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**Case Report**

# Primary Tracheal Squamous Cells Carcinoma in a Dog

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**Abstract**

Primary tracheal neoplasms are uncommon and poorly documented in veterinary medicine, being most frequently reported in domestic cats. Squamous cell carcinoma (SCC) is a malignant neoplasm that originates from the stratified squamous epithelium, considered one of the most common skin neoplasms in dogs and cats. This paper aims to report the anatomopathological and immunohistochemical findings of a clinical case of primary SCC in the trachea of a female Schnauzer canine, attended at the Veterinary Hospital Luiz Quintiliano de Oliveira of the Faculty of Veterinary Medicine of Araçatuba (FMVA – UNESP), presenting clinical signs such as choking, coughing and dyspnea for 1 month. The tracheal portion affected by the neoplasm was sent to the Veterinary Pathology department of the FMVA after surgical excision. Fragments of the neoplasm were collected and fixed in 10% formaldehyde for further histopathological and immunohistochemical analysis. Microscopically, the neoplasm was well differentiated, being characterized by the presence of keratin pearls, low degree of pleomorphism and rare mitotic figures. In the immunohistochemical analysis, there was immunoexpression of anti-cytokeratin antibodies AE1AE3, 34BE12, CK14 and CK5/6, confirming the diagnosis of squamous cell carcinoma. In about 30% of the cells there was immunostaining for Ki67 antibodies, justifying the low mitotic index of tumor cells and the few images of mitosis seen. Due to the rare occurrence of primary tracheal SCC in dogs, the use of diversified diagnostic techniques is important in order to better understand the biological behavior of this neoplasm in unusual anatomical locations.

**Key words:** neoplasm, canine, histopathology.

## Introduction

Primary tracheal neoplasms are rarely described in animals and humans. In veterinary medicine, tracheal neoplasms are most frequently reported in cats, and the clinical picture presented by these animals consists of nonspecific respiratory signs associated with lower airway obstruction (2, 10).

Squamous cell carcinoma (SCC) is a malignant neoplasm derived from the stratified squamous epithelium. It is the most common skin neoplasm in dogs and cats, and is correlated with chronic exposure to ultraviolet radiation from hairless and poorly pigmented regions (16, 17). Regarding the occurrence in the oral cavity, SCC in the gingiva is the second most frequent neoplasm in the region, with a high rate of tumor invasion of adjacent structures, commonly presenting moderate to highly differentiated, without racial or sexual predisposition (14, 7, 19).

In dogs and cats, tracheal squamous cell carcinoma is extremely rare and underreported, being most commonly described in regions adjacent to the trachea. Its occurrence

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**Table 1.** Immunohistochemical panel used in the diagnosis of squamous cell carcinoma.

| Primary antibodies | Clone | Primary antibody type | Provider | Final dilution | Antigen retrieval | pH | Incubation |
|---|---|---|---|---|---|---|---|
| AE1/AE3 | Pancytokeratin | Monoclonal | DAKO | 1:300 | Citrate | 5,6 | Overnight |
| Cytokeratin, High Molecular Weight | 34βe12 | Monoclonal | DAKO | 1 : 100 | Citrate | 5,6 | Overnight |
| CK 14 | LL002 | Monoclonal | BioSB | 1 : 400 | Citrate | 5,6 | Overnight |
| CK 5 / 6 | D5/16 B4 | Monoclonal | DAKO | 1 : 100 | EDTA | 8,9 | Overnight |
| KI67 | Mib 1 | Monoclonal | DAKO | 1:300 | EDTA | 8,9 | Overnight |

has been associated with chronic exposure of animals to environments with high concentrations of cigarette smoke (12). Primary tracheal neoplasms such as chondrosarcoma, chondroma, osteochondroma, poorly differentiated carcinoma and lipoma have been described in dogs (9, 23, 22).

This work aims to present the anatomopathological alterations of a canine with primary tracheal squamous cell carcinoma.

