The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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CONFERENCE 1
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Conference Moderator: COL William Inskeep, II
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Washington, DC 20306-6000

CASE I – 99-3217 (AFIP 2694765)

Signalment: 6-month-old, crossbred, barrow, hog (*sus scrofa domestica*)

History: The animal presented for necropsy was a pig from a group of unvaccinated, market weight, mixed breed pigs. Approximately 100 pigs developed lethargy with irregular areas of hyperemic skin prior to shipment to market. Affected pigs all died unless treated with penicillin.

Gross Pathology: The integument of the market weight pig had multiple 5 mm to 4 cm diameter, irregular hyperemic foci. No other significant gross lesions were present.

Laboratory Results: *Erysipelothrix rhusiopathiae* was isolated from the skin.

Contributor's Morphologic Diagnoses: 1. Haired skin, dermis; arteritis, suppurative, multifocal, moderate, chronic with multifocal vascular and epidermal necrosis and fibrinocellular thrombi; etiology, *Erysipelothrix rhusiopathiae*.
2. Skin; subcorneal pustules.
3. Haired skin; apocrine sweat gland adenitis, suppurative, multifocal, mild, acute; etiology, *Erysipelothrix rhusiopathiae*.

Contributor's Comment: Haired skin - There is diffuse to perivascular neutrophilic to mixed inflammation of the superficial dermis. A few arterioles in this region are necrotic and subjacent to regions of epidermal necrosis. There are multifocal pustules in the epidermis. The remaining dermis contains variable numbers of arterioles with transmural and perivascular infiltrates of neutrophils, macrophages, and lymphocytes. A few vessels contain fibrinocellular thrombi with perivascular hemorrhage. A few sections contain infiltrates of mixed inflammatory cells adjacent to apocrine sweat glands.

Erysipelas, caused by the gram positive, facultative anaerobic, bacillus *Erysipelothrix rhusiopathiae*, has a wide geographic distribution and a wide host range. It commonly causes disease in pigs, lambs, and birds and sporadic disease in numerous other species including humans. Porcine erysipelas is manifested as an acute septicemic disease in epidemic outbreaks and chronic disease or sporadic septicemias in endemic herds. Erysipelas occurs most commonly in pigs 2-12 months of age, but can affect animals of any age.

Of the known 28 serotypes, pigs are susceptible to 15. Serotypes 1 and 1a are most prevalent in acute erysipelas and serotype 2 is most common in the chronic form of disease. In the United States, serotype 1a is most frequently isolated from clinically ill pigs followed by serotypes 2, 5, and 1b. Serotypes 1b, 2, 6, and 8 have been isolated from pigs in Denmark.

The most common route of infection is believed to be oronasal exposure from chronically infected pigs. However, contamination of the environment from feral animals, other domesticated animals and possibly insects may also be source of infection.

The cutaneous lesions in acute erysipelas take three to four days to develop. By this time, there may be other clinical, gross, and microscopic signs of septicemia. The skin lesions are caused by acute cellular infiltration of neutrophils into arteriolar walls with cellular and fibrin thrombi. Other lesions such as sweat gland adenitis, renal cortical petechiae, renal medullary hemorrhage, fibrinoid necrosis of glomerular tufts, synovitis, and splenomegaly are often seen, but are not always present.

Chronic erysipelas is characterized by either endocarditis, arthritis, or both. *Actinobacillus suis* can also cause similar clinical signs and skin lesions as acute erysipelas and should be considered as a differential diagnosis.

AFIP Diagnoses: Haired skin and subcutis: Dermatitis and panniculitis, subacute, diffuse, moderate with orthokeratotic hyperkeratosis, intraepidermal pustules, and sweat gland adenitis, mixed breed, porcine.

Conference Comment: The characteristic lesions of *Erysipelothrix rhusiopathiae* include necrotizing vasculitis, thrombosis, and epidermal necrosis. Because of variation in sections, many of these diagnostic lesions were absent in the examined sections resulting in a broader differential diagnosis for conference participants. In many sections a diffuse, often perivascular, neutrophilic and lymphoplasmacytic dermatitis with a suppurative superficial component was the primary lesion. Based on these lesions the differential diagnosis included Porcine Juvenile Pustular

Psoriasiform Dermatitis, Dermatosis Vegetans, and Staphylococcal infection. The contributor's culture results were considered to be important diagnostic information.

