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Case Report

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Managing epiglottal chondrosarcoma of a dog: A case report

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ABSTRACT

A primary chondrosarcoma was found in the epiglottis of a 6-year-old, neutered, male Boxer cross-breed dog. Clinically, there was upper respiratory noise, and a 3.2 cm × 2.8 cm × 2.7 cm, ovoid mass involving the epiglottis was observed. No abnormalities were detected upon radiographic examinations (X-ray) of the chest or abdomen. Grossly, the excised mass was hard. On cut section, it displayed a lobular pattern of translucent gray-white color (interpreted as cartilage). Histologically, the epiglottal submucosa contained a non-encapsulated, fairly demarcated multi-nodular neoplasm composed of streams of cells admixed with moderate to abundant amounts of a pale amphophilic to eosinophilic matrix (chondroid). The neoplastic cells stained were immunohistochemically positive for vimentin and S-100 protein, and negative for pancytokeratin. The matrix stained deeply with Alcian Blue (pH 2.5)-periodic acid Schiff, which often separated cells into individual lacunae. To the best of our knowledge, this is the first report of extra-skeletal chondrosarcoma primarily arising in the epiglottis of a dog.

KEY WORDS: Chondrosarcoma, dog, epiglottis, larynx

INTRODUCTION

Primary neoplasms of the larynx are rare in the dog and cat [1-5]. In dogs, the reported laryngeal neoplasms include papilloma, squamous cell carcinoma, rhabdomyoma, rhabdomyosarcoma, oncocytoma, chondroma, chondrosarcoma, lipoma, leiomyoma, and leiomyosarcoma [1-5].

Chondrosarcoma is a malignant tumor of cartilage; it may be primary, originating inside a bone (central) or from the periosteum (peripheral), or secondary, initiating through malignant change in osteochondromas [6,7]. Microscopically, the malignant cells produce characteristic chondroid and fibrous matrix. Macroscopically, the chondrosarcoma develops on the bone surface, as large masses ranging in size from 2 to 20 cm in diameter with lobulated surfaces, firm or hard consistency, and relatively well bordered. Chondrosarcoma in dogs has a significantly higher incidence compared with osteosarcoma, where it frequently is located in flat bones [6,7]. The incidence of chondrosarcoma has been often reported in adult animals. The tumor has been particularly studied in dogs, where it account for about 10% of all bone sarcomas, pre-dominantly affecting large breed dogs especially the Boxer and German Shepherd breeds, with no sex preference. Chondrosarcomas have less tendency to metastasize compared with osteosarcomas. Metastasis of chondrosarcomas occurs via the hematogenous route mainly to the lung. In general, this occurs later in the course of the tumor development, but only in approximately 10% of the cases [6,7].

The only previous reported cases of chondrosarcoma of the larynx in veterinary literatures included a 10-year-old male Boxer and an 8-year-old male Doberman pinscher where the tumors were attached to the left arytenoid cartilage, but were not evaluated immunohistochemically [2,3]. There were no reports of chondrosarcoma of the epiglottis within veterinary literature. In this report, we present a low grade chondrosarcoma arising from the epiglottis of a dog, which was treated with surgical excision. In addition, gross, histologic, immunohistochemical, and histochemical findings of this tumor are discussed.

CASE REPORT

A 6-year-old, neutered, male Boxer cross was presented with a primary complaint of gagging when eating for a time period of approximately 1 month. The owner also reported a change in bark, changing from a sharp tone to dull. Based on the clinical examination, there was upper respiratory tract noise, and an ovoid lump involving the epiglottis was observed [Figure 1a]. No abnormalities were detected on radiographic examinations (X-ray) of the chest or abdomen. No significant abnormalities were seen in any blood samples from this animal as hematology, serum chemistry, and liver function tests were within the normal limits.

The animal was sedated with acepromazine maleate (0.03 mg/kg; autologous conditioned plasma injection; novartis animal health) and buprenorphine (0.03 ml/kg; vetergesic 0.3 mg/ml;

