

The Armed Forces Institute of Pathology
Department of Veterinary Pathology
Wednesday Slide Conference
2010-2011
Conference 14
15 December 2010

Conference Moderator:

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CASE I: 24087-09 (AFIP 3163065).

Signalment: 1-year-old, female, brown and white Boer, caprine (*Capra hircus*).

History: The animal was presented by the food animal clinic with history of icterus, abdominal pain and pigmenturia.

Gross Pathology: This juvenile brown and white female caprine weighed 49.5 kg and was well fleshed. The fascia and body fat had a pronounced yellow hue. Transparent red fluid was present in the thorax and abdomen and the spleen was diffusely enlarged, with a soft dark red appearance of the incised surface. The liver had a uniform yellow-tan color, and the gallbladder was distended. The kidneys were soft and dark red in color, bulging when incised. The inner renal medulla especially had a red granular character.

Laboratory Results: Elevated AST, GGT and CK were present in antemortem serum samples. Serum copper was elevated. Liver copper was 310 ppm on a wet-weight basis (normal 25-150ppm), and renal copper was 67 ppm (normal <12 ppm).

Histopathologic Description: Liver: Hepatic cords are poorly defined, containing dissociated cells, particularly in centrilobular and midzonal locations. Individualized cells at these and other sites have eosinophilic cytoplasm and pyknotic or absent nuclei. Some contain brown-gray cytoplasmic granules that are positive for copper by rhodanine staining. Occasional canaliculi are outlined by bile pigment. Small numbers of well-defined unstained cytoplasmic vacuoles, interpreted as lipid, also occur in hepatocytes. In most instances, areas of degeneration and necrosis are devoid of inflammation, although clusters of eosinophils and other leukocytes occasionally occur.

Kidney: Some cortical renal tubules contain flocculent to eosinophilic granular casts, often with granules possessing a more pronounced red color, while others contain degenerating cells. Renal tubular epithelial cells are attenuated, coagulated and pyknotic, or absent. Similar casts occur in the medulla, with less tubular damage. Thrombi occur in small, thin-walled interstitial blood vessels.

Special stains revealed bile retention in hepatic canaliculi and granules positive for copper by rhodanine were found in the liver but not the kidney. Iron stains were positive for sloughed cells in renal tubules, but the liver contained little iron pigment.

Contributor's Morphologic Diagnosis: 1. Liver: Hepatocellular degeneration and necrosis, acute, predominantly centrilobular, with cytoplasmic granularity, mild lipidosis and mild bile stasis.
2. Kidney: Acute renal tubular degeneration and necrosis, severe, with hemoglobinuric casts and interstitial thrombosis.

Contributor's Comment: Copper intoxication is somewhat divisible into an acute form, which is generally associated with gastrointestinal disturbance, or a chronic form, which is more often associated with a precipitous hemolytic crisis, although both diseases are characterized by a precipitous onset of clinical signs. Chronic copper intoxication is most frequently seen in sheep, in which chronic consumption of feed designed for use in other species is a principal cause, although exacerbating factors such as low dietary molybdenum or sulfur, exogenous copper from other sources (including swine or poultry litter) or pre-existing liver damage (often due to exposure to hepatotoxic plants) may be involved. Although they are less susceptible than sheep, acute hemolytic crises have occurred in adult Boer goats associated with increased levels of tissue copper.¹ Hepatic accumulation of copper due

to defects in metabolism or excretion occurs during the preclinical hepatopathy, with abrupt acute hepatocellular degeneration that is often precipitated by stress. Massive release of copper, thought to result from degeneration of copper rich lysosomes, precipitates hemolysis. In sheep, copper loading is characterized proteomically by a reaction to oxidative challenge, with evidence of oxidative stress injury occurring even prior to the hemolytic crisis.⁸

Hemolytic crisis has been reported to follow acute exposure of pre-ruminant kid goats exposed to milk replacer intended for calves,⁶ but an outbreak of copper intoxication in lactating dairy goats was characterized by hepatopathy without hemolytic crisis.² However, copper intoxication with hemolysis is becoming more recognized in adult and juvenile Boer goats. In this particular animal, the lesions are consistent with a post-hemolytic phase of disease, in which renal damage associated with hemoglobinuria is evident.

Liver damage in copper intoxication is thought to result from a combination of hepatocyte lysis due to the effects of the toxin and anoxia secondary to anemia. Renal damage may likewise have hemoglobinuria and anemia as cofactors affecting the lesion development. Ultrastructurally, the preclinical phase is morphometrically associated with hepatocyte and Kupffer cell swelling at the expense of the sinusoids and space of Disse, with pronounced lysosomal proliferation. At the time of crisis, lysosomes become even more enlarged and contain many residual bodies.³

Copper concentrations should not be above 230 mg/kg in liver, 12 mg/kg in kidney or 1.2 mg/kg in blood as measured by flame atomic absorption spectrometry.