## Case Description

A 9-year-old female Schnauzer canine, cardiopathic, and 8.1 kg, treated at the Small Animal Medical Clinic of Hospital Veterinário Luiz Quintiliano de Oliveira (UNESP /Araçatuba), presenting clinical signs such as choking, coughing and dyspnea for 1 month. Radiographic examination in ventrodorsal projection was performed and showed tracheal collapse secondary to a probable peritracheal neoplasm, affecting seven tracheal rings in the cervical region, cranially at the entrance to the thorax. The animal was sent to the Small Animal Surgical Clinic and underwent surgery for tracheotomy and excision of a probable peritracheal neoplasm. The surgically removed tracheal segment was fixed in 10% formaldehyde and the slides for microscopy of the lesion were submitted to the routine staining technique with hematoxylin and eosin. One of the paraffin blocks containing representative fragments of the lesion was sent to a subcontracted veterinary pathology laboratory for immunohistochemical analysis with monoclonal antibodies AE1AE3, 34BE12, CK14, CK5/6, and Ki67, according to the available and indicated panel (Table 1). The processed tissue sections were placed on previously silanized slides. Antigenic recovery by the wet heat method was carried out in a steam pan for 20-30 min. Incubation with the primary antibodies was done overnight at 4ºC. The Advance system was used for development. Staining was performed with 3,3-diaminobenzidine and counterstaining with hematoxylin. External and/or internal controls were used to validate the reaction.

In the macroscopic analysis, the tracheal fragment measured 4.5 x 3.0 x 2.0 cm, with a firm increase of volume, with a multinodular aspect, involving the tracheal rings of the entire fragment sent (Fig. 1). The surface, it was soft, white, with reddish and white areas, which sometimes creaked when cut (Fig. 2). Microscopically, the respiratory epithelium showed moderate multifocal necrosis associated

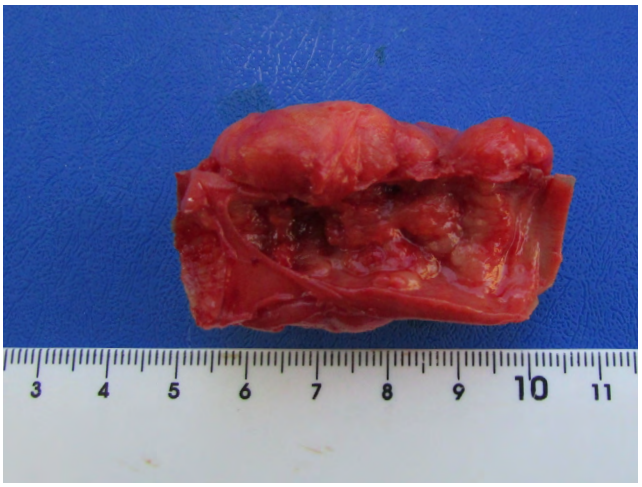


**Figure 1.** Surgically removed tracheal segment. Dog. Firm, irregular and multinodular increase of volume involving the tracheal rings. measuring 4.5 cm x 3.0 cm x 2.0 cm.

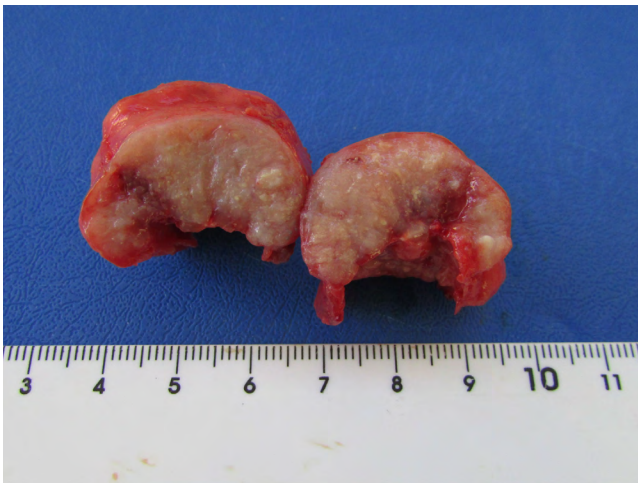


**Figure 2.** Surgically removed tracheal segment. Dog. Smooth, whitish cut surface with reddish and white areas that sometimes creaked when cut.