Contributor: Kansas State University Diagnostic Laboratory, Manhattan, KS, 66506

References: 1. Friendship R: Erysipelas: New disease or old problem revisited?. Comp Food Anim (Suppl) Feb, pp. 60-63, 1999
2. Palmer N: Bones and Joints. *In:* Pathology of Domestic Animals, eds. Jubb K, Kennedy P, Palmer N, 4th ed., vol. 1, pp. 164-167. Academic Press, San Diego, CA, 1993
3. Wood R: Erysipelas. *In:* Diseases of Swine, ed. Straw B, 8th ed. pp 419-429, 1999

CASE II – 00N3358 (AFIP 2788677)

Signalment: 12-year-old, gelding, Quarterhorse, equine.

History: This horse had lived in Salinas (Central Coastal California) for the past 7 years. The animal had a 40-day history of depression, respiratory distress with increased respiratory rate, crackles on auscultation of lung fields and fever. Previous to admission at the VMTH the horse was treated with several different antibiotics for a presumed pleuropneumonia.

Gross Pathology: The horse examined was a 475 kg, adult Quarterhorse gelding in excellent post mortem condition and fair nutritional state. The lungs failed to collapse and the visceral pleura had a prominent vascular pattern. The lung was diffusely firm and had a finely nodular texture. On section of all lung lobes, the parenchyma was bright red and diffusely obliterated by 0.1-0.2 cm miliary, white to light grey, firm nodules. In the cranioventral lobes the nodules often coalesced to form 0.3-0.4 cm irregular, firm, patches.

The thoracic, pulmonary hilar, perihilar and periaortal lymph nodes were enlarged and varied from 1 x 2 to 2 x 5 cm. On section, the lymph nodes were wet, mottled light tan and red, and had bulging cortices. The thoracic duct was engorged with white opaque fluid (lymph).

Laboratory Results: Radiological examination revealed a miliary interstitial pattern in the lungs.

Complete Blood Counts revealed a leukocytosis of 27,500/ul with neutrophilia:

WBC	27,500/ul
Neut	24,660/ul (90%
Lymph	1,096/ul (4%)
Mono	822/ul (3%)
Eosin	548/ul (2%)
Baso	274/ul (1%)

Other parameters were:

Platelets	208,000/dl
Plasma protein	8.1gm/dl
Plasma fibrinogen	500mg/dl
Protein: fibrinogen	15
Icterus index	20

Tracheobronchial lavage diagnosis: Mild to moderate purulent inflammation.

Bacterial cultures (from tracheobronchial lavage): No organisms seen on direct smear or acid-fast stained slides. No aerobic or anaerobic growths including Mycoplasma.

Coccidiomycoses titer (*Coccidioides immitis* immunodiffusion test on venipuncture): negative.

Contributor's Morphologic Diagnoses: 1. Lung: Severe, diffuse, miliary, granulomatous pneumonia (Adiaspiromycosis).
2. Lymph nodes (pulmonary hilar, periaortic, thoracic inlet): Marked reactive hyperplasia and plasmacytosis.

Contributor's Comment: The morphology, histology, EM, and staining properties of this fungus are most consistent with adiaspiromycosis. The etiologic agents of adiaspiromycosis are members of the *Emmonsia* (or *Chrysosporium*) genus, which primarily cause respiratory disease in rodents and small mammals. Relatively few human infections have been reported and numerous, sporadic animal infections are described, some of which have a gross distribution similar to that seen in this horse. Inhalation appears to be the main route of infection, with the severity and extent of the disease dependent upon the amount of dust borne conidia inhaled. Soil is the principal reservoir for *Emmonsia* spp. and these organisms have an almost worldwide distribution. The owner of this horse reported that the pasture was heavily populated with ground squirrels and striped skunks, both susceptible hosts of *E. crescens*. These animals are known to have infection rates of 80% and may play a role in distributing the organisms.

Infectious spores of *Emmonsia* do not multiply in the respiratory tract but the inhaled conidia enlarge in lung tissue and can reach diameters of up to 400

microns. The main criterion for a diagnosis of adiaspiromycosis is the presence of large, thick walled, round to oval adiaspores in tissue section. The adiaspores in tissues resemble the parasitic spherules of *Coccidioides immitis* but differ in that they lack internal structures known as endospores. Of the two described species that are respiratory pathogens, this horse had an infection most consistent with *Emmonsia crescens*, since the diameters of some of the spores seen in the lung were greater than the largest size seen in the *Emmonsia parva var. parva* (40 microns) variety.

