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Veterinary Pathology Services
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CASE I: A2010-02 (JPC 3164123).

Signalment: 7-year-old female Golden Retriever, canine (*Canis lupus familiaris*).

History: An incisional biopsy of a right mandibular mass is submitted by the referring veterinarian for histopathology.

Gross Pathologic Findings: Several fixed sections of a bony oral mass are submitted.

Histopathologic Description: Infiltrating the submucosa and extending to tissue borders is a non-encapsulated, poorly delineated epithelial neoplasia. There are coalescing islands and cords of epithelial cells supported by a variably dense collagenous stroma with few foci of osseous metaplasia. The epithelial cells have prominent intercellular junctions and often display palisading around the periphery with polarization of nuclei away from the associated basement membrane. There is moderate anisocytosis and anisokaryosis.

Contributor's Morphologic Diagnosis: Acanthomatous ameloblastoma.

Contributor's Comment: The mass is consistent with an acanthomatous ameloblastoma (acanthomatous epulis, peripheral ameloblastoma). This is a common tumor in dogs of odontogenic epithelial origin.[2] These gingival tumors arise in the oral cavity on the mandible or maxilla and seem to exhibit a predilection for the mandibular incisor-premolar region (adjacent to the canines).[7] The masses present as exophytic, verrucous lesions and histologically the coalescing cords of neoplastic odontogenic epithelium have peripheral palisading with reverse polarity of the nuclei (away from basement membrane) and prominent intercellular bridges centrally typical of stellate reticulum.[2,4,5] Clinically, these tumors are characterized by rapid growth, invasive infiltration and repeated recurrences following incomplete removal (91% recurrence with marginal excision). Bone invasion is routinely described although metastasis is not considered to be a feature of this neoplasia.[2,4,5,6,7] Complete removal, hemimandibulectomy,

and chemotherapy with bleomycin appear to be effective treatment options for acanthomatous ameloblastomas.[7]

Another common name for this tumor is acanthomatous epulis although acanthomatous ameloblastoma correctly identifies this tumor as a neoplasia of odontogenic epithelial origin. Epulis is a non-specific, clinical designation used to describe localized, exophytic, non-neoplastic and neoplastic gingival growths.[4,6,7] Epulides are generally classified as fibromatous, ossifying and giant cell types with fibromatous epulides occurring most frequently. Fibromatous, ossifying, and giant cell epulides are thought to be developmental, inflammatory and/or hyperplastic in origin and often develop in association with chronic inflammation (periodontal disease) whereas acanthomatous ameloblastomas are invasive, recurrent and generally occur in animals with milder dental plaque and inflammation. Shetland sheepdogs and mixed breed dogs appear to develop all types of epulides and acanthomatous ameloblastomas. Fibromatous, ossifying and giant cell epulides most commonly develop from the gingiva around the maxillary and mandibular premolars whereas acanthomatous epulides (71%) arise from the gingiva around the maxillary and mandibular canines. Marginal excision is generally curative for fibromatous, ossifying and giant cell epulides (90%) although acanthomatous ameloblastomas persistently exhibit invasive growth, bone infiltration and recurrence following marginal excision.[7]

JPC Diagnosis: Gingiva: Acanthomatous ameloblastoma.

Conference Comment: Ameloblastomas are tumors of odontogenic origin that arise from dental lamina rests, the developing enamel organ, the epithelial lining of an odontogenic cyst, or the basilar epithelial cells of the gingival surface epithelium. Microscopically, ameloblastomas resemble the enamel organ of a developing tooth, mimicking its inner enamel epithelium and central stellate reticulum.[1]

Conference participants discussed the variation in odontogenic tumor naming and classification schemes in veterinary and human medicine. In humans, ameloblastomas are classified into several clinicopathologic subtypes: conventional solid or multicystic, unicystic and peripheral.[1] The vast majority of human ameloblastomas are of the conventional solid or multicystic type, which appear grossly as intraosseous growths with both solid and cystic areas. Microscopically the growth pattern of these tumors are divided into the more common follicular and plexiform patterns and the less common acanthomatous, granular cell, desmoplastic, and basal cell patterns. The follicular variants appear as islands of odontogenic epithelium within a mature fibrous stroma, often with cyst formation. The plexiform variant is composed of long anastomosing cords and sheets of odontogenic epithelium, on a more loosely arranged, vascular stroma, with cysts formation much less common. Other, less common variants include the acanthomatous variant, in which squamous differentiation with keratinization or keratin pearl formation occurs within the central regions of the tumor islands, and the granular cell variant, which is composed of cells with abundant eosinophilic, granular cytoplasm within the center of the tumor islands. In the desmoplastic variant the stroma is composed of dense collagen, and the epithelial component is relatively sparse. The basal cell variant closely resembles basal cell carcinomas of the skin; they exhibit nests or islands of basaloid epithelial cells, with less evident peripheral nuclear palisading and reverse polarization and no stellate reticulum. It is possible to have multiple types

