

# Zygomatic gland adenoma in a dog: histochemical and immunohistochemical evaluation

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## Abstract

Orbital epithelial tumors in dogs are rare and most frequently malignant. Distinguishing their origin from the lacrimal or zygomatic gland is often challenging and is based mostly on tumor location. A case of adenoma involving the orbit in a 13-year-old, female, standard Schnauzer is reported. Histologically, the neoplasm was characterized by nests and cords of epithelial cells mostly forming small glandular structures. The origin of the tumor from the zygomatic gland was determined by histochemical characteristics (alcian blue pH 1 positive staining) of a small remnant of normal gland included within the tumor capsule. The benign nature of our finding was confirmed by follow-up information: 2 years after complete surgical removal of the mass no tumor recurrence or metastases was recorded.

**Key Words:** adenoma, dog, orbit, zygomatic gland

## INTRODUCTION

Epithelial tumors involving the orbit are rare in dogs. Usually these tumors have a distinct locally invasive behavior and high recurrence rate after resection.<sup>1</sup> Primary orbital epithelial tumors originate from lacrimal glands (orbital and nictitans). Secondary extension of zygomatic salivary gland tumors in the orbit has been reported in dogs.<sup>2–5</sup> Although normally located extraorbitally, the canine zygomatic gland is separated from the orbital cavity only by a thin connective tissue lamina (orbital floor).<sup>6</sup> Most of the lacrimal and zygomatic neoplasms reported in the literature are malignant,<sup>1</sup> although a neoplastic syndrome called 'lobular orbital adenoma' has recently been described.<sup>7</sup> This entity presents clinically and biologically as a benign neoplasm, although it is prone to local recurrence. Its origin from the lacrimal or zygomatic gland has not been determined.

## CASE REPORT

A 13-year-old, female standard Schnauzer presented with a progressive bulging of the ventral part of the left orbit. The left eye was exophthalmic and displaced dorso-medially but still visual. Retropulsion of the globe was impossible and not painful. A serous ocular discharge and protrusion of the third eyelid were noted. The posterior segment of the eye was normal by ophthalmoscopy.

Radiographic examination of the skull revealed the presence of a mass located in the ventro-medial portion of

the orbit, with moderate osteolysis of the zygomatic arch. Multiple biopsies of the mass were obtained and submitted for histology.

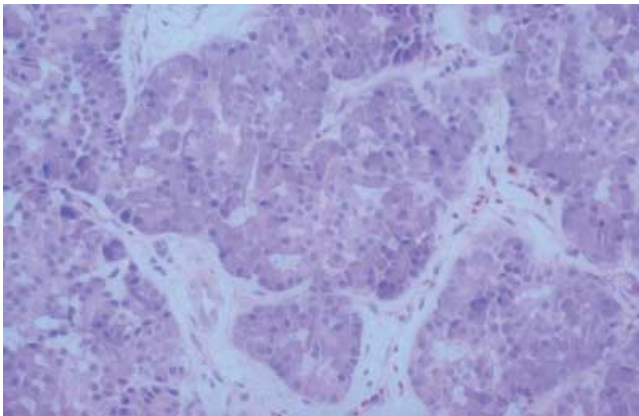
The biopsies were composed of well differentiated epithelial cells organized in adenomers and a provisional diagnosis of adenoma was made. Due to the small size of the biopsies, origin from either the lacrimal or the zygomatic gland was not determined at this stage.

The owner refused further diagnostic imaging investigations and a complete surgical excision was performed by lateral orbitotomy, in order to preserve the eyeball. The mass was easily isolated by blunt dissection from the orbital contents, the third eyelid did not appear to be involved, and a neoplasm of the zygomatic gland was suspected.

A 5 × 2 × 3-cm, grayish, well-circumscribed mass was submitted for histology. A cut section of the mass revealed a large central area of coagulative necrosis, with an internal cavity containing a small amount of sero-hemorrhagic fluid. The neoplasia was cut through transversal, sagittal and equatorial planes, fixed in 10% neutral buffered formalin and routinely processed for histology. Five-micrometer sections were obtained and stained with hematoxylin and eosin (HE), alcian blue pH 2.5, alcian blue pH 1 and periodic acid Schiff (PAS). Immunohistochemistry using the avidin-biotin-peroxidase complex (ABC) procedure was performed. Specific antisera used and antigen retrieval methods are listed in Table 1. The same staining procedures were applied to sections obtained from normal lacrimal and zygomatic glands from a control dog.