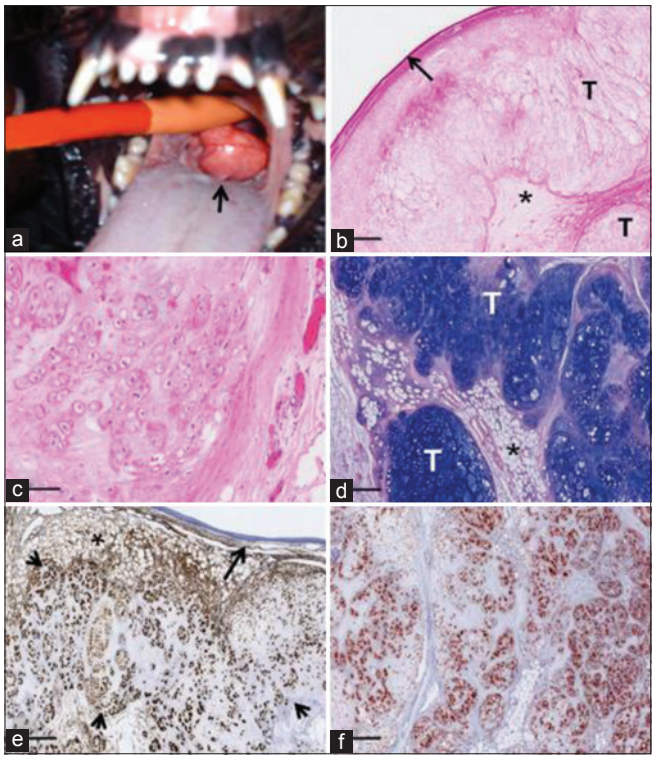

Figure 1: (a) Oral cavity; dog. An ovoid, epiglottic mass lodged against posterior wall of pharynx. (b) Epiglottal mass; dog. An expansile tumor (T) arising subjacent to the mucosa (arrow) which compresses and focally infiltrates the adjacent adipose tissue (asterisk). hematoxylin and eosin (H and E) stain. Bar = 250 µm. (c) Epiglottal mass; dog. A higher magnification of Figure 1b shows well-differentiated chondroid area where neoplastic cells are located within lacunae surrounded by elastic cartilage matrix. HE stain. Bar = 50 µm. (d) Epiglottal mass; dog. Immunohistochemistry for vimentin. The neoplastic cells within chondroid lobules in the mass (arrow heads) and bordering adipose tissue (asterisks) are strongly immunolabeled. The overlying mucosa (arrow) show negative staining for vimentin. Bar = 250 µm. (e) Epiglottal mass; dog. Immunohistochemistry for S-100. The neoplastic cells within chondroid lobules in the mass are strongly immunolabeled. Bar = 250 µm. (f) Epiglottal mass; dog. Cartilage matrix of the chondroid lobules (T) stained deeply with Alcian blue-PAS with negative staining of the adjacent adipose tissue (asterisk). Bar = 150 µm

alstoe limited.) and induced with propofol (0.4 ml/kg; propoflo; Abbott Animal Health), and was maintained on isoflurane anesthesia (Isoflo; Abbott Animal Health). Simple interrupted stay sutures of 2-0 polydioxanone suture were placed through the epiglottis.

The mass appeared attached caudally to the epiglottis by a pedicle and was ligated with polyglactin 910 (vicryl; ethicon). The epiglottis was sectioned caudal to the mass and the mass was totally excised. The bleeding was very minimal and was stopped with gentle pressure. The stay sutures were then removed. The animal also was given METACAM solution for subcutaneous injection, betamox LA 150 mg/ml suspension for injection (amoxicillin trihydrate 172.1 mg/ml) and ketamine during recovery. Routine post-operative treatment consisted of metacam oral suspension and nisamox tablets 250 mg (1 tablet twice daily) for 2 weeks. Adjuvant chemotherapy was not recommended for this animal. Post-operative checks were routinely performed at 1 day post-surgery then 1 week, 1 month, 3 months, 6 months and a year post-surgery then in general at 6 month intervals after this.

Grossly, the excised mass measured 3.2 cm × 2.8 cm × 2.7 cm, was firm to hard, and had a lobular pattern of cartilage with its translucent grayish-white color on the cut surface.

The removed epiglottal mass was fixed in 10% buffered formalin, embedded in paraffin wax, and sectioned at the thickness of 4 μm. To better characterize the neoplasm, deparaffinized sections were stained histochemically with hematoxylin and eosin, Alcian Blue (pH 2.5)-periodic acid Schiff (PAS), and van Gieson's stain. In addition, immunohistochemistry for vimentin and S-100 protein, and pan-cytokeratin were performed.