within the same tumor, and the microscopic diagnosis is made based on the dominant growth pattern. All subtypes of conventional ameloblastomas tend to be locally aggressive and tend to recur with conservative treatment. A second subtype of ameloblastoma in humans is the unicystic ameloblastoma; these tend to occur in younger patients and exhibit less aggressive biological behavior than their conventional counterparts. Microscopically these tumors appear as a single cystic sac. The third subtype, peripheral ameloblastoma, arises within soft tissue, as opposed to the other intraosseous forms. Clinically these tumors may look like a fibroma, granuloma or papilloma. Microscopically, they are similar to conventional ameloblastomas and follicular, plexiform, acanthomatous and basal cells patterns are possible. Odontogenic epithelium is often exhibited but stellate reticulum-like differentiation may not be evident. Neoplastic epithelium may be continuous with overlying surface mucosal epithelium. Peripheral ameloblastomas exhibit a less aggressive behavior and have a lower recurrence rate than their intraosseous counterparts.[1]

Tumors of odontogenic epithelium without odontogenic mesenchyme in animals are classified as peripheral or central ameloblastomas, amyloid-producing odontogenic tumors, or acanthomatous ameloblastomas.[3] Peripheral and central ameloblastomas are defined as tumors occurring from gingival soft tissue or deeper tissue within the bone of the jaw, respectively. Acanthomatous ameloblastomas are more aggressive and can be differentiated from peripheral ameloblastomas by an increased amount of stroma that resembles periodontal ligament connective tissue (characterized by abundant fibrillar collagen, regularly-positioned stellate mesenchymal cells, and regularly dispersed empty blood vessels) and a plexiform pattern consisting of interconnecting cords and sheets of epithelium. Cysts often form in acanthomatous ameloblastomas, but keratinization or keratin pearl formation is rare. Keratinization is more common in ameloblastomas, and heavily keratinized ameloblastomas may be difficult to differentiate from squamous cell carcinoma.[3]

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CASE II: T12-13569 (JPC 4019453).

Signalment: Unknown age, male neutered domestic shorthair, feline (*Felis catus*).

History: The cat had a clinical history of drooling for about two months. An oral examination was made to determine the cause and a 1 x 2 x 2 cm growth was present on the upper gingiva near the left incisor. Neoplasia or gingival hyperplasia was suspected. The mass was surgically removed and submitted to the lab in 10% buffered formalin solution for histopathological evaluation.

Gross Pathology: Grossly, the tissue contained a multilobulated mass.

Histopathologic Description: The gingival submucosa is expanded by a poorly demarcated, unencapsulated, multilobulated neoplasm. The neoplasm is composed of trabeculae, cords, islands and nests of basaloid to cuboidal/columnar and polygonal cells supported on ample fibrovascular stroma. The neoplastic cells occasionally exhibit a palisading pattern on the periphery, often surround light eosinophilic amorphous to fibrillar material and centrally located fragments of laminated keratin. The neoplastic cells have distinct cell borders, scant to ample eosinophilic cytoplasm, and round to oval nuclei with finely stippled nuclear chromatin. The nucleoli are variably distinct. Scattered mitotic cells (0-2/HPF) are observed. Multifocally, the polygonal cells exhibit squamous differentiation. Multifocal areas of osteoid formation and mineralization are evident in the mass. The light eosinophilic material stains positive for Congo red dye, remains congophilic after treatment with potassium permanganate and exhibits apple-green birefringence under polarized light.

Contributor's Morphologic Diagnosis: Amyloid-producing odontogenic tumor (APOT).

