the lesion was easily excised. Local excision can be curative with small lesions.<sup>10</sup>

The clinical history together with gross, histological, and immunohistochemical findings confirmed the diagnosis of conjunctival SCC. P53 expression using immunohistochemistry is suggestive that a p53 mutation may have caused this primary conjunctival SCC. This corroborates the p53 changes also observed in bovine, equine, and canine ocular SCC and in the human SCC of the conjunctiva and eyelids.<sup>8,11–14</sup> The malignancy of the tumor was reflected by the high proliferation index obtained using the cellular proliferation marker Ki-67.

In conclusion, to the authors' knowledge, conjunctival squamous cell carcinoma has not been reported previously in reindeer and seems to have similar characteristics to the one existing in bovine species. Early diagnosis is important to prevent local invasion that would imply more aggressive therapeutic alternatives, like enucleation or exenteration. Thus, it should be advisable that annual exams of reindeer include an ophthalmic examination to identify these lesions as soon as possible.

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