

Laryngeal Squamous Cell Carcinoma in a Mangalarga Marchador Horse

André Lang¹, Gustavo Carvalho Cobucci^{2,3}, Beatriz Ibrahim Miranda Antunes³, Felipe de Oliveira Palermo¹,
Isabella de Souza Sampaio¹ & Antonio Carlos Cunha Lacreta Junior²

ABSTRACT

Background: Squamous cell carcinoma (SCC) is the second most frequent malignant tumor in horses and the major tumor of the upper respiratory or gastrointestinal tract. Most common locations in horses are the periorbital region, external genitalia, urogenital tract, oesophagus, stomach, head and neck especially over mucocutaneous junctions where the skin is not pigmented. The presentation of the disease located in the larynx is rare and clinical signs include dyspnea, respiratory snores, cough, nasal exudate, dysphagia, nasal reflux, weight loss and sore throat. This study aims to report a case of occurrence of laryngeal SCC diagnosed by laryngoscopy and histopathological evaluation.

Case: A 14-year-old mangalarga-marchador dark bay colored horse gelding was treated in the municipality of Prados, state of Minas Gerais, Brazil. The physical examination revealed tachycardia, dyspnea and tachypnea, pale oral mucosa, apathy and dysphagia, mucous bilateral nasal secretion with food particles, respiratory noises with inspiratory snoring and increased dyspnea and cough at exercise. At endoscopic examination, a small amount of food material was observed in the nasal cavity, a large tumor was localized in the laryngeal region, with a nodular aspect and an irregular surface, several reddish focal areas and small diffuse bloody ulcerations, extending over the entire surface of the epiglottis and caudally to the arytenoid cartilages, asymmetrically with predominance in the left arytenoepiglottic fold and left arytenoid cartilage. Partial obstruction of approximately 80% of the lumen and left laryngeal hemiplegia was diagnosed. Histopathological examination showed malignant neoplasm diffusely invading the sampled tissue, characterized by "islands" of epithelial cells with large, pleomorphic nuclei and exuberant multiple nucleoli and the presence of numerous mitotic figures, formation of horny pearls and fibrous stroma. Due to the poor prognosis the horse was euthanized.

Discussion: The diagnosis of tumors has increased due to the dissemination of information and the care taken by specialized veterinarians. This fact is especially relevant when there is a need for diagnostic tools and expertise as the endoscopy and histopathology. The SCC is a tumor that occurs more frequently in areas with thinning hair and lack of pigmentation of the skin, whether on the head, especially around the nostrils, rostral portion of the face and periocular region, on the external vulvar or preputial genitalia and on the penis itself. Ultraviolet radiation can damage DNA, triggering cellular mutation. As the region of the larynx is well protected from solar radiation, other factors are necessary to induce neoplastic proliferation. Humans, as passive smokers, may develop the disease and horses in constantly smoky environments could be predisposed. Other factors like advanced age, family history of skin cancer and random individual genetic mutations must be considered. Equine papillomavirus type 2 belongs to the family of papillomavirus, which are host specific and have a pronounced tropism for cutaneous and mucosal keratinocytes and is implicated as a causal agent of SCC. The endoscopic examination proved to be essential for the diagnosis of this laryngeal SCC, allowing the macroscopic characterization, with estimation of severity due to airway obstruction, and biopsy for its histopathological conclusion. Despite the possibility of local infiltrative treatments associated with assisted surgical resection, the severity of the tumor was the basis for the decision for euthanasia.

Keywords: endoscopy, laryngoscopy, tumors, image diagnosis.

DOI: 10.22456/1679-9216.130688

Received: 9 July 2023

Accepted: 10 November 2023

Published: 7 December 2023

¹Private Veterinary Practitioner, Viçosa, MG, Brazil. ²Department of Veterinary Medicine, Lavras Federal University (UFLA), Lavras, MG. ³GammaVet Image Center, Rio de Janeiro, RJ, Brazil. CORRESPONDENCE: G.C. Cobucci [gucobucci@hotmail.com]. Rua Domingos Ferreira n. 149. CEP 22050-011 Rio de Janeiro, RJ, Brazil.

