

**Case Report****Oral squamous cell carcinoma with lung and kidney metastases in a hoary fox (*Lycalopex vetulus*)**

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Abstract

Oral squamous cell carcinoma is a common malignant tumor of epithelial cells in dogs. This tumor metastasizes slowly and primarily affects the gingiva. Despite its importance, few studies document this cancer in wild canids. A young adult female hoary fox (*Lycalopex vetulus*) kept in captivity at Associação Mata Ciliar (Jundiaí, SP, Brazil) exhibited clinical signs such as weakness, prostration, and severe oral bleeding. After anesthetizing the animal, a reddish, friable verrucous formation was observed on the upper and lower lip, as well as on the maxillary and mandibular gingiva on the right side of the mouth, extending towards the bony palate. A biopsy of this mass was performed, followed by histopathological and immunohistochemical evaluation. Histopathology indicated an atypical squamous proliferation with inflammatory features suggestive of poorly differentiated squamous cell carcinoma, which was confirmed by immunohistochemistry through the positive expression of antibodies AE1/AE3, p63, and CK14. The proliferation marker ki67 was used to assess the aggressiveness and malignancy of the tumor, and it was found in approximately 80% of the neoplastic cells. Because of the poor prognosis, the hoary fox was euthanized, and a necropsy was performed. Histopathological analysis of the tissues revealed lung metastasis, suppurative bronchopneumonia, and renal metastasis associated with neutrophilic interstitial nephritis and tubular necrosis. The present report describes this type of neoplasm for the first time in a hoary fox and one of the few observed in wild canids. The scarcity of research on neoplasm incidence in wild canids in Brazil emphasizes the need for further investigations to understand tumor development in these species better.

Key-words: pathology, neoplasm, wildlife disease, canids

Introduction

The hoary fox (*Lycalopex vetulus*) is a canid species endemic to Brazil (4). This species is found in the Cerrado and ecotones with the Pantanal in the central-south region of the country, where it frequently encroaches upon agricultural and livestock pastures (4). The hoary fox is classified as Near Threatened on the International Union for Conservation of Nature Red List (11). The main threat to the hoary fox is habitat destruction caused by expanding industrial

and commercial activities and the rapid growth of urban centers (12).

Few studies have examined the occurrence of neoplasms in wild canids, including the hoary fox. However, it is believed that these animals can develop tumors like those found in domestic dogs (9). A recent study identified tumors or tumor-like lesions in five out of 1890 red foxes (*Vulpes vulpes*). Among these, two were collagenous hamartomas, two were adenomas of the meibomian gland, and one was a teratoma in the cryptorchid testicle, all considered benign

(17). However, the incidence of tumors in these foxes is lower than that in dogs (17).

One of the most essential tumors in domestic dogs is oral squamous cell carcinoma, a locally aggressive and generally invasive malignant tumor of epithelial cells. This tumor metastasizes slowly and most commonly develops on the gingiva, affecting the maxillary and mandibular gingivae almost equally (8, 10, 23). A previous study found that melanoma was the most prevalent oral tumor in dogs, followed by oral squamous cell carcinoma (21). In the latter, the gingiva was affected in most cases (74.7%; 44/59), with a higher occurrence in males than females, and the average age of affected animals was 11 years old (21). The cause of oral squamous cell carcinoma in dogs remains unclear. In humans, this neoplasm was associated with factors such as tobacco and alcohol use, as well as infection with high-risk human papillomaviruses (HPVs) (7). In dogs, some studies suggest an association between canine papillomavirus infection and the development of squamous cell carcinoma (14).

This case report presents the gross, histopathological, and immunohistochemical findings of an oral squamous cell carcinoma in a captive hoary fox (*Lycalopex vetulus*) with lung and kidney metastases.

