CASE I: H03-0754A 03-0782 (AFIP 2935567).

**Signalment:** 4-year-old castrated, male Australian terrier (*Canis familiaris*).

**History:** The dog presented with a history of a mass in the right axilla occurring approximately one year prior to presentation. The mass had recurred after drainage and biopsy. Antibiotics, including clavulanic acid/amoxicillin, chloramphenicol, and doxycycline, had been prescribed over several months without significant response. Physical examination indicated extensive pyogranulomatous skin disease extending cranial, ventral and caudal to the right axilla with involvement of the prescapular lymph node. The owners were unable to afford the biopsy and culture procedures suggested for diagnosis, and based on previous histopathology results showing a pyogranulomatous deep dermatitis with filamentous Gram-positive organisms, the dog was placed on a trial of potentiated sulphonamides for suspected *Actinomyces* infection. After one month, the lesions showed significant improvement, but five months later there had been recurrence and the owners asked for the animal to be euthanized.

**Gross Pathology:** A castrated male Australian terrier. There was focally extensive alopecia and ulcerated multinodular swelling of the right axillary region extending over the right forelimb (fig 1-1). Subcutaneous dissection revealed multinodular to coalescing, focally extensive accumulations of light brown semifluid purulent material with black flecks throughout (fig 1-2). This extended 10-15 mm deep into the subcutis, passing over the lateral aspect of the thorax, medial to the scapula almost to its dorsal limit, and cranially to the level of the thoracic inlet, plus distally to the level of the right elbow. The right retropharyngeal lymph node was enlarged to 2 cm in length and appeared soft and edematous. The right prescapular and axillary nodes were unable to be located amongst the purulent pockets. There were no other significant findings.

**Laboratory Results:** Bacterial culture of both fresh tissue and a deep tissue swab from the affected area yielded a scanty to light growth of a Gram-positive filamentous bacterium. The isolate formed pearl-colored, shiny, waxy, adherent colonies, with Gram stain showing Gram-positive long filaments. There was pure growth on the agar and a molar tooth appearance to the colonies on aging. Partial DNA sequencing of the isolate was undertaken. A total of
404 bases were sequenced and there was a 100% match with the *Streptomyces cyaneus* 16s ribosomal RNA gene (partial sequence-Locus AY232254) held on the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/entrez).

**Contributor's Morphologic Diagnosis:** Right axilla and forelimb: Focally extensive, severe, chronic pyogranulomatous dermatitis and cellulitis, with numerous intralesional filamentous pigmented bacteria (*Streptomyces cyaneus*).

**Contributor's Comment:** The section provided was taken from the right lateral thoracic wall. Beneath focally parakeratotic, focally ulcerated, and acanthotic epidermis, there is a severe, focally extensive infiltrate of neutrophils and macrophages, the latter often containing light brown pigment, with numerous intralesional club colonies. The colonies have an eosinophilic centre and a black pigmented periphery (fig. 1-3). Filamentous organisms can be seen within the colonies. The infiltrate extends through deep dermis and hypodermis, down to underlying fascia and muscle. The organisms are negative for Ziehl-Neelsen
clearly demonstrates a genuine intralesional organism. The original source of the infection is not clear in this case, although at this site a perforating and contaminated traumatic injury is a possible inciting cause. These organisms are associated with lesions that are reported to have a low cure rate, often requiring protracted antibiotic treatment. The case reported here indicates that the isolation of Streptomyces from veterinary cases should not necessarily be considered a contamination.

AFIP Diagnosis: Haired skin and subcutis: Dermatitis and cellulitis, pyogranulomatous, diffuse, severe, with large colonies of pigmented, filamentous bacteria.

Conference Comment: Considerable discussion surrounded the brown intracytoplasmic pigment within macrophages in this case. Ultimately, conference participants concluded that this was bacterial pigment; however, many considered hemosiderin a possibility. Some participants’ sections contained an area of epidermal ulceration overlain by a serocellular crust, and some sections had small amounts of intralesional Splendore-Hoepli material.