INTRODUCTION

Neoplasms in horses are uncommon and it is related to functional, economics and well-being losses. SCC is the second most frequent malignant tumor in the species and the major tumor of the upper respiratory or gastrointestinal tract. Originating from keratinocytes of the skin or mucosa, most common locations in horses are the skin and mucous membranes of the periorbital region, external genitalia, urogenital tract, esophagus, stomach, head and neck especially in areas of thinning hair and mucocutaneous junctions where the skin is not pigmented [15, 20].

The presentation of the disease located in the larynx is rare and clinical signs include dyspnea, respiratory snores, cough, nasal exudate, dysphagia, nasal reflux, weight loss and sore throat [2,6]. As a diagnostic procedure, visual inspection of the site through endoscopy is imperative, which allows biopsy followed by histopathological study and evaluation [4].

The aim of this study is to report a case of laryngeal SCC diagnosed by laryngoscopy and histopathological evaluation.

CASE

A 14-year-old mangalarga-marchador dark bay colored horse gelding was treated in the municipality of Prados, in the region of Campo das Vertentes, state of Minas Gerais, Brazil. According to the owner's report, the horse had progressive weight loss, respiratory noises, reduced appetite and nasal discharge for many months, no injury to the skin or mucocutaneous junction has been reported.

The physical examination revealed tachycardia of 45 beats per minute (BPM), dyspnea and tachypnea of 30 movements per minute (MPM). Pale oral mucosa, apathy and dysphagia, mucous bilateral nasal secretion with food particles, respiratory noises with inspiratory snoring and increased dyspnea and cough at exercise.

A nasogastric tube was passed and esophageal obstruction associated with dysphagia was ruled out. No skin or mucocutaneous junction wound were identified. As complementary examination a complete blood count was performed, with results indicating anemia and leukocytosis and respiratory endoscopy of the upper respiratory tract suggested.

Endoscopy of the upper respiratory tract was performed with the horse in a quadrupedal station, restrained and sedated with xylazine cloridrate¹ [Ken-

sol® - 0.8mg/kg IV]. A flexible endoscope² with 140 cm length and 7.8 mm diameter and 2.8 mm biopsy instrumentation channel was used.

At endoscopic examination, a small amount of food material was observed in the nasal cavity, a large tumor was localized in the pharyngolaryngeal region (Figure 1 A, B, C, D), with a nodular aspect and an irregular surface with a mixed whitish to pink color, with several reddish focal areas and small diffuse bloody ulcerations, extending over the entire surface of the epiglottis and caudally to the arytenoid cartilages, asymmetrically with predominance in the left arytenoepiglottic fold and left arytenoid cartilage, with arytenoid thickening and local lymphoid hyperplasia. Partial obstruction of approximately 80% of the lumen and left laryngeal hemiplegia was diagnosed. Intense vascularization was observed by the presence of superficial blood vessels and abundant bleeding consequent to tissue collection with the forceps during endoscopic biopsy.

Histopathological examination showed malignant neoplasm diffusely invading the sampled tissue, characterized by "islands" of epithelial cells with large (Figure 2 A, B), pleomorphic nuclei and exuberant multiple nucleoli and the presence of numerous mitotic figures (18 figures in 10 fields/40x), formation of horny pearls and fibrous stroma. The histopathological diagnosis was laryngeal squamous cell carcinoma.

Due the poor prognosis for surgery resection of a large and disseminated tumor and the possibility of recurrence, the cost involved and suffering for the animal, the veterinary made the decision for euthanasia.

DISCUSSION

Despite neoplasms being uncommon in horses, especially compared to humans and dogs, the diagnosis has increased due to the dissemination of information and the care taken by specialized veterinarians [16]. This fact is especially relevant when there is a need for diagnostic tools and expertise that are not commonly available as the endoscopy and histopathology [17].