Contributor's Comment: Two broad groups of epithelial odontogenic tumors are recognized: tumors lacking inductive properties on connective tissue and those having inductive properties on connective tissue. Ameloblastoma and calcifying epithelial odontogenic tumors (amyloid-producing odontogenic tumors) are considered non-inductive. In contrast, ameloblastic fibroma, dentinoma, ameloblastic odontoma, complex odontoma, and compound odontoma have inductive influence on the oral mesenchyme.[7] Amyloid-producing odontogenic tumor (APOT) is a rare oral neoplasm reported in dogs and cats and contains variable amounts of amyloid deposition in the neoplastic mass.[2] Amyloid is comprised of a heterogeneous group of proteins derived from any of at least 25 different precursor molecules, and is considered a pathologic substance that appears histologically as an extracellular, amorphous, congophilic protein with green birefringence under polarized light.[5]

In humans, a neoplasm similar to amyloid producing odontogenic tumor in animals is named a calcifying epithelial odontogenic tumor (CEOT). Amyloid-producing odontogenic tumor was originally referred to as the counterpart of human CEOT. However, it was later described that APOT in dogs and cats is not a counterpart of CEOT in humans. Human CEOTs consist of sheets of eosinophilic epithelial cells showing considerable nuclear pleomorphism and invasive growth, whereas APOTs in animals mostly show basal cells with hyperchromatic nuclei, are arranged in palisades, and are benign masses that grow by expansion.[5] Due to such differences, amyloid-producing odontogenic tumor (APOT) was proposed as an appropriate alternative term for CEOTs in animals.[4]

Amyloid-producing odontogenic tumor is characterized by dental epithelium, with deposits of amyloid and sometimes prominent trabeculae of osteoid (dentinoid). The epithelium may be arranged in strands, nests or masses. Occasionally, there may be areas of mineralization of the epithelium or stroma in the form of small nodules or amorphous masses.[2] The distinctive features of calcifying epithelial odontogenic tumors in cats and dogs are the spherical amyloid-like deposits, which may undergo concentrically laminated calcification within the epithelial islands and stroma.[7]

It is believed that APOT passes through various developmental stages in which the neoplastic epithelium degenerates, forming amyloid globules which coalesce and calcify. However, the amyloid component seen in animal odontogenic tumors has not been thoroughly examined. Whether it is a secreted substance or a degenerative product remains controversial.[8] The neoplasm is suggested to originate from oral gingival epithelium or odontogenic epithelium within the connective tissue of the gingiva or within bone.[4] The amyloid in these tumors is suggested to be a secretory product of the neoplastic cells and possibly reflects an attempt to produce enamel by neoplastic ameloblasts.[1] Hirayama and co-workers examined the immunohistochemical profile of the amyloid protein from canine APOT using antibodies to ameloblastin, sheathlin, and amelogenin.[4] The neoplastic epithelial cells of APOT were focally reactive with antibodies to ameloblastin, sheathlin, amelogenin, and canine APOT amyloid. The similarity in amino acid sequence of the amyloid protein of canine APOT to that of enamel proteins, such as ameloblastin, sheathlin, and amelogenin, and the expression of these antigens in both APOT amyloid and in the neoplastic cells suggest that the amyloid of canine APOT is derived from enamel proteins secreted by ameloblasts. Based on these findings, the authors concluded that the precursor protein of amyloid fibrils in canine APOT may be derived from enamel proteins produced by ameloblasts and proposed that canine amyloid-producing odontogenic tumor (APOT) would more properly be named as canine amyloid-producing ameloblastoma (APA).[5] If this would apply to feline amyloid-producing odontogenic tumor remains to be determined.

In animals, almost all epithelial odontogenic tumors warrant a good prognosis. None has ever been reported to metastasize. They remain localized in the mandible or the maxilla, where they cause swelling and distortion. Complete surgical excision is usually curative. There is one exception to this rule: the calcifying epithelial odontogenic tumor (amyloid-producing odontogenic tumor) in cats and dogs, which although histologically benign is usually a locally invasive neoplasm and can cause destruction of bone and displacement of teeth.[7,8] Some cases

were described to recur after excision.[4] In most cases, the neoplasms are considered as low grade malignancy and rarely metastasize.[8]

JPC Diagnosis: Amyloid-producing odontogenic tumor.

