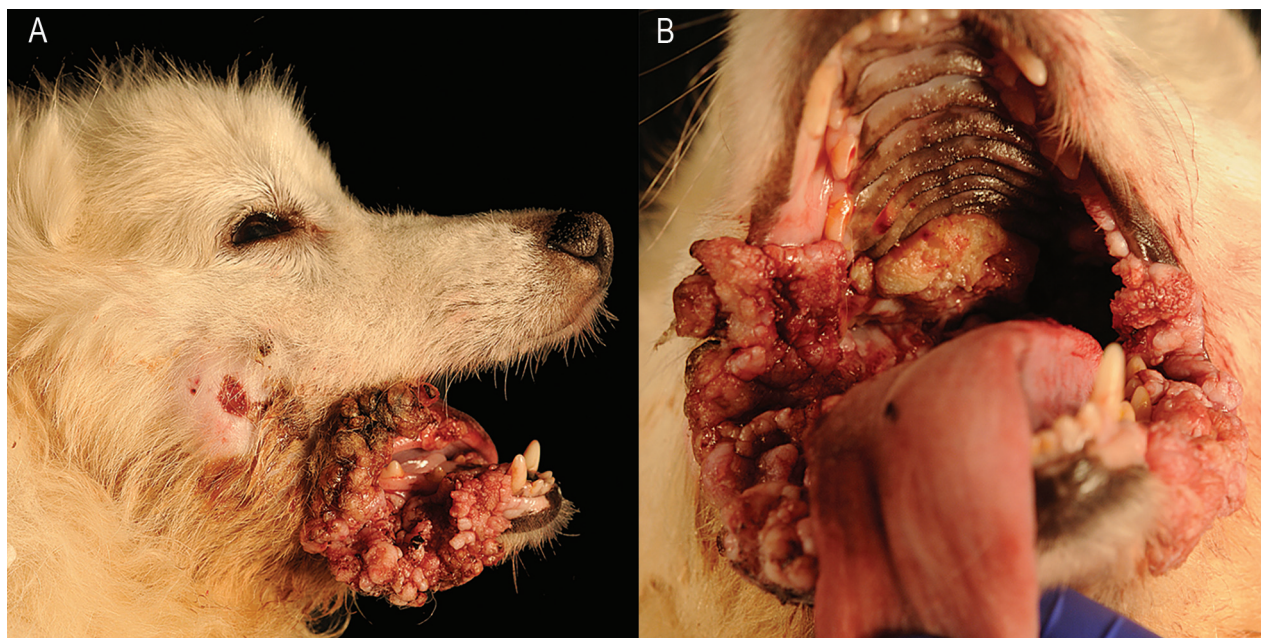# Pathology in Practice

In collaboration with the American College of Veterinary Pathologists

Figure 1—Photographs of the head (A) and oral cavity (B) of an 8-year-old American Eskimo Dog that was presented for evaluation of multiple oral and mucocutaneous junction masses, a ruptured facial abscess, and lethargy. A—Caudal to the right lip commissure is a draining tract. B—Numerous multifocal to coalescing papillomatous masses ranging from 0.5 to 4.0 cm in diameter are present on the oral mucosa and along the mucocutaneous junction of the lips, with a larger mass at the right lip commissure.

History

An 8-year-old castrated male American Eskimo dog was presented to the North Carolina State University Veterinary Teaching Hospital for evaluation of multiple oral and mucocutaneous junction masses, a ruptured facial abscess, and lethargy. The first mass had developed approximately 1 year earlier. It was papillated, approximately 1 cm in diameter, and located on the right lower lip near the lateral commissure. There were also 3 smaller (< 3 mm in diameter), gray, exophytic masses on the buccal mucosa.

