Joint Pathology Center
Veterinary Pathology Services
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CASE I: C1707 (JPC 4033366).

Signalment: 11-month-old male Border Collie dog (*Canis familiaris*).

History: The dog had a history of poor growth, weight loss, chronic upper respiratory infection and cyclical pyrexia.

Gross Pathology: There were multifocal pale yellow to white firm irregular nodules (2-3 mm in diameter) within the myocardium and on the epicardial and endocardial surfaces. Similar nodules (1-2 mm in diameter) were present on the capsular and cut surfaces of both kidneys. The lungs were diffusely mottled dark red and there was a focal firm mass (1.5 x 1.5 x 1 cm) with a reddish grey cut surface within one lung lobe. On the meningeal surface of the brain, there were multiple pale red foci.

Laboratory Results: Fungal culture of lung and brain yielded *Scedosporium prolificans*.

Histopathologic Description: Heart: Within the myocardium, there are multiple 1-2 mm diameter foci of acute coagulative necrosis characterized by large aggregates of fibrin admixed with mostly degenerate neutrophils, fewer macrophages, karyorrhectic debris and numerous intralesional fungal hyphae. The hyphae are approximately 5 µm in diameter, septate, with parallel walls and haphazard or dichotomous branching; multifocally there are ovoid conidia (approximately 5 x 8 µm). Within these areas, cardiomyofibres are fragmented and exhibit deeply eosinophilic sarcoplasm with loss of cross-striations. Within the surrounding myocardium cardiomyofibres are frequently separated by clear spaces (edema), or dissected by abundant fibrin aggregates with scattered neutrophils and macrophages. Interstitial blood vessels are often lined by plump (reactive) endothelial cells.

Contributor's Morphologic Diagnosis: Heart: Myocarditis, suppurative and necrotizing, multifocal, moderate, acute with intralesional fungal hyphae (*Scedosporium prolificans*).

Contributor's Comment: Histological examination of further tissues from this case showed necrotic foci with fungal hyphae within the lungs, kidney, liver, pancreas, pituitary gland and cerebral cortex. The fungal morphology was compatible with *Scedosporium prolificans* as obtained by fungal culture from both lungs and brain.

Scedosporium prolificans (previously *Scedosporium inflatum*) is a filamentous fungus within the family Microascaceae.[1] In the environment it has been isolated from soil and potted plants.[1] The organism is increasingly recognized as a cause of disseminated fungal infections in immunocompromised human patients. Treatment of infections is challenging due to the resistance of *S. prolificans* to most antifungal agents.[1] There are rare reports of *S. prolificans* infection in animals. In dogs *S. prolificans* has been isolated from a German Shepherd dog with a disseminated infection involving kidney, heart, bone marrow, skeletal muscle, liver, lung, spleen, lymph nodes and pancreas.[5] In addition one case has been reported of a beagle with osteomyelitis in which *S. prolificans* was found to have disseminated to the lungs.[7]

One case of *S. prolificans* infection in a horse associated with osteomyelitis and arthritis has also been described.[9] Musculoskeletal infections are also a common presentation in humans.[1] Further isolates characterized as *S. prolificans* were obtained from eye scrapings of two horses and from a draining sinus in a cat.[8]

In tissues the morphology of *Scedosporium* spp. is similar to that of *Aspergillus* spp. although *Scedosporium* spp. exhibit haphazard branching with less frequent dichotomous branching.[6] Culture of the fungus allows more definitive identification; the colonies grow rapidly and exhibit a moist, felty appearance with initially a white color that becomes olive-grey to black. Microscopically the conidiophores display distinctly swollen bases (hence the previous name *S. inflatum*) with ovoid conidia.

If material for culture is not available, identification of *Scedosporium* spp. by PCR is also possible.[4]

Disseminated infections with opportunistic fungi in dogs have been associated with a number of different fungal species. In particular, systemic infections with *Aspergillus terreus* have commonly been described in German Shepherd dogs. Breed-associated abnormalities in IgA levels or function have been reported but have not been conclusively proven to be the cause of the increased susceptibility to fungal infections.[2,11]

The Border collie presented in the current case had shown poor growth from birth and persistent upper respiratory disease; however, an underlying immunodeficiency was not established.