Case Description

A female hoary fox (*Lycalopex vetulus*), was found orphaned in an oil refinery in Paulínia, São Paulo state, in 2019. The animal was rescued and sent to the Mata Ciliar Association (Jundiá, SP, Brazil). In December 2021, after two years in captivity, the hoary fox exhibited weakness, prostration, progressive weight loss, and oral bleeding. The animal was anesthetized for a clinical examination. A multilobulated, reddish, friable, verrucous, and locally extensive mass was observed on the lower and upper lip on the right side of the animal's mouth. The mass extended over the entire maxillary and mandibular gingival area and affected the bony palate (Fig. 1). Additionally, there were signs of ulceration. A biopsy was taken from the mass. The tissue was fixed in a 10% phosphate-buffered formalin solution (pH 7.4), processed for standard paraffin embedding, cut into 5- μ m sections, and stained with hematoxylin and eosin.

The histopathological examination of the mass revealed a proliferation of epithelial cells with an exophytic lesion characterized by atypical squamous epithelium, marked hyperkeratosis, and hypergranulosis with coarse keratohyalin granules and preserved cell polarity (Fig. 2A). Neoplastic cells were arranged in nests and anastomosing trabeculae and were in a discrete fibrovascular stroma. Individually, the cells had indistinct shapes and boundaries and exhibited abundant eosinophilic cytoplasm, rarely strongly keratinized. The nuclei were round to oval, of medium size, with chromatin varying from coarsely to finely clustered. Nucleoli were evident and sometimes multiple. There was

moderate to severe anisokaryosis, karyomegaly, and nuclear pleomorphism, with a count of five mitotic figures in 10 fields at the magnification 40x, in 2.37 mm², with ocular field number (FN) 22. A moderate neutrophilic inflammatory infiltrate was also observed (Fig. 2B). No lamellar keratin (keratin pearls) areas were observed. The suggested diagnosis was an



Figure 1. A multilobulated, verrucous, friable, and diffusely reddish mass located on the lower and upper lip on the right side of the animal's mouth, affecting the entire maxillary and mandibular gingiva on the same side and advancing to the bony palate.

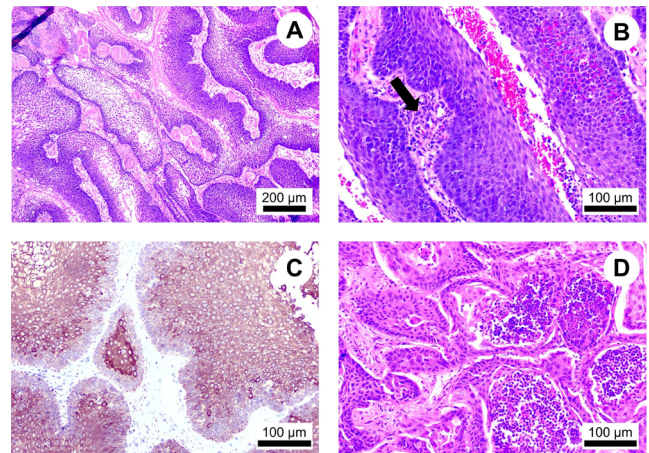


Figure 2. Histopathological examination of oral squamous cell carcinoma with metastasis in a young adult female *Lycalopex vetulus*. A- and B- Atypical squamous epithelium trabeculae with marked hyperkeratosis and hypergranulosis with coarse keratohyalin granules. Note the presence of neutrophilic inflammatory infiltrates (black arrow). (H&E, A- bar = 200 μ m, B- bar = 100 μ m). C- Immunohistochemical analysis of oral squamous cell carcinoma. The neoplastic cells exhibit positive staining for AE1/AE3. (IHC, bar = 100 μ m). D- Histopathological evaluation of the lungs. Note neoplastic epithelial cells. (H&E, bar = 100 μ m).

atypical squamous proliferation with inflammatory features suggestive of squamous cell carcinoma.

Immunohistochemical analysis confirmed the presence of squamous cell carcinoma through the positive expression of the antibodies pan-cytokeratin AE1/AE3 (AE1AE3) (Fig. 2C), protein p63 (p63), and cytokeratin 14 (CK14). The proliferation marker ki67 was found in approximately 80% of the neoplastic cells. Standard tissue processing was followed by paraffin embedding and mounting on silane-treated slides for immunohistochemistry. Antigen retrieval was performed using the heat-induced epitope retrieval (HIER) method in a steam cooker for 20-30 minutes. Primary antibodies were incubated overnight at 4°C using the Advance^R system. Staining was performed using 3,3'-diaminobenzidine, followed by counterstaining with hematoxylin. Additional information is summarized in Table 1.