Conference attendees discussed the differential diagnosis for the presence of intralesional colonies of filamentous bacteria, and the contributor provided a very good rubric for refining the differential diagnosis based on atmospheric oxygen requirements and the presence or absence of acid-fast staining. Importantly, most Nocardia sp. only stain

Streptomyces are of the family Streptomycetaceae, within the order Actinomycetales. Other bacteria within this order are Nocardia, Mycobacteria, and Actinomyces. Streptomyces are aerobic (unlike Actinomyces) and non acid-fast organisms (unlike Nocardia and Mycobacteria) comprising several hundred species. Most are found in the soil and most produce pigments. Many antibiotics, such as the aminoglycosides, tetracyclines and macrolides, are derived from these species. The bacteria are regarded as pathogenic in humans but rarely pathogenic for animals, and are a common laboratory contaminant. The genus, however, is clearly pathogenic, as indicated by various reports in the human and veterinary literature. Invasive Streptomyces infections of humans are uncommon and have been reviewed recently.

The isolation of this organism on two separate occasions, each time from two different sites plated onto different plates, makes contamination most unlikely. The bacteriology samples collected at necropsy were taken through shaved and ethanol scrubbed skin with sterile instruments to minimize contamination. Additionally, the presence of filamentous bacteria on the histology section clearly demonstrates a genuine intralesional organism. The original source of the infection is not clear in this case, although at this site a perforating and contaminated traumatic injury is a possible inciting cause. These organisms are associated with lesions that are reported to have a low cure rate, often requiring protracted antibiotic treatment. The case reported here indicates that the isolation of Streptomyces from veterinary cases should not necessarily be considered a contamination.

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acid-fast positive with a modified acid-fast stain (e.g. Fite-Faraco method), which may be useful in differentiating Nocardia sp. from opportunist Mycobacteria sp., which are strongly positive with routine acid-fast stain. However, not all species of Nocardia are acid-fast positive; in such cases, culture is necessary for differentiation from Actinomyces sp. Other notable members of order Actinomycetales include Dermatophilus congolensis and Streptobacillus moniliformis. Most members of the order are Gram positive; however, Mycobacteria sp. possess a complex cell wall that precludes Gram staining, while Nocardia sp. are only weakly Gram-positive. Compared with Actinomyces sp. and Nocardia sp., Streptomyces sp. and Actinomadura sp. are infrequent causes of disease. Clinically, the lesions caused by these four actinomycetes are indistinguishable, with the classic lesion being an actinomycotic mycetoma. By definition, a mycetoma is a destructive lesion of the skin, subcutis, fascia, and sometimes bone characterized by the triad of tumefaction, draining tracts, and tissue grains (i.e. “sulphur granules”) of various colors (e.g. white, black, yellow, red, or brown) corresponding to the etiologic agent. Actinomycotic and eumycotic mycetomas are caused by the aforementioned actinomycetes and opportunistic fungi, respectively. Nocardia sp. produces tissue grains less frequently than does Actinomyces sp. Scedosporium apiospermum (Pseudoallesheria boydii) and Acremonium hyalinum cause white-grain eumycotic mycetomas, while Curvularia geniculata, Madurella grisea, and Phaeococcus sp. cause black-grain eumycotic mycetomas.

Pseudomycetomas are characterized by the same triad of clinical signs as mycetomas, but lack a cement substance unique to true mycetomas. Dermatophytic pseudomycetomas are caused by dermatophytic fungi, most classically in Persian cats. The reader is referred to WSC 2008-2009, Conference 9, Case III for an example of dermatophytic pseudomycetoma. Bacterial pseudomycetoma results when non-branching bacteria (e.g. Staphylococcus sp., Streptococcus sp., Pseudomonas sp., or Proteus sp.) cause the Splendore-Hoeppli reaction in tissue, resulting in nodules composed of pyogranulomatous inflammation with or without fistulous tracts.