Conference Comment: As the contributor discussed in an excellent overview of amyloid-producing odontogenic tumors, recent studies have suggested the protein in canine APOTs is derived from an ameoblastin-like peptide (AAmel), in contrast to the odontogenic amyloid ameloblastic-associated protein (ODAM) found in human CEOTs.[3] In an even more recent study, Delaney and co-workers analyzed the amyloid of three feline APOTs, and found the amyloid from all three feline APOTs to contain an ameloblastin peptide identical to the AAmel that had previously been identified in APOTs from a cat, a dog, and a tiger. Furthermore, the presence of ameloblastin is consistent with the findings of a similar enamel protein in the amyloid of APOTs from a Shih Tzu and eight other dogs, all of which were immunoreactive to rat ameloblastin, porcine amelogenin and sheathlin; however, unlike their canine counterparts, neither the feline amyloid nor the feline neoplastic epithelium exhibited positive immunoreactivity for amelogenin. Ameloblastin (formerly called sheathlin) is an enamel matrix protein that maintains the differentiation state of the ameloblast and is essential for enamel formation. Ameloblastin and amelogenin (another enamel protein) are both produced by ameloblasts during amelogenesis. In addition to ameloblastin, the feline APOTs in this study also showed positive immunoreactivity with laminin antibodies. Laminins are found in dental basement membrane during early tooth development; hence its presence in the amyloid of APOTs further suggests APOTs are ameloblastic in origin.[3]

Additionally, in Hirayama and co-workers' study of canine APOTs, the neoplastic cells exhibited positive immunoreactivity for cytokeratins (CK) AE1/AE3, CK9 and CK14. In the feline study, neoplastic cells were positive for CK AE1/AE3, CK14 and CK19. CK14 and CK19 are type I intermediate filaments of odontogenic epithelium; however, they are not specific, as they are also expressed in inner and outer enamel epithelium, cells from the stellate reticulum, stratum intermedium, dental lamina and Serres rests of the developing tooth.[3]

Overall, these results suggest that ameloblasts are the cell of origin of canine and feline APOTs, and ameloblasts produce the amyloid found in APOTs. Furthermore, feline and canine APOTs both apparently produce a similar type of amyloid which is distinct from the ODAM found in human CEOTs.[3]

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CASE III: 11-1832 (JPC 4019823).

Signalment: 5-month-old male Great Dane, canine *(Canis lupus familiaris)*.

History and Gross Pathology: A 5-month-old male Great Dane was euthanized for multiple congenital heart defects. Necropsy confirmed a clinical suspicion of a ventricular septal defect, pulmonic stenosis, tricuspid dysplasia and severe right ventricular dilation. In addition, both mandibles were uniformly expanded by marked, diffuse bony proliferation that preserved mandibular anatomy. Multiple axial and appendicular bones including the radius, ulna, humerus and femur were sectioned and were normal.

Histopathologic Description: Bone, mandible: Diffusely filling and expanding the mandible by 4 to 5 times the normal size, is a well-organized subperiosteal proliferation of bone that extends from the periosteum into the medullary cavity and to the lamina dura of teeth. The cortex is not evident. This bone proliferation is composed of approximately 90% woven bone and 10% lamellar bone, with a concentration of lamellar bone at the periphery. Haversian canals can be seen on cross sections of the trabeculae. Trabeculae are frequently lined by a single layer of well-differentiated osteoblasts that are occasionally interrupted by osteoclasts within Howship's lacunae. Trabeculae are densely packed with small areas separated by moderate amounts of loose fibrous connective tissue mixed with many plump fibroblasts that are punctuated by rare aggregates of degenerative neutrophils and eosinophils, mixed with small amounts of necrotic cellular debris. There is an absence of hematopoietic elements. Multifocally, the cambium is

irregularly expanded up to 3 times normal by dense granulation tissue that merges with the bony trabeculae. There is a moderate increase in osteoclastic activity along this junction. Centrally, a single tooth is present and surrounded by periodontal ligament that merges with dentin, with an absence of cementum.

Contributor's Morphologic Diagnosis: Bone, mandible: Hyperostosis, diffuse, marked.

Condition: Craniomandibular osteopathy.