The masses did not appear to cause the dog any discomfort, and the owner had elected to monitor them. Additional persistent papillated masses in the oral cavity and along the mucocutaneous junction developed over the next 5 to 7 months, and the mass on the right lower lip continued to increase in size. Two weeks prior to presentation, a mass that was draining purulent exudate was noted by the owner on the right side of the dog's face. The owner also reported that the dog had progressive hyporexia and increased lethargy and respiratory noises. The oral lesions and purulent drainage did not respond to oral treatment with amoxicillin–clavulanic acid or clindamycin.

Clinical and Clinicopathologic Findings

On physical examination, the dog was quiet but responsive. Intermittent upper respiratory sounds were prominent; however, auscultation of the lungs revealed normal bronchovesicular sounds. Attempts to open the mouth elicited signs of pain, and bony crepitus

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was palpable during movement of the right mandible and temporomandibular joint. Severe halitosis and hypersalivation were present. Numerous multifocal to coalescing papillomatous masses ranging from 0.5 to 4.0 cm in diameter were present on the oral mucosa and along the mucocutaneous junction of the lips **(Figure 1)**. In addition, a large (4 X 5 cm), exophytic mass was located at the right lip commissure. Soft tissue swelling overlying the masseter muscle and a draining tract 2 cm caudal to this mass were also visible.

A CBC revealed mild leukocytosis (12,090 WBCs/µL; reference range, 4,360 to 11,900 WBCs/µL) with moderate monocytosis (2,297 monocytes/µL; reference range, 75 to 860 monocytes/µL) and mild, regenerative, normocytic, normochromic anemia (Hct, 37.9% [reference range, 40.2% to 61.2%]; reticulocytes, 140,000 reticulocytes/µL [reference range, 8,040 to 93,730 reticulocytes/µL]). Serum biochemical testing revealed moderate hypoalbuminemia (2.4 g/dL; reference range, 3.2 to 4.3 g/dL), with serum globulin concentration at the high end of the reference range (3.5 g/dL; reference range, 1.8 to 3.5 g/dL). CT of the head and neck revealed a mandibular mass with associated extensive bone lysis. Owing to the severity of the invasive and destructive mass, the extent of the patient's pain and discomfort, the absence of acceptable medical or surgical treatments, and the poor prognosis, the owner elected euthanasia.

Formulate differential diagnoses, then continue reading.

Gross Postmortem Findings

On postmortem examination, papillomatous nodules and masses were present on the oral mucosa, lips, middle portion of the tongue, palatoglossal arches, hard and soft palates, oropharynx, and pharynx **(Figure 2)**. Growths located on the right laryngeal saccule and ventral aspect of the epiglottis partially occluded the larynx. Most nodules were superficial, exophytic, tan-white, and small, but some nodules had coalesced into large, ulcerated masses. A large (4.5 X 5.0 X 2.0-cm), ulcerated, deeply invasive mass was found to extend from the buccal mucosa at the level of the right mandibular and maxillary second premolar teeth caudally to the right labial commissure. This mass nearly completely effaced the caudal portion of the right mandibular body and ramus and most of the right masseter muscle. A draining tract over the right masseter muscle had a circular opening (0.5 cm in diameter) and communicated with a focus of necrosis and abscess formation in the mass.

Histopathologic Findings

Most of the masses were histologically consistent with viral papillomas and were composed of papillary projections of hyperplastic mucosa supported by a fibrovascular stroma. The diagnosis of papillomas was based on the combination of papillomatous

epithelial hyperplasia, hypergranulosis, numerous koilocytes, and magenta intranuclear inclusion bodies in keratinocytes in the stratum granulosum (Figure 2). Koilocytes in the stratum spinosum and stratum granulosum were recognized as individualized, round, large keratinocytes that had a clear to amphophilic cytoplasm and a large nucleus. This combination of features suggested the masses were viral papillomas, rather than nonviral squamous papillomas or papillary squamous cell carcinomas. Epithelial dysplasia was present and consisted of hyperchromasia, nuclear crowding, mild anisocytosis, mild anisokaryosis, and disordered epithelial stratification. Less specific features were hyperkeratosis and lymphoplasmacytic and neutrophilic inflammation in the supportive fibrovascular stroma.