Other fungal species isolated from dogs with disseminated infections include *Penicillium* sp., *Paecilomyces* sp., *Chrysosporium* sp and *Pseudoallescheria boydii* or *Scedosporium apiospermum* (the asexual form of *P. boydii*).[10]

JPC Diagnosis: Heart: Myocarditis, necrotizing, acute, random, marked with numerous fungal hyphae and conidia.

Conference Comment: Fungi capable of causing disseminated disease are generally divided into two groups: the truly pathogenic, such as the dimorphic fungi *Blastomyces dermatitidis*, *Histoplasma capsulatum* or *Coccidioides immitis*, and the opportunistic pathogens, such as *Aspergillus fumigatus* or *Candida albicans*. By and large, the opportunistic fungi are ubiquitous, saprophytic organisms which tend to cause disease in immunocompromised individuals.[10] As noted by the contributor, disseminated, opportunistic fungal infections in dogs (especially German shepherds), are generally attributed to *Aspergillus* sp., such as *Aspergillus terreus*, however in recent years *Scedosporium prolificans* has gained notoriety as an emerging opportunistic pathogen in both humans and animals. In addition to *Aspergillus* sp., other differential diagnoses for *S. prolificans* include: *Candida* sp., *Zygomycetes* such as *Absidia*, *Rhizopus* and *Mucor* sp., or non-fungal agents like *Pythium insidiosum*.

S. prolificans is a filamentous, non-pigmented, parallel-walled fungus with septate, 3-5 µm, haphazardly branching hyphae (sometimes described as a "letter-H" pattern) with lemon-shaped conidiophores from which a small cluster of single-cell conidia emerges.[1] *Scedosporium* will produce conidia in solid non-aerated tissues,[5] such as the myocardium in the present case. *Aspergillus* has a similar size and tissue morphology, and it also produces conidiophores, or fruiting bodies, but unlike *Scedosporium*, these are generally more round than lemon-shaped and they only occur in aerated tissues like ectatic bronchi or the surface of skin wounds.[6] *Candida* sp. appears in tissue in both hyphal and budding yeast forms, which could be confused with *Scedosporium*, however the presence of pseudohyphae is relatively common in candidiasis and rare in *S. prolificans* infection.[6] Like *S. prolificans,* zygomycete hyphae may also appear in a "letter-H" pattern,[6] though their hyphae are more broad (6-25 µm) and pauciseptate with non-parallel walls and non-dichotomous branching.[3] *S. prolificans* may also be mistaken for *Pythium insidiosum,* which, although it is an oomycete rather than a true fungus, produces 2-10 µm, pauciseptate hyphae with non-parallel walls and non-dichotomous branching.[3]

S. prolificans is resistant to many anti-fungal drugs and generally carries a grave prognosis, so differentiating it from other opportunistic fungi or fungal-like organism is imperative.[6] Although *Scedosporium* has several unique morphologic characteristics, definitive diagnosis with histopathology alone is often difficult to achieve, so culture and/or PCR are critical.

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CASE II: NCAH 2013-2 (JPC 4033380).

Signalment: Adult male Jersey bull (*Bos Taurus*).

History: The bull was presented for slaughter at a US federally-inspected slaughter plant, passed ante-mortem inspection, and was retained for further diagnostic testing after lesions resembling tuberculosis were identified during post-mortem examination.

Gross Pathology: Multifocally within the lung there were firm, homogenous, white to tan nodules up to 2.5 cm in diameter scattered throughout the parenchyma. The medial retropharyngeal and thoracic lymph nodes were enlarged and edematous with multifocal to coalescing yellow caseous granulomas.

Laboratory Results: *Mycobacterium avium* complex was isolated by culture. PCR from formalin-fixed, paraffin-embedded tissue was negative for IS6110 (*Mycobacterium tuberculosis* complex), 16S rDNA (*M. avium* complex), and IS900 (*M. avium paratuberculosis*).