The SCC is a tumor that occurs more frequently in areas with thinning hair and lack of pigmentation of the skin, whether on the head, especially around the nostrils, rostral portion of the face and periorbital region, on the external vulvar or preputial genitalia and on the penis itself [10,11]. Sun exposure is one of the main causes for the development of SCC, since ultraviolet radiation can damage DNA, triggering cellular mutation [7].

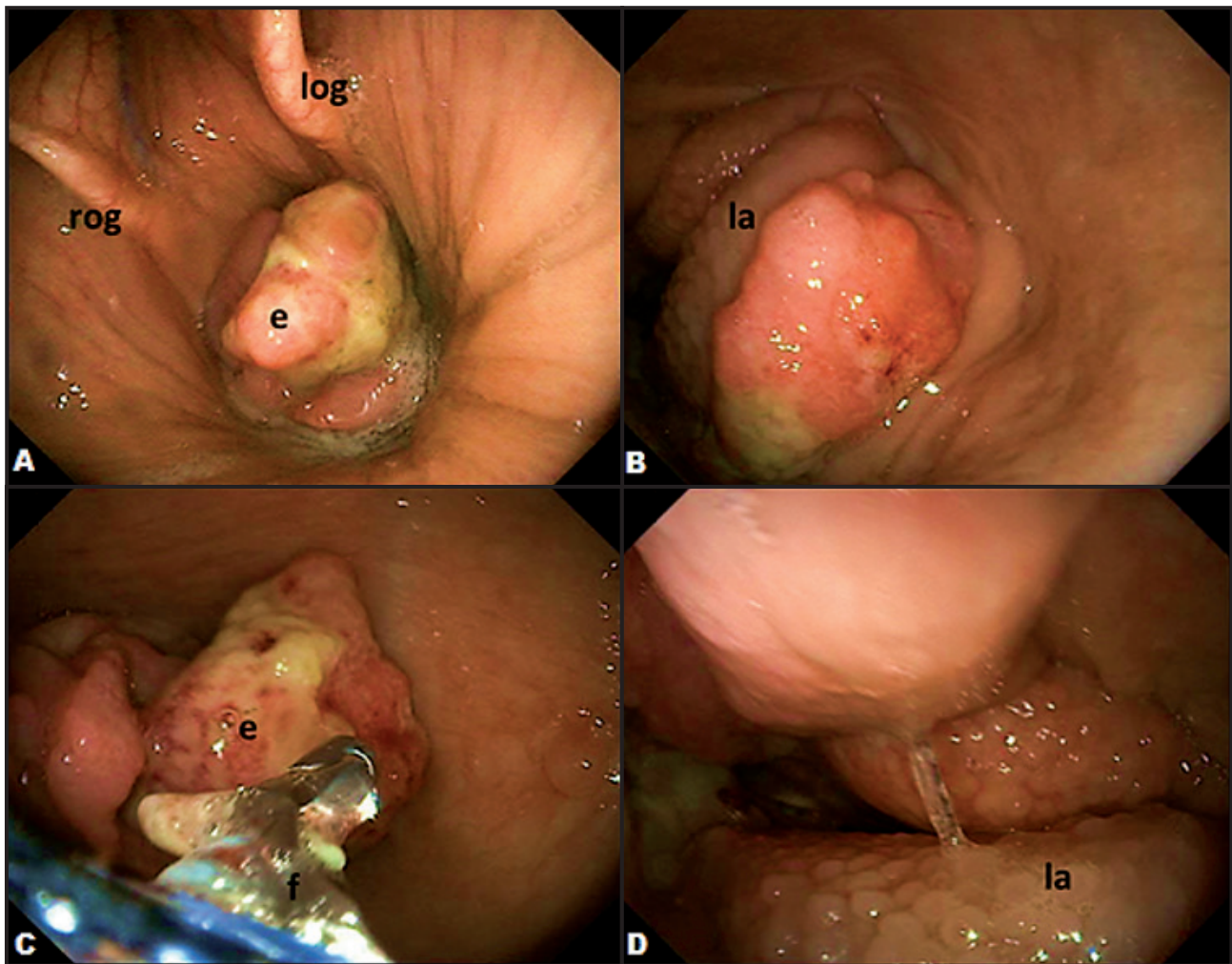


Figure 1. A- Nodular mass of irregular surface over entire epiglottis. B- Nodular mass of irregular surface over mucous membrane of arytenoepiglottic fold. C- Instrumentation to biopsy of the mass over the epiglottis. D- Lymphoid hyperplasia over the arytenoid mucosa. Epiglottis (e), log (left opening of guttural pouch), rog (right opening of guttural pouch), la (left arytenoid), f (forceps).

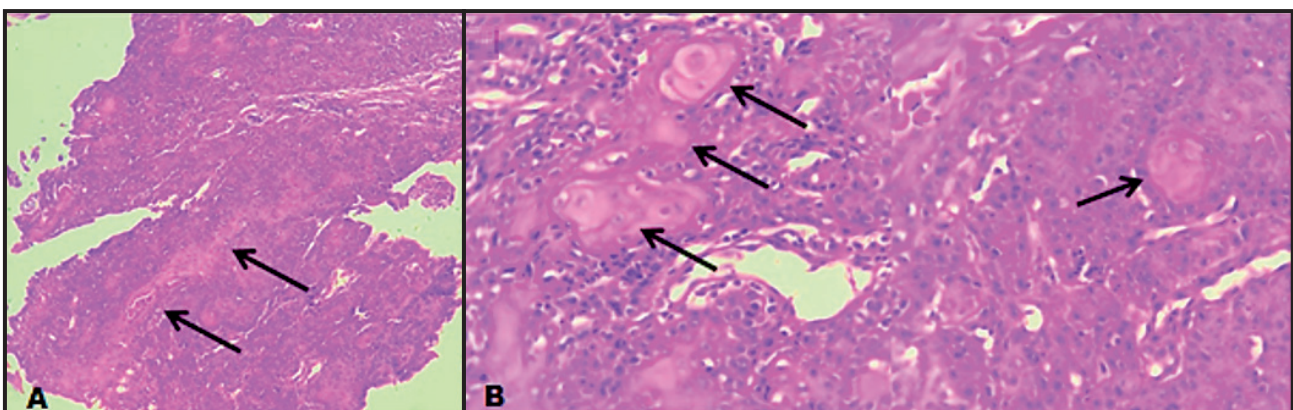


Figure 2. A- Formation of pleomorphic cell lines containing keratin beads (black arrow) [H&E; obj.40x]. B- Mass formed by islands of polyhedral cells with eosinophilic cytoplasm, with sometimes indistinct cell boundaries, euchromatic nuclei of variable sizes and shapes (moderate pleomorphism). Formation of islands of pleomorphic cells containing keratin pearls (black arrow) [H&E; obj.400x].

As the region of the larynx is well protected from solar radiation, other factors are necessary to induce neoplastic proliferation. Factors such as toxic particles inhaled in the form of smoke are quite incrimi-

nated in humans due to smoking, but not consistently in horses, although just as passive smokers may develop the disease, horses in constantly smoky environments could be predisposed. Other factors like advanced age,

family history of skin cancer and random individual genetic mutations must be considered [3].

Equine papillomavirus type 2 belongs to the family of papillomavirus, which are host specific and have a pronounced tropism for cutaneous and mucosal keratinocytes. Since it was first reported in 2010, there is increasing evidence that equine papillomavirus type 2 plays an active role in the development of equine squamous cell carcinoma and precursor lesions such as plaques and papilloma [8,9,13,14,18].