Contributor's Comment: This is a classic case of craniomandibular osteopathy, with bony expansion of the jaw characterized by filling of the medullary spaces with woven bone. Craniomandibular osteopathy (CMO) or "lion jaw" is a nonneoplastic, proliferative bony disease of the dog affecting primarily the mandible, tympanic bullae, and occasionally other bones of the head, and rarely long bones of unknown etiology.[1] The disease predominates in Scottish terriers, West Highland White Terriers, and Cairn Terriers; however, other dog breeds such as boxers, Shetland sheepdog, Great Danes, Doberman pinschers and Labrador retrievers have also been reported in the literature.[1,2,4,5,8,9] The disease is seldom recognized until signs of discomfort due to chewing and eating are observed. This usually occurs when the dogs are 4 to 7 months old. It is likely that this dog was euthanized before clinical signs developed. In addition, the peripheral replacement of woven bone by lamellar bone in this case is suggestive of resolution of the disease. The pathogenesis of CMO is unknown but is likely multifactorial. Some cases resolve spontaneously and in others the pain is so great that owners request euthanasia. This animal has multifocal areas of subperiosteal bone resorption with granulation tissue. This is interpreted to be secondary to trauma or inflammation, rather than as part of the primary disease process.

Long-bone involvement has been reported mainly in West Highland White Terriers. In a 1995 retrospective study of 10 terriers, 2 terriers had long-bone involvement.[7] Our dog was a Great Dane and did not have long bone involvement.

JPC Diagnosis: Mandible: Subperiosteal new bone growth, diffuse, marked, with medullary fibrosis.

Conference Comment: CMO is characterized by intermittent and concurrent bone resorption and production involving the endosteum, periosteum, and trabecular bone of the skull (most often the mandible).[6] As the contributor states, the pathogenesis of CMO is unknown. The occurrence of this condition in multiple breeds may suggest more than one cause.[6] In West Highland White and Scottish Terriers there is some evidence that CMO is an autosomal recessive inherited trait. Alternatively, the presence of inflammation in many cases has raised the suspicion of an infectious etiology, although none has yet been identified. It is also possible that both an inherited predisposition followed by an additional factor or agent is necessary to initiate disease.[6]

Conference participants compared and contrasted CMO and hypertrophic osteopathy (HOD), another syndrome that is characterized by periosteal new bone formation. HOD is often associated with thoracic cavity chronic inflammation or neoplasia, as well as with botryoid rhabdomyosarcoma of the urinary bladder in dogs and ovarian tumors in horses.[6] In contrast to

the distribution of CMO, which is usually confined to the bones of the skull, the periosteal hyperostotic lesions associated with HOD generally occur along the diaphyses and metaphyses of certain bones (predominantly the radius, ulna, tibia, metacarpals, metatarsals); however, HOD bony proliferation can occasionally occur in the mandible as well. Like CMO, the pathogenesis of HOD is poorly understood, and although several mechanisms have been proposed to explain the increased blood flow to the limbs that appears to consistently occur early in HOD, the exact cause remains unknown.[6]

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CASE IV: 11-233 (JPC 4015808).

Signalment: 16-year-old, neutered male, mixed breed horse (*Equus caballus*).

History: The horse was donated and become part of a research study. The horse received a commercially available *Escherichia coli* O55:B5 lipopolysaccharide (LPS) solution infused via intravenous catheter at a dose of 5 ng/kg/hr for 8 hours. Twenty-four hours later the horse was given 5g/kg of oligofructose (OF) via a nasogastric tube and was euthanized 27 hours later with an Obel laminitis score of 2+.

Gross Pathology: Grossly all tissues were within normal limits. Laminar tissue was obtained by sectioning with a bandsaw as previously described.[1] Mid-dorsal laminar sections were trimmed into 2 cm × 1 cm × 0.5 cm strips using a scalpel, formalin-fixed and then processed routinely.

Histopathologic Description: Slides were stained with periodic acid-Schiff preparation to highlight the basement membranes. Histologically, there is tapering and retraction of the secondary epidermal lamina leaving empty sleeves of basement membrane trailing off the tips of the secondary epidermal lamina. There is also some retraction of the basement membrane and secondary dermal lamina from between the secondary epidermal lamina, which creates the appearance of a thicker primary epidermal lamina.

Contributor's Morphologic Diagnosis: Multifocal degeneration and loss of epidermal lamina.