Histopathologic Description: Lung: Effacing 70-90% of the section are multiple, often coalescing, granulomas centered on homogenous eosinophilic cellular debris (caseous necrosis) that contain central basophilic granular material (mineral). Necrotic foci are surrounded by large numbers of epithelioid macrophages, multinucleated giant cells (Langhans' type), and smaller numbers of lymphocytes, plasma cells, and neutrophils. Langhans' giant cells range from 25 µm to over 100 µm in diameter and may contain over 50 nuclei. Granulomas are surrounded by fibrocytes, fibrous connective tissue, free erythrocytes, edema, and cellular debris. Similar inflammatory cells (without giant cells) and exudates expand alveolar septa and peribronchiolar areas. The interlobular septa and pleura are expanded by edema, and small amounts of inflammatory exudates. Very small numbers of acid-fast bacilli (AFB), up to 5 µm long, were present within necrotic foci and the cytoplasm of multinucleated giant cells of New Fuchsin-stained sections; bacteria fluoresced in Acridine Orange/Auramine O-stained sections.

Lymph node: Granulomatous lesions are similar to those described in the lung.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, granulomatous, multifocal to coalescing, chronic, severe, with intra- and extra-histiocytic acid-fast bacilli.

Lymph node: Lymphadenitis, granulomatous, multifocal to coalescing, chronic, severe, with intra- and extra-histiocytic acid-fast bacilli.

Contributor's Comment: *Mycobacterium bovis* causes tuberculosis in many mammals, including cattle and humans and is a zoonotic disease. Cattle slaughtered in USDA-inspected abattoirs undergo inspection by Food Safety Inspection Service (FSIS) personnel to insure that resultant products are safe and wholesome for entry into the retail market. In accordance with the USDA's Bovine Tuberculosis Eradication Program, FSIS personnel retain carcasses when granulomas resembling tuberculosis are identified. Suspect granulomas from these cattle are collected and submitted to the National Veterinary Services Laboratories (NVSL) for histopathology and culture.[12]

Microscopic lesions consistent with tuberculosis in cattle are often multicentric and coalescing with central areas of caseous necrosis and mineral. Epithelioid macrophages surround the necrosis and often

included are small to moderate numbers of multinucleated giant cells with smaller numbers of lymphocytes, plasma cells, and occasional neutrophils.[2] The typical tuberculous lesion caused by *Mycobacterium bovis* has small to occasionally moderate numbers of acid fast bacteria present within the cytoplasm of macrophages and giant cells as well as areas of necrosis.[2]

Bovine cases fitting this description undergo additional testing using PCR on formalin-fixed, paraffin embedded (FFPE) tissue to test for mycobacterial DNA. Because the scope of the TB eradication program is focused on identifying *M. bovis*, primers used in the PCR are limited to those for *M. tuberculosis* complex (MTBC, of which M. bovis is a member), *M. avium* complex (MAC, common environmental mycobacteria) and *M. avium* subsp. *paratuberculosis* (MAP, the organism that causes Johne's disease in cattle). A recent report[12] of mycobacteria cultured from clinical samples submitted to the NVSL stated that the majority of mycobacteria cultured from cattle were *M. bovis* (32%) followed by *M. avium* complex (25.5%). The next most common species, *M. fortuitum*/*M. fortuitum* complex, comprised 10.1%.[12]

The microscopic features of the current case were consistent with bovine tuberculosis. FFPE tissue was tested by PCR for mycobacterial DNA using our primers for MTBC, MAC, and MAP, and tests were negative. False negative results for mycobacterial DNA can occur for a couple of reasons. First, formalin fixation causes irreversible cross-linking between DNA and protein, which increases as the tissue fixes over time. Over-fixation in formalin can reduce availability of DNA for the PCR, and result in a false negative finding.[5] Tuberculosis-suspect submissions from FSIS are shipped to the NVSL overnight; samples are cut-in immediately upon receipt and processed overnight for microscopic exam the next day. Because this case was an FSIS submission, over-fixation is unlikely as a cause for the false-negative PCR results. Second, false negative results may occur when there are extremely low numbers of AFB, as there were in this case.