The large invasive mass in the right mandible consisted of a neoplasm composed of solid islands and cords that recapitulated features of stratified squamous epithelium (Figure 2). Neoplastic epithelial cells were smaller, more basophilic, and cuboidal at the periphery of islands and were larger, polygonal, and eosinophilic (keratinization) in central areas, sometimes with circular foci of brightly eosinophilic lamellated keratin, called keratin pearls. Intercellular spinous projections were present, similar to the projections on stratum spinosum cells of the mucosal epithelium. Cellular pleomorphism was limited; anisocytosis and anisokaryosis were both mild to moderate. Large, round to irregular nuclei had finely stippled chromatin and 0 to 2 distinct large, basophilic nucleoli. Thirteen mitotic figures were present in 10 hpf (2.37 mm^2). Some areas of the neoplasm shared histologic differentiation features with the viral papillomas. Specifically, the neoplasm retained epithelial stratification similar to that seen in the papillomas as well as keratohyalin granules and large, round clear cells that resembled koilocytes in the papillomas. Large areas of mandibular bone invasion by the neoplasm were associated with extensive bony lysis, necrotic bone fragments, and production of reactive woven bone. A deep oral ulcer extended to an area of central tumor necrosis, with bacterial colonization and abscess formation that communicated with the cutaneous draining tract.

