WEDNESDAY SLIDE CONFERENCE 2009-2010

Conference 4

30 September 2009

Conference Moderator:

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CASE I: Case II (AFIP 2936428).

Signalment: 7-year-old gelding quarter horse (*Equus caballus*).

**History:** Examined by a local veterinarian for skin lesions on the back, which were then biopsied.

Gross Pathology: Firm mass present on the back of the saddle region of horse.

Histopathologic Description: Microscopically, collagen degeneration with multifocal regions of dystrophic mineralization is observed in regions of the deep dermis with degranulating and degenerative eosinophils (**fig. 1-1**). Superficial and deep perivascular dermatitis with marked eosinophilia and smaller numbers of macrophages, lymphocytes and plasma cells are noted (**fig. 1-2**). Endothelial reactions (i.e. hyperplastic and hypertrophic endothelial cells) are present with infiltrates of eosinophils and severe edema. Thrombus (not present in all submitted slides) with chronic infiltrates of eosinophils, lymphocytes, macrophages, and marked fibroplasia is observed.

Contributor's Morphologic Diagnosis: Skin: Dermatitis, moderate, eosinophilic granulomatous with collagenolysis and mineralization, Quarter Horse (*Equus caballus*), equine.

Contributor's Comment: This lesion, frequently encountered in skin biopsies from asymptomatic horses, is known as nodular necrobiosis, nodular collagenolytic granuloma, acute collagen necrosis, or eosinophilic granuloma.[5] There is no apparent breed, age, or sex predisposition for this disease.[4,5] The etiology of this lesion is unknown, but a hypersensitivity reaction to an arthropod injury is suspected because the lesion occurs more commonly in warmer months.[5,7] Due to lesions commonly occurring in the saddle region, it also has been suggested that trauma may be a contributing factor.[4,5] Furthermore, atopy has been suggested as a predisposing factor based on positive skin test results in a horse with collagenolytic granulomas.[4] More than one causal agent is likely responsible for a seemingly identical clinicopathologic entity.[5]

With eosinophilic granulomas, single or multiple lesions, ranging from 0.5 to 10 cm in diameter, are most commonly found on the withers and back, but can also be seen in the girth area, the mane, the rump, and the face. The lesions are often round, firm, and well-circumscribed with no
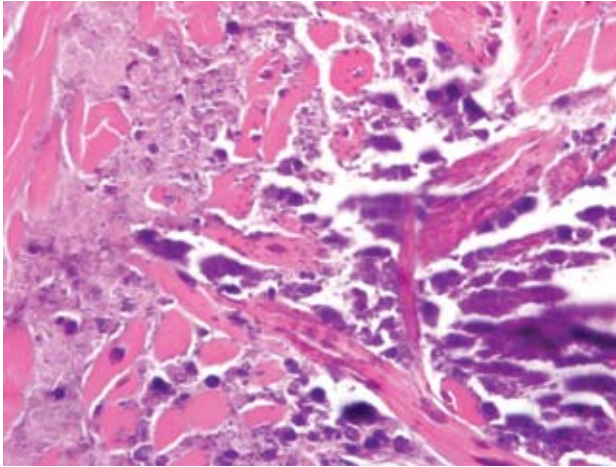

1-1. Dermis, horse. Multifocally dermal collagen fibers are degenerate or mineralized, and surrounded by numerous degenerate eosinophils and collagenolytic debris. (HE 400X)
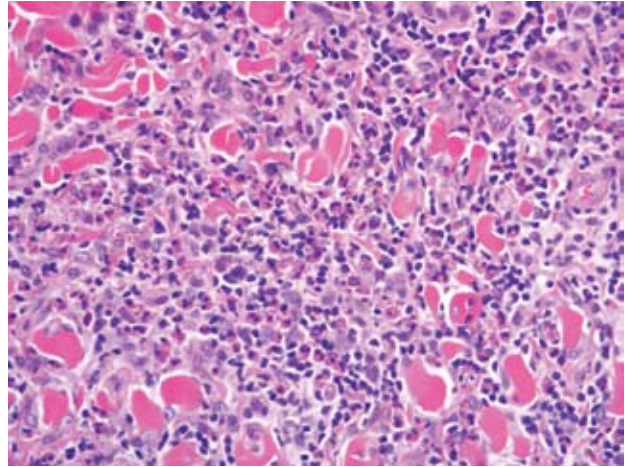
1-2. Dermis, horse. Multifocally surrounding and separating dermal collagen are nodular cellular infiltrates of high numbers of eosinophils and fewer macrophages, lymphocytes, and plasma cells. (HE 400X)
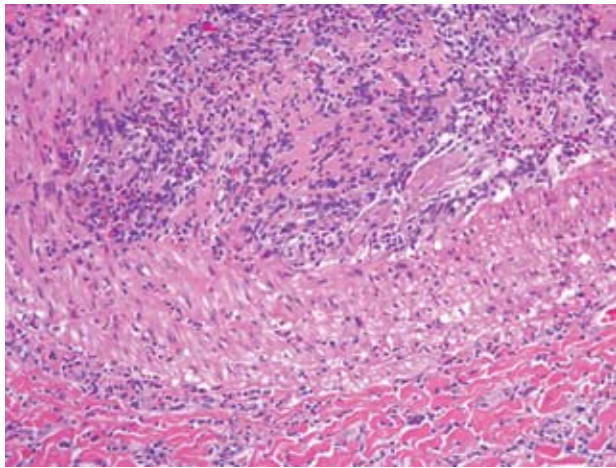
1-3. Dermis, horse. Multifocally occluding arteries are organizing fibrin thrombi which are composed of lamellations of collagen, eosinophils, and fibroblasts. The tunica intima and tunica media of affected vessels is disrupted by transmigrating eosinophils; myocytes within the tunica media are often degenerate. (HE 200X)
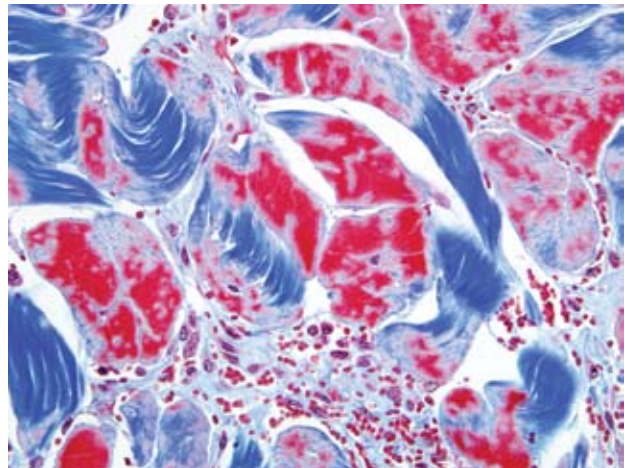
1-4. Dermis, horse. Masson's trichrome staining revealed abnormal, red-staining collagen cores in over 90% of the dermal collagen bundles. (Masson's 400X)

ulceration or alopecia. Pain and pruritus are not present. Some lesions can be cystic or plaque-like with a central caseous core.[4,5]