Multiple attempts to grow the fungus from lung tissue were unsuccessful and *Emmonsia* specific primers were used to amplify fungal DNA and the sequenced purified PCR product was consistent with published sequences of *E. crescens*.

There is no reliable serological test available, although sera from animals infected with *Emmonsia crescens* has been shown to cross react with antigens from *Histoplasma* sp. by immunoprecipitation analysis. Despite the fact that the inflammatory reaction of this horse appeared effective in killing most of the organisms (multiple empty or mineralized adiaspores), it is likely that the initial dose of inhaled conidia was extremely high and such severe inflammation compromised the air/blood gas exchange surface area. The severe plasmacytosis and reactivity of the lymph nodes within the thoracic cavity is likely a reflection of both the antigenic stimulation and chronicity of the lung infection.

AFIP Diagnosis: Lung: Pneumonia, pyogranulomatous, multifocal to coalescing, severe, with fungal spherules, Quarterhorse, equine.

Conference Comment: *Emmonsia crescens* (*Chrysosporium parvum var. crescens*) is classified as an adiaspore; a fungal organism that, while in tissue, grows in size without replicating. Infection is generally confined to the lungs, although rare dissemination via the lymphatics to local lymph nodes has been reported. Infection results in the formation of characteristic granulomas (adiaspiromas) within the pulmonary parenchyma while often sparing the intervening pulmonary tissues. Discussion during conference centered on the difficulty in distinguishing *Emmonsia* from the primary differential of *Coccidioides immitis*. The lack of endospores is a discriminating feature that helps differentiate *Emmonsia* from the endosporulating organisms *C. immitis*, *Chlorella* spp., *Prototheca* spp., and *Rhinosporidium seeberi*. Often endospores within *C. immitis* are indistinct or absent, making differentiation between the two organisms difficult. Although *Emmonsia* is reported to grow as large as 400 um, many of the organisms in the examined sections were 40 to 60 um in diameter which is again consistent with *C. immitis*. In this case PCR was a useful tool in making the definitive diagnosis. Both GMS and PAS techniques highlighted the organisms well in section.

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References: 1. Adiaspiromycosis. *In:* Color Atlas and Text of the Histopathology of Mycotic Diseases, eds. Chandler F, Kaplan W, Ajello L, pp. 30-33, 138-141. Year Book Medical Publishers Inc. Chicago, IL, 1980
2. Albassam M, Bhatnager R, Lillie L, Roy L: Adiaspiromycosis in striped skunks in Alberta, Canada. J Wildlife Dis **22**(1):13-18, 1986
3. Peterson S, Sigler L: Molecular genetic variation in *Emmonsia crescens* and *Emmonsia parva*, etiologic agents of adiaspiromycosis, and their phylogenetic relationship to *Blastomyces dermatitidis* and other systemic fungal pathogens. J Clin Microbio **36:**2918-2925, 1998
4. Sigler L: Agents of Adiaspiromycosis. *In*: Medical Mycology, eds. Ajello L, Hay R, 9th ed., pp. 571-583. Oxford University Press, New York, 1998

CASE III – D00-50961 (AFIP 2787850)

Signalment: 4-week-old, Holstein, female, bovine, *Bos taurus*

History: This calf presented with severe hypothermia, hypoglycemia, and diarrhea (the animal is from a farm with cryptosporidiosis). The calf was treated intensively with fluids, plasma, and tube feeding. Recently, the owner switched from whole milk to milk replacer but with the cold weather, increased metabolic requirements may not have been met. Despite treatment, the calf died.

Gross Pathology: The calf was thin and had no subcutaneous, coronary, or perirenal fat deposits. There was a moderate amount of serosanguinous fluid in the pleural space. Both lungs had severe anterior and ventral consolidation of lung lobes. The affected lobes were purple, tan, and firm. Major airways, including the trachea, contained cheesy, whitish exudate. The tracheobronchial lymph nodes were moderately enlarged.

Laboratory Results: Bacteriology: Many mixed non-hemolytic coliforms and mixed alpha-*Streptococcus sp*. were cultured from the bronchi, lung, liver, ileum, lymph nodes, cecum, and abdominal cavity. No *Salmonella sp*. were isolated.
Electron microscopy: EM of a virus grown in tissue culture from the lung was positive for Herpes virus only. Herpes virus particles were present in large numbers.