Additional Clinicopathologic Findings

Prior to presentation, use of a PCR assay to test a fresh-frozen sample of the masses revealed canine papillomavirus 1 (CPV1), and the tissue was used to produce an autologous vaccine. Postmortem, CPV1-specific in situ hybridization of an exophytic oral papilloma and the invasive neoplastic mass confirmed strong hybridization signals in epithelial cells of the papilloma and the neoplasm (Figure 2). The in situ hybridization chromogen signal was cytoplasmic and nuclear in epithelial cells of both the exophytic
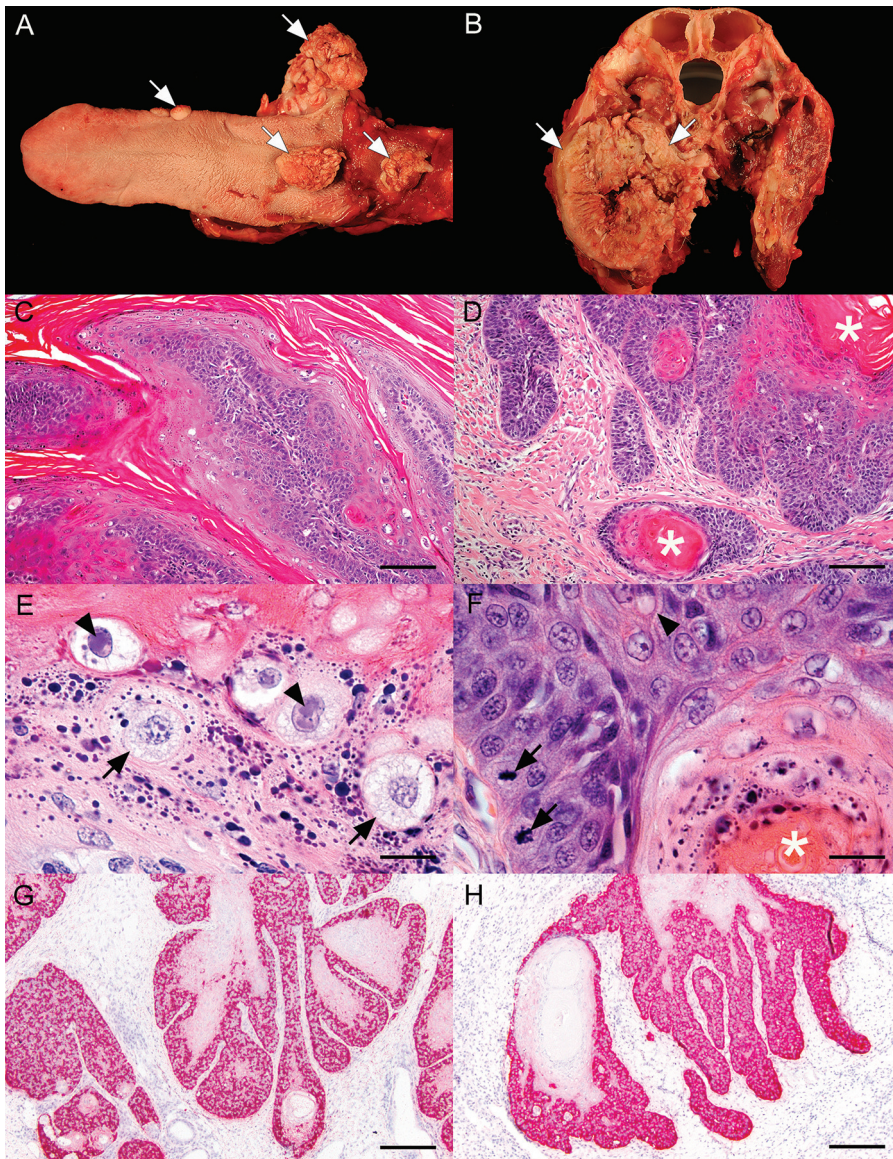
Figure 2—Postmortem photographs of the tongue (A) and a cross-section of the head (B), along with photomicrographs of sections of the masses (C–H). A—Widespread papillomatosis (arrows) of the caudal aspect of the tongue, the right laryngeal saccule, and the ventral aspect of the epiglottis partially occludes the larynx. B—An invasive, large mass (arrows) nearly completely effaces the caudal portion of the right mandibular body and ramus and infiltrates most of the right masseter muscle. C—Photomicrograph of an oral papilloma reveals papillary exophytic projections of hyperplastic epithelium with hyperkeratosis. H&E stain; bar = 100 μm. D—Photomicrograph of a section of the large invasive mass reveals a well-differentiated squamous cell carcinoma with keratin pearl formation (asterisks). H&E stain; bar = 100 μm. E—Higher-magnification image of a papilloma, taken at the stratum granulosum–stratum corneum interface, illustrates typical viral papilloma features of koilocytes without inclusion bodies (arrows) and koilocytes with magenta intranuclear inclusion bodies (arrowheads). Numerous, large, blue-black keratohyalin granules are visible (hypergranulosis). H&E stain; bar = 25 μm. F—Higher-magnification image of a section of the squamous cell carcinoma reveals mitotic figures (arrows), intercellular spinous projections (arrowheads), and a portion of a keratin pearl (asterisk). Blue-black keratohyalin granules are present at the margin of the keratin pearl. H&E stain; bar = 25 μm. G—Photomicrograph of a papilloma after in situ hybridization for canine papillomavirus 1 shows strong hybridization signals in the cytoplasm and nucleus of epithelial cells. Fast red chromogen stain; bar = 100 μm. H—Photomicrograph of a section of the squamous cell carcinoma after in situ hybridization for canine papillomavirus 1 shows strong hybridization signals in the cytoplasm and nucleus of epithelial cells. The morphology at the base of the papilloma resembles the morphology of the squamous cell carcinoma, suggesting an association between these lesions. Fast red chromogen stain; bar = 100 μm.

papilloma and the squamous cell carcinoma and confirmed the presence of CPV1 gene expression in both lesions.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: multifocal to coalescing epithelial papillomas of the oral cavity with koilocytosis and intranuclear inclusion bodies and an invasive squamous cell carcinoma associated with a pathologic mandibular fracture, intratumoral abscess, and cutaneous draining tract.

Case summary: canine papillomavirus 1–associated oral papillomatosis with focal transformation to invasive squamous cell carcinoma in an adult dog without overt immunosuppression.