Massive central necrosis consisting of degranulating, degenerative eosinophils is a key element of this lesion.[6] The amount of collagenolysis varies among lesions. To date, the reason for the absence of collagenolysis in some lesions is not known. Collagen degeneration has been hypothesized to be the result of toxic products derived from degranulating eosinophils, such as major basic protein.[6] Some more chronic lesions can present with dystrophic mineralization and thus be misdiagnosed as calcinosis circumscripta or tumoral calcinosis.[5]

Histologically, the lesions of nodular collagenolytic granulomas (eosinophilic granulomas) need to be differentiated from those of habronemiasis and hypoderma nodules.[7] Cutaneous habronemiasis (summer sores) is caused by the larval activity of three nematodes that inhabit

the horse's stomach, especially *Draschia megastoma*, during the summer months. The larvae are deposited on the skin by flies, which are attracted to a pre-existing wound. Lesions are particularly common in the skin of the pectoral region, between the forelegs. The larvae penetrate deeply into the dermis and elicit an eosinophilic and granulomatous response. The alopecic ulcerated skin becomes encrusted by a serous exudate that oozes from the surface.[3] In hypodermiasis, by contrast, there is the presence of a breathing pore for the parasite. Cutaneous habronemiasis was considered as the primary differential diagnosis; however, due to the absence of larvae in the sections, the lack of skin ulceration and alopecia, and the presence of degenerate collagen, eosinophilic granuloma (nodular collagenolytic granulomas) was diagnosed.

AFIP Diagnosis: Haired skin and subcutis: Dermatitis, eosinophilic and granulomatous, multifocal to coalescing, moderate, with collagenolysis, mineralization, and eosinophilic arteritis.

Conference Comment: Conference participants discussed the diagnostic approach to dermatitis in domestic animals, beginning with a brief review of pattern analysis and its diagnostic utility in dermatopathology. Participants discussed the following ten non-neoplastic

reaction patterns:[1]
1. Perivascular dermatitis
2. Interface dermatitis
3. Vasculitis
4. Nodular and diffuse dermatitis
5. Intraepidermal vesicular and pustular dermatitis
6. Subepidermal vesicular and pustular dermatitis
7. Perifolliculitis, folliculitis, and furunculosis
8. Fibrosing dermatitis
9. Panniculitis
10. Atrophic dermatitis

In addition to the entities well-described by the contributor (i.e. eosinophilic granuloma, habronemiasis, and hypodermiasis), the differential diagnosis for eosinophilic dermatitis in horses includes mast cell tumor, cutaneous pythiosis, multisystemic eosinophilic epitheliotropic disease, sterile eosinophilic folliculitis and furunculosis, unilateral papular dermatosis, and axillary nodular necrosis. For most of these, differentiation from eosinophilic granuloma is relatively straightforward; however, axillary nodular necrosis and unilateral popular dermatosis bear many histomorphologic similarities to eosinophilic granuloma, often creating a diagnostic challenge. These conditions were discussed at length, and their key features are summarized in the table below:[1,5]

Condition	Clinical and Gross Findings	Histomorphology
Eosinophilic granuloma (nodular necrobiosis, collagenolytic granuloma)	Most common equine cutaneous eosinophilic nodular disease; 0.5-10 cm diameter nodules anywhere on the body (often withers and back); trauma or hypersensitivity to insect bites or silicone coating on needles suspected	Dermal collagen flame figures surrounded by eosinophilic granulomatous inflammation; +/- eosinophilic folliculitis or furunculosis; +/- dystrophic mineralization; +/- lymphoid follicles; true collagen degeneration is rare
Axillary nodular necrosis	Usually unilateral; few (3-10) cutaneous and subcutaneous, 1-10 cm diameter nodules; truncal, caudal to axilla ("girth galls")	Eosinophilic coagulative necrosis surrounded by eosinophilic granulomatous dermatitis and panniculitis; dermal collagen flame figures; dystrophic mineralization of collagen; lymphoid nodules; eosinophilic or necrotizing vasculitis with endothelial cell hypertrophy, vessel lumen occlusion, and intimal mucinosis
Unilateral papular dermatosis	More common in spring and summer; Quarter Horses over-represented; numerous (30 to 300) unilateral, truncal, 2-10 mm diameter cutaneous nodules and papules; ectoparasite hypersensitivity suspected	Eosinophilic folliculitis and furunculosis; eosinophilic dermal inflammation; +/- dermal collagen flame figures

While the anatomic location is most consistent with eosinophilic granuloma, the vascular lesions observed by several conference participants in this case are strongly suggestive of axillary nodular necrosis (**fig. 1-3**). Throughout the deep dermis, all arterioles contain abundant, pale, basophilic, finely-granular subendothelial material (intimal mucinosis). Additional histologic findings present in some sections include hair shafts embedded in the deep dermis, recanalization of the thrombus in a large arteriole, few lymphocytes and plasma cells in the superficial dermis and periadnexally, and mild acanthosis, spongiosis, and orthokeratotic hyperkeratosis of the overlying epidermis.

Finally, conference participants discussed the terms "collagenolysis" and "flame figure". Collagenolysis or collagen necrobiosis, which implies true collagen degeneration, is the term classically used for the characteristic intense, acellular, eosinophilic material centered on collagen fibers in a variety of eosinophilic dermatoses. However, in many of these conditions Masson's trichrome staining and ultrastrucutural examination are inconsistent in demonstrating true collagen degeneration. Often, abnormal (i.e. red) staining of collagen fibers with Masson's trichrome is as likely to be present in areas of eosinophil degranulation as it is in other, apparently unaffected areas of the tissue section; therefore, many prefer the term "flame figure" to describe the histologic features, particularly when true collagen degeneration cannot be definitively demonstrated. In this case, Masson's trichrome staining revealed abnormal, red-staining collagen cores in over 90% of the dermal collagen bundles, but not areas containing flame figures (**fig. 1-4**). Major basic protein from degranulating eosinophils is now thought to be the source of the intense eosinophilic material seen in flame figures.[2]

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CASE II: HN2108 (AFIP 2943303).

Signalment: 6-year-old, male Siberian husky (*Canis familiaris*).

History: This dog was presented to the veterinary teaching hospital initially with hind limb paraparesis. Mild atrophy of hind limb muscles was also observed. Abdominal radiographs revealed severe calcification in the abdominal aorta. Despite treatment with levothyroxine, the rear legs became paralyzed. Gangrene of the extremities appeared and worsened. The dog was euthanized due to poor prognosis and necropsied. This animal had not been given a high-cholesterol diet.