M. avium complex was isolated from the tissues of this animal. Further subspeciation of the isolate was not performed. Culture is the gold standard for definitive diagnosis of bovine tuberculosis[1] and takes up to 10 weeks to complete with slow-growing mycobacteria.

Members of the *M. avium* complex are slow growing saprophytes commonly found in water, soil, and decaying vegetation.[4,6] MAC can cause tuberculosis-like disease in humans (particularly those who are immunocompromised) and birds.[4] Additionally, naturally occurring MAC infections have been reported in a tiger,[3] dogs,[7,9] pigs,[8] and a ferret with lymphoma.[10] Acid-fast bacilli are frequently numerous in lesions caused by MAC organisms,[6] but were uncommonly sparse in this case.

JPC Diagnosis: Lung and lymph node: Pneumonia and lymphadenitis, granulomatous, multifocal to coalescing, marked, with rare intra-histiocytic acid-fast bacilli.

Conference Comment: Although most sections contain vague, disorganized, poorly formed pulmonary granulomas, there is some slide variation, with the occasional presence of more distinct, classic granulomas. A classic granuloma has central core of necrosis, surrounded by epithelioid macrophages and multinucleated giant cells, variable numbers of lymphocytes and plasma cells, and a rim of reactive fibroblasts producing fibrous connective tissue.[1] One of the most striking features of this case is the massive size of the Langhans'-type multinucleated giant cells, which occasionally exceed 100 µm in diameter. Additionally, the few acid-fast bacilli present stain poorly with Ziehl-Neelsen and are more easily visualized with the modified acid-fast stain (Fite-Faraco), which the moderator noted is a common finding in mycobacteriosis.

Mycobacteria are broadly characterized as obligate and opportunistic pathogens. Obligate pathogens include the tuberculosis complex (MTBC: *M. tuberculosis* and *M. bovis*) and the leprosy group (*M. leprae*

and *M. lepraemurium*), while opportunistic mycobacteria are subdivided into rapid-growing (e.g., *M. fortuitum*, *M. chelonae* and *M. smegmatis*) and slow-growing (e.g., *M. avium* complex (MAC)) mycobacteria.[1] Another classic taxonomic division, which excludes the tuberculosis complex, is the Runyon system. This divides mycobacteria into four groups based on pigment and growth rate; these are summarized in Table 1. Mycobacteria have a protective lipid-rich cell wall with mycolic acid; once phagocytized, they inhibit phagosome-lysosome fusion, thus preventing oxygen radical formation, disrupting cytokine production, and avoiding proteolytic enzymes produced by the host cell.[8]

Some species, such as MTBC in cattle and *M. avium* subsp. *avium* in birds, generally produce a T_H1 (tuberculoid) reaction, which results in the formation of granulomas with low numbers of AFB (i.e., paucibacillary granulomas). The immunologic basis for this reaction is delayed-type (i.e., type IV) hypersensitivity, with a T_H1-type, or cell mediated lymphocytic response; in cattle, this often begins in the lungs, since the respiratory tract is the most common portal of entry for MTBC. Specifically, antigen presenting cells release IL-12, which induces naïve CD4+ T-lymphocytes to enter the T_H1 pathway. Once committed, T_H1 cells synthesize and release IL-2, which activates additional T_H1-lymphocytes; IFN-γ and TNF-β, which activates and attracts macrophages; and TNF-α, which promotes an inflammatory response. Interferon-γ also inhibits activation of the T_H2 pathway.[1] This T_H1 response results in "classical" macrophage activation, which influences the structure of the chronic inflammatory response. Specifically, classical macrophage activation via IFN-γ triggers the expression of MHC II, respiratory burst, and release of NO as well as the cytokines IL-1, IL-6 and TNF. The end result is microbial killing, granuloma formation, cellular immunity and delayed type hypersensitivity,[1] which accounts for the microscopic features observed in this case.