Comments

This patient presented with apparent extensive oral papillomatosis; however, additional atypical clinical signs of lethargy, pain, bone crepitus, and a draining tract led to the presumed diagnosis of CPV1–induced squamous cell carcinoma with secondary abscess formation. The squamous cell carcinoma led to mandibular bone lysis and a pathologic fracture; a secondary oral ulcer presumably led to deep bacterial infection, abscess formation within the squamous cell carcinoma, and a cutaneous draining tract.

Additional differential diagnoses considered at the time of presentation for the draining tract and signs of pain when opening the mouth were tooth root abscess, osteomyelitis, osteosarcoma, and middle ear disease (eg, cholesteatoma). However, except for osteomyelitis, the underlying cause for any of these other conditions would have been unrelated to the chronic papillomatosis. Diagnostic imaging demonstrated the extent of the papillomas and the local invasiveness of the larger mass and helped to guide medical decision-making.

Oral papillomas have a typical appearance of exophytic masses, can be single or multiple, and are common in young dogs as a result of papillomavirus infection. Currently, 23 canine papillomaviruses have been reported.[1,2] Most oral and mucosal papillomas in dogs are caused by CPV1, previously referred to as canine oral papillomavirus. Additionally, CPV1 can cause inverted papillomas and cutaneous horns on haired and nonhaired skin.[3,4] In most dogs, oral papillomas spontaneously regress in 4 to 8 weeks and only rarely result in dysphagia or respiratory obstruction.[1] Rarely, cutaneous and mucosal CPV1-associated papillomas do not regress and instead become more numerous, extending to the larynx and esophagus. This specific clinical presentation is named papillomatosis. Affected animals have high antiviral antibody titers, but a weak cytotoxic cellular immune response and do not successfully manage immunologic rejection of the papillomas.[5]

Nonregressing, occasionally extensive, viral papillomas in older dogs are often associated with immunosuppression, either iatrogenic (such as with cyclosporin, oclacitinib, glucocorticoid, or mycophenolate administration) or secondary to host immunodeficiencies.[4,6-12] Reported papilloma treatments are surgical excision, cryotherapy, laser ablation, oral azithromycin, interferon (intralesional or systemic), topical imiquimod (nonmucosal lesions), and vaccines (autologous or recombinant).[13-17] However, the efficacy of these interventions is difficult to establish because viral papillomas can regress spontaneously. Retrospective investigation of the case reported here revealed that prior to the dog's presentation to our institution, multiple oral and mucosal masses had been removed by the referring veterinarian and sent for virus identification and autologous vaccine production. CPV1 was identified with a PCR assay (detailed method unknown), but no histopathologic examination was performed at that time. The dog received 4 injections of the autologous vaccine, with no positive effect prior to the time of presentation.

Papillomaviruses carry oncogenes that can interfere with cell cycle control and induce malignant transformation in people and domestic animals via inactivation of the p53 gene and retinoblastoma protein.[18,19] CPV1 has rarely been detected in oral neoplasms, including squamous cell carcinomas, and transformation of oral papillomas into squamous cell carcinoma can occur but is considered rare.[19-22] A 3-year-old Labrador Retriever was recently reported with a CPV1-associated oral papilloma that had progressed to squamous cell carcinoma and resulted in osteolysis of the left mandible,[21] similarly to the present case. Historically, that patient reportedly had demodicosis, a possible indicator of impaired cell-mediated immunity, which if present could have contributed to uncontrolled papilloma growth and malignant transformation.[21,23]

In our case, immunosuppression was not overt. No immunosuppressive medications or other treatments (other than autologous vaccination) or comorbidities were reported by the owner or diagnosed on clinical or postmortem examination. Hematologic and histologic bone marrow evidence of immunosuppression was not found; however, these are not sensitive methods of identifying immunosuppression, which cannot be fully excluded in this case. In summary, transformation of persistent CPV1-associated oral papillomas can rarely occur and should be considered in dogs with papillomatosis and evidence of a concurrent invasive mass.

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