Contributor's Comment: In horses, the hoof is attached to the underlying distal (third) phalanx (P3) by the interdigitation of epidermal and dermal lamina. Laminitis refers to separation of the epidermal and dermal lamina in the hoof, which results in lameness and in chronic cases, rotation of P3. Laminitis can be caused by a variety of insults, including, but not limited to: obesity, endocrinopathies, colic, sepsis, toxemia, diarrhea, shock, lush pastures, excess carbohydrates, drug therapy, intense training, and black walnut shavings.[3,4] Laminitis is typically induced experimentally with oligofructose. This horse was involved in a research project investigating the 'two-hit' hypothesis, which proposes that sequential exposure to inflammatory stimuli (LPS and OF) can exacerbate the laminitic response. Clinically the degree of laminitis (Obel score) has been correlated to the degree of histologic lesions using Politt's grading scheme.[3]

The pathogenesis of laminitis remains complex and poorly understood, but matrix metalloproteinases are present at increased levels in laminitic tissues and are thought to mediate the dissolution of cell-cell and cell-basement membrane adhesion.

The earliest changes involve transformation of the normally club-shaped ends of the secondary epidermal lamina with elongation and attenuation of the tips of the secondary epidermal lamina and detachment from the underlying basement membrane (Grade I). Where the epidermal cells detach from the basement membrane, small teat-shaped bubbles may form. In Grade II lesions, these changes progress and there is retraction of the basement membrane and secondary dermal lamina from between the bases of the secondary epidermal lamina.[4] As the dermal lamina are retracted, the epidermal cells become further from their blood supply, predisposing to subsequent ischemia.[2] Retraction of the capillaries also results in increased resistance to blood flow, which is noticed clinically as 'bounding pulses'. These changes in blood flow can also result in arteriovenous anastomoses or shunts.[3,4] In grade III lesions there is almost complete separation of the epidermal lamina from the basement membrane with loss of distinction between primary and secondary epidermal lamina. Ultimately there is retraction of the tip of the primary epidermal lamina as well.[4] The lesions in this case are most consistent with grade II laminitis.

JPC Diagnosis: Hoof lamina: Epidermal laminar degeneration and necrosis with multifocal basement membrane retraction and edema.

Conference Comment: As the contributor states, the pathogenesis of equine laminitis is complex and not fully understood. Historically, laminitis has been thought to be due to an ischemic event caused by a vascular condition that constricted blood flow to the hoof.[2] Recent research suggests that degeneration of the primary epidermal lamellae and basement membrane loss may be the initial lesion, with vascular events and subsequent ischemia being an important consequence of the initial laminar degeneration. This "enzymatic theory" of laminitis is based on the findings of significantly increased amounts of matrix metalloproteinase-2 (MMP-2 aka gelatinase A) and matrix metalloproteinase-9 (MMP-9 aka gelatinase B) in lamellar tissues affected by laminitis, as well as the finding that in the developmental phase of laminitis, vessels in the feet are actually dilated rather than constricted. MMPs are found in normal lamellar tissues and are thought to play a role in the required remodeling of the epidermal lamellae that occurs as a normal part of hoof growth. MMPs are produced locally, and function to release epidermal cell-to-cell and cell-to-basement membrane adhesions, maintaining the correct shape and orientation of the hoof lamellae as the hoof grows. The increase of MMP-2 and MMP-9 and the subsequent destruction of the lamellar attachment apparatus has been shown to be a key feature in acute laminitis, although the trigger factors have yet to be elucidated. Interestingly, epidermal cells of some non-equine species, including humans, readily increase their production of MMPs when exposed to cytokines such as TNF, IL-1, and TGH-1; however, in-vitro studies have shown that equine MMPs are not activated on exposure to these cytokines. Additionally, laminitic changes in equine lamellae have not been triggered experimentally by the administration of endotoxin, prostaglandins, black walnut extract, or anaerobic conditions. The one exception is a factor present in the supernatant from cultures of *Streptococcus bovis* isolated from the horse cecum; this factor has been found to activate equine hoof MMP-2 and has experimentally resulted in lamellar separation. Thus the *S. bovis* MMP activator may play a role in naturally-occurring carbohydrate-overload laminitis.[2] Perhaps with continued research, the complete pathogenesis of equine laminitis will finally be revealed.

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