Gross Pathology: 1. Systemic arteriosclerosis showing marked calcification in the arterial wall and luminal narrowing.
2. Thrombosis of the internal iliac, femoral, popliteal, and right superficial branchial arteries.
3. Atrophy of the pituitary and thyroid glands.
4. Dry gangrene in the tail, bilateral hind limbs and right forelimb.

Laboratory Results: Serum biochemical profile before starting levothyroxine treatment revealed elevated cholesterol, triglyceride, alkaline phosphatase and creatine kinase (total cholesterol, 1560 mg/dL; triglyceride, 596 m/dL; ALKP, 8434 U/L; CK, 1178 U/L) and subnormal levels of thyroid hormones (T3, 0.3 ng/mL; T4, 0.2 ng/mL; free T4, 0.3 ng/mL).

Histopathologic Description: Grossly, epicardial coronary arteries were prominently thickened, firm, yellow-white, and appeared as cord-like structures. Multiple whitish foci were recognized within the ventricular walls by incisions after formalin fixation. Histologically, massive deposition of cholesterol clefts and

calcification with infiltration of foamy macrophages was noted in the thickened wall of epicardial arteries (**figs. 2-1 and 2-2**). Staining for elastin demonstrated the structural disorganization, such as the destruction of internal and external elastic membranes, intimal thickening, and the disappearance of the medial smooth muscle layer. Small foci of granulation tissue were scattered in the ventricular wall (**fig. 2-3**). Atherosclerotic lesions were also found in many organs, including aorta, spleen, kidney, lung, prostate, and brain. Both elastic arteries and large to middle-sized muscular arteries were involved.

Contributor's Morphologic Diagnosis: Heart (left ventricle): Coronary atherosclerosis, with small multifocal myocardial infarcts, Siberian husky, canine.

Contributor's Comment: Atherosclerosis occurs only infrequently in animals and rarely leads to clinical diseases, such as infarction of heart or brain.[6] Naturally occurring atherosclerosis has been reported in aged pigs and birds, and in dogs with hypothyroidism that develop an accompanying hypercholesterolemia.[6] Hypothyroidism in most dogs results from progressive loss of functional thyroid tissues due to primary dysfunction of the gland.[2] Histological changes in the thyroid glands of this dog

were replacement of parenchyma by adipose tissue with the absence of inflammatory cell infiltrates. Acute degeneration and necrosis of secretory cells, including TSH immunoreactive cells, were observed in the adenohypophysis; however, the cause and relationship of this lesion with the thyroid atrophy were unclear.

AFIP Diagnosis: Heart: Coronary arterial atherosclerosis, transmural and circumferential, diffuse, marked, with mineralization and multifocal chronic myocardial infarcts.

Conference Comment: Conference participants engaged in a lively discussion regarding the areas of myocardial infarction in the submitted sections. Specifically, opinions on the age of the lesions varied widely, with participants who felt the lesions consisted of immature granulation tissue favoring a subacute process, while those who felt there was increased collagen deposition favored a chronic process. We consulted with the AFIP Department of Cardiovascular Pathology and estimate the infarcts to be over one month old. The evolution of the histomorphologic changes in myocardial infarction in humans is well-characterized, and is summarized below:[5]

Time	Light Microscopic Findings
0 to 0.5 hr	None
0.5 to 4 hrs	None, or myofiber waviness at the border of the lesion
4 to 12 hrs	Early coagulation necrosis with hemorrhage and edema
12 to 24 hrs	Coagulation necrosis; myocyte hypereosinophilia and pyknosis, contraction band necrosis; early neutrophilic infiltrate
1 to 3 days	Coagulation necrosis; loss of cross striations and loss of nuclei; increased neutrophilic infiltrate
3 to 7 days	Early myofibers disintrigration; necrosis of neutrophils; macrophages at the border of the lesion
7 to 10 days	Increased macrophage infiltrate with phagocytosis of necrotic cells; early granulation tissue at infarct margins
10 to 14 days	Well-developed granulation tissue
2 to 8 weeks	Increased collagen deposition; decreased cellularity; regression of granulation tissue capillaries
>2 months	Dense collagenous scar

In addition to hypothyroidism-induced hypercholesterolemia, diabetes mellitus is often associated with atherosclerosis in dogs. In one study, dogs with atherosclerosis were over 53 times more likely than dogs without atherosclerosis to have diabetes mellitus, and over 51 times more likely to have hypothyroidism than dogs

without atherosclerosis, but were not more likely to have hyperadrenocorticism than dogs without athoersclerosis.[1] Dogs are otherwise considered relatively atheroresistant, as are cats, cattle, goats, and rats. While this condition is only rarely clinically significant in domestic animals, nonhuman primates and pigs are the most popular animal
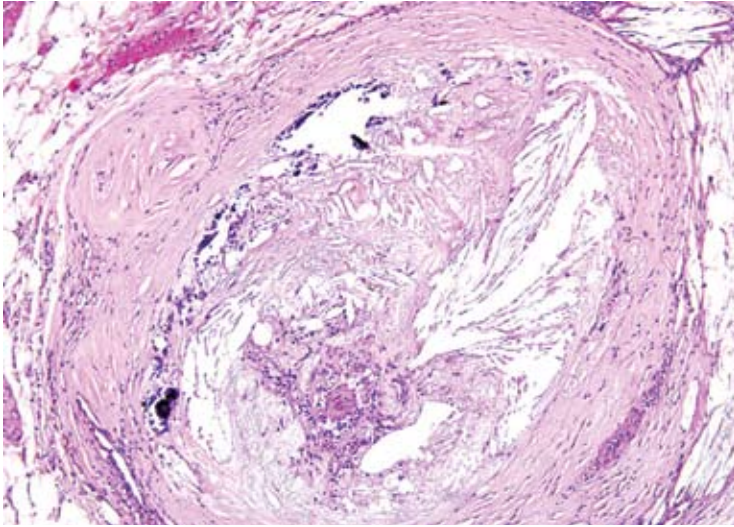
2-1. Heart, dog. Within extramural coronary arteries, the tunic media is markedly expanded and the vessel lumens are nearly or completely occluded. The tunica media is disrupted by acicular clefts and mineralization. (HE 100X)
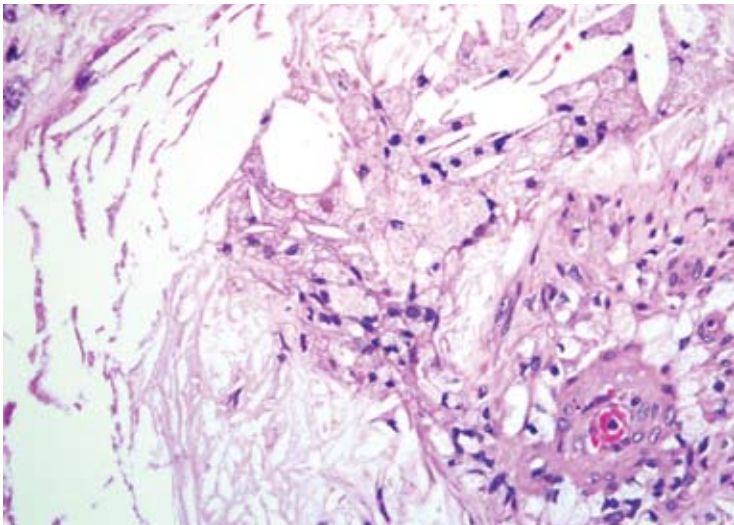
2-2. Heart, dog. The tunica media of affected vessels is expanded by many lipid-laden macrophages (foam cells), myofibroblasts, and large, clear, acicular clefts (cholesterol). (HE 400X)
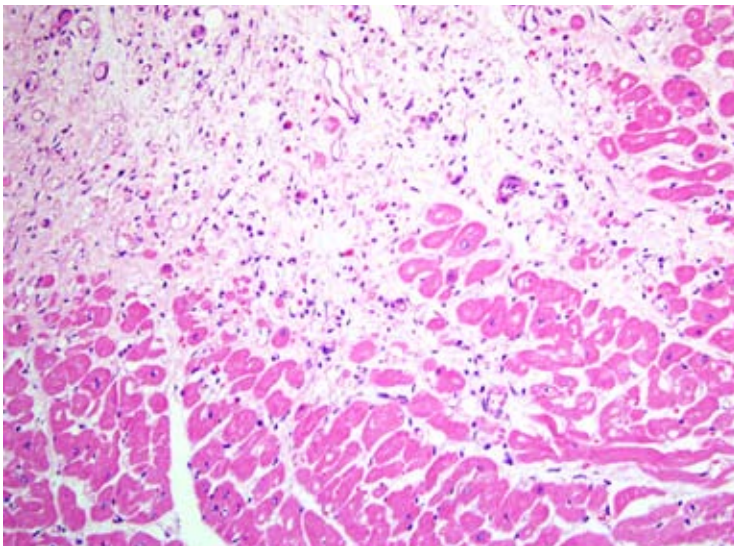
2-3. Heart, dog. Multifocally within the surrounding myocardium are large zones of regressing mature granulation tissue and chronic fibrosis rimmed by occasional degenerate and necrotic myocytes (chronic infarct). (HE 200X)