In addition, cranial and thoracic X-rays were performed. The cranial radiographs revealed irregularities and osteolysis in the bony palate region and increased heterogeneous radiopacity in the right nasal cavity. However, the nasal septum was preserved. The thoracic radiography revealed increased radiopacity in the lung with a nodular interstitial and bronchial-interstitial pattern. Moreover, multiple rounded nodules with soft tissue radiopacity were observed throughout the lung parenchyma, indicating metastases.

The animal continued to show progressive weight loss and intense prostration and was euthanized due to the poor prognosis. A necroscopic examination revealed a tumor in the oral cavity with the same characteristics as previously described and observed during the animal's anesthesia (Fig. 3A). In addition, there were multiple firm yellowish formations in the lungs, which deepened on cutting (Fig. 3B). The kidneys had irregular surfaces, medullary expansion, and cortical atrophy, as well as yellowish nodular foci in the cortex. Hydronephrosis was also observed.

The histopathological examination revealed lung metastases characterized by neoplastic epithelial cells like those found in the oral cavity. Additionally, suppurative bronchopneumonia, emphysema, and alveolar edema were observed (Fig. 2D). The kidney also exhibited metastasis, with neoplastic cells forming nests, accompanied by acute tubular nephritis, necrosis, and tubular atrophy. The spleen exhibited atrophy of the lymphoid follicles. The trachea exhibited neutrophilic and lymphoplasmacytic inflammation, along with emboli of neoplastic cells in the blood vessels.

Discussion

To the best of our knowledge, this is the first documented case of a hoary fox (*Lycalopex vetulus*) exhibiting a malignant neoplastic growth: a poorly differentiated oral squamous cell carcinoma affecting the gingiva and lip on the right side, beyond the bony palate. This condition was associated with pulmonary and renal metastases. The animal was euthanized due to its poor prognosis and the evidence of lung metastasis detected on the thoracic radiograph. The clinical signs, such as apathy, hyporexia, and progressive weight loss, also influenced the decision. Feeding and handling could be more challenging since it was a wild animal.

Radiographic assessment is extremely important for diagnosing oral neoplasms, and the detection of osteolysis is interesting for estimating the invasion of neoplastic cells and indicating the margins for surgical excision (15). The osteolysis observed on the cranial radiograph of the hoary fox allowed us to verify the tumor's invasive potential, with a significant advancement into the bony palate.

In the histopathological evaluation, the tumor showed poor histological criteria, requiring the use of immunohistochemistry for diagnostic confirmation. Specific tumor markers for identifying epithelial cells, such as pan-cytokeratin AE1/AE3, p63, and CK14, were employed to support the diagnosis of squamous cell carcinoma. The proliferation marker ki67 was used to assess the tumor aggressiveness and malignancy, with approximately 80%

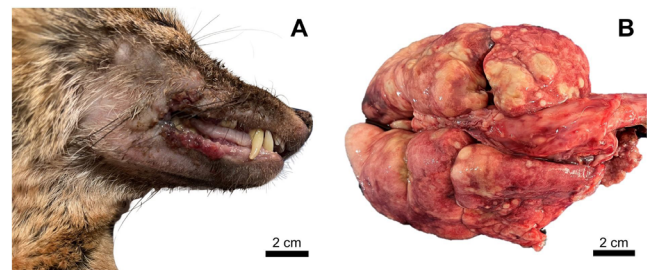


Figure 3. Postmortem examination of a young adult female *Lycalopex vetulus*. A- A multilobulated, diffuse, friable, reddish verrucous mass was observed in the lower and upper lip region on the right side of the animal's mouth. Note the signs of ulceration. B- Lungs exhibiting multiple multifocal to coalescing solid yellowish formations.

Table 1. Antibodies used for diagnosis of oral squamous cells carcinoma in a hoary fox (*Lycalopex vetulus*).