The detection of different antigens to further differentiate the immunophenotype of the tumor was performed using avidin-biotin-perioxidase complex technique using three antibodies including: A monoclonal mouse antibody against vimentin (vimentin [Code N1521], Dako Corporation, Carpinteria, CA, USA) (1:200, DAKO), S-100 protein (S-100 [Code IR504/IS504], Dako Corporation, Carpinteria, CA, USA), and polyclonal pancytokeratin (Pancytokeratin [Code AM357–5M], BioGenex, San Ramon, CA, USA) (1:100, DAKO) [8-10]. For immunohistochemical studies, additional sections from the tumor were deparaffinized using a graduated xylene and alcohol solutions, then rehydrated in distilled water using phosphate buffered saline (PBS), pH 7.4. Sections were incubated in a 3% hydrogen peroxide solution. Antigen retrieval was done using Citra solution (Antigen Retrieval Solution, BioGenex, San Ramon, CA, USA) (BioGenex), and heating in a microwave oven for 10 min on a power setting of 600 watts. Slides were allowed to cool for 20 min and rinsed in several changes of distilled water. Slides were incubated for 2 h with one of the three primary antibodies, and then washed in PBS for 5 min, and then incubated for 20 min with the secondary antibody. The slides were rinsed with buffer, incubated with the label (pre-diluted horseradish peroxidase-labeled streptavidin in PBS with a carrier protein and 0.01% thimerosal) for 20 min at room temperature, rinsed well with buffer, and 3,3'-diaminobenzidine-tetra-hydrochloride (DAB) (DAB, Biogenex, San Ramon, CA, USA) (BioGenex) was applied to the sections for 5 min at room temperature. The colour change was monitored on positive control slides and stopped by immersing all slides in deionized water. Contrast staining was performed using Mayer's hematoxylin. Slides were dehydrated through ascending concentrations of alcohol solution and xylene, and were then cover slipped. Positive control tissue and negative reagent control were run simultaneously for verification of immunohistochemical staining results.

HISTOPATHOLOGICAL FINDINGS

Microscopically, the epiglottal mass was rimmed by an outer stratified squamous non-keratinized epithelium, and the subjacent submucosa was markedly expanded by a non-

encapsulated fairly demarcated multi-lobular neoplasm [Figure 1b]. The neoplasm was composed of streams of cells admixed with moderate to abundant amounts of a pale amphophilic to eosinophilic matrix (chondroid). The neoplastic cells were spindle, stellate or polygonal in shape with indistinct cellular borders and little to moderate eosinophilic lacy to themulti-loculated cytoplasm [Figure 1c]. Nuclei were ovoid with finely stippled chromatin and one small magenta nucleolus. Anisocytosis and anisokaryosis were moderate to mark with some karyomegaly and binucleated and multinucleated cells. Mitotic figures were uncommon with only one present in 10 random high power fields (×40). Scattered areas of edema, hemorrhage, and fat necrosis were present in the adjacent epiglottal tissue.

A diagnosis of low grade (Grade I) chondrosarcoma was made based on the light microscopic examination. This tumor appeared to be completely excised, but with thin excisional margins (<3.0 mm in thickness in some areas). In addition, it apparently had involved the lingual surface and the apical portion of the laryngeal surface, as indicated by the presence stratified squamous non-keratinized epithelium with no evidence of respiratory epithelium in any of the sections examined. Immunohistochemical examination of the tumor, to further elucidate histogenesis, demonstrated strong cytoplasmic immunoreactivity to vimentin [Figure 1d], confirming their mesenchymal origin. The neoplastic cells showed moderate to marked positive cytoplasmic staining to S-100 [Figure 1e]. Conversely, no neoplastic cells showed immunoreactivity for pan-cytokeratin (for epithelial origin). For all antibodies, significant immunoreactivity was present in positive control sections, but absent in the negative controls, confirming validity of the staining technique.

Moreover, the intercellular matrix was histochemically stained dark blue with Alcian blue-PAS [Figure 1f], which often isolates cells into individual lacunae. van Gieson staining showed that the mass contained elastic fibers consistent with the presence of elastic cartilaginous tissue. Based on the histologic findings (cartilaginous differentiation of neoplastic cells and matrix), and histochemical and immunohistochemical examination, a low-grade epiglottal chondrosarcoma was definitely diagnosed.

Following the surgery, routine follow-up indicated that the animal appeared normal. There was no swelling or discomfort at the surgery site, nor any evidence of recurrence. Approximately, 2 years following the tumor's resection, radiographic examination revealed an abdominal mass present in the liver; however, the owner declined further treatment, and the animal was euthanized.