A variety of copper storage diseases occur in humans, rodents and companion animals.⁴ In Wilson's disease of humans, the rate of ceruloplasmin binding of copper is reduced, as is excretion, due to mutation of the ATP7B gene. The specific genetic defect resulting in variable breed susceptibility in other species is not known.

AFIP Diagnosis: 1. Liver: Hepatocellular degeneration and necrosis, centrilobular to mid-zonal, diffuse, moderate, with bile stasis, edema, and intracytoplasmic amphophilic granular material (copper).
2. Kidney: Tubular degeneration and necrosis, diffuse, severe, with multifocal tubular regeneration, tubular protein, granular and hemoglobin casts, and occasional fibrin thrombi.

Conference Comment: Many participants were impressed by the extent of tubulorrhesis present in the kidney. In contrast, fibrin thrombi are not evident in all sections and hence the characterization as "occasional fibrin thrombi."

Participants discussed the histomorphologic features of tubular regeneration. Approximately one week after the initial injury, evidence of regeneration is evident. Tubules are lined by attenuated epithelium with hyperchromatic nuclei, and occasional mitotic figures may be seen. As regeneration continues, cells appear smaller with increased cytoplasmic basophilia, and may be closely packed with the appearance of piling up. The moderator offered additional guidelines to aid in the interpretation of tubular regeneration, such as uneven nuclear size (anisokaryosis) and variable distance between nuclei due to cells in different stages of growth and re-epithelialization.⁷

Cases of hemoglobinuric nephrosis often have characteristic "port wine- colored" urine. There are three possible causes of such urine color: hematuria, hemoglobinuria and myoglobinuria. Identifying the cause of discoloration often provides insight as to the underlying disease process; therefore, one must be able to differentiate the three causes in the laboratory. Hematuria is easily diagnosed by centrifugation of the urine specimen; erythrocytes will be present in the urine sediment. Hemoglobinuria and myoglobinuria both lack erythrocytes in the sediment and the urine remains discolored after centrifugation. Addition of saturated ammonium sulfate will cause hemoglobin to precipitate out of the urine sample, resulting in a clear sample; myoglobin does not precipitate, and the urine remains discolored. If a blood sample is also available, centrifugation of a hemoglobinemic sample will result in pink serum, while in a myoglobinemic sample the plasma will remain clear.⁴

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CASE II: G8199 (AFIP 3168020).

Signalment: 8-month-old, Shetland pony, equine (*Equus caballus*).

History: The lethargic pony was referred to the equine veterinarian in a poor body condition, showing moderate pallor of mucous membranes, severe dyspnea and diarrhea. Thoracic radiography revealed diffuse clouding shadows of lung parenchyma with distinct vascular pattern. The pony died two days after admittance to the clinic.

Gross Pathology: Main findings during necropsy included severe pulmonary edema with multifocal hemorrhage and multifocal thrombosis, moderate to severe disseminated granulomatous pneumonia in all lobes as well as granulomatous lymphadenitis of tracheobronchial lymph nodes. Moreover, moderate chronic verminous endoarteritis of the cranial mesenteric artery with intravascular presence of numerous nematodes consistent with *Strongylus vulgaris* was present. The ileum and large intestine contained numerous nematodes consistent with *Strongylidae spp.* and multifocally revealed moderate chronic ulcerative ileitis and typhlocolitis with multiple firm transmural ulcers of 2-3 cm in diameter, partly constricting the lumen, with hemorrhagic margins as well as multifocal subserosal hemorrhagic plaques (consistent with *hemomelasma ilei*). Most mesenteric lymph nodes were moderately enlarged and firm with an irregular surface (granulomatous lymphadenitis). Liver and kidneys were diffusely moderately swollen, pale and soft with sporadic small firm white granulomatous foci.

Histopathologic Description: Lung: Expanding and replacing approximately 45 % of the normal lung architecture are multiple discrete up to 6 mm in diameter foci with marked central karyorrhectic debris (necrosis), which are sometimes accompanied by intense eosinophilic material (Splendore Hoeffli phenomenon), and surrounded by numerous degenerate neutrophils, macrophages and fewer lymphocytes and plasma cells. Often within necrotic centers few to large numbers of fungal hyphae of 3-6 µm width are present and are characterized by regular septation, thin, parallel walls and dichotomous, progressive acute angle branching.

Multifocal to coalescing alveolar lumina are expanded by homogeneous eosinophilic material (edema), fibrillar eosinophilic material (fibrin) or abundant extravasated erythrocytes (hemorrhage) in varying composition, discontinued by multifocal areas of alveolar distention and ruptured alveolar septae (emphysema). Overall, alveolar septae are slightly thickened due to multifocal to coalescing moderate engorgement of erythrocytes (hyperemia) and/or mild mixed inflammatory cell infiltrates with dominance of neutrophils accompanied by multifocal hyperplasia of type II pneumocytes and a moderate increase of alveolar macrophages (alveolar histiocytosis).