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References:
CASE II: N090-010 (AFIP 2938315).

Signalment: 26-week-old male Tg.AC hemizygous mouse (Mus musculus).

History: Mouse had a steadily enlarging mandibular mass.

Gross Pathology: Firm pale 1 centimeter mass adjacent to the incisors.

Contributor’s Morphologic Diagnosis: Mandible: Odontogenic tumor, ameloblastic, tooth.

Contributor’s Comment: The mandibular bone contains an invasive neoplasm that is composed of thin irregular anastomosing cords of cuboidal to low columnar palisading epithelial cells separated by a loose undifferentiated spindle cell stroma resembling ameloblastoma (figs. 2-1 and 2-2). The stroma contains cells with oval nuclei with finely stippled chromatin and indistinct cytoplasmic borders, consistent with ameloblasts.

Odontogenic neoplasms are the most common spontaneous neoplasms in Tg.AC mice, occurring at an incidence of approximately 13% in males and 17% in females. Grossly, they occur as firm masses in the maxilla or mandible. Microscopically, 3 morphologic types occur, the most frequent of which is the ameloblastic type as in the present case submission. The second type, which appears to arise from the periodontal ligament, is the mesenchymal type which is composed of mesenchymal cells embedded in a dense eosinophilic matrix. The third morphologic type resembles odontomas and is composed largely of irregular abortive tooth structures formed by enamel, dentin and well-differentiated ameloblasts and odontoblasts.

Other common neoplasms of Tg.AC transgenic mice include squamous papillomas of the forestomach, skin papillomas, alveolar/bronchiolar adenoma, salivary gland duct carcinoma and erythroleukemia seen primarily in the liver with involvement of the spleen, bone marrow and lymph nodes.

AFIP Diagnosis: Mandible: Odontogenic tumor, ameloblastic.

Conference Comment: Conference participants discussed odontogenesis and the distinguishing characteristics of odontogenic epithelium: 1) peripheral palisading of epithelial cells, 2) location of the nucleus at the apical pole in the palisaded cells, 3) cytoplasmic clearing at the basilar pole in the palisaded cells, and 4) prominent intercellular bridging among the internal epithelial cells.

All participants’ slides contained a pre-existing tooth at the periphery of the neoplasm. In addition, some participants’ slides contained a smaller tooth-like structure within the neoplasm. Considerable discussion centered on the origin of this structure, with some participants interpreting it as tooth recapitulation by neoplastic cells,
while other participants favored a pre-existing structure. The classification of odontogenic tumors in transgenic mice, as summarized by the contributor, is convenient and avoids the problem of interpreting this tooth-like structure. However, participants noted that classification using the World Health Organization’s (WHO’s) scheme for domestic animals would hinge on interpretation of this structure. If this structure is interpreted as recapitulation by the neoplasm, the diagnosis would be ameloblastic fibro-odontoma, while the absence of this feature would make the tumor consistent with an ameloblastic fibroma using the WHO scheme.1

While in domestic species most odontogenic tumors have similar biological behavior (i.e. local destruction by expansion, amenable to surgical excision), there are two notable exceptions. Canine acanthomatous ameloblastoma is aggressive, locally infiltrative, and prone to recurrence; fibromatous epulis of periodontal ligament origin is benign, and a surgical cure is often achieved despite incomplete surgical margins.2