DISCUSSION

Laryngeal tumors are extremely rare in small animal veterinary medicine; they include epithelial and mesenchymal types [1-5]. Previous studies have reported that squamous cell carcinoma and rhabdomyoma are the most commonly encountered types of canine laryngeal neoplasia [1,4]. Although two cases of laryngeal chondrosarcomas have been reported from a 10-year-old Boxer and an 8-year-old Doberman Pinscher, in these tumor's they were attached to the left arytenoids. Therefore, no report of epiglottis chondrosarcoma has been reported in the veterinary literature [2,3].

Histologically, the larynx cartilages include thyroid cartilage, cricoid cartilage, arytenoid cartilages, corniculate cartilages and cuneiform cartilages; all are composed of hyaline cartilage, which is the most abundant type of cartilage in the body. The epiglottis is composed of elastic cartilage. All cartilage is composed of chondrocytes located in lacunae and avascular extracellular matrix. The chondrocytes often exist in small aggregates (isogenous clusters). Elastic cartilage also has elastic fibres in the matrix [11].

In human literature, cartilaginous lesions of the larynx are classified as either neoplastic or metaplastic in origin, chondroma and chondrosarcoma being neoplastic lesions and metaplastic cartilaginous nodules (chondrometaplasia) being metaplastic in origin [12-15]. It is important to differentiate low-grade chondrosarcomas from chondromas. The symptoms are the same but chondromas are more likely to be asymptomatic. The site of origin is similar, but chondromas are almost always <2 cm in size, whereas chondrosarcomas are larger than 3 cm [12-14]. Microscopically, chondromas appear as normal cartilage with defined hypocellular lobules with no nuclear atypia and mitosis [13]. Chondrometaplasia is a common lesion composed of well-defined nodules with metaplastic activity of chondrocytes, usually with no lobular pattern of hyaline cartilage or nuclear abnormalities. They must be differentiated from neoplastic cartilaginous lesions. The nodules are <1 cm usually on ventricular bands and vocal cords; and may be multifocal [12-14].

Chondrosarcoma is a malignant tumor involving the cells that produce a cartilage matrix [16,17]. Primary chondrosarcomas are uncommon in both dogs and humans, but have been occasionally reported [14-17]. Most chondrosarcomas originate from the skeletal cartilage, whereas some occur in extra-skeletal tissues that have pre-existing cartilage tissues [18-20]. Alternately, they rarely occur in the soft tissue or parenchymal organs, where no cartilage originally existed (extra-skeletal chondrosarcomas) [6,17-20]. The age of dogs affected by chondrosarcoma ranges from 1 to 12 years, and medium-to-large breed dogs are most commonly affected [6,7,16,17]. In a previous study, Boxers accounted for 25% of all cases of chondrosarcoma; a high incidence was also found for German shepherds and mixed-breed dogs [6,7,17]. In dogs, the most frequent sites of occurrence are the pelvis, nasal cavity, sternum and ribs and less commonly in long bones [6,7,16]. Chondrosarcomas are less likely to metastasize and do so later in the course of disease than osteosarcomas [16].

Laryngeal chondrosarcomas are an extremely uncommon malignancy in humans, of which the epiglottis is the least frequent location with only five cases reported in the literature [2-4,12-15]. In this report, the existing epiglottal mass in this dog was a low-grade chondrosarcoma, which was microscopically characterized by well-developed chondroid

lobules, which was primarily originated from the elastic cartilage of the epiglottis. Histochemical staining and Immunohistochemical positivity for vimentin and S-100 protein, a standard marker for chondrogenic neoplasms [8-10], together with negativity for pan-cytokeratin confirmed the mesenchymal and chondroblastic origin. Liposarcoma could argued as a low but potential differential diagnosis for this tumor, since both chondrosarcoma and liposarcoma are positive for S-100 protein and vimentin [8,12], however, neoplastic chondrocytes in this case did not reveal clear lipid droplets in their cytoplasm.

Inadequate numbers of reports of cartilaginous tumors of the epiglottis in veterinary medical literature, and lack of archived case records and necropsy findings, impose some diagnostic and therapeutic challenges. There was no evidence of local or regional recurrence in this animal; however, an abdominal mass was observed by x-ray examination after over than 2 years of the surgical excision of this epiglottal mass. As necropsy was not allowed, we remain uncertain about a histologic diagnosis of this abdominal mass.

CONCLUSION

We conclude that the epiglottal mass in this dog represented a low-grade chondrosarcoma, a rare neoplasm reported in dogs and humans, which was primarily originated from the epiglottis and was treated surgically. Based on the above case report, the long-term prognosis of future cases of epiglottal chondrosarcomas should be considered guarded until more information has been gathered.

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