models of human disease. As atherosclerosis is a significant cause of human mortality, it merits considerable research interest. Pigs, rabbits, and chickens are atherosensitive, and develop the disease in response to high dietary cholesterol intake, particularly when the proportion of very low density lipoproteins (VLDL) is increased.[3]

The pathogenesis of atherosclerosis in humans is well-studied, and the currently-accepted model, the response-to-injury hypothesis, characterizes atherosclerosis as a chronic response of the arterial wall to endothelial injury. An exhaustive review of the pathogenesis is beyond the scope of this report. Briefly, hypothyroidism or diabetes mellitus results in dyslipoproteinemia (i.e. increased low density lipoprotein (LDL) cholesterol, decreased high density lipoprotein (HDL) cholesterol, and/or increased levels of abnormal lipoprotein (a)). This causes endothelial damage by a variety of mechanisms, including oxygen free radical production that speeds the decay of nitric oxide and decreases its vasodilator activity. The damaged endothelium allows lipoproteins to accumulate in the intima, where they are oxidized by free radicals generated by macrophages and endothelial cells. Oxidized LDLs, which are directly toxic to endothelium and smooth muscle cells, are ingested by macrophages

and smooth muscle cells, which become characteristic so-called "foam cells". Damaged endothelium also allows the adhesion of platelets and expresses vascular cell adhesion molecule 1 (VCAM-1), which binds monocytes and T lymphocytes. Platelets and migrating leukocytes elaborate platelet-derived growth factor, interleukin-1, and interferon-gamma, which promote smooth muscle proliferation and extracellular matrix synthesis.[4] The resulting characteristic histologic lesion is the deposition of lipid in the vessel wall (i.e. "atheroma"), which is overlain by fibrosis and mineralization. The lesion in dogs differs from that of humans with respect to the location of lipid, which is primarily within the tunica media and adventitia in dogs, but primarily within the tunica intima in humans. With chronicity, atheromas may cause luminal narrowing and/or ulceration, leading to thrombosis, hemorrhage, or aneurysm.[3]

Most affected are the aorta, iliac, carotid, coronary, and femoral arteries. Grossly, affected vessels are enlarged and firm, with prominent yellow-brown nodules that project into the lumen. Other degenerative arterial diseases in domestic species include arteriosclerosis, and medial calcification, summarized in the table below.[6]

Name	Clinical and Gross Findings	Histomorphology
Atherosclerosis	Pigs, rabbits, chickens are atherosensitive; dogs, cats, cattle, goats, and rats are atheroresistant; affects large elastic and large and medium muscular arteries; seen most commonly in dogs with hypothyroidism or diabetes mellitus; gross = vessel wall thickened and firm with yellow-brown nodules projecting into the lumen	Fibrofatty plaque or atheroma in the vessel wall (tunica intima and media) and lipid-laden macrophages and/or smooth muscle cells (i.e. "foam cells") in the media and intima
Arteriosclerosis	Occurs in many species but rarely clinically significant; age-related, degenerative change; means "arterial hardening"; results in loss of elasticity; abdominal aorta most frequently affected; also occurs at arterial branching sites (turbulent blood flow); gross = raised, firm, white plaques	Intimal thickening by mucopoly-saccharides (early) or medial thickening by proliferating smooth muscle cells and fibrosis that infiltrates into the intima (later); splitting of the internal elastic lamina
Arterial medial calcification	Common; affects elastic and muscular arteries; caused by certain plant toxins (e.g. *Cestrum diurnum, Solanum malacoxylon, Trisetum flavescens*), vitamin D toxicosis, renal disease (aged guinea pigs and rats), and paratuberculosis; spontaneous in rabbits; gross = solid, pipe-like vessels with white, solid, raised intimal plaques	Prominent, granular, basophilic material on medial elastic fibers; form a circumferential ring of mineralization in muscular arteries

In horses, intimal bodies in small arteries and arterioles, and siderocalcinosis of cerebral vessels are usually incidental findings of no clinical significance.(3,6)

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CASE III: V07-00718 (AFIP 3067153).

Signalment: 9-year-old, male castrated domestic shorthair cat (*Felis catus*).

History: The cat had a history of anorexia, weight loss, mild anemia and dyspnea. The animal had been treated with antibiotics without improvement.

Gross Pathology: The cat was in poor body condition. Multifocally in the lung and spleen, there were numerous, 3-6 mm diameter, firm, white nodules.

Laboratory Results: Aerobic culture from the lung

and spleen was negative. Fluorescent antibody test (FA) for plague was negative.