Monoclonal antibodies	Target tissue	Manufacturer	Clone	Dilution	Antigen retrieval	PH	Incubation
AE1/AE3	Epithelial cell cytoplasm	DAKO	Pan-cytokeratin	1:300	Citrate	5,6	Overnight
Ki67	Proliferating cell nuclei	DAKO	Mib 1	1:300	EDTA	8,9	Overnight
P63	Epithelial basal cells nucleus	DAKO	DAK-p63	1:400	EDTA	8,9	Overnight
CK14	Cell membrane and cytoplasm	BioSb	(LL002)	1:100	Citrate	5,6	Overnight
Cocktail melanoma	Melanocytes cytoplasm	CellMarque	Pool (Hmb45 + Mart -1 + Tyrosinase)	1:400	EDTA	8,9	Overnight

expressing it. This marker predicts cell proliferation more accurately than the mitotic index (15).

The hoary fox in this report was a young adult (3 years old), which is consistent with the findings of a recent study in Croatia, where most red foxes (*Vulpes vulpes*) with neoplasms were young individuals (17). In contrast, tumors in domestic dogs usually manifest in animals over nine years old (23). Furthermore, unlike the case described here, metastases to internal organs are rare in domestic dogs, which could indicate that the behavior of oral squamous cell carcinoma may be different in wild canids, such as the hoary fox, with more aggressiveness, since metastases occurred in two organs, the kidney, and the lung. However, bone involvement and invasion are common in domestic dogs (18). This pattern is consistent with the case of the hoary fox, which exhibited osteolysis, identified by radiographic images, particularly affecting the bony palate.

Reports of naturally occurring malignant tumors in wild canids are rare. In Brazil, neoplasms have been reported in bush dogs (*Speothos venaticus*), specifically a lymphoma (3) and a cutaneous mast cell tumor (20). However, most reports of neoplasms in Brazilian wild canids have been described in the maned wolf (*Chrysocyon brachyurus*). These reports consist of mammary tubulopapillary carcinomas (6) and ovarian tumors, such as granulosa cell tumors, ovarian adenopapillomas, and dysgerminomas (16). Other tumors previously reported in the maned wolf include a urinary bladder teratoma (5). In a captive 17-year-old male crab-eating fox (*Cerdocyon thous*), a colonic papillary adenocarcinoma was recently described (19). Only one pancreatic adenoma tumor has been reported in the hoary fox (*Lycalopex vetulus*) (22). Our report represents the second tumor described in this species.

In wild canids, oral squamous cell carcinoma was reported in a 19-year-old captive female coyote (*Canis latrans*). The coyote had a dark red mass measuring 2.0 × 2.0 cm on its soft and hard palate and adjacent gingiva, which led to euthanasia (2). Neoplasms in wild canids are mainly documented in captive animals (6, 19). Captive animals are monitored continuously throughout their lives, and studies are often conducted posthumously, as was the case with the hoary fox in this study.

The cause of tumor formation in this hoary fox is unknown. However, the hoary fox was rescued as a cub in an oil refinery where pollutants were discharged into the environment (1,10). These pollutants are mainly polycyclic aromatic hydrocarbons (PAHs), which are released into the atmosphere as byproducts of organic matter combustion (10). Petroleum PAHs are toxic, mutagenic, and carcinogenic (1). Notably, benzene, a component of gasoline and other petroleum products, is a human and animal carcinogen. Previous studies with rats and mice have shown that exposure to benzene can induce lymphoma, leukemia, as well as tumors in the oral cavity, mammary glands, ovaries, and uterus (13). The duration of the hoary fox's exposure to

environmental carcinogens is unclear, making it impossible to confirm whether tumor development is related to this exposure. In our case, this is purely a hypothesis.

In conclusion, squamous cell carcinoma can develop on the gingiva and lip of hoary foxes. Therefore, it is essential to consider this type of neoplasm as a potential diagnosis for other tumors in the same region. In our case, we identified an atypical squamous proliferation suggestive of squamous cell carcinoma. Diagnostic confirmation was obtained using tumor markers. The high level of Ki67 indicated that the tumor was highly aggressive and could potentially metastasize. The present report highlights the importance of pathology as a diagnostic tool in wild animals. It also emphasizes the need for further research to understand the occurrence of tumors in wild canids and their relationship with genetic and environmental factors.

Conflict of Interest

The authors declare no competing interests.

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