Molecular diagnostics: PCR examination of tissues was negative for BVD virus.

Virology: Electron microscopic examination of the feces revealed many coronavirus particles. Tissue culture was positive for CPE and FA results were positive for IBR, a herpes virus. Results of virus isolation attempts from lung tissue sent to the National Veterinary Services Laboratory (NVSL) revealed bovine herpes virus-1 (IBR) and parainfluenza-3 virus (PI3).

Contributor's Morphologic Diagnoses: Lung; severe, acute, necrotizing bronchointerstitial viral pneumonia (IBR and PI3 positive).

Contributor's Comment: Infectious Bovine Rhinotracheitis Virus (IBRV) and Parainfluenza-3 (PI3) are viruses commonly associated with Bovine Respiratory Disease Complex (BRDC). BRDC includes both shipping fever and enzootic pneumonia of calves. BRDC is caused by a variety of viral, bacterial, and Mycoplasma pathogens along with a variety of host risk factors and environmental conditions. Other viruses commonly associated with BRDC are bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), and bovine respiratory coronavirus (BRCV).

Enzootic Calf Pneumonia usually occurs in calves housed under intensive husbandry conditions. Calves are usually 1 week to 6 months of age. It is most common in winter months among dairy calves housed indoors. Pneumonia most often develop during environmental stress such as during times of temperature fluctuations (results in decreased ciliary activity), high humidity (increases the number of organisms and leads to chilling of calves), poor ventilation (results in high numbers of bacterial organisms and pneumonia). Respiratory infections are likely to occur when mixing animals from different sources. There is also a higher incidence in calves that have not received sufficient colostrum.

In this three-week old calf, we demonstrated co-infection with both IBR and PI3 by several methods:
1) Histopathological examination of lung revealed eosinophilic intranuclear inclusions in bronchial and alveolar epithelium suggestive of infection with a herpes virus (IBR).
2) Histopathological examination of lung revealed eosinophilic intracytoplasmic inclusions in bronchial and alveolar epithelium suggestive of infection with a non-herpes virus (PI3)
3) Virus isolation of IBR from the lung (CPE and FA positive), with confirmation by EM of numerous herpes virus particles.
4) Virus isolation of PI3 from the lung at the National Veterinary Services Laboratory (NVSL).

IBR is bovine herpes virus-1. The respiratory subtype is BHV-1.1. Infection with BHV-1 causes necrosis of epithelium of the respiratory tract. This compromises the ciliary clearance of bacteria from the upper respiratory tract.

BHV-1 infection also impairs macrophage, neutrophil, and lymphocyte function. This impaired function leads to increased respiratory infections. The acidophilic, intranuclear viral inclusions in respiratory epithelial cells are usually present 2 to 3 days following infection.

PI3 is a member of the *Paramyxoviridae* family. It is ubiquitous and worldwide. PI3 infection also impairs the function of the immune cells, particularly alveolar macrophages and lymphocytes, and to a lesser extent, the activity of neutrophils. The acidophilic, intracytoplasmic viral inclusions are often noted in bronchiolar epithelium 2 to 4 days following infection. Infection with PI3 itself often results in subclinical infection to mild clinical signs; its importance then lies in mixed infections as part of the bovine respiratory disease complex.

There is evidence that IBR and PI3 infections act synergistically to suppress the immune system, thus, increasing the animal's susceptibility to secondary infections.

AFIP Diagnosis: Lung: Pneumonia, bronchointerstitial, necrosuppurative, diffuse, severe, with intranuclear and intracytoplasmic inclusion bodies and many bacilli, Holstein, bovine.

Conference Comment: Enzootic pneumonia was the first choice for most conferees. The contributor has provided a concise review of this entity.