Histopathologic Description: There are multifocal to coalescing foci where alveoli are filled with numerous epithelioid macrophages and smaller numbers of neutrophils, lymphocytes, and a few plasma cells. Many of the macrophages contain small numbers of cytoplasmic 2-5 micron diameter yeast that have a 1-3 micron diameter central basophilic body surrounded by clear halo (capsule) (**fig. 3-1**). The interstitium of the lung is variably infiltrated by mixed leukocytes.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, granulomatous / pyogranulomatous, multifocal to coalescing, moderate with intrahistiocytic yeast, domestic shorthair, feline (*Felis catus*), etiology consistent with Histoplasma capsulatum.

Contributor's Comment: Histoplasmosis is caused by *Histoplasma capsulatum*, which is an intracellular parasite of the monocyte and macrophage system and involves the reticuloendothelial system.[7] The organism is a soil-borne dimorphic fungus, and in the environment it exists as a mycelial form that produces small spores (2-3 um in size, microconidia) and large, thick-walled chlamydospore with surface projections (8-12 um, macroconidia). In the parasitic phase inside the body it transforms to a yeast-like form. In the soil, the organism appears as white-brown mold.[3] The organism prefers warm and humid areas, and proliferates best in nitrogen-rich soil.[2] A relationship has been established between bird and bat excrement and occurrence of the disease.[3,7]

The disease affects a wide variety of mammals.[3] In North America, histoplasmosis is often diagnosed in the Mississippi, Ohio, Missouri River areas. The disease is mostly non-contagious in humans, dogs, cats, swine, horse, wild animals, and cattle. In companion animals, it is most often found in dogs and less frequently in cats.[7] True rate in companion animals is difficult to measure due to subclinical infections.[2] The infection is initiated by inhalation or ingestion of dust from soil. The majority of infections occur without signs and lesions, whereas when infection becomes clinically apparent, it is disseminated, and always fatal.[7] It is known now that in humans and lower animals, an acute, nonfatal form is more prevalent than the disseminated, fatal, rare form. The infection is connected to common environmental source rather than contagion from host to host.[3]

The genus *Histoplasma*, besides the three conventionally accepted species (*capsulatum*, *duboisii*, and *farciminosum*), was reported to have eight clades from different geographic
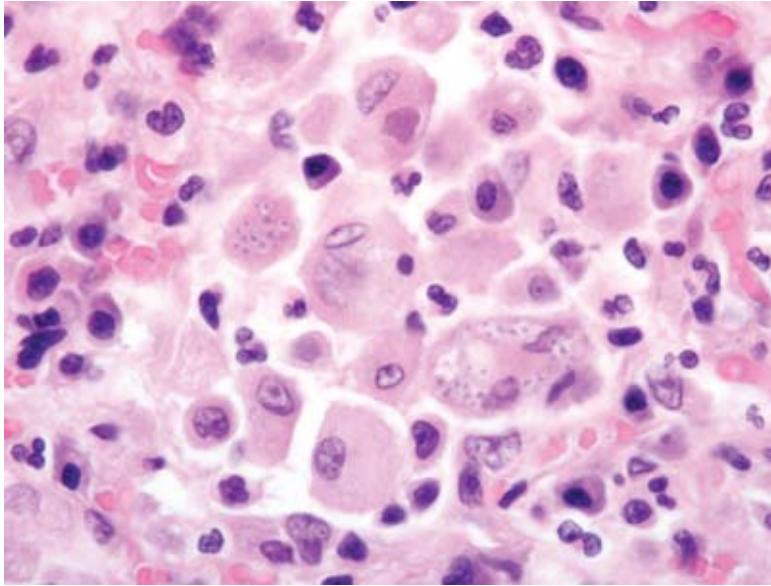
3-1. Lung, cat. Multifocally within alveoli are large numbers of alveolar macrophages which contain up to 20 circular yeasts which are 3-4um in diameter with a 1um basophilic central nucleus and a 1um thick clear halo. (HE 1000X)

regions, suggesting that genetically distinct geographical populations exist.[1,4] Genetic polymorphisms in the same areas were 100% similar.[6] *Histoplasma capsulatum* is the cause of classic histoplasmosis worldwide. *H. duboisii* is the cause of African histoplasmosis and *H. farciminosum* is the cause of epizootic lymphangitis in horses. This is characterized by chronic indurative ulceration of the skin with enlargement of regional lymph nodes.[3]

When inhaled, the microconidia are able to reach the lower respiratory tract. The incubation period is usually 12-16 days, during which the microconidia transform into yeast phase and reproduce by budding. The yeast is phagocytosed by mononuclear phagocytes and replicates further inside the cell.[2] This route accounts for the infection in the cervical and bronchial lymph nodes, but the usual intestinal lesions were also suggested to occur directly via ingestion, or by secondary infections like in the case of many other organs. The latent infections might persist without causing illness for months to years in different species. The signs of advanced disease include diarrhea, pyrexia, emaciation, hepatomegaly, splenomegaly, a lymphadenopathy, and a nonregenerative, normochromic, normocytic anemia. In later stages the leukocyte counts are low, and toxic changes occur, such as the appearance of Doehle bodies. The disease usually causes lymphopenia and eosinopenia. Diagnosis can be made from fine-needle aspirations of liver, spleen, enlarged lymph nodes, bone marrow, or skin with microscopic examination. Cytologically, the organism can be detected in the cytosol of macrophages.[7]

If the spore dose is high or the host's immune system is compromised, the infection can cause severe disease. The host's cellular immune system, mainly involving cytokine-mediated macrophage killing, can control the infection. In some instances, the infection is not completely cleared and if immune suppression takes place, a reactivation can occur. The yeast form is more resistant to host defense due to its more invasive nature as it can severely impair phagocyte function. Moreover, the fungus can induce nonspecific anergy by overproducing interleukin-4, thus interfering with cellular immune response.[2]

In dogs, the primary disease in the lung appears as classic granulomas containing epithelioid and multinucleated giant cells carrying the organisms. After recovery, these regress to fibrocalcareous nodules present in the lungs for many years. Sometimes, similar lesions may be found in other organs.[3] Pathologically, the pulmonary lesions are 1-2 cm grayish nodules.

In the intestines, the lesions most frequently occur in the lower part of the small intestine as nodular thickenings of the mucosa in the lamina propria and submucosa that are the result of infiltration of lymphocytes, plasma cells and macrophages. In rare instances, ischemic ulcerations might occur when the thickening is extreme.[7] In the intestine the lymph nodules and adjacent lymph nodes are greatly enlarged.[3]

Lymph nodes are firm and dry and greatly enlarged. In histological preparations, coalescing granulomas that replace portions of the cortex can be seen.[7] In lymph nodes, the predominant infiltrating cell type is histiocytic, with a less frequent plasma and lymphoid cell presence.[3] The spleen is also enlarged, grey, and firm, characterized by sinus expansion and colonization with macrophages

containing the organism.