Several small, medium-sized or large arterial and venous blood vessels contain thrombi of different size and extension composed of homogeneous (serous) to fibrillar (fibrinous) eosinophilic material, erythrocytes, necrotic debris, degenerate neutrophils, occasionally admixed with the fungal hyphae described above, accompanied by moderate to marked infiltration of vessel walls by fibrinous to necrotic debris, degenerate neutrophils and sporadically by fungal hyphae. Multifocally, but not present in all slides, there is segmental to circumferential necrosis of bronchial/bronchiolar walls with sloughed epithelial cells, mixed inflammatory cells, erythrocytes, fibrin and necrotic debris within the lumina in varying composition and sometimes admixed with the fungal hyphae described above. Subpleural, interlobar, perivascular, as well as, peribronchial/peribronchiolar interstitial connective tissue is moderately separated by clear space or homogeneous eosinophilic material (edema) with few mixed inflammatory cell infiltrates.

Contributor's Morphologic Diagnosis: 1. Lung: pneumonia, severe, multifocally extensive, acute to subacute, necrosuppurative to pyogranulomatous with intralesional hyphae consistent with *Aspergillus sp.* and bronchitis/bronchiolitis (not present in all slides), moderate to severe, multifocal, acute, necrosuppurative.
2. Lung: vasculitis, severe, multifocal, acute, fibrino-necrotizing with intramural hyphae consistent with *Aspergillus sp.*, thrombosis, alveolar and interstitial edema as well as pulmonary hemorrhage.

Contributor's Comment: The genus *Aspergillus* encompasses more than 200 species of which only approximately 19 cause disease in humans.¹ Aspergilli reproduce asexually by forming conidia-bearing multicellular structures ('conidiophores') which release millions of uninucleate cells called conidia into the air that are easily dispersed by the wind and have a diameter small enough (2.5 to 3.5 μm) to reach peripheral airways.^{1,25} *Aspergillus* conidia occur in soil, air, water and greatest numbers are found in hay and straw enriched with leaf and grass compost. They are considered to be the main vehicle for infective transmission, and when they get the chance to germinate inside the body producing branched septate hyphae that invade tissues, different forms of aspergillosis can develop.^{1,25}

Aspergillosis refers to a variety of diseases caused by several species of *Aspergillus*, which are relatively uncommon in humans and mammals but represent a major cause of mortality in birds. The diseases vary in severity and clinical cause, depending on the species and organs affected as well as the immunocompetence of the host. Members of the *A. fumigatus* group cause most cases of aspergillosis.^{9,23} However, several other *Aspergillus* species, particularly *A. flavus*, *A. terreus*, *A. nidulans* and *A. niger* have also been described as causative agents.^{7,9,17,26}

In horses, equine guttural pouch mycosis is the predominant form of aspergillosis and is considered a rare, life-threatening opportunistic infection, with *A. fumigatus* being most frequently isolated. There is no breed or gender predisposition and the pathogenesis remains nearly unknown. However, predisposing factors might be soft tissue trauma and environmental conditions such as poor ventilation, high humidity and warm temperatures that encourage conidial germination. In the majority of cases, the dorsomedial aspect of the guttural pouch is affected, showing necro-hemorrhagic to fibrinous inflammation accompanied by angioinvasion, erosion of cranial nerves and tissue necrosis as common sequelae.^{17,23}

Pulmonary or invasive aspergillosis is very uncommon in horses and usually comprises hematogenous spread of fungal hyphae. Typical lesions are multifocal embolic pneumonia, often centered on pulmonary vessels, and include neutrophilic and fibrin exudate in alveoli, hemorrhage, necrosis and leukocytoclastic vasculitis with resultant thrombosis and infarction,⁸ all of which is also seen in this case. More chronic lesions can appear as classic granulomas with central necrotic cores. Although hyphae are often present within the lesions and may be readily seen with hematoxylin and eosin stains, special stains, such as Periodic Acid-Schiff (PAS) reaction or Gomori-Methenamine Silver (GMS) stain are helpful to visualize their characteristic morphology. In this case, we used PAS reaction to show the characteristic frequent dichotomous branching and hyphal morphology, which is described in table 1.