The high incidence of odontogenic tumors in Tg.AC transgenic mice is attributed to the expression of the ras oncogene. Odontogenic tumors appear in up to 100% of dual transgenic mice expressing both ras and myc oncogenes, and appear as early as 4 weeks of age.3 Oncogenes are constitutively active genes that promote autonomous cell growth in cancer cells in the absence of normal growth-promoting signals. Oncogenes result from mutations in proto-oncogenes, and their products are called oncoproteins. Normal ras proteins are bound to the cytoplasmic aspect of the plasma membrane, and are activated only upon growth factor binding to plasma membrane receptors. Activated ras stimulates the mitogen-activated protein kinase cascade, resulting in signals to the nucleus for cell proliferation. Mutated ras proteins are continually activated, usually due to point mutations that impair guanosine triphosphate (GTP) hydrolysis, resulting in continuous stimulation of downstream proliferation signals. The mechanisms by which myc oncogene expression influences cell proliferation are less clear. Myc modulates myriad cellular activities, and is thought to be involved in carcinogenesis via some of its many targets, including ornithine decarboxylase and cyclin D2.5

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References:

CASE III: D03-33535 (AFIP 2944782).

Signalment: 14-week-old male Toggenburg goat (Capra hircus).

History: This goat kid presented to the veterinarian with hind limb paresis of 6 weeks duration with progression to ataxia, recumbency, facial tremors, and strabismus. The goat kid was euthanized.

Gross Pathology: The goat was in good postmortem and nutritional condition. Musculoskeletal: There was redness and swelling of the tendons and muscles of the caudal aspect of the right rear leg extending from the hoof to the distal femur. There were two areas of hemorrhage and dark friable muscle along the left lateral thorax and focally in the left cervical muscles. Respiratory: There was a mild amount of green purulent fluid in the frontal sinuses. There was redness, wetness and consolidation of 50% of the left cranial, left middle and accessory lung lobes. Nervous: The lateral ventricles were slightly

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Caprine arthritis-encephalitis virus (CAEV) is a retrovirus in the subfamily Lentivirinae. Phylogenetic analyses demonstrate similarity to maedi/visna virus (MVV) of sheep, one of the first lentiviruses to be discovered. Both MVV and CAEV are characterized clinically by slow progressive and debilitating illnesses of sheep and goats. Subclinical infections with CAEV occur commonly in goats and subclinically infected animals can be sources of virus transmission to others in the herd. Infection with CAEV results in persistent infection of macrophage-monocyte cells, and virus is expressed and then shed upon transition of the precursor monocyte to its mature macrophage type.

Several clinical entities of CAEV infection are recognized: progressive arthritis in adults, progressive weight loss in adults, indurative mastitis or hard bag in adult does, interstitial pneumonia and a leukoencephalomyelitis in kids 2 to 6 months of age. The pathological feature of each is lymphocytic/lymphoproliferative inflammation due to a host immune response with the expressed viral proteins.

The goat kid in this case, in addition to having leukomyelitis, had focal area of lymphoplasmacytic and histiocytic inflammation of the cerebral white matter; moderate, multifocal, interstitial pneumonia with lymphoplasmacytic and histiocytic thickening of the alveolar septa, perivasculitis and peribronchitis; and mild multifocal lymphoplasmacytic and histiocytic synovitis. Postmortem diagnosis can be confirmed through detection of virus, with immunohistochemistry and polymerase chain reaction tests being more rewarding than virus isolation.

Antemortem diagnosis is commonly achieved by detecting antibodies using agar gel immunodiffusion (AGID) or ELISA and much of the disease control efforts are focused on isolation or culling of seropositive animals.  

AFIP Diagnosis: Spinal cord, white matter: Meningomyelitis, necrotizing and lymphohistiocytic, focally extensive, severe, with spheroids and gliosis.

Conference Comment: There is significant slide variation, with the distribution ranging from unilateral and confined primarily to the white matter to bilateral and nearly diffuse. Predominantly within the white matter, but also extending into the adjacent gray matter and overlying leptomeninges, a perivascular infiltrate composed of numerous lymphocytes and fewer histiocytes and plasma cells expands Virchow-Robin space and extends variably into the adjacent neuropil. Affected blood vessels are lined by hypertrophied reactive endothelium. Affected white matter is often lost and replaced by myriad macrophages with abundant foamy cytoplasm (gitter cells), astrocytes that often contain phagocytic debris, and fewer lymphocytes and reactive microglia (fig. 3-1). Remaining myelin sheaths are frequently dilated, occasionally mineralized, and either empty or expanded by swollen axons (spheroids) (fig. 3-2). There is mild gliosis and rare neuronal necrosis within the gray matter. Numerous lymphocytes and fewer histiocytes and plasma cells multifocally expand the leptomeninges.