In contrast, some types of mycobacteriosis are characterized by a T_H2 (lepromatous) response; examples include leprosy (*M. leprae*) and Johne's disease (MAP). Here, commitment to the T_H2 immune response is induced by IL-4, and T_H2-lymphocytes release IL-4, IL-5, IL-10, IL-13, IL-17 and IL-19, which result in B-lymphocyte activation, antibody production (humoral immunity) and alternative macrophage activation. This is an ineffective method of killing mycobacteria, so histologically there is a multibacillary, disseminated granulomatous response, generally in the gastrointestinal tract and mesenteric lymph nodes.[1]

Although MAC can produce both multibacillary, lepromatous lesions and paucibacillary, tuberculoid lesions,[4] *M. bovis* is more commonly isolated from bovine pulmonary granulomas, so it is somewhat surprising that *M. avium* was isolated from the submitted tissue and that PCR for MTBC was negative. As the contributor noted, the gross and microscopic features of this case were more consistent with bovine tuberculosis.

Table 1: Runyan system of mycobacterial classification[11]

I	slow-growing photochromogens that turn yellow when exposed to light
II	slow-growing scotochromogens that appear yellow in the dark and after exposure to light
III	slow-growing non-photochromogens are non-pigmented
IV	rapid growers, which show visible growth within seven days

Contributing Institution: National Centers for Animal Health
http://www.ars.usda.gov/
http://www.aphis.usda.gov/

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CASE III: N2012AFIP938 (JPC 4032817).

Signalment: 1-year-4-month-old male rock hyrax (*Procavia capensis*).

History: This rock hyrax was found dead without premonitory signs. There were no other deaths in the group in the preceding four months. A second, four month old, rock hyrax was euthanized due to similar lesions 10 days after this individual died.

Gross Pathology: There are multifocal to coalescing, thick, tenacious, white to pale yellow plaques adhered to the mucosal surfaces of the aryepiglottic folds, the vocal folds, the laryngeal pouches and the nasopharynx and extending into tonsillar crypts. The aryepiglottic folds are markedly thickened, resulting in severe reduction in the diameter of the glottis (pinpoint). There is multifocal red discoloration of the mucosa in areas not covered by plaques. There were no other significant findings.

Laboratory Results: Aerobic culture, larynx: moderate coagulase negative *Staphylococcus* spp., moderate *Pasteurella multocida*

Anaerobic culture, larynx: Few *Prevotella* spp.

Histopathologic Description: Larynx/pharynx: There is multifocal and extensive ulceration of the laryngeal mucosa, a thick overlying pseudomembrane and dense inflammatory infiltrates in the

submucosa. Intact epithelium adjacent to areas of ulceration is infiltrated by variable numbers of neutrophils, is mildly hyperplastic and expanded by edema. These areas often contain large syncytial cells which, in addition to individual epithelial cells, have large amphophilic intranuclear inclusion bodies that either fill the entire nucleus leaving a thin band of peripheralized chromatin, or are surrounded by a clear halo encircled by peripheralized chromatin. The overlying pseudomembrane is composed of fibrin, wispy basophilic material (mucin), viable and intact neutrophils, cellular debris, sloughed epithelial cells (occasionally with intranuclear inclusion bodies) and large, dense colonies of mixed bacteria which are most prevalent on the surface. Inflammation expanding the submucosa directly subtending ulcers is composed of predominantly neutrophils with fewer lymphocytes and plasma cells; the latter predominate in the deeper submucosa and more peripherally. There is multifocal marked submucosal edema and submucosal capillaries are markedly congested.

Contributor's Morphologic Diagnosis: Larynx/Pharynx: Laryngitis/pharyngitis, fibrinosuppurative, ulcerative, subacute, multifocal to coalescing, extensive, marked, with intralesional intranuclear viral inclusion bodies, intralesional viral syncytia and superficial mixed bacteria.

Contributor's Comment: The histological findings in this case, specifically mucosal ulceration with intralesional intranuclear inclusion bodies and syncytia formation, were consistent with infection by the previously described hyrax herpesvirus.[1] The severity of lesions resulted in near-obstruction of the larynx and presumably contributed to the death of this hyrax.