Important predisposing factors for the development of invasive aspergillosis in horses seem to be prolonged antibiotic, glucocorticoid or NSAID administration,^{13,18,22} immunosuppression associated with leukaemia,⁵ neutropenia,⁴ pituitary adenoma,⁶ heavy exposure to conidia from mouldy environmental material^{7,13} or prolonged and intense periods of stress.^{14,24} In addition, a compromised intestinal mucosa serving as the site of entry, which has been also found in this case in the form of an ulcerative enterocolitis, is considered to be an important predisposing condition for hematogeneous spread of fungi resulting in invasive, systemic aspergillosis, often with predominant pulmonary manifestation.^{4,8,13,18,22,24,26} Also mixed invasive fungal infections, such as concomitant aspergillosis and mucormycosis, have been reported in horses^{7,24} and calves.¹⁰

Besides horses, dogs, cows and dolphins also are particularly susceptible to certain forms of aspergillosis. In dogs, canine sinonasal aspergillosis predominantly affects mesocephalic or dolichocephalic breeds, where *A. fumigatus* is most commonly isolated, but *A. terreus* predominates in cases involving German shepherd dogs. Predisposing factors are unknown, and pathology is concentrated on the nasal cavity and paranasal sinuses. Sometimes the cribriform plate is invaded and CNS infection may be established.²³ Bovine mycotic abortion due to *Aspergillus* infection occurs worldwide and sporadically. Typically second or third trimester abortion is observed, with highest incidence during winter when gravid cows are indoors and fed with heavily contaminated hay or silage. *Aspergillus fumigatus* accounts for the majority of cases and suggested routes of infection are ingestion or inhalation. The pathological hallmark is placentitis with leathery appearance of the placenta (almost pathognomonic), intercotyledonary thickening and hypertrophy of cotyledons.²³ Cetacean mycotic pneumonia is the predominant

form of aspergillosis in free ranging dolphins and is considered to be a regional disease. Predisposing factors are suspected to be a combination of viral and environmental factors.²³

In contrast to the rather low incidence of aspergillosis in mammals, avian aspergillosis resembles a major cause of morbidity and mortality in birds and affects animals of all ages, whether immunocompetent or immunosuppressed in captive or free-ranging environments; captive penguins seem particularly susceptible.² In approximately 95% of the cases *A. fumigatus* is isolated, and *A. flavus* occurs second most frequently. Inhalation is the route of infection, with initial colonization in the lower respiratory tract. Susceptibility may be attributed to differences in innate and acquired immunity compared to mammals, as well as predisposing anatomic characteristics such as lack of an epiglottis and diaphragm (inability to produce strong cough), limited distribution of pseudostratified ciliated respiratory epithelium, lack of surface macrophages, and different heterophilic killing mechanisms (using cationic proteins, hydrolases and lysosomes rather than myeloperoxidases and oxidative mechanisms). A unique feature of avian aspergillosis is the presence of reproductive phases in tissue.²³

The most common forms of human aspergillosis are pulmonic and may be divided into (1) non-pathogenic saprophytic colonization, including noninvasive pulmonary aspergillomas/mycetomas or invasion of necrotic tissue; (2) hypersensitivity-induced aspergillosis, including *Aspergillus*-asthma, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis (or extrinsic allergic alveolitis), bronchocentric granulomatosis and chronic eosinophilic pneumonia; and (3) invasive disease, including pseudomembranous tracheobronchitis, acute bronchopneumonia, angioinvasive aspergillosis, chronic necrotizing aspergillosis and invasive pleural disease.^{1,25}

In general, human patients with pre-existing structural lung disease, atopy, occupational exposure or impaired immunity are susceptible.¹ Therefore, saprophytic colonization is increased in patients with advanced stages of chronic obstructive pulmonary disease (COPD), chronic asthma requiring long-term steroid therapy, primary ciliary dyskinesia syndrome and cystic fibrosis.²⁵ *Aspergillus spp.* also have a significant potential to act as powerful allergens; thus, hypersensitivity-induced *Aspergillus*-asthma in lower airways is caused by Type I anaphylactic reaction in atopic individuals upon exposure to *Aspergillus* conidia or hyphae. Allergic bronchopulmonary aspergillosis (ABPA) is caused by hypersensitivity to colonised *Aspergillus sp.*, resembling a complication of asthma, and is immunologically characterized by Type I, Type III and Type IV hypersensitivity. Hypersensitivity pneumonitis (or extrinsic allergic alveolitis) occurs primarily in non-atopic individuals. It is an inflammatory interstitial lung disease possibly resulting from Type III and Type IV hypersensitivity reactions following persistent or intense exposure to *Aspergillus* conidia with acute lung injury via complement-dependent neutrophils (due to Type III reaction) or chronic stages such as granuloma formation, interstitial lung fibrosis and distal bronchiolitis obliterans (due to Type IV reaction).²⁵

In human patients with altered local or systemic immune defense mechanisms, most severe and life-threatening invasive disease may develop.^{1,25} Invasive pulmonary aspergillosis ranks second to candidiasis in causing systemic fungal infections in immunocompromised human patients, and in most cases lung manifestation occurs with common haematogenous spread to other organs, especially the CNS. Hence, immunosuppression is considered to be the major condition with prolonged neutropenia as the leading cause.²⁵