Conference participants discussed the differential diagnosis for neurologic disease in goats:
- Copper deficiency (enzootic ataxia, “swayback”)
- Neuronal vacuolar degeneration of Angora goats
- Cerebrospinal nematodiases
- Polioencephalomalacia
- Spinal abscesses
- Listeriosis
- Rabies
- CAEV

The contributor provided a concise overview of the pathogenesis and clinical manifestations of CAEV. Small ruminant lentiviruses are enveloped, single-stranded RNA viruses that are more homologous to the feline immunodeficiency virus (FIV) than to other retroviruses, but differ from feline and primate lentiviruses in that they are not immunosuppressive. Like other retroviruses, CAEV expresses the following viral genes (1):
- gag: encodes nucleocapsid and matrix glycoproteins that are detected by antibody-based
3-1. Spinal cord, cervical, goat. White matter is replaced by many foamy macrophages (Gitter cells) and necrotic debris, and dilated axon sheaths which are either devoid of axons or occasionally contain Gitter cells. Virchow-Robin's spaces are filled with a lymphoplasmacytic infiltrate and endothelium is hypertrophied. (HE 400X)

3-2. Spinal cord, cervical, goat. Multifocally within the white matter, there are spheroids and gemistocytic astrocytes. (HE 400X)
diagnostic tests
  – *pol*: encodes a variety of viral enzymes, including reverse transcriptase
  – *env*: encodes surface glycoprotein that mediates binding to cell receptors and entry into cells

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**References:**

**CASE IV: TVDML A1 (AFIP 3134285).**

**Signalment:** 6-year-old Angus cow (*Bos taurus*).

**History:** The herd has been out at pasture with no significant abnormalities described. The owner noticed excessive hair loss on this animal several days prior to its acute death.

**Gross Pathology:** A 1050 pound adult female cow is presented for necropsy and most of the carcass is noted to be alopecic. In multifocal to coalescing foci the cardiac muscle is tan to pale yellow and slightly raised on cut section.

**Laboratory Results:** Only postmortem contaminants were isolated via bacterial culture of the myocardium.

**Histopathologic Description:** In multifocal to coalescing foci there is extensive replacement of the epicardium and myocardium by predominately a granulomatous and lymphocytic infiltrate with fewer eosinophils and plasma cells. Multinucleated giant cells containing up to 30 nuclei (both foreign body type and Langhan’s type) are common (fig. 4-1). Degenerative changes in adjacent myofibers include hypereosinophilia, loss of cytoplasmic detail (cross striations) and mineralization. A similar type of inflammatory infiltrate was found histologically in the kidney, liver, adrenal gland, and skin. PAS, Grams stain, and Acid-Fast staining failed to illustrate etiologic agents.

**Contributor’s Morphologic Diagnosis:** Myocarditis, granulomatous and lymphocytic, multifocal to coalescing, chronic, severe.