**Table 1.** Antibodies applied, source, dilution and antigen retrieval technique

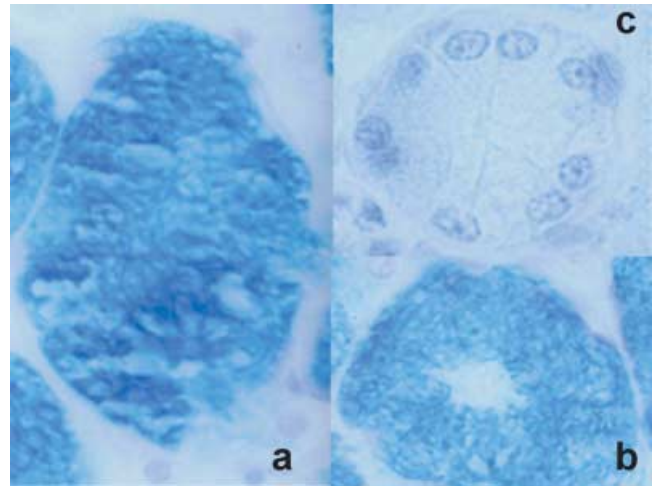
Antibody	Source	Clone	Isotype	Dilution	Ag retrieval
Pan-cytokeratin (CK AE1/AE3)	Zymed	AE1/AE3	IgG1	1 : 2000	Pepsin, 10 min, 37 °C
Cytokeratin 8/18 (CK 8/18)	Zymed	UCD/PR-10.11	IgG1	1 : 200	Pepsin, 10 min, 37 °C
Cytokeratin 14 (CK 14)	Neomarket	LL002	IgG3	1 : 2000	Microwave, buffer citrate
$\alpha$ smooth muscle actin ( $\alpha$ SMA)	Dako	1A4	IgG2	1 : 2000	None
Vimentin (VIM)	Dako	3B4	IgG2a	1 : 1000	None

**Figure 1.** Neoplastic cells were organized in lobules or cords embedded in fibrovascular stroma. Cells were arranged in a single row encompassing a small central lumen (acinar structure). H&E,  $\times 400$ .

Histologically the mass was surrounded by a fibrous capsule, 0.5–2 mm thick, occasionally projecting short branches within the neoplastic mass. A large central area of coagulative necrosis and hemorrhage, containing hemosiderin-laden macrophages, was present. Neoplastic cells were organized in lobules or cords embedded in a thin fibrovascular stroma. Cells were arranged in a single row encompassing a small central lumen (acinar structure) (Fig. 1). Cells were polygonal with well-defined borders, abundant, finely granular, lightly, or more rarely, intensely eosinophilic cytoplasm and basal or central, round to oval, small nucleus with finely dispersed chromatin and 1 or 2 small nucleoli. Mitotic figures were rare.

In one section of the mass a small peripheral lobule (2 mm), composed of acinar structures lined by a single row of large prismatic cells with abundant foamy, pale cytoplasm and small basal nuclei (mucus-secreting cells), was observed. Additionally, in the lobule, a small cluster of cells with a finely granular, intensely eosinophilic cytoplasm and small round nuclei (serous cells – serous demilunes) was recognizable in each acinus. A single layer of spindle-shaped cells with a scant cytoplasm and slender elongated dark nuclei encompassed each acinar structure (myoepithelial cells). This small lobule was included within the capsule of the neoplasm and was interpreted as the residual normal gland.

A few ductular structures lining a large empty lumen and composed of a single layer of regular cubical cells with eosinophilic, homogeneous cytoplasm and basal nucleus

**Figure 2.** Alcian blue (pH 1) staining: differentiated lobule within the neoplastic mass (a) and control zygomatic gland (b) stained intensely positive. Conversely, control lacrimal gland (c) stained negative.

were occasionally detectable; these were interpreted as normal ducts entrapped by neoplastic growth.

#### Immunohistochemical results

Neoplastic epithelial cells stained diffusely for cytokeratin AE1/AE3 (CK AE1/AE3) and had a diffuse cytoplasmic pattern; staining for Vimentin, alpha Smooth muscle actin ( $\alpha$ SMA) and cytokeratin 14 (CK 14) was negative. Approximately 60% of neoplastic cells stained intensely for cytokeratin 8/18 (CK 8/18).

In the residual, normal, lobule mucus-secreting cells stained CK 8/18 negative and CK AE1/AE3 positive (apical cytoplasmic border positivity). Serous cells stained intensely for both CK AE1/AE3 and CK 8/18 (diffuse cytoplasmic pattern). Both acinar cell types stained Vimentin,  $\alpha$ SMA and CK 14 negative, while spindle-shaped basal cells around acini stained only CK 14 and  $\alpha$ SMA positive (myoepithelial cells).