Investigations into this virus performed by Galeota et al.[1], including biological behavior and molecular characteristics, supported the inclusion of hyrax herpesvirus in the *Alphaherpesvirinae* subfamily and *Simplexvirus* genus. At our institutions we have seen oral ulcers on numerous occasions in rock hyraxes (often as incidental lesions), and previous molecular investigations from individuals in this colony have shown the offending organism to have 100% shared identity with published hyrax herpesvirus DNA polymerase gene sequences.

As with multiple other members of the *Alphaherpesvirinae*, lesions associated with the hyrax herpesvirus are the result of epithelial necrosis and ulceration with secondary inflammation and, in some cases, bacterial infection. Systemic lesions associated with this virus have not been confirmed, but one published case was described as having nonsuppurative meningoencephalitis[1] and we have seen neuronal necrosis in one of our cases. As with other herpes viruses, it is suspected that the hyrax herpesvirus causes long-term infection with occasional episodes of recrudescence. As no new animals were recently introduced into this group prior to the death of these two animals, recrudescence was suspected in these cases. The colony had recently been transferred to their indoor winter housing which likely introduced stressors and may have induced shedding of the virus in previously infected animals and overt lesions in these younger individuals.

JPC Diagnosis: Larynx/Pharynx: Laryngitis/pharyngitis, ulcerative and fibrinosuppurative, acute, multifocal, marked, with eosinophilic intranuclear inclusion bodies, viral syncytial cells, submucosal edema and colonies of bacteria.

Conference Comment: Herpesviruses belong to the order Herpesvirales, which contains three families: *Herpesviridae* (herpesviruses of birds, mammals and reptiles), *Malacoherpesviridae* (oysters) and *Alloherpesviridae* (fish and frogs).[2] Herpesviruses are double-stranded, enveloped DNA viruses with worldwide distribution. Replication occurs within the nucleus, resulting in intranuclear inclusion bodies; the viral envelope is acquired via budding through the nuclear membrane. These viruses usually have a narrowly restricted host range and are known for the ability to establish latent infections. The family *Herpesviridae* is divided into three broad subfamilies: *alpha*, *beta* and *gammaherpesvirinae*.[2] Betaherpesviruses, also known as cytomegaloviruses, replicate slowly, have a highly restricted host range

and often produce greatly enlarged cells. When latent, they are sequestered in secretory cells, lymphoreticular organs, and the kidney, however they are more associated with continuous viral shedding than periodic reactivation (as opposed to alphaherpesviruses).[2] Betaherpesvirus is normally found in many species, however it usually only causes disease in immunosuppressed individuals, such as SIV infected monkeys. Betaherpesviruses also cause inclusion body rhinitis in swine (suid herpesvirus 2) and salivary gland inclusions and cytomegaly in guinea pigs (caviid herpesvirus 2).[2] Gammaherpesviruses, such as ovine herpesvirus-2 and alcephaline herpesvirus-1 (causative agents of malignant catarrhal fever) or saimiriine herpesvirus 2 (*Herpesvirus saimiri*) replicate in lymphoblastic cells and induce lymphoproliferative response.[2]

Alphaherpesviruses tend to lyse host cells and typically result in widespread or localized necrosis, as in this case. They grow rapidly, producing Cowdry type-A intranuclear inclusion bodies (and viral syncytial cells) and establishing lifelong latent infections in both the lymphoreticular system and the trigeminal ganglion.[4] Gallid herpesvirus 2 (Marek's disease) is a rare example of an alphaherpesvirus which acts more like a gammaherpesvirus in that it induces a lymphoproliferative response.[2] Table 1 summarizes other alphaherpesviruses of veterinary importance.[2,4]