In healthy, immunocompetent individuals, various elements of the pulmonary innate immune system are involved in recognition and elimination of inhaled *Aspergillus* conidia, thereby preventing colonization of the respiratory system. Ciliated and mucus secreting epithelial cells perform effective mucociliary clearance that is important for entrapment and elimination of inhaled conidia. Surfactant, mainly produced by Type II pneumocytes and Clara cells, has been implicated in antimicrobial activity, with surfactant protein A and D serving as collectins. Alveolar macrophages represent first line phagocytic defence by intracellular killing of swollen spores and prevention from germination. Recruited neutrophils play an essential role by extracellular (degranulation) as well as intracellular (phagocytosis) elimination of Aspergilli. Dectin-1, expressed by macrophages, neutrophils and dendritic cells, is an important receptor of innate antifungal defence, being essential for spore recognition and phagocytosis as well as production of oxygenated free radicals (fungicidal activity). Additionally, certain Toll-like receptors (TLR) have been found to play a predominant role in the recognition of *A. fumigatus* (TLR2: recognition of spores, TLR4: recognition of spores and hyphae).³

On the other hand, several pathogenicity factors were found in different *Aspergillus spp.* to overcome certain host defense mechanisms, such as endotoxins that inhibit epithelial ciliary activity, as well as a variety of proteases (incl. elastase, collagenase and trypsin) that damage epithelial cells and thus impair effective mucociliary clearance.^{1,3} Further, *A. fumigatus* produces a phospholipid capable of decreasing the binding of complement factor C3b to its

surface, resulting in disturbed complement activation.³ Also other fungal proteins of *A. fumigatus* are probably related to virulence by promoting mycelial growth into lung parenchyma or structural alterations of conidia that are resistant to host defence mechanisms.¹

Moreover, it is likely that *Aspergillus* mycotoxins can work as virulence factors due to direct cytotoxic effects. In vitro studies revealed that aflatoxin (produced by *A. fumigatus*) suppresses the function of macrophages, and ochratoxin (produced by *A. ochraceus*) is cytotoxic to lymphocytes and suppresses lymphocytic, monocytic and granulocytic activity. Other possible immunosuppressive mycotoxins gliotoxin, fumagillin, fumigacin, fumitremorgin A and Asp-hemolysin are discussed, while different mycotoxins together may have synergistic effects. However, further *in vivo* studies are needed for confirmation of direct relation to *Aspergillus* pathogenesis.¹⁵

Beyond that, melanin pigment, mannitol, catalases and superoxide dismutases are suggested as antioxidant defenses produced by *Aspergillus*.^{11,27} Although it seems that certain antioxidant molecules produced by *A. fumigatus* do not directly inhibit the oxidizing activity of phagocytes, inhibition of reactive oxygen species production by macrophages (e.g. during corticosteroid treatment) abolishes their ability to kill the spores while phagocytosis continues so that conidia can germinate and proliferate intracellularly.³ However, since pulmonary macrophages and neutrophils constitute a crucial part of first line innate host defense, neutropenia and long-term corticosteroid treatment are generally regarded as major risk factors for the pathogenesis of invasive aspergillosis.^{1,3,11,19}

Table 1: Morphological characteristics, pathogenicity factors and manifestations of common mycotic diseases caused by mycelial fungi:

Species	Hyphal morphology	Septae/ Branching	Pathogenicity factors/ Toxins	Manifestation
<i>Aspergillus spp.</i> ^{1,3,9}	hyphae width 3-6 µm; thin parallel walls (conidia not seen in tissue)	regularly septate; dichotomous progressive 45° branching	adhesins, antioxidants (e.g. melanin), various proteases; mycotoxins (e.g. gliotoxin, aflatoxin, ochratoxin)	sinonasal, placental, pulmonary, angioinvasion, systemic
<i>Fusarium spp.</i> ⁹	hyaline hyphae width 3-7 µm (similar to <i>Aspergillus spp.</i>)	septate, frequent characteristic 90° branching (sometimes 45°)	mainly plant pathogenic; mycotoxins (e.g. fumonisin B ₁ , fusaric acid)	rare mycosis; pulmonary, angioinvasion, dermatitis, keratitis
<i>Candida spp.</i> ^{9,21}	round or oval hyaline budding blastospores 3-5 µm; hyaline hyphae and pseudohyphae (excl. <i>C. glabrata</i>)	septate, irregular branching	adhesins, formation of biofilm (oxylipin farnesol), different aspartate proteinases, “phenotypic switching”; no toxins described	mucocutaneous (e.g. vaginal, oral), cutaneous, GIT, systemic (e.g. pulmonary, renal), rarely angioinvasion
Zygomycetes (<i>Mucor spp.</i> , <i>Rhizopus sp.</i> , <i>Basidiobolus spp.</i> , <i>Mortierella spp.</i>) ^{9,16}	broad hyphae (up to 25 µm width and 200 µm length), non-parallel, thin walls (often folded, collapsed or twisted); slightly visualized by PAS and GMS stain	infrequently septate; non-dichotomous, irregular branching; frequently bizarre forms, focal bulbous dilatations	free iron & acidosis play central role; adherins (subendothelial matrix), possibly secreted toxins or proteases; endo-symbiotic endotoxin-producing bacteria (Genus <i>Burkholderia</i>)	cutaneous, subcutaneous, angioinvasion, systemic (with or without pulmonary focus), rhinocerebral
<i>Pseudallescheria boydii</i> ^{12,20}	narrow hyphae, parallel walls (similar to <i>Aspergillus spp.</i>)	septate, highly branching at less acute angles, intertwined	α-Glucan, proteases, metalloproteases, superoxide dismutases (phosphatases)	cutaneous, subcutaneous, respiratory tract/ pulmonary, angioinvasion