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with neoplastic proliferation of keratinocytes with a high degree of pleomorphism, which had round to irregular nuclei of varying sizes, central to peripheral and with coarse chromatin, sometimes finely reticular and peripheral, containing evident nucleoli; the cytoplasm was moderate to severe, weakly eosinophilic and with sometimes distinct borders (Fig. 4). These cells were grouped and arranged in several cell layers, forming islands delimited by septa of fibrous connective tissue, surrounding the keratin pearls, interspersed with a discrete multifocal mononuclear inflammatory infiltrate. The neoplasm extended from the tracheal mucosa to the smooth muscle without affecting the hyaline cartilage. In smooth muscle, multifocal areas of mineralization were observed (Fig. 3), as well as focally extensive hemorrhage. Mitosis figures were observed in moderate quantity.

In the immunohistochemical analysis, neoplastic cells immunoexpressed AE1AE3, 34BE12, CK14 and CK5/6, and did not express 35BH11. The Ki67 proliferation marker was positive in approximately 30% of these cells, thus favoring the morphological diagnosis of a well differenciated squamous cell carcinoma.

## Discussion

This report describes the first case of primary squamous cell carcinoma in the trachea dog, without previous or secondary involvement in the oral region. Squamous cell carcinoma is the second most common malignancy in the oral region of dogs, but its occurrence in the trachea is rare (2, 15).

In humans, malignant tracheal neoplasms have a higher incidence, and SCC is commonly associated with smoking. In these patients, the neoplasm is generally described macroscopically as a bulky and obstructive mass, commonly with intraluminal invasion, and the symptoms presented are nonspecific, such as cough and dyspnea (1), as in the case reported, however, not can affirm that in dogs, tobacco would work as a substance that induces carcinogenesis. Previous studies have shown that dogs exposed to environments with high concentrations of tobacco smoke presented pulmonary anthracosis, in addition to an increased population of lymphocytes and macrophages in lung tissue when compared to unexposed animals, demonstrating that the passive contact with tobacco smoke is capable of causing structural changes in the airways (24). In the present case, there was a mononuclear inflammatory infiltrate, with predominance of lymphocytes. These findings also corroborate with Lobetti & Williams (1992) case report, as they observed the same inflammatory pattern in an anaplastic tracheal SCC on a cat.

In the immunohistochemical analysis of the reported case, there was immunoexpression of anti-cytokeratin antibodies AE1AE3 (Fig. 5), 34BE12 (Fig. 6), CK14 (Fig. 7) and CK5/6 (Fig. 8). In the immunohistochemical profile performed in cats with tracheal SCC, there is immunoexpression of anti-cytokeratin AE1AE3 antibodies as in the case reported. This immunostaining occurs in tissues of epithelial origin that are histologically equivalent to squamous epithelial cells associated with the formation of keratin pearls arranged in islands, cords or trabeculae, similarly to the reported case (10). The 34BE12 monoclonal antibody is able to recognize a set of cytokeratins that are commonly expressed in stratified squamous epithelium and in neoplasms such as SCC, as well as CK14 and CK5/6 (8, 26, 27). According to Jalava et al. (2006), the immunostaining for Ki67 antibodies is useful for evaluating the proliferation of tumor cells, having great importance in determining the mitotic index of these cells. In the present work, about 30% of the cells immunoexpressed for the Ki67 marker (Fig. 9), which corroborates the microscopic findings where mitotic figures were rarely seen.