**Contributor’s Comment:** The multiorgan distribution of a granulomatous infiltrate described in this case and the failure to identify causative etiologic agents is consistent with hairy vetch (*Vicia villosa*) toxicosis. Hairy vetch is a forage legume and can be commonly found in pastures in the United States and other temperate regions of the world. It is widely cultivated and used as pasturage, harvested as hay and silage, and utilized as a cover crop although it has been associated with a systemic granulomatous disease in some cattle. The specific etiologic factors and pathogenic mechanisms involved in the development of this syndrome remain unknown. It is unusual that hairy vetch is commonly ingested by cattle without the development of clinical signs and it is difficult to routinely induce the disease experimentally. It has been proposed that a plant
4.1. Lung, pig. Areas of necrosis admixed with high numbers of alveolar macrophages, fewer lymphocytes and plasma cells and necrotic leukocytes with slender, elongated, streaming nuclei ("oat cells") (arrows) that often surround small colonies of 1-2 um diameter bacilli (circle). (HE 400X)

4-2. Lung, pig. Bronchiolar epithelium is necrotic and replaced by eosinophilic cellular debris admixed with moderate numbers of histiocytes, lymphocytes, fewer plasma cell and rare neutrophils. Bronchiolar lumina often contain exudate composed of cellular and inflammatory debris. (HE 400X).
constituent (lectins) induces a type IV, or cell mediated hypersensitivity reaction or potentially directly activates T lymphocytes initiating the systemic granulomatous reaction described with the syndrome. Furthermore, the low incidence of disease and disease occurrence when availability of vetch is limited suggest duration of exposure or repeated exposure to the plant help support a hypersensitivity theory. As the syndrome mainly occurs in Holstein and Angus cattle a genetic predisposition has also been suggested.\textsuperscript{2,3}

Cattle that develop clinical disease usually have been grazing a pasture that contains a mixture of hairy vetch and small grains, including rye, wheat, and oats and outbreaks typically occur during the season of maximal vetch growth.\textsuperscript{2} Additionally, development of the disease occurs after grazing an affected pasture for at least 2-6 weeks. Vetch associated disease is typically more severe in adult cattle greater than 3 years old.

The clinical syndrome associated with Vetch toxicosis varies and three different clinical manifestations have been described. One syndrome involves acute neurological signs and death consistent with cyanogenic glycosides contained in the seeds. A second syndrome involves subcutaneous swellings of the head, neck, and body with ulcers of the oral mucous membranes, purulent nasal discharge, rales, cough and congestion. The third syndrome involves the systemic granulomatous response and presents clinically as a dermatitis and conjunctivitis with diarrhea and weight loss.\textsuperscript{3} With the systemic granulomatous syndrome gross lesions include a multifocal to coalescing, soft, and gray to yellow infiltrate disrupting the normal architecture of the involved tissues (liver, kidney, spleen, heart, adrenal gland, skin). Histologically, the salient feature is a multifocal to coalescing granulomatous infiltrate disrupting the affected tissue architecture. The infiltrate is typically characterized by epitheloid macrophages, multinucleated giant cells, lymphocytes, plasma cells and typically eosinophils.

\textbf{AFIP Diagnosis:} 1. Heart: Myocarditis, granulomatous and eosinophilic, multifocal to coalescing, severe, with myocyte degeneration and necrosis.  
2. Heart, myocardium: Sarcocysts, few.

\textbf{Conference Comment:} The contributor provided an excellent overview of hairy vetch toxicosis. As the contributor noted, the pathogenesis of hairy vetch toxicosis remains somewhat unclear, and cattle exposed to the plant do not consistently develop disease. Therefore, hairy vetch toxicosis remains a diagnosis of exclusion. Conference participants discussed the importance of ruling out other causes of granulomatous inflammation, even when affected cattle are known to be exposed to hairy vetch. Special stains for bacterial and fungal etiologies were performed by the contributor, and repeated at AFIP, with no causative organisms seen. Some slides contained low numbers of sarcocysts that were not associated with inflammation, and were considered incidental (fig. 4-2).

Other compounds that may induce the production of lesions indistinguishable from vetch toxicosis include diureido-isobutane (DUIB) and citrus pulp.\textsuperscript{1} In horses, vetch toxicosis causes lesions similar to those in cattle, with the notable exceptions being the relative lack of eosinophils in the infiltrate and the lack of heart involvement.\textsuperscript{1}

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\textbf{References:}  