AFIP Diagnosis: Lung: Pneumonia, necrohemorrhagic, multifocal to coalescing, severe, with necrotizing vasculitis, intra-alveolar edema and fibrin, and myriad angiocentric fungal hyphae, etiology consistent with *Aspergillus* spp.

Conference Comment: The contributor provides an excellent summary of the comparative pathology and pathogenesis of aspergillosis. Like the contributor, participants noted the close association of fungal hyphae with blood vessels and the presence of angioinvasion, and thus favored the use of the term “angiocentric” in the histomorphologic diagnosis.

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CASE III: D09-30400 (AFIP 3166595).

Signalment: Adult, female, warmblood, equine (*Equus caballus*).

History: This mare presented to the referring veterinarian for increased respiratory noise. On physical examination a mass was noted in the area of the left arytenoid cartilage. This mass was surgically removed and sent for histopathologic examination. This mare had been imported to Canada from Argentina in apparently good health several years prior to examination for this problem.

Gross Pathology: The received surgical biopsy is a 2.5 cm x 1.5 cm wide, white with brown-black areas, cerebriform mass with a small elliptical base.

Laboratory Results: Polymerase chain reaction (PCR) for the 18S rRNA gene sequence of *Rhinosporidium seeberi* was performed using the formalin fixed, paraffin embedded biopsy material. The resulting amplified DNA was sequenced and the nucleotide sequence was confirmed to be identical to samples of *Rhinosporidium seeberi* present within GENBANK.

Histopathologic Description: Larynx (per contributor): From a cartilaginous base numerous papillary projections of markedly hyperplastic epithelium extend with dramatic down growths into a fibrovascular stalk. Variably sized clusters of glandular tissue are noted within focal regions at the periphery of the biopsy, occasionally exhibiting dilation with small amounts of basophilic granular material within the lumen. Multifocally throughout the submucosa are numerous variably sized, often exceeding 100 µm in diameter, spherical structures (sporangia) with a variably prominent cuticle-like lining and filled with abundant variably sized and colored endospores. Few of the sporangia are ruptured and associated with a marked neutrophilic inflammatory response. Within the fibrous tissue and dissecting between sporangia there is a marked inflammatory infiltrate composed of plasma cells, lymphocytes and fewer macrophages. Although few, sporangia are noted within 1 mm of the lateral margins. No sporangia are within the cartilage of the deep margin.

Contributor's Morphologic Diagnosis: Larynx: Laryngitis, lymphoplasmacytic, histiocytic, severe, multifocal to coalescing, chronic, with marked epithelial hyperplasia, and intralesional sporangia consistent with *Rhinosporidium seeberi*.

Contributor's Comment: Once thought to be a fungus, *Rhinosporidium seeberi* is an unusual organism now included in the class Mesomycetozoa, where it is the only member known to cause disease in mammals and birds.⁷ Other members of this class are known to cause disease in aquatic animals, primarily fish.⁷ Most commonly reported as a pathogen in tropical regions of the world, particularly India, Sri Lanka and Argentina, it has been sporadically reported in numerous locations, including Europe and North America.⁶ Typically, these cases are thought to have been acquired in endemic areas and the animals then brought to non-endemic countries, as exemplified in a group of four polo ponies in the United Kingdom thought to have been infected in their native Argentina and not diagnosed until sometime after arrival into the United Kingdom.⁵ However, both human and canine cases apparently acquired within Canada have been reported.^{2,5}

Most lesions appear as pale to white, single or multiple, polypoid masses; these are most typically found in the nasal cavity.² Cases of laryngeal rhinosporidiosis are seemingly extremely rare, although one case has been reported in a Belgian Warmblood.⁸ In horses, affected animals may present with epistaxis due to bleeding of traumatized nasal polyps, increased respiratory noise, or they may be asymptomatic.

It is presumed that the infection is most commonly acquired through exposure to contaminated water. The organism is thought to enter the body through preexisting damage to the normal mucosal barriers. Once in the body, endospores are released from the sporangia where they enlarge and mature to become juvenile, intermediate and finally mature sporangia filled with new endospores which are then available to repeat the cycle.⁷

AFIP Diagnosis: Squamous mucosa with supporting cartilage, larynx (per contributor): Laryngitis, lymphoplasmacytic and neutrophilic, proliferative and polypoid, multifocal, moderate, with fibrosis and numerous intralesional sporangia and endospores, etiology consistent with *Rhinosporidium seeberi*.