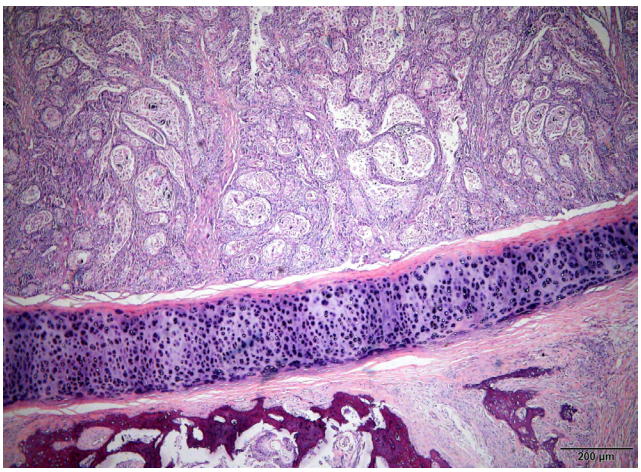


**Figure 3.** Tracheal squamous cell carcinoma showing mucosa, submucosa and smooth muscle with islands of neoplastic keratinocytes with central keratin pearls, separated by fibrous connective tissue septa. In the smooth muscle, multifocal areas of mineralization (HE).

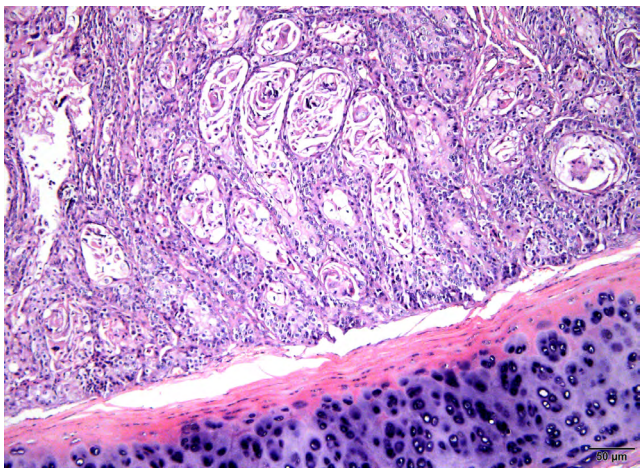


**Figure 4.** Tracheal SCC with higher magnification of keratin pearls (HE).

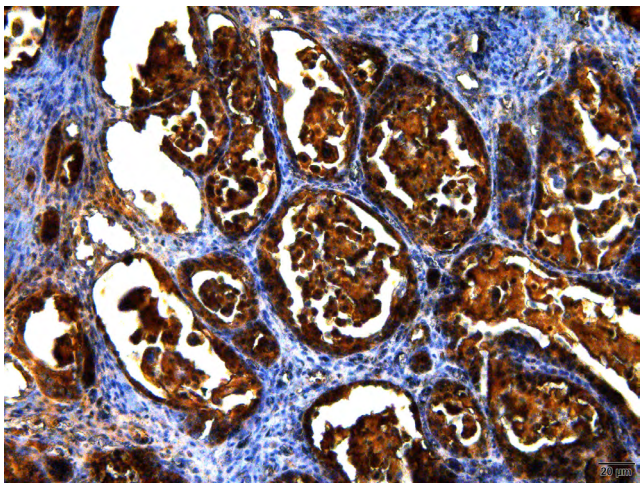

**Figure 5.** Tracheal squamous cell carcinoma. Dog. Positive immunoexpression of anti-cytokeratin AE1AE3 antibodies.

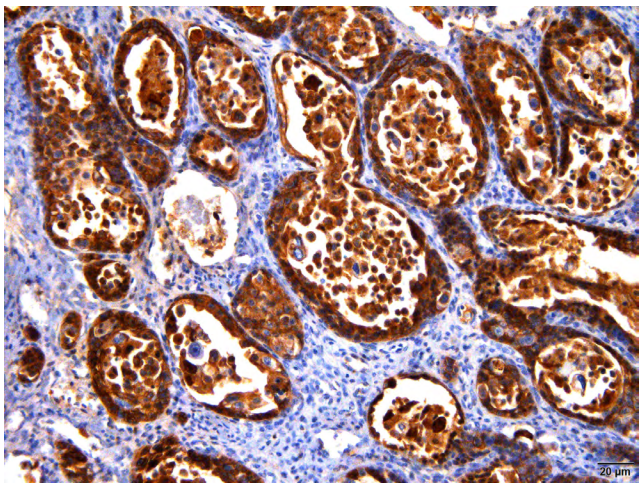

**Figure 6.** Tracheal squamous cell carcinoma. Dog. Positive immunoexpression of 34BE12 anti-cytokeratin antibodies.

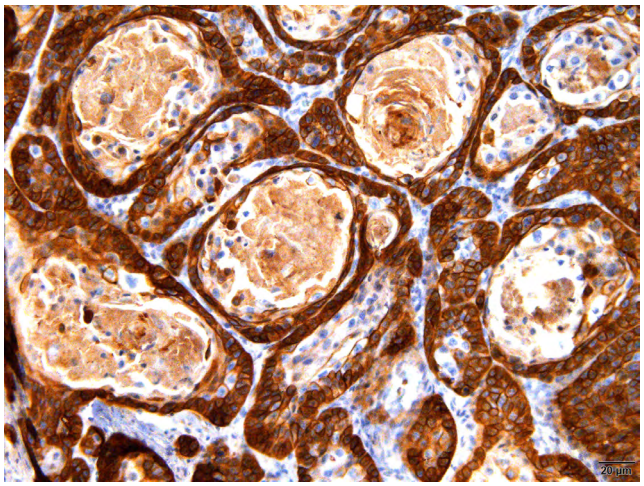