No distinctive immunohistochemical result was obtained for lacrimal and zygomatic glands: in both glands serous cells stained diffusely CK AE1/AE3 and CK 8/18 positive, mucus-secreting cells were CK 8/18 negative and CK AE1/AE3 positive (positivity confined to cytoplasmic borders), and myoepithelial cells were only CK 14 and  $\alpha$ SMA positive.

#### Histochemical results

Histochemical results obtained for neoplastic tissue and normal lacrimal and zygomatic glands are compared in Table 2.

**Table 2.** Histochemical results

	Neoplasm			Normal zygomatic gland		Normal lacrimal gland	
	Residual normal gland cells		Neoplastic cells	Mucous cells	Serous demilunes	Mucous cells	Serous cells
	Mucous	Serous					
PAS	+	+	+	+	+	+	+
Alcian blue pH 2.5	+	-	-	+	-	+	-
Alcian blue pH 1	+	-	-	+	-	-	-

Approximately 80% of the neoplastic cells were PAS positive and alcian blue (both pH 2.5 and pH 1) negative; in the residual, normal gland mucous cells stained faintly PAS and intensely alcian blue (both pH 2.5 and pH 1) positive, serous cells were only PAS positive. Ductular cells were both alcian blue and PAS negative.

In the normal zygomatic glands, mucus-secreting cells stained PAS and alcian blue positive at both pHs tested (sulfated acid glycoprotein secretion), serous demilunes stained only PAS positive (neutral glycoproteins). Normal lacrimal acinar cells were PAS and alcian blue pH 2.5 positive but pH 1 negative (sialylated acid glycoprotein) (Fig. 2). Based on histochemical results the diagnosis of zygomatic gland adenoma was made.

Follow-up information confirmed the benign nature of the neoplasm: after 2 years the dog was healthy, the eye was still visual, and the limited osteolytic change in the zygomatic bone had decreased.

## DISCUSSION

The canine zygomatic gland is normally located outside the orbital cavity, ventral to the zygomatic arch of the temporal bone, with its base lying against the ventral part of the orbit.<sup>6</sup> It is a tubulo-acinar salivary gland with mixed sero-mucus secretion, and its mucous is mainly composed of sulfated acid glycoprotein (PAS+, alcian blue pH 2.5 and alcian blue pH 1+) as opposed to the predominant sialylated secretion (PAS+, alcian blue pH 2.5+ but alcian blue pH 1-) of lacrimal glands.<sup>8,9</sup>

In our sample mucous cells were PAS as well as alcian blue positive. PAS staining was poorly specific, identifying all glycoproteins (neutral in serous cells and acid in mucous cells). An alcian blue pH 2.5 positive reaction, being specific for acid glycoproteins, was restricted to mucous cells but only alcian blue pH 1 staining was conclusive, allowing the distinction between the two major groups of acid glycoproteins (sialylated and sulfated) secreted by mucous cells. According to the literature<sup>8,9</sup> and as confirmed by our normal controls, histochemical features of the residual normal gland, included in the neoplasm, were consistent with zygomatic origin.

Immunohistochemical examination of the tumor did not allow a further characterization of neoplastic cells: diffuse cytokeratin positivity and vimentin negativity confirmed

neoplastic cell epithelial nature, while the lack of  $\alpha$ SMA and CK14 positive cells excluded the neoplastic activation of myoepithelial cells. It has previously been reported<sup>10</sup> that mixed type salivary tumors are rare in dogs while they have a high incidence in humans. Staining for cytokeratin in neoplastic and normal serous cells was more intense than in mucous cells, probably because cytoskeletal structures are not obscured by large secretory granules in serous and neoplastic cells as occurs in mucous cells.

The literature reports of zygomatic gland tumors in dogs describe adenocarcinoma as the most common neoplasm,<sup>1,2,4,5</sup> while zygomatic adenoma has only occasionally been reported.<sup>3</sup> Recently, a new syndrome called 'canine lobular orbital adenoma' has been described in 15 dogs.<sup>7</sup> In this syndrome the orbit is invaded by an epithelial neoplasm with histologic features of well differentiated acinar tissue, lacking ductal structures, consistent with both lacrimal and zygomatic gland origin. Lobular orbital adenoma is considered a benign tumor, though very likely to recur if an accurate and complete excision is not performed.

In our case, on histologic examination the neoplasm was composed of well differentiated acinar structures, consistent with a diagnosis of adenoma. The zygomatic origin was histochemically demonstrated by the presence of a small lobule of acinar cells that maintained their original secretory features.

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