Conference Comment: As noted by the contributor, conference participants identified neutrophilic inflammation primarily in association with ruptured sporangia. The aspect of this case that makes it somewhat challenging is tissue identification, and most participants identified the specimen as nasal mucosa. Given the histomorphologic features of the tissue and lesion, and combined with the fact that nasal rhinosporidiosis is much more common and reports of laryngeal infection are exceedingly rare, nasal cavity is a reasonable conclusion as the source of the affected tissue.

Based on the histomorphology of the organism, the etiologic differential diagnosis includes *Coccidioides immitis* and *Emmonsia parva* or *E. crescens* (formerly *Chrysosporium* spp.). The organisms of *C. immitis* are typically smaller, with spherules ranging from 20-200 µm in diameter, and endospores measuring 2-5 µm and more uniform in size and shape throughout the spherule. *Emmonsia* spp. form adiaspores in tissue that are characterized as large, spherical, uninucleate conidia that measure 10-20 µm in diameter for *E. parva* and up to 300 µm for *E. crescens*. *Emmonsia* spp. have a thick wall which is PAS-positive and helps distinguish them from *C. immitis*.¹

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CASE IV: S1001225 (AFIP 3167243).

Signalment: Seven-day old, female, Quarter horse, equine (*Equus caballus*).

History: Acute onset of diarrhea, colic and death in less than 24 hours from the beginning of the clinical signs.

Gross Pathology: The carcass was in a very good state of postmortem preservation and the tail and perineal region were soiled with abundant yellow, dried feces. Diffusely, the jejunum and ileum were thickened (~3-4 mm) and had dark red, edematous mucosa with multifocal pseudomembrane formation, which was more predominant in the ileum and terminal jejunum. The serosa of the jejunum and ileum had multifocal petechiation and a few, focal, larger (up to 1 cm in diameter) subserosal ecchymoses.

The small intestinal content was composed of scant, red to brown fluid. The large colon had a focally extensive area (~50 cm long and involving the left ventral portion, the pelvic flexure and left dorsal portion of the colon) with multifocal to coalescing areas of necrosis and pseudomembrane formation. The cecum had a focal, small (~3 cm in diameter) area of dark red mucosa with mural edema adjacent to the ileocecal valve. The colonic and cecal content was composed of a moderate amount of light brown fluid.

Laboratory Results: The small intestine and colon contents were positive by the ELISA method for *Clostridium perfringens* toxins alpha and beta and *Clostridium difficile* toxins A/B. *Clostridium perfringens* type C (CPE and beta 2 negative) was isolated from the small intestine and colon and *C. difficile* was isolated only from the colon. *Salmonella* PCR and cultures were negative from small intestine.

Histopathologic Description: Jejunum, ileum and colon: There is diffuse, marked, coagulative necrosis of the mucosa, characterized by severe disruption of the architecture with diffuse mucosal hypereosinophilia, loss of the mucosal epithelial lining, attenuation or collapse of the intestinal villi and crypts, and occasional multifocal loss of the mucosa/submucosal boundaries. On the mucosal surface, admixed with superficial necrotic debris and within the pseudomembrane, there are numerous clusters of bacteria, predominantly thick and short rods. Within the lamina propria and in the submucosa, there is moderate to marked hyperemia and hemorrhage, abundant fibrin and cellular and necrotic debris with very rare neutrophils. There is multifocal vascular thrombosis of small to mid-size caliber blood vessels in the mucosa and submucosa and the submucosal lymphatics are markedly dilated. Within the muscular layer, in one section, there are a few multifocal hemorrhages. The serosa is moderately expanded by clear spaces (edema) and there is mild hemorrhage.

Contributor's Morphologic Diagnosis: Enterocolitis, necrohemorrhagic, acute, diffuse, severe, with pseudomembrane formation, mucosal/submucosal vascular thrombosis, edema and massive numbers of *Clostridia*-like bacilli attached to the mucosal surface.

Contributor's Comment: Equine enteritis and enterocolitis, manifested with diarrhea and colic, are important causes of morbidity and mortality of foals and adult horses. These syndromes have been associated with a variety of etiologies, including *Clostridium* spp., *Salmonella* spp., *Ehrlichia risticii*, *Aeromonas* spp, *Lawsonia intracellularis*, cantharidin toxicity, and larval cyathostomiasis.¹⁵ Although the first reports associating clostridia with enteritis in the foal were published in the 1930's,¹³ only in the past few decades *Clostridium perfringens* (*C. perfringens*) and *Clostridium difficile* (*C. difficile*) have been increasingly reported as relevant pathogens involved in cases of enteritis and enterocolitis in horses.^{2,3,4,8,12,15,16} *C. perfringens* is classified into five types (A, B, C, D, and E) based on the production of one or more of four so-called major toxins, namely alpha (CPA), beta (CPB), epsilon (ETX) and iota. Two other major toxins, namely enterotoxin (CPE) and Beta 2 (CPB2), can also be produced by all types of *C. perfringens*, but they are not used in the classification of this microorganism. *C. perfringens* type B and C have been associated with severe necrotizing, hemorrhagic enterocolitis in foals, although type C is considered the most commonly reported clostridial enteric pathogen in foals in North America.⁶