Contributor: Minnesota Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108

References: 1. Briggs R, Kehrli M, Frank G: Effects of infection with parainfluenza-3 virus and infectious bovine rhinotracheitis virus on neutrophil function in calves. American Journal of Veterinary Research. **49**(5):682-686, 1988
2. Brown T, Ananaba G: Effect of respiratory infections caused by bovine herpesvirus-1 or parainfluenza-3 virus on bovine alveolar macrophage functions. American Journal of Veterinary Research. **49**(9):1447-1451, 1988
3. Ghram A, Reddy P, Morril J, Blecha F, Minocha H: Bovine herpesvirus-1 and parainfluenza-3 virus interactions: clinical and immunological response in calves. Canadian Journal of Veterinary Research. **53**(1)62-67, 1989
4. Dungworth D: The Respiratory System. *In:* Pathology of Domestic Animals, eds. Jubb K, Kennedy P, Palmer N, 4th ed., vol. 2, pp. 613-615, 628-629. Academic Press, San Diego, CA, 1993
5. Kapil S, Basaraba R: Infectious bovine rhinotracheitis, parainfluenza-3, and respiratory coronavirus. Veterinary Clinics of North America - Food Animal Practice. **13**(3):455-469, 1997
6. Radostits O, Gay C, Blood D, Hinchcliff K: Veterinary Medicine, 9th ed, pp. 1160-1168. WB Saunders, London, England, 2000

7. Virtala A, Grohn Y, Mechor G, Erb H: The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during the first 3 months of life. Preventative Veterinary Medicine. **39**(1):25-37, 1999

CASE IV – D01 11762 (AFIP 2789018)

Signalment: 8-year-old, spayed female, Border collie, canine

History: Five-month history of a progressive, proliferative, red lesion involving the cornea of the right eye. A slightly raised, red lesion, with peripheral vascularization and edema, was noted at the nasal limbus of the right eye. The left eye was unremarkable. Fluorescein stain uptake was negative. The lesion was unresponsive to therapy with topical dexamethasone, oral prednisone and subconjunctival methylprednisolone acetate. The right globe was enucleated due to the poor response to therapy. The lesion involved one-half of the right cornea at the time of enucleation.

Gross Pathology: The extirpated right globe was egg-shaped and 3.0 cm front-to-back and 2.5 cm side-to-side. The cornea was thickened and a brown lesion occupied one-half of the cornea.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Unilateral corneal hemangiosarcoma.

Contributor's Comment: A poorly demarcated, expansile mass comprised of variably sized vascular channels lined by a single layer of cells was present in the cornea. Scant to moderate numbers of erythrocytes were present in the vascular channels. The nuclei of the cells lining the vascular channels were slightly pleomorphic (round to oval to oblong). Anisokaryosis was moderate. Mitotic figures were rare but abnormal arrangements were noted. The cells lining the vascular channels stained positively with factor VIII related antigen (VIII:RAg; von Willebrand's factor). The histologic findings and the positive staining for factor VIII related antigen warranted a diagnosis of hemangiosarcoma.

Hemangiosarcomas can occur anywhere in the conjunctiva and in the dog, they most frequently involve the perilimbal bulbar conjunctiva. Solar radiation may play a role in their pathogenesis. Hemangiosarcomas of the cornea are uncommon in the dog but are fairly destructive when they do occur. They are commonly accompanied by extensive corneal vascularization and perilesional edema. Enucleation or a freehand corneal scleral graft are the preferred therapies. Factor

VIII related antigen, a vascular endothelial marker, can be a useful adjunct in establishing a diagnosis of vascular neoplasia.

AFIP Diagnosis: Cornea and subconjunctival connective tissue: Hemangiosarcoma, well-differentiated, Border collie, canine.

Conference Comment: While cutaneous, splenic, and cardiac hemangiosarcoma are relatively common neoplasms in dogs, hemangiosarcoma of the cornea is not. Because of the lack of atypia, conference participants initially debated whether this neoplasm was benign or malignant. However, the mitotic rate (up to 2 per high power field in some fields) and the apparent infiltration of the conjunctiva were considered important diagnostic features. The Department of Ocular Pathology at the AFIP remarked that to their knowledge this condition has never been reported in humans.

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References: 1. Hargis A, Lee A, Thomassen R: Tumor and tumor-like lesions of perilimbal conjunctiva in laboratory dogs. JAVMA **73**(1):1185-1190, 1986
2. Gelatt K: Veterinary Ophthalmology, 3rd ed., pp. 667. Lippincott Williams and Wilkins, Philadelphia, PA, 1999
3. Moore P, Hacker D, Buyukmichi N: Ocular angiosarcoma in the horse: morphological and immunohistochemical studies. Vet Pathol **23**:240-244, 1986
4. Wilcock B: The Eye. *In:* Pathology of Domestic Animals, eds. Jubb K, Kennedy P, Palmer N, 4th ed., vol.1, pp. 515. Academic Press, San Diego, CA, 1993

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