The diagnosis was possible due to the endoscopic examination of the upper airways with flexible fiberoptic endoscopy, through which a biopsy was performed and subsequent histopathological examination of the fragments [4]. These techniques are the golden standard for confirming the diagnosis, as seen in most publications on the subject. Isolated laryngoscopy may already be enough to detect the tumor mass and indicate the diagnosis, due to the epidemiology of SCC as the most prevalent tumor in the region. Histopathology allows definitive confirmation and is also important to determine possible safety margins when small lesions allow treatment and to estimate the prognosis [1]. Radiographic examination may be useful tool in the diagnosis of laryngeal SCC, identifying increased pharyngeal opacity and reduction of pharyngeal lumen or a clearly delineated mass, as additional radiographic findings. Lymphadenopathy suggestive of metastatic

spread may be highlighted by Ultrasonography. Computed tomography can be more useful to detect bone lysis than radiography [21].

As a treatment, surgical resection associated with topical cryotherapy or intralesional chemotherapy is indicated, however systemic chemotherapy, as doxorubicin, is rarely reported in horses. COX-2-derived prostaglandins are related with tumor, metastatic growth, and tumor angiogenesis. Thereby, piroxicam is thought to have an antitumor effect, potentially through the influence of COX-2 inhibition [5,11,12,19]. Although anti-inflammatories were used in this horse, obviously the area to be resected was too wide and the prognosis was poor.

The endoscopic examination proved to be essential for the diagnosis of this laryngeal SCC, allowing the estimation of severity due to partial obstruction and favoring biopsy for its histopathological conclusion. Despite the possibility of systemic and local infiltrative treatments associated with assisted surgical resection, the severity of the tumor was the basis for the decision for euthanasia.

MANUFACTURERS

¹König Brasil - Indústria Farmacêutica. Mairinque, SP, Brazil.

²Medical Flex. Belo Horizonte, MG, Brazil.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- 1 **Armando F., Godizzi F., Razzuoli E., Leonardi F., Angelone M., Corradi A., Meloni D., Ferrari L. & Passeri B. 2020.** Epithelial to Mesenchymal Transition (EMT) in a laryngeal squamous cell carcinoma of a horse. *Future Perspectives Animals*. 10(2318): 13. DOI: 10.3390/ani10122318
- 2 **Brinsko S.P. 1998.** Neoplasia of the male reproductive tract. *Veterinary Clinics of North America: Equine Practice*. 14(3): 517-33. DOI: 10.1016/s0749-0739(17)30184-0.
- 3 **Curado M.P. 2004.** Fatores de risco para o carcinoma espinocelular da laringe e hipofaringe. 118f. São Paulo, SP. Tese (Doutorado) - Programa de Pós-Graduação, Fundação Antônio Prudente.
- 4 **Dixon P.M. 1995.** A review of the role of the epiglottis in equine upper airway obstruction. *Equine Veterinary*. 7(3): 131-139. DOI: 10.1111/j.2042-3292.1995.tb01210.x.
- 5 **Fortier L.A. & MacHarg M.A. 1994.** Topical use of 5-fluorouracil for treatment of squamous cell carcinoma of the external genitalia of horses: 11 cases (1988-1992). *Journal of the American Veterinary Medical Association*. 205: 1183-1185.
- 6 **Jones D.L. 1994.** Squamous cell carcinoma of the larynx and pharynx in horses. *Cornell Veterinary*. 84: 15-24.
- 7 **Kusewitt D.F. 2013.** Neoplasia e biologia tumoral. In: Zachary J.F. & McGavin M.D. (Eds). *Bases da Patologia em Veterinária*. Rio de Janeiro: Elsevier, pp.733-815.
- 8 **Knight C.G., Munday J.S., Peters J. & Dunowska M. 2011.** Equine penile squamous cell carcinomas are associated with the presence of equine papillomavirus type 2 DNA sequences. *Veterinary Pathology*. 48(6): 1190-1194. DOI: 10.1177/0300985810396516.