Directional spread of alphaherpesviruses within the nervous system and the establishment of latency is a critical component of the viral lifecycle. The virus initially replicates peripherally in the skin or mucus membranes, where the innate immune response provides the first line of defense. Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs) expressed by the virus. Activated TLRs result in the production of cytokines, which recruit macrophages and/or induce proteins that degrade mRNA and inhibit translation. The adaptive immune response also works to prevent viral spread via viral specific CD8+ cytotoxic T-cells.[3] Although the initial infection is typically cleared within a few weeks, the virus spreads along axons to the sensory nerve ganglion, where the viral genome is maintained indefinitely. During latency, normally productive viral genes are quiescent and unproductive and LATs (latency associated RNA transcripts) accumulate in neuronal nuclei. LATs are Bcl-2 analogs (Bcl-2 is an anti-apoptotic regulator protein) that confers resistance to apoptosis, allowing viral persistence in sensory neurons.[5] Thus, in times of stress or immune suppression, the virus can reactivate and cause clinical disease.

Table 1: Alphaherpesviruses of veterinary importance.[2,4]

Bovine herpesvirus 1	Infectious bovine rhinotracheitis, infectious pustular vulvovaginitis
Bovine herpesvirus 2	Bovine mammillitis/pseudo-lumpy skin disease
Bovine herpesvirus 5	Bovine herpes meningoencephalitis
Porcine herpesvirus 1	Pseudorabies/Aujeszky's Disease
Equine herpesvirus 1	Equine abortion
Equine herpesvirus 3	Equine coital exanthema
Equine herpesvirus 4	Equine rhinopneumonitis
Equine herpesvirus 5	Multinodular pulmonary fibrosis
Gallid herpesvirus 1	Avian infectious laryngotracheitis
Gallid herpesvirus 2	Marek's disease
Psittacid herpesvirus	Pacheco's disease
Anatid herpesvirus 1	Duck Plague
Feline herpesvirus 1	Feline viral rhinotracheitis
Canine herpesvirus 1	Canine herpesviral disease
Macacine herpesvirus 1	B-virus of macaques
Saimiriine herpesvirus 1	Herpes tamarinus

Contributing Institution: Wildlife Conservation Society
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CASE IV: 2105011 (JPC 4017829).

Signalment: 13-year-old neutered female Labrador retriever dog (*Canis familiaris)*.

History: This dog had a two-day history of rear limb edema and an activity level that had declined over several months. Abdominal ultrasound revealed 2 large masses in the region of the liver, one of which was compressing the vena cava. It was suspected that postcaval compression by this mass had caused the limb edema.

Gross Pathology: Bilaterally, the subcutaneous tissues of the rear limbs are thickened up to 1.5 cm by gelatinous pale yellow fluid, and this fluid became red, with discoloration of the adjacent tissue below the hock. A 13x12x8cm firm red mass is present on the left lateral liver lobe, and a smaller similar mass is present on the left medial lobe. The liver weighed 6.3% of the body weight. An externally firm 8x7x5 cm mass is present on the right lobe of the pancreas. When sectioned, the mass is soft and dark red continuing several pale nodules that measure 0.5 cm or less in diameter. The pancreatic lymph nodes were firm, tan and measure 3 cm in length.

Histopathologic Description: Pancreas: Compressing the adjacent pancreatic parenchyma and expanding the fibrous capsule is a multi-lobular, moderately cellular mass arranged in acini and tubules. These are separated by fibrovascular stroma that is expanded by amorphous eosinophilic hyaline material. Individual neoplastic cells are cuboidal or polygonal, with distinct cytoplasmic borders and abundant amphophilic to granular eosinophilic cytoplasm. The basal nuclei are round, possessing finely stippled chromatin and a variably distinct nucleolus. No mitotic figures are found in ten 400X fields. Focal necrosis is apparent. Neoplastic cells do not infiltrate the capsule of the organ, but lymphatic emboli occurred in some sections.

Contributor's Morphologic Diagnosis: Pancreas: Exocrine pancreas: adenocarcinoma, hyalinizing.

Contributor's Comment: The pancreatic mass is consistent with pancreatic exocrine adenocarcinoma of hyalinizing type. Overall, exocrine pancreatic tumors are most frequent in dogs amongst the domestic

species, with a higher prevalence in older females according to some studies. Extensive implantation and metastases to lymph nodes and liver are often evident by the onset of clinical signs.