Clostridium perfringens type C produces two major toxins, CPA and CPB. The CPA toxin is a lecithinase, which is considered the main virulence factor in *C. perfringens* type A gas gangrene of humans and animals. However, the contribution of CPA to the virulence of type C isolates is negligible.

The CPB toxin, on the other hand, is a necrotizing toxin that forms pores in the membrane of susceptible cells. This toxin is considered to be responsible for the intestinal necrosis and systemic alterations seen in type C infections of several animal species, including horses. Lethal disease caused by *C. perfringens* type C in many mammalian animal species and humans originates when type C strains proliferate and produce toxins in the intestine.¹⁴ Because CPB is highly susceptible to the action of trypsin, neonatal animals are considered to be particularly susceptible to type C infections due to the low level of intestinal trypsin during the first days of life. Although *C. perfringens* type C causes severe intestinal damage, death in affected animals is thought to primarily result from toxemia following absorption of toxins from the intestine into the circulation.^{10,11} Therefore, type C infections are considered to be true enterotoxemias, i.e. diseases produced by toxins generated in the intestine, but that are absorbed into the general circulation and act on organs distant from the gastrointestinal tract. A presumptive diagnosis of *C. perfringens* type C enterotoxemia can be established based on clinical history, i.e. acute onset of diarrhea, colic or sudden death, and gross and microscopic lesions. This presumptive diagnosis can be reinforced by isolation of large numbers of *C. perfringens* type C from the small and/or large intestine, because this microorganism is rarely isolated from the gut of normal horses, as opposed to *C. perfringens* type A which is frequently isolated from clinically normal horses (contributor's unpublished observations). However, failure to isolate *C. perfringens* type C from the gut does not preclude a diagnosis of type C enterotoxemia because confirmation of a diagnosis of type C infection should be based on detection of CPB in intestinal contents.¹¹ Demonstration of CPB in the gut content can be achieved by *in vivo* assays in mouse and guinea pig (less common nowadays) or *in vitro* methods based on enzyme immunoassays, such as ELISA.

To date, the only published reports of enterotoxemia by *C. perfringens* type C in horses confirmed by toxin detection are limited to sporadic cases.^{3,5,7,8,9} On the other hand, a few reports have been published that describe a larger

number of animals in which a diagnosis was based on pathology and isolation of *C. perfringens* type C but without toxin detection.^{4,16} To our best knowledge, there is no published information of the pathological findings of *C. perfringens* type C enterotoxemia based on a large number of cases with a diagnosis confirmed by CPB detection in intestinal contents.

Currently, we are working on a manuscript to be submitted for publication in which the lesions of the intestinal tract in several horses with *Clostridium perfringens* type C enterotoxemia confirmed by the detection of the beta toxin in the intestinal contents are characterized. We believe that the lesions present in the submitted slides are very characteristic of, but not diagnostic for, equine type C enterotoxemia.

AFIP Diagnosis: Small intestine; and colon (per contributor): Enterocolitis, fibrinonecrotic, diffuse, severe, with transmural edema, hemorrhage, fibrin thrombi, and myriad surface bacilli.

Conference Comment: As mentioned by the contributor, *Clostridium perfringens* types A-E are distinguished from one another based on their exotoxin profiles. A summary of the toxins produced by each type follows:¹

- *C. perfringens* type A: α
- *C. perfringens* type B: α, β, ϵ
- *C. perfringens* type C: α, β
- *C. perfringens* type D: α, ϵ
- *C. perfringens* type E: α, ι

Each type produces different syndromes based on the species of animal affected.¹

	<i>C. perfringens</i>				
	Type A	Type B	Type C	Type D	Type E
Piglet	White scours		Hemorrhagic enteritis		
Cow/calf	Acute intravascular hemolysis (calf); Hemorrhagic bowel syndrome (dairy cattle)	Calf: similar to lamb	Struck-like syndrome in feedlot cattle; Hemorrhagic enteritis (calf)	Enterotoxemia (calf)	Hemorrhagic enteritis (calf)
Sheep/lamb	Acute intravascular hemolysis (lamb)	Lamb dysentery	Struck (adults); Hemorrhagic enteritis (lamb)	Enterotoxemia (pulpy kidney, overeating disease)	
Horse/foal	Necrotizing enteritis (foal)		Hemorrhagic necrotizing enteritis (foal)		
Dog	Hemorrhagic canine gastroenteritis				

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