The liver becomes enlarged, and diffuse grey discoloration, due to capsular thickening without focal lesions, occurs.[7] Liver enlargement is caused by diffuse interlobular and intralobular proliferation of mononuclear phagocytes, leading to displacement of liver parenchyma, and causing liver dysfunction.[3]

The disease often involves the adrenal gland's cortex, medulla, or both.[7] The involvement of adrenal glands in fatal cases is very striking, characterized by the replacement of the gland by macrophages. This phenomenon is most likely connected to the terminal stage of the disease, as it is not seen in animals sacrificed earlier.[3]

The organ enlargements are caused by intense infiltration of extensively proliferating monocytes and epitheloid macrophages carrying the organisms in their cytoplasm. The 2-4 um yeast bodies appear as basophilic dots surrounded by a halo (part of the cell wall) on sections stained with eosin and hematoxylin. The halo can be stained for bound glycogen, appearing as a ring, that helps distinguish the organism from cellular debris.[7] The cell wall can be stained selectively by PAS, Bauer, GMS or Gridley fungus method, resulting in a red or black ring appearance.[3]

When the organism is present in abundance, the staining is not necessary. However, in case of occult infection, isolation of organism from tissues must be done for diagnosis. Care must be taken during isolation to prevent inhalation of chlamydospores. The organism can only be held responsible for focal, nonprogressive lesions if it can be histologically demonstrated from tissue sections. For biopsy, enlarged lymph nodes or aspiration biopsy of bone marrow can be used.[7] Also, biopsies of tonsils and liver, which are places of extensive mononuclear phagocyte proliferation, or in certain cases, smears of circulating blood, can be used. Serologic tests are not reliable.[3]

Differential diagnosis is necessary from *H. farciminosum* by culturing the organism, as in tissue sections they appear the same, though the geographic and anatomical locations of the two diseases can reduce this difficulty. Differentiating from other protozoan and mycotic organisms can be done by immunologic staining techniques or can be based on morphological differences. *Leishmania donovani* contains a bar-shaped kinetoplast that is not present in histoplasmosis. *Toxoplasma gondii* is smaller and does not have rings when stained for bound glycogen. *Blastomyces dermatitidis* yeasts are larger and the budding base is different. Differentiating from *Sporothrix schenckii* requires immunologic staining. Some malignant neoplasms such as lymphoma can lead to uptake of tissue debris by macrophages; however, in these cases the absence of organism can differentiate from histoplasmosis.[3]

Public Health considerations: Animals and people traveling through endemic areas might be at risk. Direct host-to-host transmissions have not been reported. Also, transplanting kidneys of infected donor can carry the disease to the host.[2] In humans, the disease can affect people with AIDS or on immunosuppressive therapy. The disease can be the result of reactivation from a latent infection in both humans [8] and dogs.[5] (Reviewed in 1)

AFIP Diagnosis: Lung: Pneumonia, pyogranulomatous, multifocal to coalescing, moderate to marked, with numerous intrahistiocytic yeasts.

Conference Comment: In some sections there is pyogranulomatous pleuritis and/or reactive pleural mesothelium. The contributor provided a thorough overview of this entity and the differential diagnosis. Currently, the accepted nomenclature for the organisms described above is *Histoplasma capsulatum* var. *capsulatum, H. capsulatum* var. *farciminosum*, and *H. capsulatum* var. *duboisii*.[2,7]

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http://128.123.206.6/animal-and-plant-protection/veterinary-diagnostic-services

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CASE IV: CASE I (AFIP 3134286).

Signalment: 1-year and 11-month-old female Springer spaniel (*Canis familiaris*).

History: The dog collapsed and died after having shown declining exercise tolerance over 5 weeks and tachypnea, dyspnea, inappetence and pyrexia of several days duration.

Gross Pathology: The thoracic cavity contained approximately 100 mL of hemorrhagic turbid fluid. The mediastinum and parietal pleura were markedly thickened with velvety proliferations, dull and reddish discolored. Multiple small yellow soft granules, which measured up to 0.3 cm in diameter, were present within the pleural tissue (interpreted as sulfur granules). The lungs were decreased in size and the overlying visceral pleura was wrinkled (interpreted as compression atelectasis) (**figs. 4-1 and 4-2**). Tracheobronchial lymph nodes were enlarged, measuring up to 6 x 3 x 3 cm.

Laboratory Results: Pleural tissue and a sterile swab obtained from the pleural effusion were submitted for bacteriology. A heavy mixed growth of *Actinomyces viscosus* and a *Bacteroides* sp. was isolated from the pleural fluid. Bacteriology on the pleural tissue revealed a moderate mixed growth of *Actinomyces viscosus* and a *Bacteroides* sp.