**Figure 7.** Tracheal squamous cell carcinoma. Dog. Positive immunoexpression of anti-cytokeratin CK14 antibodies.

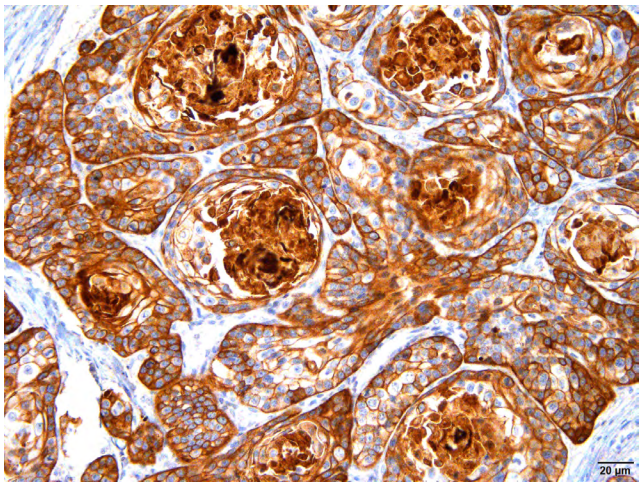

**Figure 8.** Tracheal squamous cell carcinoma. Dog. Positive immunoexpression of anti-cytokeratin CK5/6 antibodies.

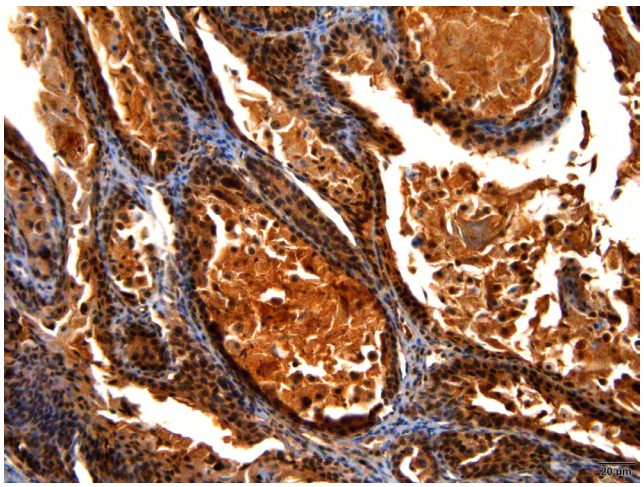

**Figure 9.** Tracheal squamous cell carcinoma. Dog. Positive immunoexpression of Ki67 proliferation marker.

## Conclusion

Primary tracheal neoplasms in dogs are not commonly reported, with squamous cell carcinoma being rare.

## Declaration of Conflicts of Interest

The authors declared that there is no conflict of interest in relation to the research, authorship or publication of this article.

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