- 9 **Lale D., Geyer A., Jindra C., Cavalleri J.M.V. & Ramsauer A.S. 2023.** Equine papillomavirus type 2-associated, carcinomatous lesions of the penis and laryngopharynx of an elderly Icelandic horse gelding. *Veterinary Records Case Reports*. 11: e508. DOI: 10.1002/vrc2.508.
- 10 **MacFadden K.E. & Pace L.W. 1991.** Clinical manifestation of squamous cell carcinoma in horses. *Compendium on Continuing Education for the Practising Veterinarian*. 13: 669-676.
- 11 **Moore A.S., Beam S.L., Rassnick K.M. & Provost P. 2003.** Long-term control of mucocutaneous squamous cell carcinoma and metastases in a horse using piroxicam. *Equine Veterinary Journal*. 35: 715-718. DOI: 10.2746/042516403775696320
- 12 **Paterson S. 1997.** Treatment of superficial ulcerative squamous cell carcinoma in three horses with topical 5-fluorouracil. *Veterinary Records*. 141: 626-628. DOI: 10.1136/vr.141.24.626.
- 13 **Ramsauer A.S., Wachoski-Dark G.L., Fraefel C., Tobler K., Brandt S., Knight C.G., Favrot C. & Grest P. 2019.** Paving the way for more precise diagnosis of EcPV2-associated equine penile lesions. *BMC Veterinary Research*. 15(1): 356. DOI:10.1186/s12917-019-2097-0.
- 14 **Scase T., Brandt S., Kainzbauer C., Sykora S., Bijmolt S., Hughes K., Sharpe S. & Foote A. 2010.** Equus caballus papillomavirus-2 (EcPV-2): an infectious cause for equine genital cancer? *Equine Veterinary Journal*. 42(8): 738-745. DOI: 10.1111/j.2042-3306.2010.00311.x.
- 15 **Scott D.W. & Miller W.H. 2010.** Neoplasms, cysts, hamartomas, and keratoses. In: *Equine Dermatology*. 2nd edn. St. Louis: Elsevier/Saunders, pp.468-516.
- 16 **Silva S.C.G., Alves A.D.F., Rönna M. & Torres M.B.A.M. 2015.** Carcinoma de células escamosas em equino com metástase pulmonar: relato de caso. *Revista Brasileira de Higiene e Sanidade Animal*. 9(4): 689-698.
- 17 **Sprenger L., Gabardo L., Risolia L., Molento M., Silva A. & Sousa R. 2014.** Frequência de neoplasias cutâneas em equinos: estudo retrospectivo do laboratório de patologia veterinária da universidade federal do Paraná. *Archives of Veterinary Science*. 19(3): 81-86. DOI: 10.5380/avs.v19i3.35774.
- 18 **Sykora S. & Brandt S. 2017.** Papillomavirus infection and squamous cell carcinoma in horses. *Veterinary Journal*. 223: 48-54. DOI: 10.1016/j.tvjl.2017.05.007.
- 19 **Theon A.P., Pascoe J.R., Galuppo L.D., Fisher P.E., Griffey S.M. & Madigan J.E. 1999.** Comparison of perioperative versus postoperative intratumoral administration of cisplatin for treatment of cutaneous sarcoids and squamous cell carcinoma in horses. *Journal of the American Veterinary Medical Association*. 215: 1655-1660.
- 20 **Valentine BA. 2006.** Survey of equine cutaneous neoplasia in the Pacific Northwest. *Journal of Veterinary Diagnostic Investigation*. 18(1): 123-126. DOI: 10.1177/104063870601800121.
- 21 **Vandersmissen M., Etienne A.L., Dancot M., Evrard L., Beccati F., Grulke S. & Busoni V. 2022.** Conventional imaging is useful for assessment of equine pharyngeal squamous cell carcinoma but underestimates bone involvement. *Equine Veterinary Education*. 35(4): e331-e338. DOI: 10.1111/eve.13713.