4-1, 4-2. Thorax and thoracic pluck, dog. The mediastinum and parietal pleura are dull red and markedly thickened with velvety proliferations (asterisk). There are multiple yellow soft granules which measure up to 0.3 cm in diameter within the pleural tissue, and lungs are decreased in size with thickened and wrinkled pleura (arrowhead). Photograph courtesy of The Royal Veterinary College, Department of Pathology and Infectious Diseases, Hawkshead Lane, North Mymms, Hatfield, Herts AL97TA, United Kingdom, sschoeniger@rvc. ac.uk
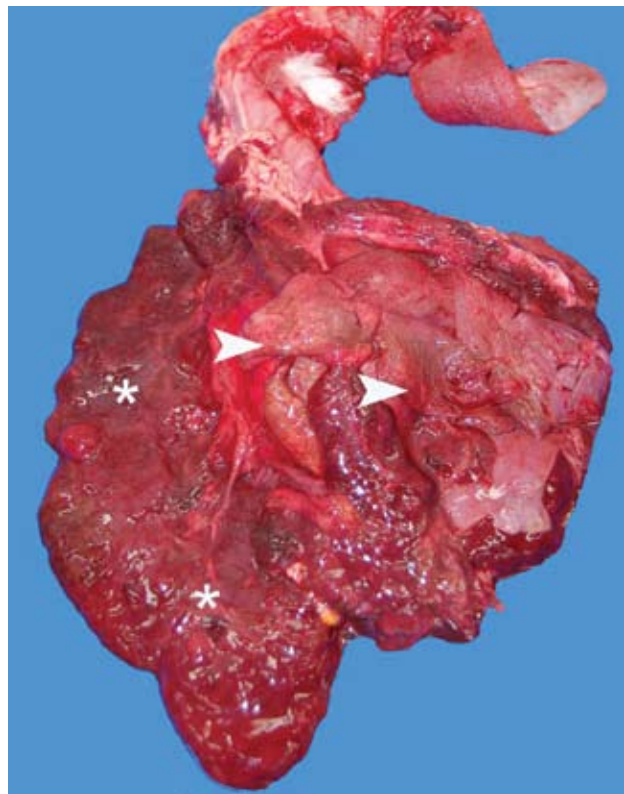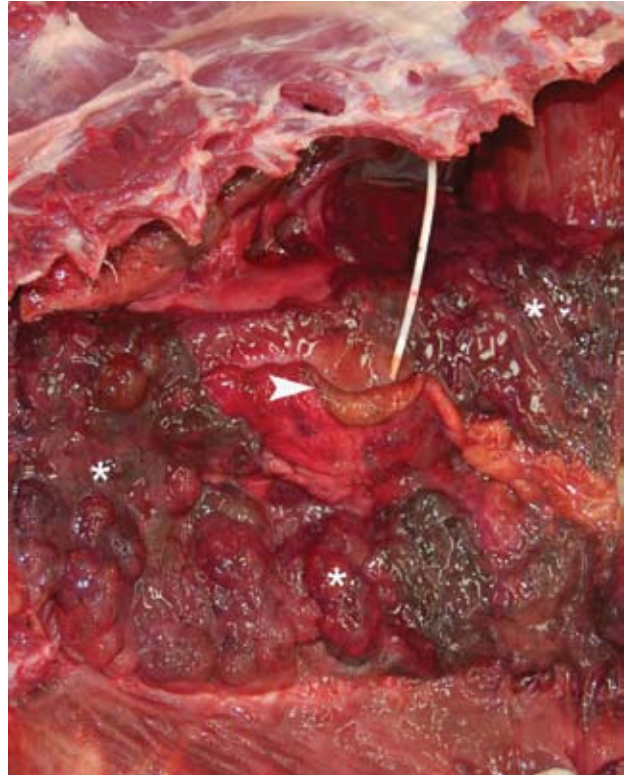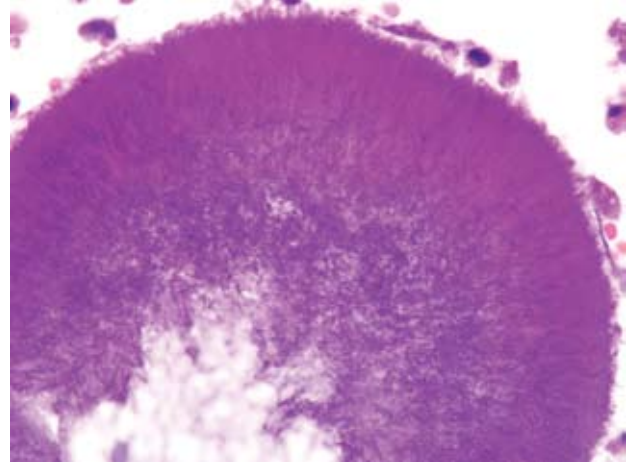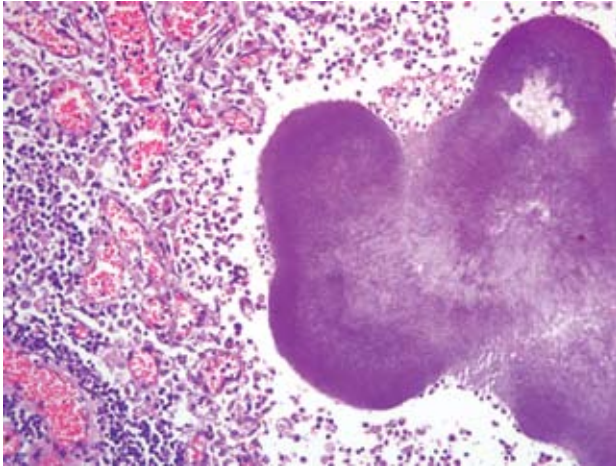
4-3, 4-4. Pleura, dog. Pleura are markedly expanded by abundant granulation tissue and pyogranulomatous inflammation, often centered on large colonies of filamentous bacteria which are embedded in a brightly eosinophilic proteinaceous material. (HE 400X, HE 1000X)

Histopathologic Description: **Parietal pleura:** The pleura was diffusely expanded by infiltration with numerous histiocytes and neutrophils (pyogranulomatous inflammation), which surrounded intralesional large bacterial colonies composed of radiating filamentous bacteria. In addition, the pleura contained numerous small-sized congested vessels, mild fibroblast proliferation and multifocal infiltration with lymphocytes and plasma cells (**figs. 4-3 and 4-4**).

Lungs: The visceral pleura was expanded by infiltration with numerous lymphocytes, plasma cells, epithelioid macrophages and scattered neutrophils, and contained numerous small-sized congested vessels and mild fibroblast proliferation. In the subpleural parenchyma, alveoli were lined by cuboidal cells (hyperplasia of type II pneumocytes). Scattered alveoli contained intraluminal macrophages and/or a few erythrocytes. Numerous alveolar spaces were decreased in size (atelectasis). There was mild anthracosis. Some pulmonary capillaries contained intraluminal megakaryocytes.

Contributor's Morphologic Diagnosis: 1. Parietal pleura: Proliferative and pyogranulomatous pleuritis with intralesional large colonies of filamentous bacteria.
2. Lungs: Pyogranulomatous pleuritis and pulmonary atelectasis.

Contributor's Comment: The main pathological finding was proliferative and pyogranulomatous pleuritis with intralesional large colonies of radiating filamentous bacteria. Intralesional large colonies of filamentous bacteria can be observed in Actinomycosis and Nocardiosis. In

the present case, a mixed growth of *Actinomyces viscosus* and a *Bacteroides* sp. was cultured from the pleura and the thoracic effusion.

Infection with either *Actinomyces* or *Nocardia* is associated with similar clinical signs and macroscopic and microscopic pathological findings.[10] Accurate ante mortem diagnosis of the agent involved by bacterial culture and/or molecular methods is important, since *Actinomyces* and *Nocardia* spp. require different antibiotic treatments.[3]

Actinomyces spp. are anaerobic or facultative anaerobic, Gram-positive filamentous bacteria, which are non-acid-fast.[3,7,13] They are commensal bacteria of the mucosa of the oral cavity, urogenital-, upper respiratory- and gastrointestinal tracts. Certain *Actinomyces* spp., e.g. *A. hordeovulneris*, are saprophytes, which are mostly attached to plant material such as grass awns.[3,7,10,12,13] Actinomycosis is caused by either endogenous or exogenous infection.[3,13] Endogenous infections are initiated by mucosal injury, through which *Actinomyces* spp. gain access to the underlying soft tissues. Exogenous infections result from contaminated bite wounds and inhaled or ingested grass awns with attached bacteria. The grass awns can migrate in tissues causing infections at distant sites.[3,7,13] Within infected tissues, *Actinomyces* usually spreads by direct extension; hematogenous dissemination is rare.[7] Bacterial spread is facilitated by proteolytic enzymes released from neutrophils and macrophages.[7] Actinomycosis in dogs usually causes (sub)cutaneous and intracavitary infections.