Carcinomas may be manifest as single or multiple masses in the organ. They are histologically variable and are classified as acinar, which look like normal exocrine tissue, tubular, which appear more similar to ducts, or undifferentiated, when cells occur in sheets. Hyalinizing carcinomas contain interstitial glassy eosinophilic matrix in tubular lumina or expanding the matrix.[1] This substance is not congophilic and fails to stain immunohistochemically with reagents to serum amyloid A, amylin, α1-antitrypsin or immunoglobulin light chain. The nature of this matrix is not known. In a small case series, these carcinomas tended to favor the acinar pattern and patients that were not euthanized immediately after surgery had a somewhat longer survival than expected.[1] A case with clear cell morphology and similarly increased interstitial matrix has been recently reported in a dog, in which the matrix was PAS+.[4] Our case has a mixed pattern with sheets of undifferentiated cells as well as acini.

Cytokeratin labeling has been disappointing[2] for use in identifying pancreatic carcinomas in dogs, and has the draw-back of staining cytokeratin on other organs when utilized to identify metastases. In cats pancreatic exocrine neoplasms are often positive for one or both reagents, as is normal pancreas. In dogs, pancreatic ducts are reported to express cytokeratin 7, but acini were negative, while acinar tissue reacted with neither reagent. Four pancreatic exocrine neoplasms tested in this series were also uniformly negative. Recently claudin-4 has been suggested as a reagent useful in negative poorly differentiated exocrine tumors and ductular tumors from between differentiated acinar neoplasms, which are positive.[3]

The patient had very severe rear leg swelling with marked subcutaneous edema, which histologically was found to contain numerous neutrophils. Two of the cases in reference 1 also had one or more areas of suppurative panniculitis, although the lesions were in other locations. This patient had severe postcaval compression from one of its hepatocellular carcinomas, and this may have been the cause of edema.

JPC Diagnosis: Exocrine pancreas: Hyalinizing pancreatic adenocarcinoma.

Conference Comment: The most notable characteristic of this neoplasm, and the feature that distinguishes it from the more common variants of canine exocrine pancreatic carcinoma (EPC), is the extensive extracellular deposition of homogenous to globular eosinophilic material.[1] This substance is consistent with the microscopic appearance of amyloid, however, as noted by the contributor, it is generally not congophilic or birefringent and is immunohistochemically negative for serum amyloid A, amylin, α1-antitrypsin and immunoglobulin light chain; the origin of this material remains unknown.[1] Interestingly, in this case, the application of Masson's trichrome stained the hyaline eosinophilic material blue.

Canine hyalinizing pancreatic adenocarcinoma is a well-differentiated, solitary mass most commonly seen in the right limb of the pancreas. The most frequent clinicopathological abnormalities are elevated serum amylase and lipase.[1] Hyalinizing pancreatic adenocarcinoma is a form of EPC, and the main differential diagnoses are other variants of EPC, such as anaplastic (undifferentiated) EPC or pancreatic acinar or ductal carcinomas.[4] Pancreatic acinar cell carcinomas are further classified histologically as well-differentiated, which are less invasive, or poorly-differentiated, which tend to metastasize or invade adjacent tissue. One recent report found that loss of expression of claudin-4, a tight junction integral protein normally expressed in canine pancreatic acinar cell membranes, may lead to cellular detachment, disorientation and invasion in poorly-differentiated EPCs. The same study suggested immunohistochemical staining for claudin-4 as a marker to distinguish well-differentiated from poorly-differentiated acinar cell carcinomas.[3] Canine hyalinizing pancreatic adenocarcinomas behave less aggressively than the other, more common variants of canine EPC. Dennis, et al. speculate that this more

benign behavior may be secondary either to the hyaline matrix material mechanically or biochemically impeding malignancy, or to the degree of tumor differentiation (or both).[1]

In the sections submitted for the conference, there is a diffuse loss of cellular detail and differential staining within the tumor, and because the contributor observed that some sections contained neoplastic emboli within lymphatics this led several conference participants to speculate that the entire neoplasm could be infarcted.

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