Subcutaneous lesions are often located in the cervico-

facial or interdigital areas. Regional lymphadenopathy may be present. Intracavitary infections are mostly located in the thoracic cavity; infections of the abdominal cavity and the retroperitoneal space are uncommon.[4,7,10,12] In dogs, *Actinomyces* has also been reported as a rare cause of meningoencephalitis and endophthalmitis.[6,2] *Actinomyces*-induced pyogranulomatous meningo–encephalitis has been reported in a 1-year-old German Shepherd dog without history of trauma or involvement of other organs by *Actinomyces* infection.[6] A migrating foxtail was considered as a possible cause for the meningoencephalitis.[6] Concurrent endophthalmitis and pneumonia due to Actinomyces infection have been described in a Rottweiler dog.[2] *Actinomyces* spp. were also isolated from canine corneas with ulcerative keratitis.[11]

Diseases caused by infection with *Actinomyces* spp. in other species include osteomyelitis of the mandible and maxilla in cattle (lumpy jaw; *A. bovis* and *A. israelii*), supra-atlantal ("poll evil") and supraspinal ("fistulous withers") bursitis of horses (*A. viscosus* and *A. bovis* together with *Brucella suis* or *Brucella abortus*), mastitis, abortion, pneumonia, cystitis and pyelonephritis in pigs (*A. bovis, A. suis*) and abscesses in brain and temporal bone in a goat.[1,3,8,13]

Actinomyces spp. are often isolated together with other commensal bacteria [7], particularly *Bacteroides* spp., *Escherichia coli* and *Fusobacterium* spp. The presence of additional bacteria results in an increased pathogenicity, since it facilitates the formation of bacterial aggregates, which are relatively resistant against phagocytosis and bactericidal enzymes released by inflammatory cells. Bacterial aggregates are formed by the attachment of fimbriae present on the surface of *Actinomyces* spp. to surface receptors of other bacteria.[7]

In comparison to *Actinomyces* spp., *Nocardia* spp. are aerobic and partially acid-fast Gram-positive bacteria, which exist in coccobacillary (resting phase) to filamentous (active growing phase) forms.[3] Infection with *Nocardia* is always exogenous; *Nocardia* spp. are present as saprophytes in the environment, where they are attached to soil, dust and plant material. Infection can be acquired by ingestion, inhalation and wound contamination.[3,7,13] In cattle, mastitis is usually caused by infection through the teat canal.[13] The primary localized infection may be followed by hematogenous dissemination.[3,7,13] The most common agent for nocardiosis in dogs is *N. asteroides*.[7] *Nocardia* infection causes three main disease manifestations: (sub)cutaneous, thoracic, and disseminated.[13] The (sub)cutaneous form is usually associated with lymphadenopathy of regional lymph nodes.[10] The thoracic form is characterized by the

pleuritis and/or pneumonia.[3,13] Disseminated nocardiosis develops often secondary to the pulmonary disease.[7] Nocardial infections in other species include mastitis and abortion in cattle; pneumonia, lymphadenitis and abortion in pigs; and wound infection, mastitis and pneumonia in sheep, cattle and horses.[3,13]

The pathogenicity of *Nocardia* spp. is dependent on bacterial factors (strain and growth phase) and host immunity. Certain strains of *Nocardia* are more virulent due to an ability to survive in phagocytic vacuoles of neutrophils and macrophages.[3,7] Disease caused by *Nocardia* spp. is often associated with immune suppression or heavy bacterial exposure.[3,7]

Since infections with *Actinomyces* and *Nocardia* spp. cause similar lesions, the distinction between actinomycosis and nocardiosis is not possible based on macroscopic and microscopic pathological findings.[3,7,10] Macroscopically, (sub)cutaneous actinomycosis and nocardiosis are characterized by the presence of skin edema and inflammation. Ulceration and draining tracts are common.[10] In the thoracic form, the parietal and visceral pleura are inflamed and thickened by velvety proliferations. Similar lesions may be present within the mediastinum or pericardial sac. The thoracic cavity often contains a reddish-brown turbid effusion due to accumulation of sanguinopurulent fluid, which can cause compression atelectasis of the lungs.[3,5,13] Grossly, infected tissues might contain yellowish granules measuring about 0.1 cm in diameter ("sulfur granules", tissue grains). Sulfur granules are common in actinomycosis, but rare in nocardiosis.[3,9,10] Occasionally sulfur granules might be observed in infection with *N. caviae* and *N. braziliensis*, whereas they are usually absent in infection caused by *N. asteroides*.[10]

The microscopic hallmark of nocardiosis and actinomycosis is pyogranulomatous inflammation with intralesional large colonies of filamentous bacteria.[5] If the infection exists over a longer time, granulation tissue formation, fibrosis and/or infiltration with lymphocytes and plasma cells is usually present. A modified acid-fast stain (e.g. Fite-Faraco modification) will stain *Nocardia* spp., but not *Actinomyces* spp.[3] On cytological and histopathological examination, the sulfur granules are composed of large bacterial colonies surrounded by eosinophilic clubbed material. The eosinophilic clubbed material is considered to be caused by an antigen-antibody reaction (Splendore-Hoeppli reaction).[6,9,10]

Proliferative pleuritis with reddish-brown turbid thoracic effusion is diagnostic for actinomycosis and nocardiosis. Skin lesions of actinomycosis and nocardiosis have to be

differentiated from bacterial pseudomycetoma, which is also characterized by skin edema and pyogranulomatous inflammation. Ulceration, draining tracts and tissue grains might be present as well. In contrast to actinomycosis and nocardiosis, the intralesional bacterial colonies in pseudomycetoma are composed of non-filamentous Gram-positive or Gram-negative bacteria surrounded by Splendore-Hoeppli reaction. Possible causes of bacterial pseudomycetoma are *Staphylococcus, Streptococcus, Proteus* spp. and *Pseudomonas* spp.[10]

An incidental finding in this case, unrelated to the actinomycosis, was the presence of megakaryocytes in some pulmonary capillaries. Megakaryocytes can occasionally be observed within pulmonary capillaries of different animal species. It has been shown that megakaryocytes can exit the intact bone marrow and arrest in pulmonary capillaries, where they can release platelets in the circulation.[14]

AFIP Diagnosis: Lung: Pleuritis, proliferative and pyogranulomatous, diffuse, chronic, severe, with granulation tissue, atelectasis, and large colonies of filamentous bacteria.

Conference Comment: There is slide variation with respect to the number of bacterial colonies present intralesionally. The contributor provided a complete review of this entity, with due emphasis on the key differences between nocardiosis and actinomycosis, which were reviewed during the conference. For more information on the differential diagnosis for intralesional filamentous bacteria, sulphur granules, mycetomas, and pseudomycetomas, the reader is referred to WSC 2009-2010, Conference 1, Case I.

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